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A Spoonful of Honey Helps Antibiotic Usage Go Down

Victoria C. Nolan[1], James Harrison[1] and Jonathan A. G. Cox[1]*

[1]School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, UK, B4 7ET

*Author to whom correspondence should be addressed:

Dr Jonathan A. G. Cox, School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, UK, B4 7ET; J.a.g.cox@aston.ac.uk; 0(+44)121 204 5011

ORCID ID: 0000-0001-5208-4056

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Abstract:

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Honey is a complex sweet food stuff with well-established antimicrobial and antioxidant properties. It has been used for millennia in a variety of applications, but those most noteworthy include treatment of surface wounds, burns and inflammation. A variety of substances in honey have been suggested as the key component to its antimicrobial potential; polyphenolic compounds, hydrogen peroxide, methylglyoxal and bee-defensin 1. These components vary greatly across honey samples due to botanical origin, geographical location and the individual bee. The use of medical grade honey, Medihoney and Revamil, in the treatment of surface wounds and burns has been seen to improve the healing process, reduce healing time, reduce scarring and prevent microbial contamination. Therefore, medical grade honeys should be used for these treatments and reduce the demand for antibiotic usage. In this review, we outline the constituents of honey and how they affect the antibiotic potential of honeys in a clinical setting.

Introduction:

Honey has been established as an effective antimicrobial and antioxidant for millennia [1]. Used mainly for the treatment of surface wounds, burns and inflammation, it has since been developed into medical treatments in the form of Medihoney and Revamil [2,3]. However, the initial interest into honey as an antimicrobial therapy was drastically diminished upon the discovery and implementation of antibiotics. The widespread use of antibiotics has resulted in the vast prevalence of antimicrobial resistance, with drugs considered as a last resort now becoming threatened. This is not the only issue, multiple drug resistant (MDR) organisms are complicating treatment of infections and some MDR organisms have acquired increased virulence and transmissibility [4]. Further to this, drug discovery rates are not meeting the

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current requirement for overcoming the threat [5]. Ultimately, we are now facing the possibility of a post-antibiotic era and novel treatments are crucial for combating infection. This has reignited the interest into honey, its potential as an antimicrobial treatment and the mechanisms behind its effectiveness [6].

For millennia honey has been used in a variety of cultures, with differing applications. The ancient Egyptians used honey as a topical ointment, a wound dressing and for embalming their dead. In ancient Greece it was used as a remedy for gout, pain, fever and wound healing [7]. The mechanism behind its effectiveness was never questioned, it was simply accepted [8]. The first initial report of honeys antimicrobial activity was by van Ketel in 1892, outlining its bactericidal effect [9]. Since then, honey has been observed to have a broad spectrum of activity, inhibiting both gram positive and gram negative organisms, including: *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneuomniae, Staphylococcus aureus, Bacillus subtilis* and *Listeria monocytogens* and their multidrug-resistant counterparts [10]. Interestingly, it has been observed that no organism has gained resistance to honey [11]. Furthermore, sub inhibitory doses of honey have been shown to restore oxacillin susceptibility in methicillin-resistant *Staphylococcus aureus* (MRSA) [12]. Initial studies into honey have outlined some key factors attributing to its antimicrobial effect, these were; high sugar content, low pH, hydrogen peroxide, polyphenolic compounds and the identification of an inhibine (Figure 1) [2,8,9]. Further studies exploring why honey is a powerful antimicrobial identified the inhibine was a 1,2-dicarbonyl compound in the form of methylglyoxal, a potent antimicrobial, found mainly in Manuka honey [13]. More recent studies have also identified a bee derived protein, bee-defensin 1, as a potential antimicrobial component within honey (Figure 1) [14]. This review aims to explore the components that attribute to honey antimicrobial activity and its potential applications.

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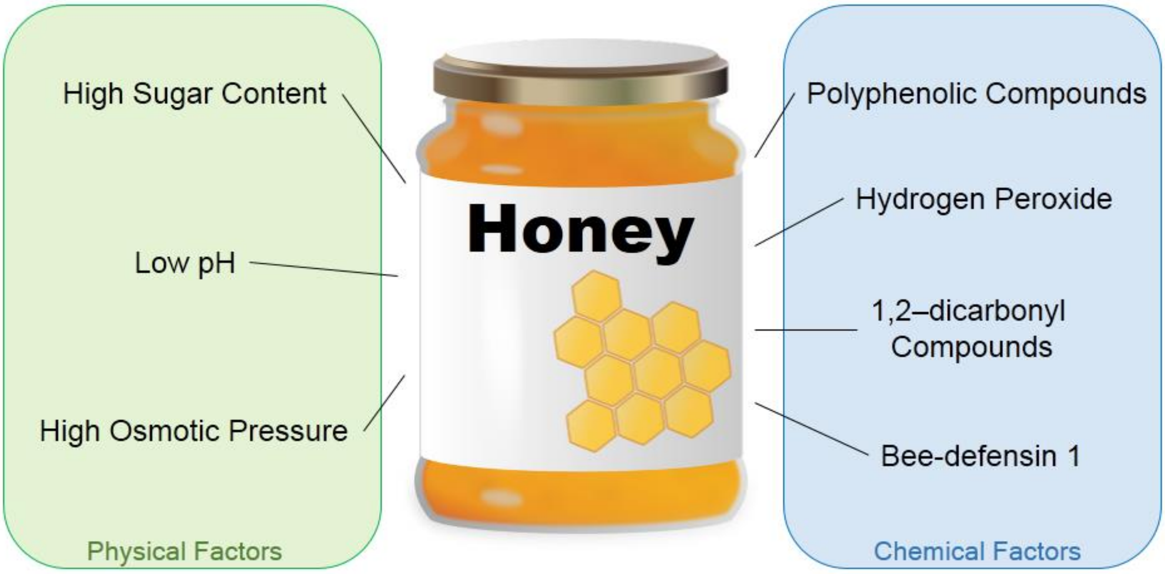

Figure 1: The main constituents attributing to honeys antimicrobial activity.

Composition and Classification:

Honey is a complex food substance, comprised of 180 to 200 different substances, including; sugar, water, proteins, vitamins, minerals, polyphenolic compounds and plant derivatives [14,15]. Depending on origin, honey can be classified as honeydew or blossom. Honeydew honey is produced by the collection of living plant, aphid and insect secretions [17]. Whereas blossom honey is produced by the collection of flower nectar and characterised by pollen content. Blossom honey can be further divided into unifloral, where the botanical origin is predominantly from one source, or multifloral, where multiple sources can be identified [18]. The botanical origin of honey can have the biggest influence on its antioxidant activity [19]. One honey that has been of great significance, due to its broad spectrum of antimicrobial activity, is Manuka honey, derived from *Leptospermum sp.* [1]. This unifloral honey is used within the pharmaceutical industry and has been developed into Medihoney. Its antimicrobial

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activity has been attributed to phytochemicals produced by the *Leptospermum sp.* plant and subsequently transferred to the honey. Recently however, honeydew honey has been investigated as a more potent antimicrobial than unifloral honey, furthering the importance of honey origin [20]. Furthermore, the composition of active compounds present within plant nectar can vary depending on geographical locations and climate conditions [19]. All of these different components can influence the quality of the honey and subsequently the antimicrobial activity.

Carbohydrates:

Carbohydrates, predominantly monosaccharides, such as glucose and fructose constitute up to 82.4% of the chemical make-up of all varieties of honey [21]. The next largest component of honey is water, ranging from 13-23% [22]. These two factors impose a stressful environment for microorganisms, as a result of low pH and high osmotic pressure, preventing food spoilage due to unsuitable growth conditions (Figure 1) [2,22]. It is considered that this unfavourable environment largely contributes to the antimicrobial activity of honey. Wahdan (1998) demonstrated that an undiluted sugar solution, mimicking the same sugar and water percentage of honey, exhibited bacteriostatic and bactericidal activity, indicating that these parameters play an important role in the antimicrobial activity of honey [23]. Conversely, Brady, Molan and Harfoot (1996) created an artificial honey, representative of sugar content and acidity, and tested it against a range of dermatophytes, pathogenic fungus that are the cause of cutaneous mycoses. They observed no inhibitory activity against any organism tested [24]. However, they did observe activity for Manuka honey, suggesting that high sugar levels and low acidity are not the sole source for antimicrobial activity. Further to this, Wahdan (1998) found significant differences between activity of the sugar solution and

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honey, indicating there are other components within honey that attribute to its antimicrobial activity [23]. Moreover, in 1937 the antimicrobial activity of honey was attributed to the presence of an 'inhibine', an unidentified component of honey that supporting sugar content alone was not responsible for the antimicrobial activity exhibited by honey [24]. Studies exploring the mechanisms behind the antimicrobial activity of honey identified a variety of other possibilities, including the presence of polyphenols, hydrogen peroxide 1,2dicarbonyls and bee-defensin 1 (Figure 1).

Polyphenolic Compounds:

Polyphenolic compounds are a diverse group of chemicals that include flavonoids and phenolic acids (non-flavonoids), defined by the presence of phenolic structures [26]. Produced as plant secondary metabolites, these bioactive compounds are transferred from the plant to the honey, and have been identified as a major component of the health-promoting properties of honey [27]. Furthermore, the phenolic acids identified in honey have been used to identify the botanical and geographical origin of a given honey sample [28]. Therefore, the botanical origin of honey is significant because it can influence the phytochemicals present, and consequently impact the antimicrobial capacity [13,28].

A variety of polyphenolic compounds have been identified in honey, examples of these can be seen in Table 1. Several polyphenolic compounds have been identified to have inhibitory effects against a variety of bacteria, these include; apigenin, quercetin, myricetin and rutin [30]. The mechanism of action varies among the compounds. Apigenin inhibits DNA gyrase, quercetin increases membrane permeability disrupting membrane potential, transport and motility, myricetin inhibits the DNA B helicase required by *E. coli* for DNA replication and rutin induces topoisomerase IV mediated DNA cleavage in *E. coli* resulting in growth

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inhibition [30–33]. Polyphenols are typically responsible for destroying free radicals and inhibiting oxidation [35].

Table 1:Common polyphenolic compounds found within honey *[25–27*,35].

Phenolic Acids	**Flavonoids**
2-*cis,A-trans* Abscisic acid	Apigenin
2-Hydroxycinnamic acid	Catechin
Caffeic acid	Chrysin
Chlorogenic acid	Galangin
Cinnamic acid	Genistein
Ellagic acid	Isorhamnetin
Ferulic acid	Kaempferol
Gallic acid	Luteolin
p-Courmaric acid	Myricetin
p-Hydroxybenzoic acid	Naringenin
Protocatechuic acid	Pinobanksin
Sinapic acid	Pinocembrin
Syringic acid	Quercetin
Vannilic acid	Rutin

Hydrogen Peroxide:

The presence of hydrogen peroxide within honey has been well established and is considered one of the main antimicrobial constituents in honey. It is produced as a by-product during nectar harvest by the honey bee (*Apis mellifera*). Upon harvest, bee derived enzymes are added, including diastase, invertase and glucose oxidase. The diastase and invertase breakdown the larger disaccharides, mainly sucrose, into monosaccharides, glucose and fructose [37]. The glucose oxidase then catalyses the oxidation of glucose, resulting in

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hydrogen peroxide production (Figure 2) [38]. Interestingly, the antimicrobial effect of hydrogen peroxide in honey increases upon dilution, this enables the glucose oxidase enzyme to bind to glucose more readily, resulting in a continuous production of hydrogen peroxide [39]. The levels of hydrogen peroxide in honey vary between samples and are dependent on two factors, the amount of glucose oxidase added and the presence of pollen derived catalase [40]. Since glucose oxidase catalyses the reaction, higher levels of glucose oxidase result in more hydrogen peroxide production. This can be influenced by honey bee health and diversity of foraged diet [41]. Additionally, catalase is known for the breakdown of hydrogen peroxide into water and oxygen, therefore, it is of no surprise that the lower the amount of catalase, the higher the hydrogen peroxide content [40].

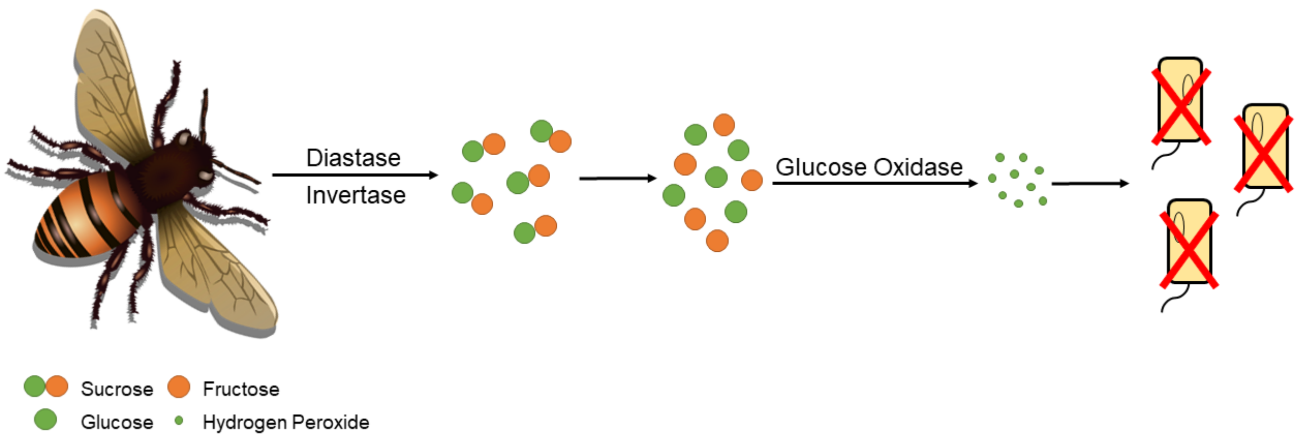

Figure 2: The production of hydrogen peroxide in honey. As nectar is harvested by the honey bee (*Apis mellifera*) several enzymes are added via the salivary glands; diastase, invertase and glucose oxidase. Diastase and invertase convert large saccharides into monosaccharides, the glucose oxidase then converts glucose into hydrogen peroxide. Hydrogen peroxide is then available to kill bacteria.

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Hydrogen peroxide is a well-established antimicrobial. Classed as an oxidative biocide, it removes electrons from chemical structures resulting in oxidation [42]. The oxidation action causes inhibition of microbial growth and irreversible DNA damage through generation of hydroxyl radicals [3,42]. Hydrogen peroxide levels within honey can range between 0.5 mM and 2.5 mM, however a minimum level of 2.7 mM hydrogen peroxide is required to cause DNA degradation in *E. coli* [38]. Regardless of this, honey containing less than 2.5 mM hydrogen peroxide can exhibit the ability to induce DNA degradation in bacteria, suggesting hydrogen peroxide is not the only antimicrobial component of honey. Furthermore, Manuka honey maintains DNA degradation after removal of hydrogen peroxide and exhibits no change in antimicrobial activity [13,37].

1,2-dicarbonyls:

Antimicrobial activity observed in honey that contains reduced hydrogen peroxide, or still exhibits activity after removal of hydrogen peroxide, have been defined as non- peroxide activity. Non-peroxide activity has been attributed to a variety of different substances, one of which is a group of compounds known as 1,2-dicarbonyls. The 1,2-dicarbonyls are highly reactive compounds, generated in carbohydrate rich foods through caramelization or Maillard reactions [44]. These are achieved through heat treatment or prolonged storage and are associated with aroma, colour and taste [45]. 1,2-dicarbonyls are formed as an intermediate of a non-enzymatic reaction with glucose and free amino groups resulting in the formation of advanced glycation end products (AGEs) [46]. Those formed by hexoses include 3-deoxyglucosone (3-DG) and glucosone, formation by disaccharides and olidosaccharides result in 3-deoxypentosone (3-DP) [44]. Breakdown products of 3-DG result in generation of

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5-hydroxymethalfurfural, indicating honey freshness [10]. Other breakdown products of more antimicrobial significance are methylglyoxal and glyoxal.

Methylglyoxal (MGO) has been identified as the main antimicrobial component of Manuka honey [47]. The presence of MGO in Manuka honey is determined by the concentration of dihydroxyacetone. Adams, Manley-Harris and Molan (2009) identified that all nectar collected from *Leptospermum sp.* contains varying levels of dihydroxyacetone and no measurable MGO [47]. To further investigate this, they added dihydroxyacetone to clover honey and observed production of MGO. Furthermore, addition of arginine and lysine resulted in greater production of MGO, consistent with findings that the non-enzymatic production of MGO requires these amino acids [49]. Within the hive, low amounts of MGO can be detected, but high levels of dihydroxyacetone are present. Once harvested the conversion of dihydroxyacetone into MGO takes place, resulting in increased MGO levels and a reduction of dihydroxyacetone [50]. Interestingly, heating of the honey to 37 °C results in increased MGO, however the heating to 50 °C causes a loss of both MGO and dihydroxyacetone [48]. Additionally, the MGO content of Manuka honey has been directly correlated to the 'Unique Manuka Factor' (UMF) rating, indicating this is the main antimicrobial component of Manuka honey [51].

Bee-defensin-1:

Bee-defensin 1 is an antimicrobial peptide (AMP) identified in bee hemolymph, the bee blood system, and hypopharyngeal glands [6]. It is one of four AMPs, others include apidaecin, abaecin, hymenoptaecin and defensin [52]. Their role within the bee is as an innate immune response, exhibiting activity against fungi, yeast, protozoa and both gram positive and gram negative bacteria [53]. Importantly, bee-defensin 1 is mainly effective against gram

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positive bacteria, most notably *B. subtilis, S. aureus* and *Paenibacillus larvae*, however it has limited effectiveness against multidrug resistant organisms [54]. Levels of bee-defensin 1 vary drastically between honey samples, this is as a result of the production from glands of individual bees whose production of AMPs varies [55]. The mechanism behind defensins is their ability to disrupt the integrity and permeability of membranes, resulting in the inhibition of synthesis of DNA, RNA and proteins [56].

Honey in Medical Settings:

The main applications of honey within a medical setting are for treatment of surface wounds and burns. Two distinct types of honey have been developed into medical grade honey, these are Medihoney and Revamil. Medihoney is developed from Manuka honey, whereas Revamil honey is produced in greenhouses under standardised conditions [55]. Interestingly, the active components of these two honeys differ. The Medihoney activity is based on MGO activity, where hydrogen peroxide activity is variable and there is an absence of bee-defensin 1 [57]. However Revamil acts primarily through hydrogen peroxide and bee-defensin 1 activity [58]. The honey can be applied directly to the surface of a wound. This provides a physical barrier between the wound and the environment, preventing contamination [59]. Secondary effects provided by application are the antimicrobial properties, including both bacteriostatic and bactericidal activity, further preventing wound contamination [55]. Additionally, an osmotic gradient is generated due to the high sugar content and low water activity, generating a flow of bacteria, necrotic tissue and debris out of the wound [60]. Finally, the phenolic content in honey aids in inflammation, helping to improve wound healing [61]. Overall, this has been observed to improve both the healing of the wound, time taken to heal and reduce scarring [62]. This can reduce the use of antibiotics, while still aiding wound treatment.

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Conclusion:

Honey is a potent antimicrobial, exhibiting a broad spectrum of activity. A variety of components attribute to the antimicrobial potential of honey, including sugar content, polyphenolic compounds, hydrogen peroxide, 1,2-dicarbonyl compounds and bee-defensin 1. All of these are present in varying levels depending on nectar source, honey bee and storage. Within a medical setting honey can be used as an effective wound treatment, removing the need for antibiotics. Honey has the potential to vastly reduce the requirement of drugs of last resort for highly drug resistant bacterial infections, since the current resistance to antimicrobial mechanisms of honey are largely unseen. This is likely due to multiple modes of action of honey resulting in a unique combination therapy, which does not allow such swift adaptation of bacteria to become resistant, as is the case with other antimicrobial methods. As the authors of this review, we feel that the use of honey will likely be expanded in the future. This is largely due to the rise in MDR organisms causing infections that are largely untreatable by numerous antibiotics, particularly since honey has been shown to be capable of reversing mechanisms of antibiotic resistance [12]. Therefore, the revival of the use of honey as an antimicrobial agent represents a promising therapeutic avenue to help curb the increasingly worrying incidence of antibiotic resistant bacterial infections.

Author Contributions:

V.C.N., J.H. and J.A.G.C. reviewed the literature and wrote the manuscript.

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Conflicts of Interest:

The authors declare no conflict of interest.

References:

1. Cokcetin, N.N., *et al.*, “The antibacterial activity of Australian Leptospermum honey correlates with methylglyoxal levels,” *PLoS One*, 2016, vol. 11, no. 12, pp. 1–13.

2. Albaridi, N.A., “Antibacterial Potency of Honey,” *Int. J. Microbiol.*, 2019, vol. 2019.

3. Saranraj, P., Sivasakthi, S., “Comprehensive Review on Honey: Biochemical and Medicinal Properties,” *J. Acad. Ind. Res.*, 2018, vol. 6, no. 10, pp. 165–181.

4. Davies, J., Davies, D., “Origins and Evolution of Antibiotic Resistance,” *Mol. Biol. Rev. Microbiol. Mol. Biol. Rev*, 2010, vol. 74, no. 3, pp. 417–433.

5. Simpkin, V. L., Renwick, M. J., Kelly, R., Mossialos, E., “Incentivising innovation in antibiotic drug discovery and development: Progress, challenges and next steps,” *J. Antibiot. (Tokyo).*, 2017, vol. 70, no. 12, pp. 1087–1096.

6. McLoone, P., Warnock, M., Fyfe, L., “Honey: A realistic antimicrobial for disorders of the skin,” *J. Microbiol. Immunol. Infect.*, 2016, vol. 49, no. 2, pp. 161–167.

7. Eteraf-Oskouei, T., Najafi, M., “Traditional and modern uses of natural honey in human diseases: A review,” *Iran. J. Basic Med. Sci.*, 2013, vol. 16, no. 6, pp. 731–742.

8. Molan, P. C., “The Antibacterial Activity of Honey,” *Bee World*, 1992, pp. 5–27.

Peer-reviewed version available at *Antibiotics* **2019**, *8*, 251; doi:10.3390/antibiotics8040251

9. Dustmann, J. H., “Antibacterial Effect of Honey,” *Apiacta*, 1979, vol. 1, no. 1, pp. 1–4.

10. Laallam, H., *et al.*, “Modeling the synergistic antibacterial effects of honey characteristics of different botanical origins from the Sahara Desert of Algeria,” *Front. Microbiol.*, 2015, vol. 6, no. NOV, pp. 1–12.

11. Maddocks, S. E., Jenkins, R. E., “Honey: A sweet solution to the growing problem of antimicrobial resistance?,” *Future Microbiol.*, 2013, vol. 8, no. 11, pp. 1419–1429.

12. Jenkins, R. E., Cooper, R., “Synergy between oxacillin and manuka honey Sensitizes methicillin-resistant Staphylococcus aureus to oxacillin,” *J. Antimicrob. Chemother.*, 2012, vol. 67, no. 6, pp. 1405–1407.

13. Mavric, E., Wittmann, S., Barth, G., Henle, T., “Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (Leptospermum scoparium) honeys from New Zealand,” *Mol. Nutr. Food Res.*, 2008, vol. 52, pp. 483–489.

14. Bucekova, M., *et al.*, “Antibacertial Activity of Different Blossom Honeys: New Findings,” *Molecules*, 2019, vol. 24, no. 8, pp. 1–20.

15. El Sohaimy, S. A., Masry, S. H. D., Shehata, M. G., “Physicochemical characteristics of honey from different origins,” *Ann. Agric. Sci.*, 2015, vol. 60, no. 2, pp. 279–287.

16. Da Silva, P. M., Gauche, C., Gonzaga, L. V., Costa, A. C. O., Fett, R., “Honey: Chemical composition, stability and authenticity,” *Food Chem.*, 2016, vol. 196, pp. 309–323.

17. Vasić, V., *et al.*, “Two aspects of honeydew honey authenticity : Application of advance analytical methods and chemometrics,” *Food Chem.*, 2020, vol. 305, no. September 2019, pp. 1–9.

18. Manivanan, P., Rajagopalan, S. M., Subbarayalu, M., “Studies on authentication of true source of honey using pollen DNA barcoding,” *J. Entomol. Zool. Stud.*, 2018, vol.

Peer-reviewed version available at *Antibiotics* **2019**, *8*, 251; doi:10.3390/antibiotics8040251

6, no. 3, pp. 255–261.

19. Silici, S., Sagdic, O., Ekici, L., "Total phenolic content, antiradical, antioxidant and antimicrobial activities of Rhododendron honeys," *Food Chem.*, 2010, vol. 121, no. 1, pp. 238–243.

20. Pita-Calvo, C., Vázquez, M., "Differences between honeydew and blossom honeys: A review," *Trends Food Sci. Technol.*, 2017, vol. 59, pp. 79–87.

21. Almasaudi, S. B., *et al.*, "Antimicrobial effect of different types of honey on Staphylococcus aureus," *Saudi J. Biol. Sci.*, 2017, vol. 24, no. 6, pp. 1255–1261.

22. Chirife, J., Zamora, M. C., Motto, A., "The correlation between water activity and % moisture in honey: Fundamental aspects and application to Argentine honeys," *J. Food Eng.*, 2006, vol. 72, no. 3, pp. 287–292.

23. Mandal, M. D., Mandal, S., "Honey: Its medicinal property and antibacterial activity," *Asian Pac. J. Trop. Biomed.*, 2011, vol. 1, no. 2, pp. 154–160.

24. Wahdan, H. A. L., "Causes of the antimicrobial activity of honey," *Infection*, 1998, vol. 26, no. 1, pp. 26–31.

25. Brady, N. F., Molan, P. C., Harfoot, C. G., "The sensitivity of dermatophytes to the antimicrobial activity of manuka honey and other honey," *Pharm. Sci.*, 1996, vol. 2, no. 10, pp. 471–473.

26. Cianciosi, D., *et al.*, "Phenolic compounds in honey and their associated health benefits: A review," *Molecules*, 2018, vol. 23, no. 9, pp. 1–20.

27. Güneş, M. E., Şahin, S., Demir, C., Borum, E., Tosunoğlu, A., "Determination of phenolic compounds profile in chestnut and floral honeys and their antioxidant and antimicrobial activities," *J. Food Biochem.*, 2017, vol. 41, no. 3, pp. 1–12.

28. Escuredo, O., Silva, L. R., Valentão, P., Seijo, M. C., Andrade, P. B., "Assessing Rubus honey value: Pollen and phenolic compounds content and antibacterial

Peer-reviewed version available at *Antibiotics* **2019**, *8*, 251; doi:10.3390/antibiotics8040251

capacity," *Food Chem.*, 2012, vol. 130, no. 3, pp. 671–678.

29. Fyfe, L., Okoro, P., Paterson, E., Coyle, S., Mcdougall, G. J., "Compositional analysis of Scottish honeys with antimicrobial activity against antibiotic-resistant bacteria reveals novel antimicrobial components," *LWT - Food Sci. Technol.*, 2017, vol. 79, pp. 52-59.

30. Das, A., Datta, S., Mukherjee, S., Bose, S., Ghosh, S., Dhar, P., "Evaluation of antioxidative, antibacterial and probiotic growth stimulatory activities of Sesamum indicum honey containing phenolic compounds and lignans," *LWT - Food Sci. Technol.*, 2015, vol. 61, no. 1, pp. 244–250.

31. Bernard, F. X., *et al.*, "Glycosylated flavones as selective inhibitors of topoisomerase IV," *Antimicrob. Agents Chemother.*, 1997, vol. 41, no. 5, pp. 992–998.

32. Griep, M. A., Blood, S., Larson, M. A., Koepsell, S. A., Hinrichs, S. H., "Myricetin inhibits Escherichia coli DnaB helicase but not primase," *Bioorganic Med. Chem.*, 2007, vol. 15, no. 22, pp. 7203–7208.

33. Mirzoeva, O. K., Grishanin, R. N., Calder, P. C., "Antimicrobial action of propolis and some of its components: The effects on growth, membrane potential and motility of bacteria," *Microbiol. Res.*, 1997, vol. 152, no. 3, pp. 239–246.

34. Ohemeng, K. A., Schwender, C. F., Fu, K. P., Barrett, J. F. "DNA gyrase inhibitory and antibacterial activity of some flavones(1)," *Bioorganic Med. Chem. Lett.*, 1993, vol. 3, no. 2, pp. 225–230.

35. Jibril, F. I., Hilmi, A. B. M., Manivannan, L., "Isolation and characterization of polyphenols in natural honey for the treatment of human diseases," *Bull. Natl. Res. Cent.*, 2019, vol. 43, no. 1.

36. Estevinho, L., Pereira, A. P., Moreira, L., Dias, L. G., Pereira, E., "Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey,"

Peer-reviewed version available at *Antibiotics* **2019**, *8*, 251; doi:10.3390/antibiotics8040251

Food Chem. Toxicol., 2008, vol. 46, no. 12, pp. 3774–3779.

37. Ball, D. W., "The chemical composition of honey," *J. Chem. Educ.*, 2007, vol. 84, no. 10, pp. 1643–1646.

38. Brudzynski, K., Abubaker, K., St-Martin, L., Castle, A., "Re-examining the role of hydrogen peroxide in bacteriostatic and bactericidal activities of honey," *Front. Microbiol.*, 2011, vol. 2, no. OCT, pp. 1–9.

39. Brudzynski, K., "Effect of hydrogen peroxide on antibacterial activities of Canadian honeys.," *Can. J. Microbiol.*, 2006, vol. 52, no. 12, pp. 1228–1237.

40. Taormina, P. J., Niemira, B. A., Beuchat, L. R., "Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power," *Int. J. Food Microbiol.*, 2001, vol. 69, no. 3, pp. 217–225.

41. Weston, R. J., "The contribution of catalase and other natural products to the antibacterial activity of honey: A review," *Food Chem.*, 2000, vol. 71, no. 2, pp. 235–239.

42. Finnegan, M., Linley, E., Denyer, S. P., McDonnell, G., Simons, C., Maillard, J. Y., "Mode of action of hydrogen peroxide and other oxidizing agents: Differences between liquid and gas forms," *J. Antimicrob. Chemother.*, 2010, vol. 65, no. 10, pp. 2108–2115.

43. Schurman, J. J., Sumner, S. S., Marcy, J. E., "Antibacterial Activity of Hydrogen Peroxide Against Escherichia Coli O157:H7 and Salmonella spp. in Fruit Juices, Both Alone and in Combination with Organic Acids," 2001, Master of Science, Virginia Polytechnic, Blacksburg Virginia, July 18 2001.

44. Degen, J., Hellwig, M., Henle, T., "1,2-Dicarbonyl compounds in commonly consumed foods," *J. Agric. Food Chem.*, 2012, vol. 60, no. 28, pp. 7071–7079.

45. Arena, E., Ballistreri, G., Tomaselli, F., Fallico, B., "Survey of 1,2-Dicarbonyl

Peer-reviewed version available at *Antibiotics* **2019**, *8*, 251; doi:10.3390/antibiotics8040251

Compounds in Commercial Honey of Different Floral Origin," *J. Food Sci.*, 2011, vol. 76, no. 8,.

46. Schalkwijk, C. G., Posthuma, N., Ten Brink, H. J., Ter Wee, P. M., Teerlink, T., "Induction of 1,2-dicarbonyl compounds, intermediates in the formation of advanced glycation end-products, during heat-sterilization of glucose-based peritoneal dialysis fluids," *Perit. Dial. Int.*, 1999, vol. 19, no. 4, pp. 325–333.

47. Roberts, A., Jenkins, R., Brown, H. L., "On the antibacterial effects of manuka honey: mechanistic insights," *Res. Rep. Biol.*, 2015, vol 6, p. 215-224.

48. Adams, C. J., Manley-Harris, M., Molan, P. C., "The origin of methylglyoxal in New Zealand manuka (Leptospermum scoparium) honey," *Carbohydr. Res.*, 2009, vol. 344, no. 8, pp. 1050–1053.

49. Majtan, J., *et al.*, "Methylglyoxal-induced modifications of significant honeybee proteinous components in manuka honey: Possible therapeutic implications," *Fitoterapia*, 2012, vol. 83, no. 4, pp. 671–677.

50. Grainger, M. N. C., Manley-Harris, M., Lane, J. R., Field, R. J., "Kinetics of the conversion of dihydroxyacetone to methylglyoxal in New Zealand manuka honey : Part II – Model systems," *Food Chem.*, 2016, vol. 202, pp. 492–499.

51. Johnston, M., Mcbride, M., Dahiya, D., Owusu-Apenten, R., "Antibacterial activity of Manuka honey and its components : An overview," *AIMS Microbiol.*, 2018, vol. 4, no. 4, pp. 655–664.

52. R. A. Ilyasov, L. R. Gaifullina, E. S. Saltykova, A. V. Poskryakov, and A. G. Nikolenko, "Review of the experssion of antimicrobial peptide defensin in honey bees *Apis mellifera*," *J. Apic. Sci.*, 2012, vol. 56, no. 1, pp. 115–124.

53. Oryan, A., Alemzadeh, E., Moshiri, A., "Biological properties and therapeutic activities of honey in wound healing: A narrative review and meta-analysis," *J. Tissue*

Peer-reviewed version available at *Antibiotics* **2019**, *8*, 251; doi:10.3390/antibiotics8040251

Viability, 2016, vol. 25, no. 2, pp. 98–118.

54. Kwakman, P. H. S., Zaat, S. A. J., "Antibacterial components of honey," *IUBMB Life*, 2012, vol. 64, no. 1, pp. 48–55.

55. Kwakman, P. H. S., te Velde, A. A., de Boer, L. , Vandenbroucke-Grauls, C. M. J. E., Zaat, S. A. J., "Two major medicinal honeys have different mechanisms of bactericidal activity," *PLoS One*, 2011, vol. 6, no. 3, pp. 3–9.

56. Ganz, T., "Defensins: Antimicrobial peptides of innate immunity," *Nat. Rev. Immunol.*, 2003, vol. 3, pp. 710–720.

57. Kwakman, P. H. S., Velde, A. A., Boer, L., Speijer, D., Vandenbrouke-Grauls, C. M. J. E., Zaat, S. A. J., "How honey kills bacteria," *FASEB J.*, 2010, vol. 24, pp. 2576–2582.

58. Carter, D. A., *et al.*, "Therapeutic manuka honey: No longer so alternative," *Front. Microbiol.*, 2016, vol. 7, no. APR, pp. 1–11.

59. Molan, P. C., "Re-introducing honey in the management of wounds and ulcers - theory and practice.," *Ostomy. Wound. Manage.*, 2002, vol. 48, no. 11, pp. 28–40.

60. Minden-Birkenmaier, B. A., Bowlin, G. L., "Honey-based templates in wound healing and tissue engineering," *Bioengineering*, 2018, vol. 5, no. 2.

61. Samarghandian, S., Farkhondeh, T., Samini, F., "Honey and health: A review of recent clinical research," *Pharmacognosy Res.*, 2017, vol. 9, no. 2, pp. 121–127.

62. Molan, P. C., "The evidence supporting the use of honey as a wound dressing," *Int. J. Low. Extrem. Wounds*, 2006, vol. 5, no. 1, pp. 40–54.