Studies on the Antimicrobial Activity of Honey - A Review

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Abstract

The biological activity of important honey, one of the most characteristics, which distinguishes it from other natural products and makes it important for the medical and therapeutic field. Its potential health benefits can be influenced both by its composition, which varies between different types of honey, and by the bioavailability of various phytochemical components and how they are absorbed and metabolized. It was found that a series of compounds contained in honey such as phenolic compounds, especially flavonoids, have an inhibitory effect against a wide spectrum of bacterial species, Gram-positive (G+) and Gram-negative (G-), but also an important antifungal and antiviral action. These important properties of honey are due both to the quality of the polyphenols contained, which is more important than their quantity, but also to the high osmolarity, acidity, H_2O_2 content and the composition of non-peroxidic components.

Keywords: honey, antibacterial, antifungal, antiviral activity.

1. Introduction

Beekeeping has seen increasing interest in recent years, both as a science and as an art, due to the many advantages related to bee products. Throughout history, people have widely consumed honey, the benefits of this product being recognized and recorded in the ancient world, in India, Egypt and Greece, it being used both as a food and for therapeutic purposes, in the treatment of various ailments [1]. Honey is a miraculous product of nature, due to its content in important bioactive compounds, such as polyphenols. Despite their variability in the chemical composition of honey, the most important polyphenols are flavonoids, such as apigenin, quercetin, chrysin, luteolin, kaempferol, galangin, genistein, pinobanksin and pinocembrin, and phenolic acids represented by gallic acid, chlorogenic acid, syringic acid , p-coumaric acid, caffeic acid, vanillic acid and p-hydroxybenzoic acid [2], respectively vitamins (vitamin C), enzymes, sugars and, to a lesser extent, volatile compounds. These compounds show important medical benefits such as anti-inflammatory action [3], antioxidant [4], antimicrobial, prebiotic [5; 6] and immunostimulators [7]. It has been observed that the quality of honey and its therapeutic properties depend on its chemical composition which, in turn, is dependent on the geographical area, the floristic composition, the season, environmental factors but also the beekeepers' practices such as processing methods, handling and storage conditions. Currently, a number of compounds present in honey, especially

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polyphenols, are of particular interest for medical and food research, mainly due to their functional properties. In addition to being considered to be strong agents of peroxide radicals, mainly due to the presence of high mobility of hydrogens in their molecular structures [8], polyphenols are both effective immune modulators, but it also exhibits important antibacterial, antifungal, antiviral and antiprotozoal properties [8-11]. Numerous studies point to the fact that honey, particularly Manuka honey, exhibits significant antibacterial and antibiofilm activity against a wide range of Gram-positive and Gram-negative bacteria, including; *Streptococcus pyogenes, Mycobacterium, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi, Salmonella enterocolitis, Shigella dysenteriae, Pseudomonas aeruginosa, Mycobacterium tuberculosis, Staphylococcus acuptococcus, Streptococcus, Methicillin-resistant Streptococcus acuptococcus* [12-18]. The strong antibacterial properties of honey are related to its high osmolarity, acidity, content in H_2O_2 and composition of non-peroxidic components [19]. On the other hand, the antibacterial capacity of honey is also linked to an improvement of the intestinal microbial balance due to the large number of contained oligosaccharides, which function as a substrate for the growth of prebiotic microorganisms, especially of the genera *Lactobacillus* and *Bifidobacterium* [20]. Also, numerous studies have highlighted the antiviral action of honey. Shahzad, A., [21] evaluated in vitro, the effect of Manuka honey and clover honey on varicella-zoster virus-infected human malignant melanoma (MeWo) cells, and the results showed reduction of viral plaques under treatment with both types of honey. A similar study revealed the anti-HIV-1 RT activity of Manuka honey, an effect that was associated with its constituents, 2-methoxybenzoic acid (2-MBA) and methylglyoxal (MGO), which inhibited HIV-1 RT activity in a dose-dependent manner [22]. In addition, studies have shown that honey also has an important antifungal activity against Candida infection (*Candida albicans, Candida glabrata, Candida dubliniensis, Candida tropicalis, Candida krusei and Candida parapsilosis*) and *Rhodotorula* sp. [23,24]. Research by Guttentag, A. et al., [25] indicated that jarrah honey has unique antifungal attributes, inhibiting and killing dermatophyte infection, making it a potentially promising candidate for the treatment of tinea [25].

Currently, the installation of the antibiotic resistance process requires the investigation of effective therapeutic solutions, regarding the action of some microorganisms. For these reasons, worldwide, there is a major concern of researchers and industry, regarding the use of the therapeutic potential of natural compounds, implicitly those found in different types of honey, for the development of effective treatment schemes, without adverse effects, cheap and ecological.

2. Antibacterial, antifungal and antiviral activity of honey

Currently, worldwide, antibiotic resistance is a serious problem, this aspect determining the need to search for new, natural antimicrobial compounds [26,27]. Numerous in vitro studies have shown the antimicrobial activity of different types of honey against a wide range of skin-colonizing bacteria and food-borne bacterial species, including antibiotic-resistant bacteria [28-30]. Also, in vivo studies have demonstrated that honey has beneficial actions against wound infections [31] so that the practice of using honey in the treatment of wounds is gaining popularity in modern medicine, as a result of its function its antimicrobial properties [32]. Moreover, certain types of honey exhibit broad-spectrum antimicrobial activity, having an important role against antibiotic-resistant bacterial pathogens [33-36]. Floral sources are responsible for differences in the type and level of antimicrobial activity [37,38]. Julie, I., et al., [39] found that honey has clinical potential and exhibits a wide range of antibacterial activities with possible therapeutic uses. The antibacterial action was mainly due to the hydrogen peroxide formed by the enzyme glucose-oxidase, secreted by the bees. The antibacterial activity of honey is largely dependent on its peroxidic activity and nonperoxidic mechanisms [40]. There is evidence that honey had broad-spectrum activity against gram-positive and gram-negative bacteria [41].

Honey is a natural product used for its anti-fungal properties, and this activity is influenced by many factors. Honey varieties from different phyto-geographical areas have been found to vary in their ability to inhibit the growth of fungal yeasts,

suggesting that the botanical origin of honey varieties influences fungal activity.

Flavonoids such as chrysin and kaempferol, present in different types of honey [42], are very active in inhibiting the replication of several herpes viruses, adenoviruses and rotaviruses [43]. A series of studies showed that quercetin and rutin exerted antiviral activity against HSV (*Herpes Simplex Virus*), syncytial virus, poliovirus and Sindbis virus [44]. These compounds exert their action by inhibiting viral polymerase and binding to viral nucleic acids or viral capsid proteins [44]. Also, Chen S, et al., [45] demonstrated the involvement of honey flavonoids, such as chrysin, acacetin, and apigenin, in inhibiting the activation of latent human immunodeficiency virus-1 (HIV-1) through a mechanism that likely includes inhibition of viral transcription.

3. The mechanism of antimicrobial activity of honey

The antimicrobial activity of honey is an extremely complex process, determined by many of its physico-chemical properties [46,13], such as low pH, high osmolarity, hydrogen peroxide [47,48], defensin – 1 and polyphenols [29]. Most often honey is acidic (pH below 7), the pH of flower honey varying between 3.03 and 5.5 – 6.0 [49,50], depending on the botanical source, nectar pH, soil or plant association, and the concentration of various acids and minerals such as calcium, sodium, potassium, and other ash constituents. In general, the lower the pH value of honey, the higher the activity of inhibiting the activity of microorganisms [13].

The high concentration of sugars (approximately 80% of the weight of this product) is an important factor in the elimination of microorganisms, mainly bacteria sensitive to high osmotic pressure Defensin-1, a peptide secreted by the hypopharyngeal glands of bees, exhibits activity against Gram-positive bacteria, including *Staphylococcus aureus, Bacillus subtilis, and Paenibacillus*. This protein could be used to treat infections and in the development of new drugs that could fight antibiotic-resistant bacteria [51].

Another constituent of honey, with a strong antibacterial effect, is the glucose-oxidase enzyme, the oxidoreductase that catalyzes the oxidation of glucose into gluconic acid. The byproduct of this reaction, hydrogen peroxide (H_2O_2), is a powerful antimicrobial agent. The concentration of hydrogen peroxide varies, however, depending on the floral source and geographical area [52], respectively environmental conditions, and these differences are reflected in the value of the minimum inhibitory concentration (MIC) required for to inhibit various bacteria (<3% - ≥ 50%) [53]. Recent studies [54] have shown that H_2O_2 is the main compound involved in conferring the antibacterial effect of European honey. However, some studies showed that the level of H_2O_2 in different types of honey did not directly correlate with antibacterial activity [51]. More importantly, through the degradation of H_2O_2 in various honeys, hydroxyl radicals are generated, involved in the generation of oxidative stress in bacterial cells [55]. In addition, the phenolic compounds or polyphenols in the structure of honey can contribute to its antimicrobial activity [56]. They represent a chemically heterogeneous group, containing approximately 10,000 compounds, which include phenolic acids and flavonoids, secondary metabolites, derived from plants [57]. Phenolic compounds such as quercetin, caffeic acids, caffeic acid phenethyl ester (CAPE), acacetin, kaempherol, galangin, chrysina, pinocembrin, pinobanksin apigenin have been detected in honey, which have a promising effect in the treatment of chronic diseases [58]. Phenolic compounds have an antioxidant effect, acting by eliminating free radicals and inhibiting lipid oxidation [59], but many researches have demonstrated that they are also responsible for the antimicrobial properties of honey. Through the hydrolysis of H_2O_2, oxygen is generated, which can accelerate the auto-oxidation process of polyphenols in honey. These, in turn, becoming pro-oxidant agents, can lead to the generation of other H_2O_2 molecules, which, in the presence of transition metals, can generate hydroxyl radicals [60]. In this way, polyphenolic compounds can significantly contribute to the modulation of honey's antibacterial effects. However, the observed differences could also be caused by differences in the activity or concentration of glucose oxidase or the concentration in defensin-1, an aspect that has not been investigated so far. The results of these studies also revealed that the concentrations of individual constituents are too low to contribute substantially to the antimicrobial activity of the product. It is possible, however, that

a combination of different ingredients, for example, phenolic compounds, could contribute significantly to the activity of honey [61]. A fairly satisfactory activity of the composition of phenolic compounds extracted from several types of honey, Malaysian and Polish, was observed by Aljadi and Yusoff [62], respectively by Mazol et al. [63].

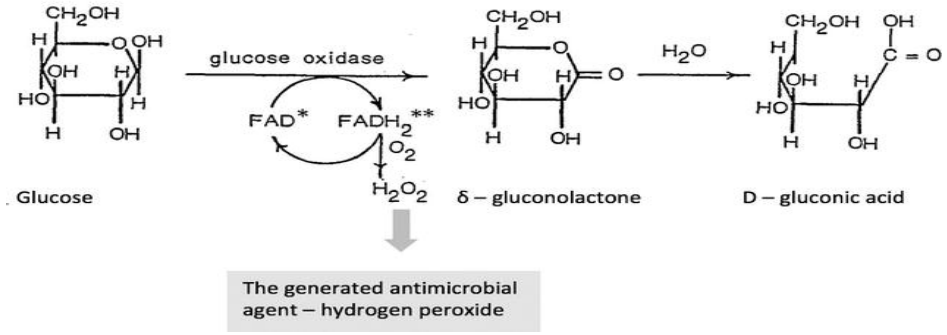

Figure 1. Glucose oxidase catalysis reaction – generation of hydrogen peroxide [64]

Interesting results in this field were presented by Mundo et al [65], who observed a non-peroxide activity against *Bacillus stearothermopnilus* in most of the 27 honey samples diluted in water containing catalase (the enzyme that degrades hydrogen peroxide). In contrast to Bacillus, neutralization of H_2O_2 with catalase resulted in loss of activity against *S. aureus* in all honey samples tested. This result could suggest the presence of non-protein components in these types of honey, which were responsible for the inhibition of *Bacillus* growth in honey suspensions not containing hydrogen peroxide [65]. The presence of antimicrobial components (combination of cationic and non-cationic substances) other than methylglyoxal, glucose oxidase and defensin-1 in Manuka honey was confirmed in studies by Kwakman et al [29].

4. Conclusions

Numerous in vitro and in vivo studies have demonstrated that some compounds contained in honey such as methylglyoxal, glucose oxidase and defensin-1 show important antimicrobial activity. Thanks to these compounds, honey can be used in the antibacterial prophylaxis of numerous bacterial species such as *Streptococcus pyogenes, Mycobacterium, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi, Salmonella enterocolitis, Shigella dysenteriae, Pseudomonas aeruginosa, Mycobacterium tuberculosis, Staphylococcus acuptococcus, Streptococcus aureus , Streptococcus acuptococcus Staphylococcus aureus, Bacillus subtilis, and Paenibacillus.* In the pus, a number of other compounds such as chrysin, kaempferol, quercetin and rutin, present in different types of honey exert an important antiviral activity, by inhibiting the replication of several herpes viruses, adenoviruses and rotaviruses, by inhibiting viral polymerase and binding to nucleic acids viral or viral capsid proteins. The antimicrobial activities of honey against certain types of microorganisms, as well as its antibiofilm activity, mechanism of action, chemical composition and clinical indications, remain largely unknown, which encourages the development of clinical trials targeting the use of honey-based products as therapy complementary.

References

1. Sharma, K., Sharma, K., Kumar, R., A review of physico-chemical and biological properties of honey, Journal of Entomology and Zoology Studies, 2024; 12(2): 153-161.
2. Jaganathan, S.K., Mandal, M. Antiproliferative effects of honey and of its polyphenols: a review. J Biomed Biotechnol, 2009, 1–13, doi:10.1155/2009/830616

3. Minden-Birkenmaier, B.A.; Cherukuri, K.; Smith, R.A.; Radic, M.Z.; Bowlin, G.L. Manuka honey modulates the inflammatory behavior of a dHL-60 neutrophil Model under the cytotoxic limit. Int. J. Biomater. 2019, 2019, 6132581.
4. Erejuwa, O.O.; Sulaiman, S.A.; AbWahab, M.S. Honey: A novel antioxidant. Molecules 2012, 17, 4400–4423.
5. Mustar, S.; Ibrahim, N. A Sweeter Pill to Swallow: A Review of Honey Bees and Honey as a Source of Probiotic and Prebiotic Products. Foods 2022, 11, 2102.
6. Fratianni, F.; Ombra, M.N.; d'Acierno, A.; Caputo, L.; Amato, G.; De Feo, V.; Coppola, R.; Nazzaro, F. Polyphenols content and in vitro α-glycosidase activity of different Italian monofloral honeys, and their effect on selected pathogenic and probiotic bacteria. Microorganisms, 2021, 9, 1694.
7. Babaei, S.; Rahimi, S.; Torshizi, M.A.; Tahmasebi, G.; Miran, S.N. Effects of propolis, royal jelly, honey and bee pollen on growth performance and immune system of Japanese quails. Vet. Res. Forum, 2017, 7, 13–20.
8. Al-Mamary, M., Al-Meeri, A., Al-Habori, M. Antioxidant activities and total phenolics of different types of honey.Nutrition Research, 2002; 22(9):1041–1047
9. Al-Kafaween, M.A.; Hilmi, A.B.M.; Al-Jamal, H.A.N. The Beneficial Effects of Stingless Bee Kelulut Honey against *Pseudomonas aeruginosa* and *Streptococcus pyogenes* Planktonic and Biofilm: Doi. org/10.26538/tjnpr/v5i10. 15. Trop. J. Nat. Prod. Res. (TJNPR), 2021, 5, 1788–1796.
10. Mohammed, S.E.A.; Kabbashi, A.S.; Koko, W.S.; Ansari, M.J.; Adgaba, N.; Al-Ghamdi, A. In vitro activity of some natural honeys against *Entamoeba histolytica* and *Giardia lamblia trophozoites*. Saudi J. Biol. Sci. 2019, 26, 238–243.
11. Rani, G.N.; Budumuru, R.; Bandaru, N.R. Antimicrobial activity of honey with special reference to methicillin resistant Staphylococcus aureus (MRSA) and methicillin sensitive *Staphylococcus aureus* (MSSA). J. Clin. Diagn. Res., JCDR 2017, 11.
12. Morroni, G.; Alvarez-Suarez, J.M.; Brenciani, A.; Simoni, S.; Fioriti, S.; Pugnaloni, A.; Giampieri, F.; Mazzoni, L.; Gasparrini, M.; Marini, E. Comparison of the antimicrobial activities of four honeys from three countries (New Zealand, Cuba, and Kenya). Front. Microbiol., 2018, 9, 1378.
13. Mandal, M.D.; Mandal, S. Honey: Its medicinal property and antibacterial activity. Asian Pac. J. Trop. Biomed., 2011, 1, 154–160.
14. Johnston, M.; McBride, M.; Dahiya, D.; Owusu-Apenten, R.; Nigam, P.S. Antibacterial activity of Manuka honey and its components: An overview. AIMS Microbiol., 2018, 4, 655.
15. Goslinski, M.; Nowak, D.; Kł¸Ebukowska, L. Antioxidant properties and antimicrobial activity of manuka honey versus Polish honeys. J. Food Sci. Technol., 2020, 57, 1269–1277.
16. Girma, A.; Seo, W.; She, R.C. Antibacterial activity of varying UMF-graded Manuka honeys. PLoS ONE 2019, 14, e0224495
17. Feknous, N.; Boumendjel, M. Natural bioactive compounds of honey and their antimicrobial activity. Czech J. Food Sci., 2022, 40, 163–178.
18. Ahmadi–Motamayel, F.; Hendi, S.S.; Alikhani, M.Y.; Khamverdi, Z. Antibacterial activity of honey on cariogenic bacteria. J. Dent., 2013, 10, 10–15.
19. Albaridi, N.A. Antibacterial potency of honey. Int. J. Microbiol., 2019, 2464507.
20. Mohan, A.; Quek, S.-Y.; Gutierrez-Maddox, N.; Gao, Y.; Shu, Q. Effect of honey in improving the gut microbial balance. Food Qual. Saf., 2017, 1, 107–115.
21. Shahzad, A. In vitro antiviral activity of honey against varicella zoster virus (VZV): A translational medicine study for potential remedy for shingles. Transl. Biomed., 2012, 3, 2.
22. Obossou, E.K.; Shikamoto, Y.; Hoshino, Y.; Kohno, H.; Ishibasi, Y.; Kozasa, T.; Taguchi, M.; Sakakibara, I.; Tonooka, K.; Shinozuka, T. Effect of manuka honey on human immunodeficiency virus type 1 reverse transcriptase activity. Nat. Prod. Res. 2022, 36, 1552–1557.
23. Irish, J.; Carter, D.A.; Shokohi, T.; Blair, S.E. Honey has an antifungal effect against Candida species. Med. Mycol. 2006, 44, 289–291.
24. Moussa, A.; Noureddine, D.; Saad, A.; Abdelmelek, M.; Abdelkader, B. Antifungal activity of four honeys of different types from Algeria against pathogenic yeast: *Candida albicans* and *Rhodotorula sp*. Asian Pac. J. Trop. Biomed. 2012, 2, 554–557.
25. Guttentag, A.; Krishnakumar, K.; Cokcetin, N.; Hainsworth, S.; Harry, E.; Carter, D. Inhibition of dermatophyte fungi byAustralian jarrah honey. Pathogens, 2021, 10, 194.
26. Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A.k., Wertheim, H.f., Sumpradit, N., Vlieghe, E., Hara, G.l., Gould, I.M., Goossens, H., Greko, C., SO, A.d., Bigdeli, M., Tomson, G., Woodhouse, W., Ombaka, E., Peralta, A.q., Qamar, F.N., Mir, F., Kariuki, S., Bhutta, Z.a., Coates, A., Bergstrom, R., Wright, G.D., Brown, E.D. &Cars, O. 2013. Antibiotic resistance-the need for global solutions. Lancet Infect Dis. 13(12): 1057-98.
27. Dixon B. Bacteria can't resist honey. Lancet Infect Dis., 2003;3:116.
28. Kwakman, P. H., te Velde, A. A., de Boer, L., Spejer, D., Vandenbroucke-Grauls, C. M., and Zaat, S. A., 2010. How honey kills bacteria. FASEB J., 24, 2576–2582.

29. Kwakman, P. H., and Zatt, S. A., 2012. Antibacterial components of honey. IUBMB Life 64, 48–55. doi: 10.102/iub.578.
30. Lusby P.E., Coombes A.L., Wilkinson J.M. Bactericidal Activity of Different Honeys against Pathogenic Bacteria. Archives of Medical Research., 2005;36(5):464–467.
31. Robson, V., Cooper, R. Using leptospermum honey to manage wounds impaired by radiotherapy: a case series. Ostomy Wound Manage, 2009; 55: 1, 38–47.
32. Ismail Z.B., Alshehabat M.A., Hananeh W., Daradka M., Ali J.H., El-Najjar E.K. Recent advances in topical wound healing products with special reference to honey: a review. Res. Opin. Anim. Vet. Sci., 2015;5(2):76–83.
33. Blair S.E., Cokcetin N.N., Harry E.J., Carter D.A. The unusual antibacterial activity of medical-grade Leptospermum honey: antibacterial spectrum, resistance and transcriptome analysis. Eur. J. Clin. Microbiol. Infect Dis., 2009;28:1199–1208.
34. Cooper R.A., Halas E., Molan P.C. The efficacy of honey in inhibiting strains of *Pseudomonas aeruginosa* from infected burns. J. Burn Care Rehabil., 2002;23:366–370.
35. Cooper R.A., Molan P.C., Harding K.G. The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. J. Appl. Microbiol., 2002;93:857–863.
36. French V.M., Cooper R.A., Molan P.C. The antibacterial activity of honey against coagulase-negative staphylococci. J. Antimicrob. Chemother., 2005; 56:228–231.
37. Price, J.N., Morgan, J.W., 2006. Variability of plant fitness influences range expansion of *Leptospermum scoparium*. Ecography, 29, 623–631.
38. Brady, N., Molan, P., Bang, L., 2004. A survey of non-manuka New Zealand honeys for antibacterial and antifungal activities. J. Apic., Res. 43, 47–52.
39. Julie, I., Shona, B., Carter, Dee A., 2011. The antibacterial activity of honey derived from Australian Flora. PLoS One 6 (3), e18229.
40. Mohd, I., Zainol, K., Mohd, Y., Mohd, Y., Mohd, Y., 2013. Antibacterial activity of selected Malaysian honey. BMC Complement Altern. Med., 13, 129.
41. Katrina, B., Calvin, S., 2014, Antibacterial Compounds of Canadian Honeys Target Bacterial Cell Wall Inducing Phenotype Changes, Growth Inhibition and Cell Lysis That Resemble Action of β-Lactam Antibiotics, PlosOne, https://doi.org/10.1371/journal.pone.0106967
42. Levites Y, Amit T, Youdim MB, Mandel S. Involvement of protein kinase C activation and cell survival/cell cycle genes in green tea polyphenol (-)-epigallocatechin 3-gallate neuroprotective action. J Biol Chem, 2002, 277: 30574-30580.
43. Singhal RL, Yeh YA, Praja N, Olah E, Sledge GW Jr, et al. Quercetin down-regulates signal transduction in human breast carcinoma cells. Biochemical and biophysical research communications, 1995, 208: 425-431.
44. Tripoli E, Guardia ML, Giammanco S, Majo DD, Giammanco ML. Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. Food Chemistry, 2007, 104: 466-479.
45. Chen S, Ding Y, Tao W, Zhang W, Liang T, et al. Naringenin inhibits TNF-α induced VSMC proliferation and migration via induction of HO-1. Food Chem Toxicol, 2012, 50: 3025-3031
46. Israili, Zafar, Antimicrobial Properties of Honey, American Journal of Therapeutics, 21(4): p 304-323, 2014.
47. Molan, P.C., Potential of honey in the treatment of wounds and burns, Am J Clin Dermatol, 2001; 2(1):13-9.
48. White J. W., Subers M. H., Schepartz A. I., 963. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide, and its origin in a honey glucose-oxidase system. Biochem. Biophys. Acta., 73, 57–70
49. Ahed Abdulkhaliq & Khalid M. Swaileh, Physico-chemical properties of multi-floral honeyfrom the West Bank, Palestine, alestine, International Journal of Food Properties, 2017, 20:2, 447-454.
50. Atrouse, O.M., Oran, S.A., Al-Abbadi, S.Y., 2004. Chemical analysis and identification of pollen grains from different Jordanian honey samples. International Journal of Food Science and Technology 39(4), 413-417.
51. Bucekova, M., Juricova, V., Monton, E., Martinotti, S., Ranzato, E., Majtan, J., 2018. Microwave processing of honey negatively affects honey antibacterial activity by inactivation of bee- derived glucose oxidase and defensin-1. Food Chemistry, 240, 1131-1136
52. 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, 2, 213.
53. Carter, C., and Thornburg, R. W. , 2004. Is the nectar redox cycle a floral defense against microbial attack? Trends Plant Sci. 9, 320–324
54. Matzen, R.D., Leth-Espensen, J.Z., Jansson, T., Nielsen, D.S., Lund, M.N., Matzen, S., The Antibacterial Effect In Vitro of Honey Derived from Various Danish Flora, Dermatology Research and Practice, Volume 2018, Article ID 7021713, 10 pageshttps://doi.org/10.1155/2018/7021713
55. Brudzynski K., Lannigan R., 2012. Mechansim of honey bacteriostatic action against MRSA and VRE involves hydroxyl radicals generated from honey's hydrogen peroxide. Front. Microbiol., 3:36 10.3389/fmicb.2012.00036.
56. Estevinho, L., Pereira, A.P., Moreira, L., Dias, L.G, Pereira, E. Antioxidant and antimicrobial effects of

phenolic compounds extracts of Northeast Portugal honey. Food ChemToxicol, 2008.46: 3774–3779.
57. Pérez-Pérez, E., Vit, P., Huq, F., Flavonoids and polyphenols in studies of honey antioxidant activity, Int. J. Med. Plant Altern. Med., 2013, 1, 063-072.
58. Istasse Thibault, Nicolas Jacquet, Thomas Berchem, Eric Haubruge, Bach Kim Nguyen, Aurore Richel. Extraction of Honey Polyphenols: Method Development and Evidence of Cis Isomerization, Anal Chem Insights. 2016 Aug 10;11:49-57. doi: 10.4137/ACI.S39739. eCollection 2016.
59. Challacombe, CA., Abdel-Aal, E. M., Seetharamana, K., & Duizer, L. M. Influence of phenolic acid content on sensory perception of bread and crackers made from red or white wheat. Journal of Cereal Science, 2012, 56, 181–188.
60. Brudzynski, K, Sjaarda, C, & Maldonado-Alvarez, L. A new look on protein–polyphenol complexation during honey storage: Is this a random or organized event with the help of dirigent-like proteins? PLoS One, 2013, 8, 1–9.
61. Weston, R.J., 2000. The contribution of catalase and other natural products to the antibacterial activity of honey: a review, Food Chemistry, 71(2): 235-239.
62. Aljadi, A.M., Yusoff, K.M., 2003. Isolation and Identification of Phenolic Acids in Malaysian Honey with Antibacterial Properties, Turkish Journal of Medical Sciences, 33(4).
63. Mazol, I., Sroka, Z., Sowa, A., Ostrowska, A., Drys, A., Gamian, A., 2016. Antiradical and antimicrobial activity of phenolic fractions obtained from honeys, Acta Pol Pharm., 73: 379-388.
64. Szweda, P., 2017. Antimicrobial Activity of Honey. In: De Alencar Arnaut De Toledo, V., Ed., Honey Analysis, Vol. 1, IntechOpen, London, 215-232.
65. Mundo, M.A., Padilla-Zakour, O.I., Worobo,R.W., 2004. Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys, International Journal of Food Microbiology, 97(1): 1-8.