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Antimicrobial Activity of Honey

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Abstract

Honey has had a valued place in traditional medicine for centuries. It was used to overcome liver, cardiovascular and gastrointestinal problems and for treatment of some types of infectious disease. Particularly, good results were achieved in the case of application of this product for therapy of infected, difficult to heal wounds. The high health-promoting properties of honey have been recently confirmed in many research investigations. The antimicrobial activity of this product is highly complex. Generation of hydrogen peroxide, bee defensin-1, high osmolarity and low value of pH seems to be crucial for its antimicrobial potential. Considering honey as a therapeutic, antimicrobial agent special attention deserves Manuka honey. Its high antimicrobial activity is caused by high concentration of 1,2-dicarbonyl compound methylglyoxal. Some authors also suggest that other phytochemicals, especially phenolic compounds, are important antibacterial ingredients of honey. The results of many *in vitro* but also *in vivo* studies confirm high antimicrobial potential of honey against some important human and veterinary pathogens: *Staphylococcus aureus*, *Helicobacter pylori*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa* and *Escherichia coli*. We do not have doubts that honey, but also other bee products, especially propolis, is promising antimicrobial agents and possibilities of their application in clinical medicine deserve consideration.

Keywords: honey, glucose oxidase, bee defensin-1, polyphenols, antimicrobial activity, infectious diseases, staphylococci

1. Introduction

1.1. Honey: a beneficial food product

Due to its unique taste, nutritional value and health-promoting properties, honey has a valued place in the human diet. Sugars, mainly fructose and glucose, and minor amounts of oligosaccharides account for about 80% of its weight. As a consequence, it is an easily digestible and high energetic food product. Consumption of 100 g of honey provides the body

with about 320 kcal. However, the health-promoting properties of this product come mainly from the presence of other than sugar components: enzymes, peptides, free amino acids, vitamins, organic acids, flavonoids, phenolic acids and other phytochemicals and minerals [1]. The beneficial effects of eating honey have been confirmed by centuries of observations. Consequently, honey has become one of the major therapeutic agents of traditional medicine. Depending of botanical source, different types of honey are proposed for prophylaxis and treatment of different health problems. According to polish traditional medicine [2],

- rapeseed honey (produced from *Brassica napus* L.) soothes liver disease, and it is also recommended in therapy of diseases of the cardiovascular system and kidneys;
- honey produced from acacia (*Robinia pseudoacacia* L.) nectar is especially recommended for diabetics, and it also helps to alleviate digestive disorders and gastrointestinal diseases;
- heather (*Calluna vulgaris* L.) honey is used for treatment prostate and liver and biliary system diseases;
- many benefits come from consumption of buckwheat (*Fagopyrum esculentum* Moench) honey; it relieves the symptoms of hypertension and atherosclerosis and promotes regeneration of bone tissue. It is also recommended to diabetics and for treatment of inflammatory conditions of the kidney, urinary tract and joints;
- good results in the treatment of depression and neuroses have been obtained by the use of honey sourced from buckwheat, linden tree (*Tilia* spp.) and also some multifloral and honeydew honeys;
- diaphoretic and antipyretic effects have been confirmed for raspberry (*Rubus idaeus* L.) and linden tree honeys; as a consequence, these honeys are popular in treatment of influenza and bacterial infections.

In particular, interesting and important issue is antimicrobial activity of honey. In fact, it is the only food product that without any technological processing, nor addition of preservatives, can be stored for a long period of time—even several years, without any negative symptoms. Interestingly, the honey is not a sterile product (**Figure 1**).

It contains some microorganisms, mostly bacteria yeast and molds. However, the specific environment of this product, high osmotic pressure (high concentration of sugars) and high acidity (low value of pH) prevents the development of microorganisms [3]. As a consequence, only some groups of bacteria and fungi are able to exist within environment of honey and the population of microorganisms is stable during storage. Moreover, it has been shown that some of bacteria that are present in the honey produce antimicrobial agents, bacteriocins, which can protect the product against development of other microorganisms and are beneficial for consumers' health [4]. Antimicrobial potential of honey has been successfully used in folk medicine, with a particularly good result in the case of therapy of infected, difficult to heal wounds [5]. Since the introduction to the clinical practice sulfonamides and next antibiotics treatment of infectious diseases with natural products, including honey was minimized. Due to observed recently rapid increase in isolations of strains resistant to a plethora of antibiotics, the possibility of using herbs, honeybee products and other natural products for the treatment of infection is again seriously considered. The aim of preparing this chapter

is presenting perspectives not only limitations of application honey for treatment but also prophylaxis of diseases caused by microorganisms, especially bacteria.

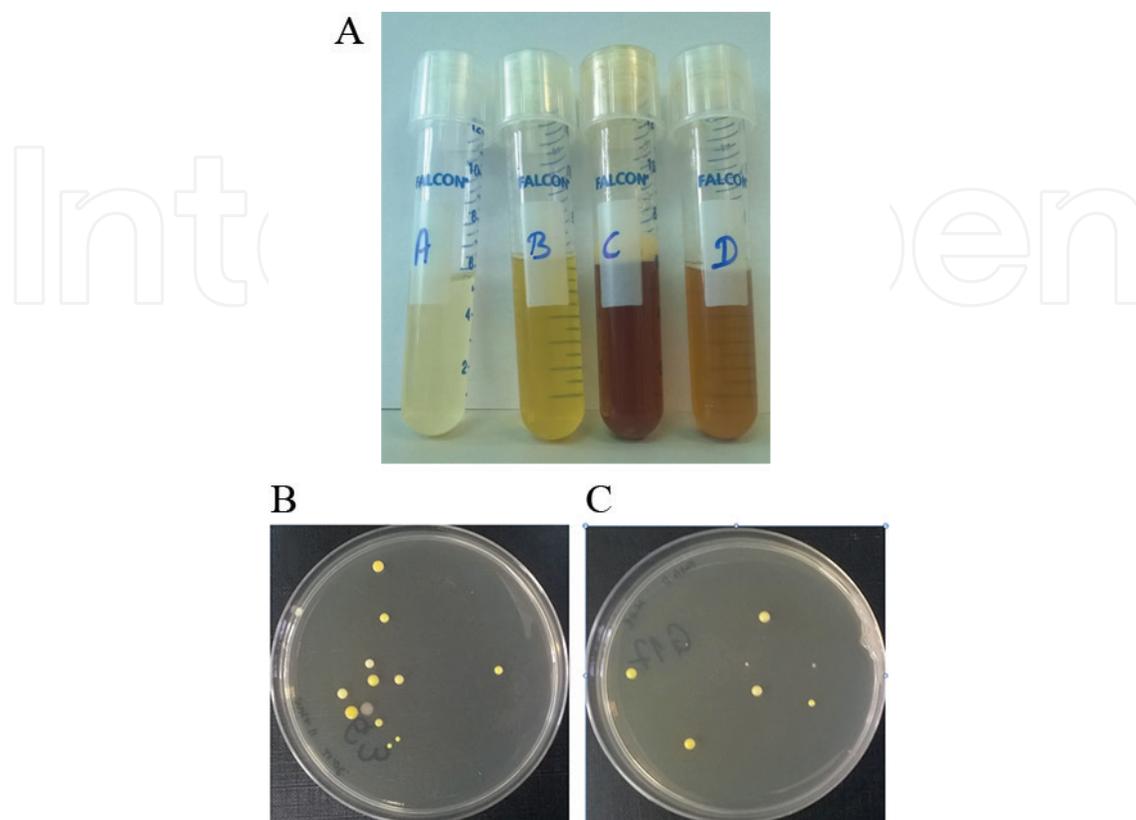


Figure 1. (A) Solutions of investigated honeys (25%, v/v): A, rapeseed honey (*Brassica napus* L.); B, multifloral honey; C, buckwheat honey (*Fagopyrum esculentum* Moench); D, Manuka honey (*Leptospermum scoparium*). Some characteristic differences in the color of the solutions are visible. (B) Result of growing of 50 μ l of solutions of honeys A and B on LA agar. In both cases, several colonies were obtained, which confirm contamination of investigated honeys with some bacteria.

2. Mechanism of antimicrobial activity of honey

The antimicrobial activity of honey is highly complex and still remains not fully recognized. To date, it has been established that several components of this product play a crucial role for its antimicrobial properties [6–11]:

- high concentration of sugars (about 80% of weight of this product) eliminates microorganisms, mainly bacteria that are sensitive to high osmotic pressure and inhibit the development of more osmotolerant microorganisms;
- low pH value—high concentration of organic acids (e.g., gluconic acid). The pH of most honey types is in the range from 3.4 to 6.1, which in combination with high osmotic pressure eliminates or enables the development of most microorganisms;
- bee defensin-1, it is a peptide secreted by the honeybee hypopharyngeal glands. As a component of royal jelly (it is also called royalysin), it probably plays a key role in the health of bee larvae. It exhibits activity against Gram-positive bacteria, including *Bacillus subtilis*,

Staphylococcus aureus and *Paenibacillus larvae* (etiological agent of important bee larval diseases American foulbrood). While high concentration of sugars and low pH are universal antibacterial factors of all honeys, strong differences have been noticed in the case of amount of this peptide in different honey and royal jelly samples. The bees also produce at least three other antibacterial peptides as important components of their innate immune system. However, to date, they have not been detected in honey;

- glucose oxidase—the enzyme, oxidoreductase that catalyzes the oxidation of glucose to gluconic acid. The side product of this reaction, hydrogen peroxide (H_2O_2), is a strong antimicrobial agent. The detailed mechanism of this reaction is presented below (Figure 2). The enzyme is produced in honeybees' salivary glands and introduced to the collected nectar. It protects the ripening honey against the development of pathogenic microorganisms. Interestingly, the enzyme is present but not active in the mature honey; this product is sufficiently protected with high osmotic pressure and low acidity. When the honey is diluted, the enzyme regains activity, which is extremely important for honeybees and especially their larvae health. Honey is the most important component of honeybees' diet; however, before consumption, it is diluted in water. The generated by the enzyme H_2O_2 is a major antimicrobial defense factor for this diluted honey. Its production is also crucial for antimicrobial potential of honey used for treatment of skin and soft tissue infections, infected wounds or eradication pathogenic bacteria located within upper respiratory tract or *Helicobacter pylori* located in human stomach.

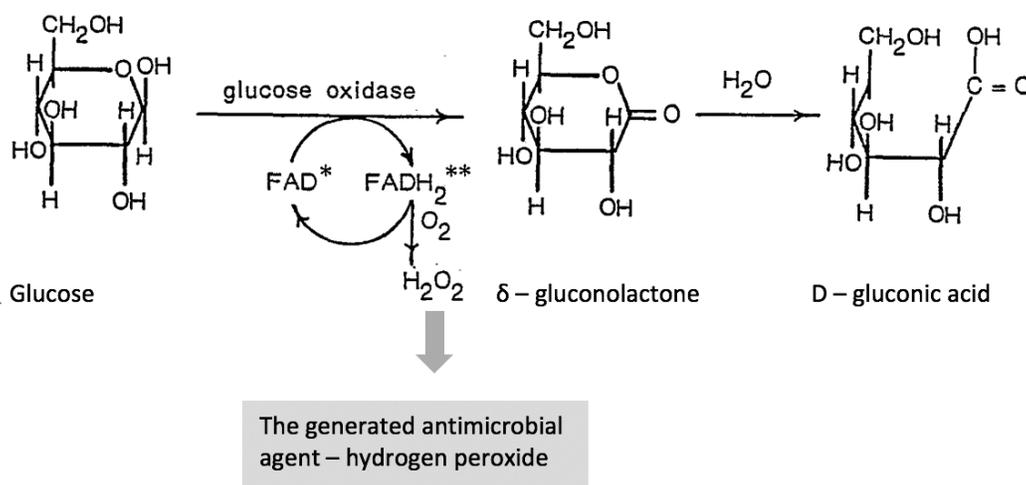


Figure 2. The reaction catalyzed by glucose oxidase—generation of hydrogen peroxide.

The unique antimicrobial properties have been identified for honey produced from the Manuka bush (*Leptospermum scoparium*) indigenous to New Zealand and Australia. In contrast to majority of other nectar and honeydew honeys, the crucial factor responsible for the bactericidal activity of this product is high concentration of 1,2-dicarbonyl compound methylglyoxal (MGO) [10–12]. Some authors also suggest that other phytochemicals, especially phenolic compounds, are important antibacterial ingredients of honey. Evident differences in activity of honeys produced from different botanical sources seem to support this hypothesis [13–15]. However, the observed differences could be also caused by differences in activity or

concentration of glucose oxidase or concentration of defensin-1, which has not been investigated to date. The presence of phenolic compounds in honey has been confirmed in several independent studies. The results of these studies also revealed that concentrations of individual constituents are too low to substantially contribute to the antimicrobial activity of the product. It is possible, however, that combination of different ingredients, for example, phenolic compounds, might significantly contribute the activity of honey [6, 11, 16]. Quite satisfactory activity of composition of phenolic compounds extracted from several Malaysian and Polish honeys was observed, respectively, by Aljadi and Yusoff [17] and Mazol and coworkers [18]. Interesting results in this area have been also presented by Mundo and coworkers (2004) [15], who observed non-peroxide activity against *Bacillus stearothermophilus* in most of 27 honey samples diluted in water containing catalase (the enzyme degrading hydrogen peroxide). In contrast to *Bacillus*, the neutralization of H_2O_2 with catalase resulted in loss of activity against *S. aureus* in the case of all tested honey samples except of two samples of horsemint honeys. This result could suggest the presence of some nonproteinaceous components in these honeys, which were responsible for inhibition of growth of *Bacillus* in the suspensions of honey not containing hydrogen peroxide [15]. The presence of antimicrobial components (combination of cationic and noncationic but not identified substances) other than methylglyoxal, glucose oxidase and defensin-1 in Manuka honey was confirmed in the studies of Kwakman and coworkers [10, 11]. These authors also investigated that the other honeys, assigned as RS (Revamil—medical grade honey) and completely opposite results, were obtained. In the case of this product neutralization of H_2O_2 , MGO, defensin-1 and subsequent titration of honey to neutral pH resulted in complete loss of antimicrobial activity [10, 11]. On the basis of current state of knowledge, it rather should be assumed that phytochemicals, except of methylglyoxal, are not crucial for antimicrobial potential of most honeys. However, in the case of honeys produced from some botanical sources, they probably substantially support the primary factors: pH, high osmolarity and defensin-1 (in the case of undiluted honey) and hydrogen peroxide in the case of diluted product. Thus, the contribution of phytochemicals to the antimicrobial activity of honey remains unclear and needs to be investigated.

The investigation carried out by Lee and coworkers [4] revealed that honey is a promising source of bacteriocinogenic bacteria strains. The mentioned authors analyzed two Manuka honey samples from New Zealand and six domestic honeys from the United States of America. The 2217 isolates out of 2398 strains (92.5%) exhibited activity at least against one of the tested microorganisms. Among them, 1655 exhibited activity against *Listeria monocytogenes* and 1605 inhibited the growth of another important human and veterinary pathogen *S. aureus* [4]. Beside of that, at the moment, it is rather difficult to classify bacteriocins as the next important antibacterial component of honey. To date, only the strains producing these peptides have been isolated from honey, the presence of bacteriocins within the product has not yet been confirmed.

3. Determination of antimicrobial activity of honey

The in vitro antimicrobial activity of most agents is usually estimated with two methods: an agar diffusion assay and a serial dilution method in microtiter plates. Both these methods have been also used for determination of antimicrobial potential of honey. The agar diffusion

assay is based on the measurement of size of growth inhibition zone around the place of loading a sample of honey (usually well, cut with a cork borer in the agar). The assay is easy and quick in performance. Unfortunately, it has several important limitations [11]:

- high viscosity of honey and problems with loading of defined volume of the product sample to the wells in the agar. It is especially problematic when the honey is crystallized;
- problems with diffusion of active components (defensin-1 and especially glucose oxidase characterize with high molecular weight) through the agar matrix. As a result, the diameters of observed growth inhibition zones are relatively low. The honeys with evidentially different activities established with other methods give similar results in agar diffusion assay (not large differences in the diameters of growth inhibition zones are observed—based on results of own studies, **Figure 3**);

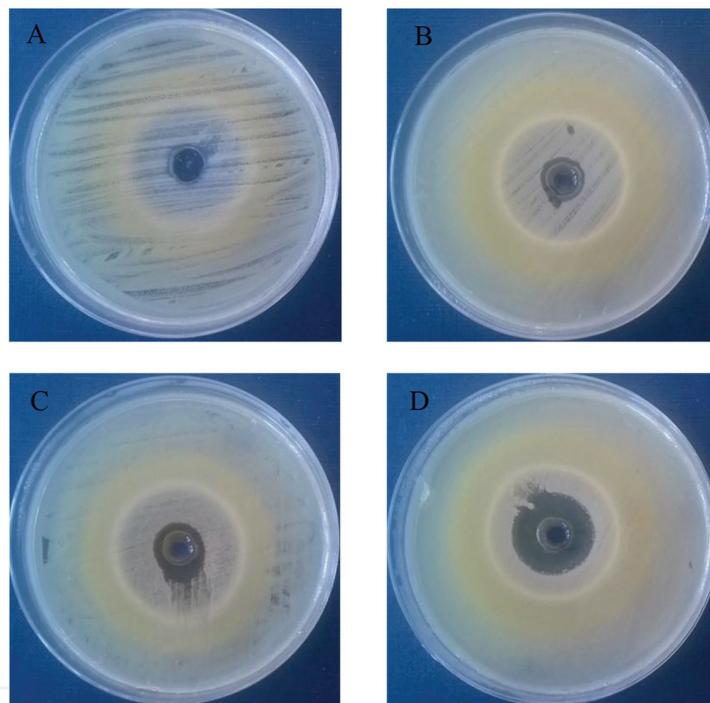


Figure 3. Results of agar diffusion assay of activity of four selected honeys: (A) rapeseed honey (*Brassica napus* L.); (B) multifloral honey; (C) buckwheat honey (*Fagopyrum esculentum* Moench); (D) Manuka honey (*Leptospermum scoparium*). Definitely the highest activity was observed in the case of Manuka honey—picture D. Some characteristic halo zones are present in all pictures. 100 μ l of 50% (v/v) was loaded to the wells in the agar.

- low reproducibility—it is difficult to get similar results (diameter of growth inhibition zone) in several independent experiments;
- low discriminatory power—consequence of relatively low sizes of observed growth inhibition zones. It is also difficult to compare the obtained results with the results of other authors;
- lack of possibilities to distinguish bacteriostatic and bactericidal activity;
- problems with interpretation of obtained results. Usually except of clear, growth inhibition zones at least one halo zone can be observed (**Figure 3**). In this halo zone, the colonies of

growing bacteria characterize with different (lower) diameter, and some changes of color of agar can be noticed, which is difficult in interpretation (**Figure 3**). In our opinion, the presence of these halo zones is a consequence of influence of low molecular components of honey on the growth of microbial cells.

The problems with high viscosity can be, at least partly, omitted by using honey dissolved in sterile water (e.g., 50%, w/w), as it has been proposed by several authors [15]. However, usually it does not solve other discussed above problems.

Based on our experience, we would rather recommend a serial dilution method for investigation of antimicrobial potential of honey [13]. This method allows quantitative determination of both bacteriostatic and bactericidal activity of tested honey samples, **Figures 4** and **5**. The bacteriostatic activity is characterized with MIC (*Minimal Inhibitory Concentration*—the lowest concentration of honey that inhibits the growth of tested strain of microorganisms) parameter, while bactericidal activity is characterized with MBC (*Minimal Bactericidal Concentration*—the lowest concentration of an antibacterial agent required to kill a particular bacterium) parameter.

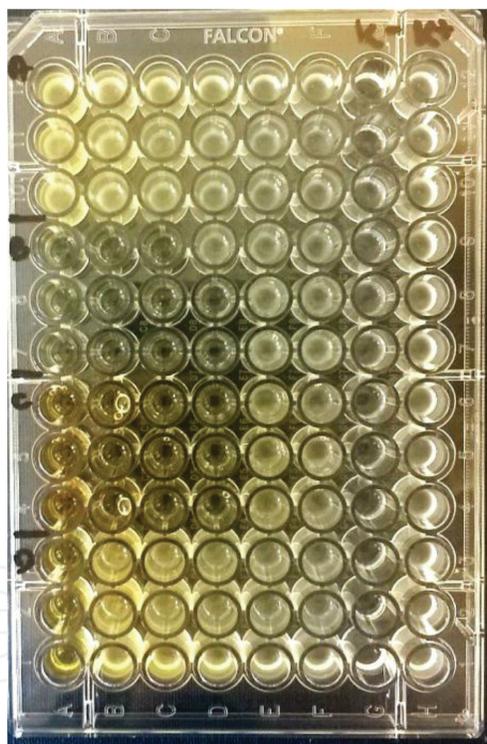


Figure 4. The results of determination of antistaphylococcal activity of four tested honeys: (A) rapeseed honey (*Brassica napus* L.)—rows 1–3; (B) multifloral honey—rows 4–6; (C) buckwheat honey (*Fagopyrum esculentum* Moench)—rows 7–9; (D) Manuka honey (*Leptospermum scoparium*)—rows 10–12. The concentrations of honeys in the wells of following columns were as follows: 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39% (v/v). The wells of column number 7 contained only growing medium (Mueller Hinton Broth II cation adjusted) neither honey nor cells of bacteria were present in these wells—negative control. The wells of column 8 did not contain honey, and they were used as a positive control of growth of bacteria in the medium not containing any antimicrobial agent. No activity was observed in the case of rapeseed honey. The reference strain of bacteria *S. aureus* PCM1051 was able to grow in all wells of rows 1–3. The MIC value for multifloral honey (the lowest concentration of honey, which caused visible inhibition of growth of *S. aureus* strain), was 3.12% in row 4 and 1.56% in rows 5 and 6. The MIC value for buckwheat honey in all three tested rows was 1.56%, and the constant value of MIC for Manuka honey, 12.5%, was observed in the rows 10–12.

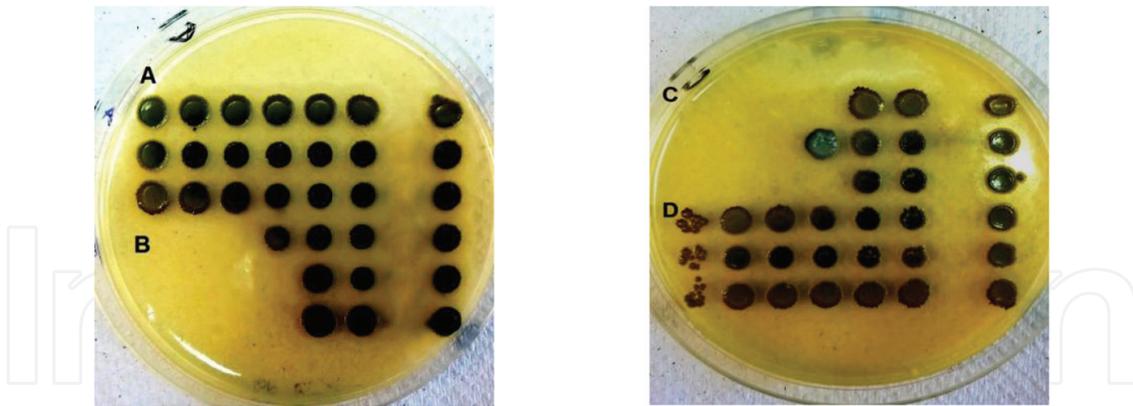


Figure 5. The results of determination of minimal bactericidal concentrations of tested honeys against *S. aureus*. The assay (carried out according to the procedure presented in **Figure 6**) confirmed the lack of activity of rapeseed honey. The MBC values of multifloral honey were exactly the same as MIC values for this product. In the case of buckwheat honey, MBC and MIC values were the same in the rows 7 and 9, and in the case of row 8, the MBC was twice of the MIC value, 3.12 and 1.56% (v/v), respectively. Interestingly, no bactericidal activity was observed in the case of Manuka honey. However, the intensity of growth of bacteria transferred from the wells with the highest concentration of this honey (12.5%) was evidently inhibited in comparison with samples presenting wells containing lower concentrations of this product. Despite the fact that the same product was tested in triplicate, in the case of determination of activity of multifloral and buckwheat honeys, some differences in the obtained MIC and MBC values were observed for different rows (e.g., MBC value for buckwheat honey was 1.56% in the case of rows 7 and 9, and in the case of row 8, the bactericidal effect was achieved at the concentration of 3.12%). However, the observed differences of determined values of the parameters of interest for particular honey were not larger than two times, which is acceptable in these assays.

The detailed description of procedure of performance of this assay as well as determination of both parameters MIC and MBC has been presented in **Figure 6**. The most problematic step of this assay is preparing the output solution of honey; in our laboratory, it is usually 25% (v/v). Because of high viscosity of honey, the determination of volume of the product used for preparing the solution has to be done extremely carefully. Other way, it can be a source of significant measurement errors. There are also several other advantages of serial dilution method in comparison with agar diffusion assay. The dilution assay gives more reproducible results, which are easy in interpretation. It also characterizes with much better discriminatory power, for example, results presented in **Figures 4** and **5**.

Slightly modified serial dilution method can be also used for determination of antibiofilm activity of honey. Minimum biofilm eradication concentration (MBEC—the lowest concentration of an antibacterial agent required to eradication of biofilm formed by a particular bacterium) of honey is determined in this assay. In general, the assay is performed identically as in the case of determination of MIC or MBC parameters. However, in the first step, the bacterial biofilm is grown in the wells of titration plates.

Preparing this chapter, we performed some assays of activity of four selected honeys: A, rapeseed; B, multifloral; C, buckwheat; D, Manuka honey against *S. aureus* PCM2054 reference strain. As it is presented in **Figures 3** and **4**, some important differences in the results of these two assays have been obtained. In the case of agar diffusion assay, definitely the highest activity was observed for Manuka honey, while in the case of dilution method, buckwheat

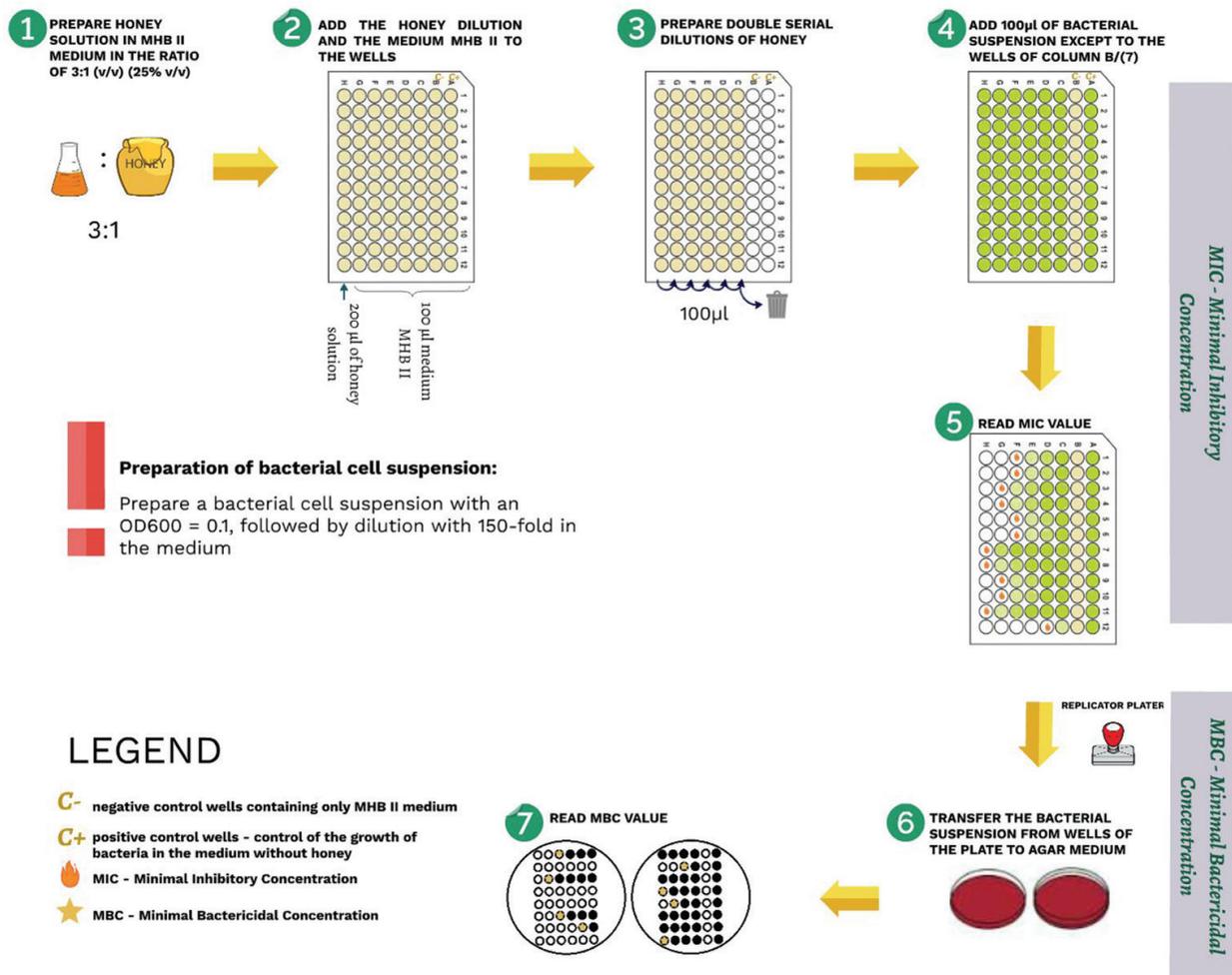


Figure 6. The procedure of performance of serial dilution method. The MHBII medium used for honey dilution should be prepared with using of only 75% of water volume recommended for this medium. The required volume of medium will be obtained in the consequence of adding of honey. MHBII medium used for serial dilution of honey and preparing of suspension of bacterial cells should be prepared according to manufacturer's procedure (with using recommended volume of water).

and multifloral exhibited much better activity in comparison with the honey produced in New Zealand. Rapeseed honey in both assays was classified as non-active. These results are in agreement with our previous observations [13]. Our previous research revealed also that activity of polish honeys is hydrogen peroxidase dependent [13]. Thus, the relatively low activity of buckwheat and multifloral honey in the case of agar diffusion method was probably a consequence of difficulties of migration glucose oxidase through the agar. Activity of Manuka honey comes mainly from high content of MGO, which is a low molecular weight component that can easily migrate through the agar generating a large growth inhibition zone. It is also worth to notice that MBC and MIC values for buckwheat and multifloral honeys are the same (1.56%, v/v). The MBC value for Manuka honey could not be determined in the tested range of concentrations; however, evident inhibition of growth of *S. aureus* in the wells containing 12.5% (v/v) of the honey is visible in both: titration plate as well as on the Petri dishes with Baird-Parker agar.

4. Antimicrobial activity of honey: in vitro and in vivo studies

High antimicrobial potential of honey has been confirmed in many in vitro tests, but also in vivo studies. Some important differences in the activity of honey produced from different botanical sources have been revealed. In general, molds and yeasts are less sensitive to the activity of this product, as high concentration as 30–50% is necessary to inhibit the growth of these groups of microorganisms [11]. Much higher activity has been observed in the case of bacteria, especially sensitive are Gram-positive bacteria. Taking into account mechanisms of activity, methods of application and dose of the product necessary for effective elimination of bacteria only some specific types of diseases could be treated with honey, for example, infected wounds, skin and soft tissue infections, infections located within upper respiratory tract, mucosa of digestive tract, vaginal mucosa and some specific disease, for example, stomach ulcers caused by *Helicobacter pylori*. This limitation was the main criterion for selection of bacterial species for presented below description concerning the results of research of antimicrobial activity of honey and possibilities of its application in clinical practice or prophylaxis of some disease.

4.1. Antistaphylococcal activity of honey

High, in vitro, antistaphylococcal activity of honey has been confirmed by many researchers—for details please see our previous review [19]. In fact, staphylococci belong to the most sensitive bacteria to the components of this product [19]. The growth of staphylococci is inhibited by proteinaceous components—defensin-1 and generated by glucose oxidase H_2O_2 , as well as by other antimicrobial ingredients: mainly polyphenols and methylglyoxal in the case of Manuka honey. Our previous research revealed high antistaphylococcal (against *S. aureus* PCM 2051—reference strain) activity of polish honeys produced from cornflower (*Centaurea cyanus* L.), buckwheat (*Fagopyrum esculentum* Moench) and thyme (*Thymus vulgaris* L.) with MIC values of 3.12 or 6.25% (v/v); some differences of activity of different samples of honey obtained from the same botanical sources were observed [13]. The obtained results (the ranges of effective concentrations) are in agreement with the results presented by other authors who investigated honeys sourced from different geographical locations, for example, from Greece [14] or Iran [20]. High antistaphylococcal activity of honey has been also confirmed for MRSA (*Methicillin-Resistant Staphylococcus aureus*) clinical isolates. Effective inhibition of growth of MRSA isolates has been revealed in the case of Chilean honey obtained from Ulmo tree [21], Malaysian melaleuca honey [22], some Thai honeys, especially from longan flower [23], Finland [24], Ethiopia [25] and several other geographical regions. Honey is also effective in eradication of staphylococcal biofilm. Lu and coworkers [26] revealed that New Zealand Manuka-type honeys, at the concentrations they can be applied in wound dressings, are highly active in both preventing *S. aureus* biofilm formation and in their eradication and do not result in bacteria becoming resistant [26]. High efficiency in elimination of bacterial biofilm confirmed also for honeys whose activity depends mainly from hydrogen peroxide generation, for example, “Medihoney”—therapeutic honey and Norwegian Forest Honey [27]. Staphylococci are often isolated from skin and soft tissue infections; they are also important etiological factor of wound infections. The group of Blaser achieved a full

healing in seven consecutive patients whose wounds were either infected or colonized with methicillin-resistant *S. aureus*. Antiseptics and antibiotics had previously failed to irradiate the clinical signs of infection [28]. Interesting results were also presented by Al-Waili [29] who used honey for treatment surgical wounds made on the dorsum of mice infected with different species of bacteria. It was found that local application of raw honey on infected wounds reduced redness, swelling, time for complete resolution of lesion and time for eradication of bacterial infection due to *S. aureus* or *Klebsiella* sp. Its potency was comparable to that of local antibiotics [29]. Because of their promising properties, the wound dressing materials containing honey (mostly Manuka honey) are already commercially available and gain popularity in treatment difficult to heal infected wounds.

4.2. Activity against *Helicobacter pylori*

In vitro anti-*H. pylori* activity of honey has been confirmed by several research groups. Using agar diffusion assay, Nzeako and Al-Namaani [30] investigated activity of eight samples of honey (four from Germany, one from Switzerland, one from Iran and two from Oman). All of them effectively inhibited the growth of *H. pylori*. The size of growth inhibition zones produced by the samples of 100 µl of undiluted honey varied from 15 mm for Blossom bee honey (Switzerland) to 29 mm for Al-Nada clove honey (Oman) [30]. Interesting results of in vitro studies of anti-*H. pylori* activity of three locally produced honeys from different regions in South Africa were presented by Manyi-Loh and coworkers [31]. The authors revealed high activity of honey but also extracts of organic, nonproteinaceous components of these products [31]. Al Somal and colleagues [32] revealed much better anti-*H. pylori* activity of Manuka honey in comparison with peroxide-dependent honey. All five isolates tested by the authors were sensitive to a 20% (v/v) solution of Manuka honey in an agar well diffusion assay, but none showed sensitivity to a 40% solution of a honey sample in which the antibacterial activity was due primarily to its content of hydrogen peroxide [32]. The observations presented by the groups of Manyi-Loh et al. [31] are especially important from the point of view of specific conditions in stomach. High concentration of HCl and low value of pH certainly affects the activity of enzymes that are present in consumed food, including glucose oxidase, which generates hydrogen peroxide and is crucial for antimicrobial activity of most types of honeys. Thus, the presence of other than H₂O₂ antimicrobial components in honey is very important for possibilities of its effective application for prophylaxis and therapy of in vivo *H. pylori* infections. Recently, Sahin [33] revealed that phenolic components of chestnut and oak honeys effectively inhibited activity of two enzymes: urease and xanthine oxidase, which are important virulence factors of *H. pylori*. These results importantly confirm that regular consumption of honey (especially the products rich in polyphenols) could prevent gastric ulcers deriving from *H. pylori* [33]. Moreover, analyzing the group of 150 dyspeptic patients, Boyanova and colleagues (2015) [34] revealed that consumption of honey at least 1 day weekly significantly reduces the risk of development of infection with *H. pylori* [34]. The in vitro susceptibility of *H. pylori* to honey is well documented. In our opinion, more studies aiming in evaluation of in vivo effects of regular consumption of honey for development of *H. pylori* infection within stomach are necessary. These researches should concentrate on selection of type of honeys (probably characterized with high content of polyphenols and/or MGO), especially effective in eradication this bacterium from the tissue.

4.3. Activity against *Mycobacterium tuberculosis*

M. tuberculosis, being the leading member of the MTB complex (*Mycobacterium tuberculosis complex*), is the main cause of tuberculosis worldwide. Over the recent past years, resistance against antituberculous drugs has emerged rapidly, resulting in MDR (*Multi Drug Resistant*) strains. In vitro activity of Beri honey (from Pakistan) was tested against 21 clinical isolates of MDR-MTB by Hannan and coworkers (2014) [35]. The obtained results clearly demonstrate that Pakistani Beri honey exhibits significant antimycobacterial potential, and three (14%) of the isolates were susceptible at 1% (v/v) honey, while at 2% (v/v) of honey, 18 (86%) isolates were found to be susceptible. All the 21 isolates ($n = 21$) were susceptible at 3% (v/v) of honey [35]. Honey was also proposed for treatment tuberculosis by Avicenna, a known ancient Persian philosopher and physician. At the beginning of twenty-first century, this hypothesis was evaluated by the researchers from Shiraz University of Medical Sciences in Iran [36]. It was demonstrated that the growth of mycobacteria was inhibited by adding 10% honey to the growing media (Lowenstein-Jenson media and L-J media were used). *Mycobacteria* did not grow in culture media containing 10 and 20% honey, while it grew in culture media containing 5, 2.5 and 1% honey. Thus, the obtained results of in vitro tests are quite optimistic [36]. However, future research of in vivo activity of honey against *Mycobacteria* located within the lung tissue would be necessary for fully evaluation of its usefulness in the treatment of tuberculosis. According to the best of our knowledge to date, such studies have not been conducted.

4.4. Activity against Gram-negative bacteria: *P. aeruginosa* and *E. coli*

The most carried out to date studies revealed that Gram-negative bacteria are a bit less sensitive to the activity of honey in comparison with Gram-positive bacteria. This situation was also observed in the research carried out in our group. The collection of over 30 Polish monofloral honeys was tested, and definitely most of them were less active against *P. aeruginosa* and especially *E. coli* reference strains in comparison with *S. aureus* PCM2051. However, the activity of most of honeys against these pathogens was on satisfactory level, with MIC values in the range of concentrations from 6.25 to 25% (v/v) and from 12.5 to 25% (v/v), respectively [13]. Honey effectively eradicates biofilm formed by *P. aeruginosa* [37]. Activity of this product against this bacterium has been also confirmed in some in vivo studies. The stingless bee honey has been successfully used for treatment of *P. aeruginosa* infected conjunctivitis in Hartley guinea pigs [38]. The investigation carried out by the group of Khoo (2010) revealed that Tualang honey-treated rats demonstrated a reduction in bacterial growth in *P. aeruginosa* inoculated wounds [39]. *P. aeruginosa* belongs to the important etiological factors of wound infections. Thus, activity of many potential wound dressing materials containing honey against this bacterium has been carried out. Most of them confirm high therapeutic antimicrobial potential of honey.

5. Antimicrobial activity of other honeybee products

In addition, honeybees also produce propolis, wax, pollen, bee bread and royal jelly. All these products exhibit some antibacterial activity. However, from the point of view of possibilities

of their application for prophylaxis or treatment of infections, definitely the most promising is propolis. It is a resinous substance produced from plants' buds and exudates, modified by addition of honeybees' salivary secretions and wax. It is a product of a very complex chemical composition, which depends on many factors; in particular, important are geographical location and plant base, which is available for honeybees when collecting their products. Some of its ingredients, mainly polyphenols and flavonoids, exhibit high antimicrobial activity. As a consequence, it is used by honeybees as a hive disinfectant [40]. Ethanolic extracts of propolis exhibit high activity against wide spectrum of human and veterinary pathogenic microorganisms. The investigation carried out in our group revealed promising activity of Polish propolis against clinical isolates of azole-resistant yeasts of the genus *Candida*. In total susceptibility of 44 strains [*C. albicans* ($n = 20$), *C. glabrata* ($n = 14$) and *C. krusei* ($n = 10$)] were tested, and in the case of one sample of propolis, the MFC (*Minimal Fungicidal Concentration*) values were in range from 0.156 to 1.25% (v/v) [41]. Many studies also revealed high activity of propolis against Gram-positive bacteria, including as dangerous pathogens as *S. aureus*, *S. epidermidis*, *Listeria monocytogenes* [42], *Bacillus subtilis* and *B. cereus* [43]. It has been also confirmed that ethanolic extracts of propolis enhance activity of some antibiotics against staphylococci [44]. Some important Gram-negative bacteria also exhibit sensitivity to the components of propolis. However, the research of propolis from different regions of the world is consistent and indicates that higher concentrations are necessary for elimination of *E. coli* or *P. aeruginosa* in comparison with Gram-positive bacteria [45–47]. Propolis belongs to the most popular products used for treatment infections in traditional medicine. During last several decades, its high antimicrobial potential has been confirmed with a large number of scientific publications. We have no doubts that possibilities of application of this product in clinical medicine deserve consideration.

6. Conclusions

Honey produced from some botanical sources exhibits high antimicrobial activity. Possibilities of application of this product for treatment infections in clinical practice should be the subject of intensive investigations in the near future. Except of high activity, the most important advantages of this product are as follows:

- lack of side effects for patients (important drawback of antibiotics);
- low costs of therapies;
- low possibility of development of resistant strains—the cells of pathogens are simultaneously affected with several factors, for example, hydrogen peroxide, bee defensin-1, methylglyoxal or other phytochemicals;
- the honey provides the body of the patient many health-promoting components, for example, antioxidants, microelements, trace elements and vitamins.

However, it has to be notice that several important problems would have to be solved for more common application of honey for treatment infections.

- only the honey classified as medical grade—with confirmed antibacterial activity, free of pathogenic microorganisms and toxic components could be used in medical applications;
- each batch of raw material (honey) would have to be tested for its biological activity;
- the method of sterilization, safe for proteinaceous antibacterial components of honey (glucose oxidase, bee defensin-1), would have to be developed (gamma-irradiation sterilization seems to be promising [48]);
- much more studies are necessary to check the *in vivo* effects of treatment of infections;
- one method of determination of antimicrobial activity of honey should be recommended, as a consequence comparison of activity of the product tested in different laboratories would be easier (based on our experience, we would recommend serial dilution method for this purpose).

Summarizing we have no doubt that honey is an interesting and promising alternative to classical antibiotics and should be more seriously considered as therapeutic agents.

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