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Editor: Diah Karmiyati

Bildung

Embracing Society 5.0 with Humanity

Editor : Diah Karmiyati
Desain Sampul : Ruhtata
Lay out/tata letak Isi : Tim Redaksi Bildung

Perpustakaan Nasional: Katalog Dalam Terbitan (KDT)
Yogyakarta: CV. Bildung Nusantara, 2022

x + 1115 halaman; 15 x 23 cm
ISBN: 978-623-6225-67-7
Cetakan Pertama: Maret 2022

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Daftar Isi

Antibiofilm Activity of Honey in Multispecies Pathogen

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Introduction

The invention of antimicrobial agents is an important contribution to the improvement of life-threatening communicable disease therapy. However, as a result of the widespread use of these useful drugs, a slew of new resistance mechanisms has developed and spread rapidly among bacteria that cause disease. Antibiotics resistance becomes more commonly found than before for instance bacteria strains that cannot be killed by Methicillin, Vancomycin, and Carbapenem [1], [2]. Besides mutations or gene resistance acquisition, this resistance could be caused by the ability of the microorganisms to form biofilms on the tissues or medical devices [2].

Microorganisms' biofilm is a complex protective mechanism structure in which microbes stick one to another to a surface. Theses aggregate cells are embedded within a slimy substance called extracellular polymeric substances (EPS) result in an increment of surface attachment ability, a higher population density, as well as pathogenicity improvement [3]. The protective effects of biofilm formation from antimicrobial agents include decreased penetration, drug molecules trap in the extracellular matrix and higher concentration microbial inactivation enzymes [4]. The Biofilm formation may cause microorganisms become more difficult to eradicate from the host due to higher tolerance to standard antimicrobial drugs and also resistance to phagocytosis [5]. Moreover, in the form of biofilms, particular fungi such as *Candida albicans* is able to produce 2.3 times higher concentration of carcinogenic compounds such as acetaldehyde [6]. To overcome this problem, it is necessary to find better alternative treatment strategies to prevent, mitigate and destroy biofilms.

In the midst of various approaches that have been studied to control biofilm formation, the use of natural products has shown a promise. Honey has been known for its potential health benefits such as anti-inflammatory, anti-cancerous, antiviral, and anti-oxidant activities since 19th century [7]. In vitro and in vivo studies have shown honey exhibited a potential as a broad-spectrum antibiotic, antiviral and antifungal activities. [8]. Honey is a complex blend of many organic and inorganic compounds such as sugars, proteins, minerals, vitamin, enzymes, phenolic acid, organic acids, pigments, minerals, phenolic acids, flavonoids and many other elements [9]. Honey's antimicrobial activity is determined by a number of bioactive substances, and one of them commonly studied such as hydrogen peroxide (H_2O_2). This substance is produced from the reaction between glucose and glucose oxidase activated when the honey is diluted. Hydrogen peroxide has been frequently suggested a major contributor for honey antimicrobial activities [10], [11].

A part from inhibition the growth of planktonic microorganism cells, honey also has demonstrated antibiofilm activity during in vitro studies. The antibacterial activities of honey on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and other bacterial biofilms have been examined [12]–[15]. Honey also reduces the extracellular polysaccharide matrix production of microorganism thus results in mature biofilm integrity disturbance [16]. However, honey antibiofilm activity against fungi such as *Candida sp.* has not been widely explored. Therefore, this narrative review will discuss about phytochemicals, antimicrobial and antifungal properties as well as honey antibiofilm effect against bacteria and fungi.

Composition of honey

The composition of honey is very complex containing at least 181-200 different substances. In general, 100g of honey contains 82.4 g of total carbohydrates, consisting of Fructose 38.5 g, Glucose 31.28 g Sucrose 1.31 g, Maltose 7.31. Total gluconic acid 0.57 g, Moisture content 17.1 g, Ash 0.169 g, Fiber 0.2 g, Amino acids / proteins 0.3 g, N 0.041 g, Iron 0.42 mg, Potassium 52 mg, Calcium 6.00 mg, Phosphorus 4.00 mg, Magnesium 2.00 mg Copper 1–100 μg / g, Zinc 0.22 mg, Riboflavin 0.038 mg, Niacin 0.21 mg, Pantothenic acid 0.068 mg, Pyridoxine 0.024 mg, Folic acid 2 μg, Vitamin C 0.5 mg. Honey also contains enzymes namely invertase, diastase, catalase, glucose oxidase, phosphatase, and protease [17], [18], [19], [20].

Bioactive components and anti-microbial mechanism of honey

Hydrogen Peroxide is produced from the results of the glucose oxidase reaction where the enzymes in honey convert glucose into gluconolactone. Hydrogen Peroxide causes the death of microorganisms [21]. The antimicrobial activities in several types of honeys depend on the endogenous concentration hydrogen peroxide known as a disinfectant and a strong oxidizing agent. Honey also contains polyphenols, including Gallic acid, Ferulic acid, Chlorogenic acid, Ellagic acid, Syringic acid and Caffeic acid and these bioactive compounds are also considered as antibacterial effect sources in honey [22]. There are more than 150 polyphenol compounds in honey. These polyphenolic compounds have function as antioxidants [23]. Honey antioxidant activity is mainly due to phenolic and flavonoid compounds. The level of honey antioxidant activity is proportional to the value of phenolic and flavonoid concentration [24].

Methylglyoxal (MGO) is a highly reactive dicarbonyl compound (1,2-dicarbonyl methylglyoxal) and it induces rapid and non-enzymatic modification of lysine and arginine residues of protein resulting generation of advanced glycation end products or AGEs [25]. Non-enzymatic conversion of dihydroxyacetone in Manuka honey is the source of MGO production. In Manuka honey stored for less than 1 year, the MGO level was from 0.102 to 0.793 mg / g, the level would increase to 1.541 mg / g when long-term storage or heat treatment [26]. At MGO levels above 0.15 mg / g, this is what causes the antibacterial properties of Manuka honey. Other types of honey have low MGO levels, ranging from 0.0004 to 0.0054 mg / g [27], [28].

Water is the main constituent of living things and honey only contains approximately 15% - 21% water. The water concentration in honey is low enough to allow bacteria or other microorganism growth and the activity of unbound water molecules ranges from 0.562 to 0.62 [29]. The high osmosis power of honey is because 84% of the components contained in honey consist of glucose and fructose). Osmolarity results in strong interactions between sugar molecules and water molecules and leaves very few water molecules. This osmotic pressure will cause the microorganisms to become dehydrated so that they cannot grow. Also, the high osmosis effect of honey inhibits bacterial growth [29], [30], [31]. The acidity level of honey has a pH of 3.2 - 4.5 and this characteristic will inhibit bacterial metabolism and cause bacteria to easily undergo lysis and bacteria to die [32]. The optimum pH for bacterial growth ranges from pH 7.2 to 7.4 [33].

Honey has the potential to inhibit the activity of many pathogenic bacteria including gram-negative and gram-positive bacteria [34], [35]. Manuka honey has activity bactericidal against planktonic cells and biofilms of *Pseudomonas aeruginosa* and *Staphylococcus aureus* honey and their antibiotic activity against *Pseudomonas aeruginosa* was higher than that of *Staphylococcus aureus* [36]. In addition, it was also reported that planktonic *S. aureus* cells exposed to Manuka honey enlarged and had more septa [37]. Manuka honey interacts synergistically with vancomycin antibiotics in the formation of *S. aureus* biofilms and interacts additives with gentamicin antibiotics in the formation of *P. aeruginosa* biofilms [38]. Manuka and clover honey showed good activity against planktonic cells and biofilms from *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella spp.,* and *Proteus mirabilis* [39].

The mechanism of honey as an anti-fungal is still being debated, but several studies say that the mechanism is almost the same as honey as an antibacterial with active substances H_2O_2, flavonoids, methylglyoxal, as well as high sugar content that can affect fungal growth [40], [41], [42], [43].

Different types of honey anti-biofilm activity

Biofilms are a major mode of microbial growth and are essential to development of infection. Cell adhesion, microcolonies formation and biofilm maturation are important stages of biofilm growth. From these structures, bacteria can spread and develop colonies in new environments [44]. Biofilm can be identified using biopsy as a standard procedure of diagnosis in wounds. Often, staining of the biopsy samples is able to identify microcolonies, extracellular polysaccharide matrix, and inflammatory cells [45].

Not only bacteria, various clinically significant fungi also form biofilms such as *Candida, Aspergillus, Cryptococcus, Trichosporon, Coccidioides*, and *Pneumocystis* [46]. Even though *Candida albicans* is one of commonly studied fungi as biofilm producers but *Cryptococcus neoformans* and *Aspergillus fumigatus*, have been known also play significant role in biofilm-related infections. Adhesion, colonization, maturation and dispersal are the developmental stages of fungal biofilm formation which are controlled by complex molecular events [47].

A biofilm is a complex structure made of aggregate of microbial cells and extracellular polymeric substances (EPS) on any surface [48], which can prevent the healing process [49]. A biofilm formation may create a physical barrier that can inhibit infiltration of antibiotics and decrease the chance of wound disinfection [50]. Antibiotic resistance of bacteria in the existence of biofilm has significant contribution to the chronicity of infections. While the exact cause the resistance remains uncertain, several studies have shown that it might be multimodal strategies [51], [52]. Bacterial biofilms may lead to chronic infections due to the increment of tolerance to antibacterial agents and chemical disinfectant, and phagocytosis prevention. It might be associated also with an increased level of mutations as well as with quorum-sensing pathways. Other factors such as common mechanisms of resistance, efflux pumps upregulation and antibiotic target molecules mutations in bacteria may also promote to the formation of biofilms [53]. It has been suggested that over 75% of microbial infections in the human body is characterized by biofilm-related infections [54].

It has been shown by studies that systemic infection is initiated by biofilm dispersal since start from this point, the bacteria exit from biofilm structure and disseminates inside of the host [55], [56]. The release of singular cells and/or multicellular aggregates of bacteria controlled by an active phenotype change involving the sensing of environmental signals and their transduction via complex regulatory networks to final effectors [57]. Similar to the ability to form biofilms, the dispersion of microorganism seems to be a common property shared by most bacteria that cause them suitable for colonization of new niches [58].

It is well established that chronic wounds such as diabetic foot ulcers, pressure ulcers, and venous leg ulcers are difficult to treat and the evidence of bacterial biofilm was abundantly found in specimens from chronic wounds [59]. Evidence from studies suggests that the formation of polymicrobial biofilm in skin and wound infections could be a risk factor for relapse and the treatment become more challenging because of antibiotic resistance [60]. Risk of infection and chronic inflammation increase since biofilm may prolong and prevent healing in both acute and chronic wounds [45]. Mechanical debridement followed by topical antimicrobial agents are crucial to mitigate biofilm reformation because of commercially available antimicrobials and wound dressings are often ineffective in managing biofilm [61]. Efficacious novel antibiofilm agents' development become an area of interest in wound care. The multimodal anti-biofilm mechanism of different types honey and their efficacy against multidrug-resistant bacteria would make it an exciting prospect for forthcoming study [62].

Antibacterial properties of honey have been associated to various bioactive ingredients and mechanisms. These properties include methylglyoxal (MGO), hydrogen peroxide, defensin-1, flavonoids, bee peptides, dehydration of the wound and a low pH and high osmotic pressure [63]–[66]. Specific mechanism might be predominantly involved for particular microorganism for instance the osmotic effect of honey demonstrates significant antibacterial effect for *Helicobacter pylori*, while hydrogen peroxide only exhibits a minor contribution [63]. On the other hand, Egyptian honeys have mostly show antimicrobial activity against *Escherichia coli* mainly by hydrogen peroxide production [67]. Furthermore, the antibacterial efficacy of Manuka honey towards *Escherichia coli* and *Staphylococcus aureus* was found directly to the presence of methylglyoxal [68]. However, the bactericidal activity of Menuka honey is still effective on *Escherichia coli* even after neutralization of its methylglyoxal component due to several unknown factors [69]. Methylglyoxal has demonstrated the antibacterial activity of manuka honey against *Bacillus subtilis* and *Staphylococcus aureus* and but not against *Pseudomonas aeruginosa* and *Escherichia coli* [69]. Among the aforementioned antibacterial mechanism of actions, Methylglyoxal (MGO) appeared to be associated to Manuka honey's efficacy to prevent biofilm formation. Moreover, Manuka honey has shown "significant partial detachment" at 50% concentration of *Proteus mirabilis* biofilms after 24 hours [70]. However, though Manuka honey can infiltrate the biofilm and kills bacterial cells but MGO administration alone is not responsible for this effect, highlighting the importance of other bioactive substances in honey's antibacterial activity, especially on *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains [71], [72].

Even though in vitro studies have demonstrated prospective anti-biofilm efficacy of different types of honey [14] but current randomized controlled trial (RCT) has found no superiority of honey in preventing biofilm on infections when compared with standard care in some settings [73]. Perhaps, antibiofilm activity reported by in vitro assay studies might have inadequate clinical relevance [71], [72]. A recent review study found that there is evidence with limited quality of honey's beneficial effect for partial thickness burns healing. However, inconclusive evidence regarding the use of honey for other wound-related indications. For partial thickness burns, treatment with honey may heal faster compared to conventional dressing [74].

Conclusion

Honey exhibits antibacterial activity against a wide variety of gram-positive and gram-negative bacteria, antibiotic-resistant as well as antibiotic-sensitive bacteria. Several honey bioactive components may contribute not only to the antibacterial efficacy but also antifungal effect. These bioactive substances provide a synergy that result in modulation of the resistance to antimicrobial drugs. Honey demonstrates activities against both planktonic and biofilm phenotype states of microorganisms. Microorganism's planktonic and biofilm states play important role in delaying healing of wounds. There is a very large variation in terms of the anti-microorganism's potency of different types of honey. Manuka is one of the most studied honey which has in vitro activity against both planktonic and biofilm microorganisms. It is clinically important, particularly since most traditional antibiotics or antifungal lack activity against biofilms. However, further clinical studies are required to test the efficacy of the prospective anti-biofilm efficacy of honey as a treatment for wounds and other health conditions.

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