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Antimicrobial Efficacy of Honey's Flavonoids and Phenolic Acids: Implications for Wound Healing and Pharmaceutical Development

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Abstract

The antimicrobial nature of honey has been appreciated for thousands of years; however, the molecular mechanism through which its function is only now being unraveled. This article discusses the importance of flavonoids and phenolic acids in terms of their contribution to the anti-microbial nature of honey. Various flavonoids, such as quercetin, kaempferol, chrysin, and galangin, along with phenolic acids like caffeic acid, gallic acid, ferulic acid, and p-coumaric acid, use a combination of various modes of actions in order to act upon pathogenic microorganisms. Their mode of action includes disruption of bacterial cell membrane, interference with nucleic acid synthesis, protein denaturation, inhibition of quorum sensing, elimination of established biofilm and induction of oxidative stress. The antimicrobial activity extends to various species of both Gram-positive pathogens like methicillin-resistant Staphylococcus aureus, Gram-negative bacteria like Pseudomonas aeruginosa and Escherichia coli, and fungal pathogens such as Candida albicans. Additionally, phenolic acids are capable of modulating inflammatory response and have proangiogenic effects as well as stimulate collagen deposition, enhancing the process of tissue repair. The rise of antibiotic resistance among pathogenic bacteria has once again led to a resurgence in research on honey as a possible source of new medicines. There is clinical proof that honey-based treatments work well for burns, diabetic ulcers, and post-operative infections. However, there are many hurdles, such as the inconsistent composition of honey, lack of standardization, poor bioavailability, and legal concerns that are now impeding the development of drugs. Possible areas of exploration in the future could be extraction of bioactive ingredients, chemical modification to increase potency, use of nanoparticles for delivery, and inclusion in antimicrobial stewardship initiatives.

Introduction

The usage of honey for healing purposes has been documented throughout history by nearly all human civilizations. Wound treatments using honey are mentioned in ancient Egyptian texts from 2000 BCE. Additionally, Greek physicians like Hippocrates and Dioscorides recommended honey for various uses, such as healing infections, sore throat, and digestive disorders. Similarly, honey is considered useful for treatment in both traditional Chinese medicine and Ayurveda [1]. The historical appreciation of honey can be attributed to its consistent effectiveness due to a lack of knowledge about its mechanism of action. Since the discovery of penicillin in 1928 and the ensuing rapid development of the antibiotic era, conventional treatments like honey had been replaced by the more effective, specific, and relatively safe antibiotics in modern medicine. Nevertheless, the increasing problem of antimicrobial resistance has drastically changed the situation [2]. Multidrug-resistant pathogens like methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE), carbapenem-resistant Enterobacteriaceae (CRE), and extensively drug-resistant Pseudomonas aeruginosa (XDRPA) have emerged as major health problems. According to the WHO report of 2019,

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antimicrobial resistance is one of the ten main public health threats that humanity is facing today. Approximately 1.27 million people died directly because of multidrug-resistant bacterial infections in 2019 alone [3,4].

The above-mentioned crisis has led to renewed interests in the antimicrobial properties of natural products, one of which is honey. Unlike monotherapy using antibiotics that can induce resistance due to selective pressure, honey consists of various biologically active substances acting in various ways, thus, making it less likely to cause the development of resistance. Even though the antimicrobial effect of honey can be attributed to the presence of high sugar concentration, low pH, and enzyme-induced hydrogen peroxide, recent studies have identified the essential role of phenols, such as flavonoids and phenolic acids, especially in honey types with low hydrogen peroxide activity, like manuka honey. Secondary metabolites that are synthesized by plants include flavonoids and phenolic acids. Such compounds are characterized by phenolic rings that provide for antioxidant, anti-inflammatory, and antimicrobial effects [5]. Honey contains an extensive range of such compounds with considerable variation depending on the type of nectar used, geographic origin of flowers, and other factors. The content of total phenolic compounds in honey varies between 50 and 1000 mg/kg, with dark honeys having more flavonoids than light-colored varieties [6].

This review offers an exhaustive study of the antibacterial effect of honey-based flavonoids and phenolic acids, paying attention especially to their mode of action, range of efficacy, function in wound healing, and pharmaceutical prospects. This paper aims to summarize present understanding of the molecular modes through which these chemicals act to prevent and destroy microorganisms, to evaluate the proof for their effectiveness against important pathogens, both multidrug-resistant and otherwise, to investigate the double purpose they serve as antibacterials and wound healers, to analyze their present and potential use in pharmaceuticals, and to recognize future research opportunities in this domain.

Composition of Honey Relevant to Antimicrobial Activity

❖ Major Bioactive Compounds

This review offers an exhaustive study of the antibacterial effect of honey-based flavonoids and phenolic acids, paying attention especially to their mode of action, range of efficacy, function in wound healing, and pharmaceutical prospects. This paper aims to summarize present understanding of the molecular modes through which these chemicals act to prevent and destroy microorganisms; to evaluate the proof for their effectiveness against important pathogens, both multidrug-resistant and otherwise, to investigate the double purpose they serve as antibacterials and wound healers, to analyze their present and potential use in pharmaceuticals, and to recognize future research opportunities in this domain [7].

Although chemically analogous to quercetin except for the absence of a 3'-hydroxyl moiety, kaempferol possesses reduced antimicrobial activity but better stability and bioavailability. Kaempferol is abundantly present in rosemary honey and propolis in amounts of 5–15 mg/kg. Antimicrobial action of kaempferol is largely mediated via the interference with bacterial membranes and DNA gyrase of bacteria [8]. Chrysin is a flavonoid that exists mainly in propolis and some types of honey and does not have any hydroxyl group on its B ring but contains a 5,7-dihydroxy group on its A ring. Chrysin has been shown to have a strong antibacterial effect on Gram-positive bacteria such as S. aureus, with its minimum inhibitory concentration varying between 15 and 60 micrograms per milliliter. It works by interfering with the integrity of the bacterial cell wall and blocking efflux pumps [9].

Galangin, which is a flavanol found in propolis and manuka honey, possesses a 5,7 dihydroxy group at its A ring and a 3-hydroxy group in its C ring but without any substituents in the B ring. Galangin has been found to possess an exceptional effect against methicillin resistant Staphylococcus aureus at minimum concentrations as low as 10 micrograms/milliliter. The special feature that galangin displays is its ability to inhibit cell wall synthesis by binding penicillin binding proteins [10]. Phenolic acids also include caffeine acid, which is considered highly important among other compounds. The caffeic acid phenethyl ester, found in both propolis and manuka honey, has shown wide-spectrum antimicrobial activity with minimal inhibitory concentrations in the range of 5 to 50 microliters per milliliter against different organisms. The caffeic acid prevents bacterial DNA synthesis by targeting topoisomerases, while blocking virulence factor production by suppressing NF-kappa B activity [6], [11].

Gallic acid, a trihydroxybenzoic acid, occurs abundantly in dark honeys such as buckwheat and chestnut honey. The content of gallic acid varies between 2 and 50 mg/kg, with high levels present in honeys obtained from areas containing plants rich in gallic acid. Mechanisms of action of gallic acid on microorganisms include its capability to disrupt cell membranes, precipitate proteins, and chelate iron. Ferulic acid and p-coumaric acid are members of the class of hydroxycinnamic acids which are commonly found in honey sourced from various flowers. The amounts of ferulic acid usually fall between 0.5 to 5 milligrams per kilogram and p-coumaric acid between 0.2 to 3 milligrams per kilogram. These chemicals possess moderate antimicrobial activities by themselves but have crucial synergistic effects on the action of other chemicals [12].

❖ Variation Based on Floral and Geographical Origin

The profile of flavonoids and phenolic acids in honey is mainly influenced by the flower sources visited by honeybees. In monofloral honeys sourced from a single flower species, certain phenolic profiles can be used to identify authentic

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products. For instance, Manuka honey from New Zealand is made from the nectar of Leptospermum scoparium flowers and has relatively higher levels of methylglyoxal, leptosperin, and flavonoids such as leptosin and manuka flavanone [6], [13]. These compounds offer superior anti-pathogen activities, even against strains with antibiotic resistance capabilities. Buckwheat honey, made from Fagopyrum esculentum flowers, is one of the most potent sources of total phenols compared to other commercially available types, with total phenolic content varying between 500 and 1500 mg/kg (equivalent to gallic acid). Buckwheat honey has high quercetin and kaempferol contents, which contribute to its dark brown color and antimicrobial effects on Gram-negative microorganisms like Pseudomonas aeruginosa [14].

Gallic acid and caffeic acid derivatives are abundant in European and Asian chestnut honeys, while total phenolic content is similar to that of buckwheat honey. The high antibacterial effect of chestnut honey against biofilm-producing bacteria, such as Staphylococcus epidermidis and Candida albicans, is attributed to the synergistic effect of phenolic acids and high hydrogen peroxide content. Acacia honey, obtained from Robinia pseudoacacia flowers, possesses lower total phenolic content but high amounts of p-coumaric acid and particular flavonoids like kaempferol and isorhamnetin [15]. Although acacia honey shows lower total phenolic content compared to other types, it still demonstrates strong antimicrobial activity due to synergy between compounds and high osmolarity. Geographical location can result in significant differences in phenolic content even among the same floral species, depending on variations in soil composition, climate, altitude, and agricultural conditions. Mountain honey has greater phenolic content than lowland honey, perhaps because of higher UV radiation and temperature stress promoting secondary metabolism in plants. Organic honey production, which restricts synthetic pesticides and fertilizers, may increase phenolic content in certain cases but is not uniformly observed for all floral sources [6].

- **Synergistic Interaction with Other Honey Components**

The antimicrobial effect of honey is not based on the presence of only phenolic compounds because these phenolics work together with other honey constituents in order to exert a wide spectrum antimicrobial effect typical of honey as a whole. To develop pharmaceutical products with antimicrobial properties, knowledge of these interactions is crucial. The hydrogen peroxide produced in honey as a result of glucose oxidase action is formed due to adding glucose oxidase enzyme in honey production by bees [16]. Glucose oxidase enzyme oxidizes glucose in diluted honey to gluconic acid and hydrogen peroxide is formed in the process. Hydrogen peroxide is formed in amounts of 0.1 to 3 millimolar in diluted honey which is enough to prevent development of most bacteria but not lethal for bacteria. Flavonoids and phenolic acids improve the antimicrobial effect of hydrogen peroxide via multiple ways. They stabilize hydrogen peroxide protecting it from catalysis by reducing metal ions, can produce additional oxidative stress in microorganism cells due to redox reactions, or inhibit catalases that protect bacteria from hydrogen peroxide effects [6, 17, 18].

Low pH value of honey ranges between 3.2 and 4.5. Low pH makes honey anti-microbial due to the creation of unfavorable conditions for bacteria. Most disease-causing bacteria are optimally active in environments with neutral pH and inhibited or die when pH is below 4.5. At low pH, phenols are effective as undissociated forms can pass through bacteria membrane easily compared to dissociated forms. The two factors (low pH and phenolic compounds) combine in a synergistic way to increase effectiveness in fighting microorganisms with the effect of the particular phenolic compounds growing by 2 to 10 folds when pH changes from 7 to 4 [19, 20].

High osmolarity caused by sugar content of 70 to 80 percent in honey leads to the formation of osmotic pressure that extracts water from bacteria cells leading to plasmolysis and inhibiting their growth. Osmolarity alone cannot kill most bacteria but increases sensitivity to other anti-microbial compounds such as phenols. Dried bacteria cells are weak in repairing themselves and have weak membranes that can be attacked by flavonoids and phenolic acids [21].

Methylglyoxal, a dicarbonyl which occurs in high quantities in manuka honey, but at lower levels in other types of honeys, has strong antimicrobial properties irrespective of the presence of hydrogen peroxide. Methylglyoxal inhibits bacteria by reacting with the proteins and DNA to cause cross-linking. Flavonoids and phenolic compounds boost the effects of methylglyoxal by working via different means such as by blocking the actions of glyoxalase enzymes which degrade methylglyoxal. The synergistic effect of methylglyoxal and phenolics in manuka honey results in the ability to fight off resistant strains [22].

Antimicrobial Mechanisms of Flavonoids and Phenolic Acids

- **Disruption of Bacterial Cell Membrane Integrity**

Bacterial cell membranes represent major targets of honey flavonoids and phenolic acids. Honey flavonoids and phenolic acids are amphiphiles consisting of hydrophobic aromatic rings as well as hydrophilic hydroxyl groups that allow them to intercalate into the lipid bilayers of bacterial cell membranes. Intercalation results in the disarrangement of bacterial cell membranes and leads to an increase in their fluidity and permeability, which consequently causes loss of intracellular components such as potassium ions, nucleotides, and proteins, thus causing cell death [23]. Studies indicate that molecules like quercetin and kaempferol intercalate into the bacterial membrane bilayer using hydrogen bonds established between their hydroxyl group and phospholipid polar heads of the membranes. Simultaneously, aromatic rings orient along the hydrophobic acyl tails of the phospholipids. Such interactions cause membrane destabilization and increase its permeability. Atomic force microscopy studies show that quercetin, at concentrations of 10 to 50 micrograms per

milliliter, results in disruption of bacterial membrane structure characterized by pore and bleb formation [8].

Galangin shows very strong membrane disruption effects against Gram-positive bacteria, because such types of bacteria have no outer membrane, so are easier targets for substances that target membranes. For example, the minimum inhibitory concentration of galangin against S. aureus is about 15 to 30 μg/mL, and the complete disruption of membranes is achieved at concentration levels higher than 50 μg/mL [24]. It seems that the existence of the 5,7-dihydroxy substitution pattern within the A-ring of flavones is essential for their membrane disruption properties, because flavones without such substitutions have less membrane activity. The difference in sensitivity of bacterial and mammalian cells to membrane disrupting effects of polyphenols gives grounds for using such compounds therapeutically. There are higher concentrations of negatively charged phospholipids, such as phosphatidylglycerol and cardiolipin, in the bacterial membranes compared to the mammalian ones. Flavones become positively charged when placed into acidic conditions, and their cationic character allows them to interact electrostatically with the membranes of bacteria, and not those of mammals with a larger fraction of zwitterions [8, 25].

❖ Inhibition of Nucleic Acid Synthesis

Several compounds found in honey affect the processes involved in the synthesis of bacteria's DNA and RNA. Caffeic acid and some of its metabolites act as inhibitors of topoisomerase enzymes in bacteria. The enzymes involved include DNA gyrase and topoisomerase IV, which play a critical role in replication and transcription of bacterial DNA [26]. The enzymes make a temporary double-strand break within bacterial DNA, pass a different DNA strand through the break and seal the break back up again. Inhibition of the topoisomerase enzymes involves stabilization of the enzyme-DNA complex such that the break cannot be sealed, resulting in the formation of double-strand breaks within DNA that are ultimately fatal to the bacteria. Quercetin acts as an intercalator of DNA strands; it inserts itself into DNA by fitting into the space between two bases of DNA, thereby distorting the DNA strand and affecting DNA replication and transcription by interfering with the action of DNA polymerase and RNA polymerase on the DNA [27].

The DNA synthesis is inhibited by the gallic acid by oxidative damage of DNA strands and bases. Gallic acid autoxidizes in the presence of oxygen, forming hydrogen peroxide and other reactive oxygen species, resulting in DNA damage. The concentration of the gallic acid necessary to observe DNA damage is about 50-100 μg/mL, and although it is much higher than gallic acid concentrations usually found in honey, this level can be achieved in concentrated preparations based on honey. Chrysin has been shown to inhibit the activity of bacterial RNA polymerase, an enzyme which transcribes DNA into mRNA molecules [28]. Chrysin interacts with the switching region of RNA polymerase, thus making the conformational changes required for the transcription initiation impossible. The mechanism of action is similar to that of antibiotic rifampicin, though chrysin targets a different region, and thus its activity against rifampicin-resistant strains has been proven [29].

❖ Protein Denaturation and Enzyme Inhibition

Flavonoids and phenolic acids can interact with proteins in bacteria via various modes such as hydrophobicity, hydrogen bonding, and chemical modification. Such an interaction can result in protein denaturation and loss of their secondary and tertiary structure or inhibition of enzymatic action due to specific binding of these organic molecules at active sites or allosteric sites. Hydrogen bonds are established between the phenolic hydroxyl group of such organic molecules and amide groups in the protein backbone or with the polar side chains such as serine, threonine, tyrosine, and glutamine. Many hydrogen bonds between a flavonoid and protein lead to stabilization of such a binding event that results in structural distortion of the protein [30].

It was discovered that kaempferol acts as an inhibitor of bacterial enoyl-acyl carrier protein reductase, an enzyme involved in fatty acids biosynthesis. In addition, the antibiotic triclosan also acts through its inhibition, targeting the same site in the enzyme, but with stronger binding. Disruption of fatty acid biosynthesis leads to impaired formation of the cell membrane and thus increased sensitivity to compounds that interfere with the cell membrane. Ferulic acid acts as an inhibitor of bacterial dihydrofolate reductase, which is necessary for tetrahydrofolate biosynthesis [31]. Tetrahydrofolate is needed for nucleotides formation; it acts similar to antibiotic trimethoprim. However, ferulic acid is much weaker than trimethoprim, having minimum inhibitory concentration about 100 times higher. At the same time, ferulic acid shows synergistic activity with sulfonamide antibiotics acting through inhibition of another enzyme from this pathway [32].

❖ Anti-Quorum Sensing Activity

The phenomenon of quorum sensing represents a process of bacterial cell-to-cell communication for coordinating gene expression based on population density. The regulation of virulence gene expression in pathogenic bacteria via quorum sensing mechanisms includes toxins, proteases, and biofilm synthesis. Suppression of quorum sensing decreases the virulence of bacteria, and thus may help avoid developing drug-resistant strains [33]. Quorum sensing activity of several compounds found in honey has been described. For instance, quercetin interferes with the LasI/LasR and RhlI/RhlR quorum sensing systems of Pseudomonas aeruginosa and inhibits the synthesis of enzymes such as elastase, pyocyanin, and rhamnolipids. At subinhibitory levels ranging from 5 to 10 micrograms per milliliter, quercetin suppresses quorum sensing-regulated gene expression by 50 to 80 percent [34].

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The compound caffeic acid phenethyl ester has been observed to interfere with the function of agr quorum sensing in Staphylococcus aureus. Agr quorum sensing mediates the regulation of various virulence genes such as alpha toxin, phenol soluble modulins, and proteases. Exposure to caffeic acid phenethyl ester at a concentration of between 10 and 20 micrograms per milliliter significantly reduces alpha toxin production to less than ten percent and weakens the hemolytic activity of the supernatant. The flavonoid galangin has shown antimicrobial activity against the AI-2 quorum sensing pathway, which is involved in interspecies communication of both gram-negative and gram-positive bacteria. The molecule binds to the LuxP receptor protein that receives AI-2 signals thus interfering with signaling and subsequent gene transcription [3].

- **Biofilm Inhibition and Eradication**

Biofilms consist of microbial communities attached to one another within an environment containing an extracellular matrix of substances produced by bacteria themselves. They are highly resistant to both antibiotic therapy and the actions of the body's immune system. Honey polyphenols such as flavonoids and phenolic acids have been shown to have preventive and antimicrobial effects against bacterial biofilms [35]. Quercetin prevents biofilm formation in S. aureus at concentrations ranging from 10 to 25 micrograms per milliliter by interfering with biofilm formation mechanisms, namely bacterial attachment to surfaces and production of EPS. This occurs via suppression of polysaccharide intercellular adhesion genes and suppression of the accessory gene regulator system. Caffeic acid and ferulic acid prevent biofilm formation in P. aeruginosa by dispersing biofilm cells and increasing the sensitivity of bacteria to antibiotics [36]. These effects occur due to interference with second messenger systems involved in regulating the transition between biofilm and planktonic forms of growth. In particular, reduction of cyclic diguanylate results in conversion of bacteria to planktonic form. Gallic acid has exhibited a high effectiveness in treating Candida albicans biofilms. Treatment with gallic acid ranging from 50 to 100 µg/mL causes a reduction of more than 80 percent in biofilm biomass along with an inhibition of yeast to hypha conversion, which is crucial for the formation of biofilm. Treatment with gallic acid in conjunction with fluconazole leads to a synergistic effect on fluconazole-resistant Candida albicans biofilms [37].

- **Oxidative Stress Induction in Microbial Cells**

The formation of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals, by flavonoids and phenolic acids occurs via several pathways. In the process of redox cycling, these molecules are reduced from their parent structure into a semiquinone radical, followed by the reaction with oxygen to generate superoxide and return the parent molecule back. Redox cycling may produce massive amounts of superoxide that, subsequently, dismutate to hydrogen peroxide and produce hydroxyl radicals, one of the most active and dangerous ROS [38, 39]. The efficiency of redox cycling in quercetin is relatively high due to the presence of two substitutions at the 3'- and 4'-positions that provide stability for the semiquinone radical through resonance interactions. The rate of superoxide formation by quercetin is about ten times higher compared to that of kaempferol without the 3'-hydroxyl substituent and about 100 times higher compared to that of chrysin lacking both hydroxyl groups in the B-ring [40].

Oxidative stress mediated through flavonoids and phenolic acids targets several bacterial cellular structures such as lipids, proteins, and DNA. Oxidation of lipids resulting from free radicals acting upon unsaturated fatty acid lipids of the bacterial cell membrane is another way to damage the membrane integrity of the bacterium. Oxidation of proteins results in protein carbonylation and cross-linkage and thereby deactivation of enzymes and protein denaturation. Oxidative damage to DNA includes strand breakage, alteration of DNA bases, and DNA-DNA cross-linkage, which are potentially lethal to bacteria [41, 42]. Bacteria have several ways to defend against oxidative stress, for example, superoxide dismutases, catalase, and peroxidase enzymes. Also, bacteria have DNA repair mechanisms to protect against any DNA damage. However, both the oxidative stress generated through flavonoids and the inhibition of the enzyme activity of bacteria's antioxidant system due to other honey constituents work synergistically to neutralize the defense mechanism of bacteria [43].

Spectrum of Antimicrobial Activity

- **Gram-Positive Bacteria**

The minimum inhibitory concentration of manuka honey on clinical isolates of methicillin-resistant Staphylococcus aureus usually falls within 3% - 10% weight per volume with concentrations of flavonoids amounting to about 10 µg/ml – 30 µg/ml. The minimum inhibitory concentration of individual flavonoids, such as galangin, quercetin, and chrysin, against methicillin-resistant Staphylococcus aureus is about 15 µg/ml – 60 µg/ml [24]. Flavonoids can exert their antibacterial effect against Staphylococcus aureus through targeting several aspects including the bacterial membrane structure, cell wall synthesis, and production of virulence factors. Flavonoid-induced cell killing occurs in a concentration-dependent manner, where the bactericidal effect may be achieved when the concentration used is 2- to 4-fold greater than the minimum inhibitory concentration. In addition, the use of flavonoids in combination with oxacillin or vancomycin against methicillin-resistant Staphylococcus aureus leads to 4- to 16-fold reduction in antibiotic minimum inhibitory concentration [44].

The Streptococcus pyogenes bacteria that cause streptococcal pharyngitis and necrotizing fasciitis can also be killed by honey flavonoids. Growth of the Streptococcus pyogenes bacteria can be inhibited by quercetin and kaempferol at a minimum inhibitory concentration of 20 to 40 micrograms per milliliter, while the formation of the streptolysin O and other virulence factors can also be inhibited. Propolis flavonoids such as chrysin and galangin can inhibit the growth of the

Streptococcus pneumoniae bacteria that are responsible for community-acquired pneumonia at a minimum inhibitory concentration of 10 to 30 micrograms per milliliter [28]. The Enterococcus faecalis and Enterococcus faecium bacteria, including the vancomycin-resistant enterococci, are not as easily inhibited by honey flavonoids compared to staphylococci and streptococci, as the minimum inhibitory concentration is 50 to 200 micrograms per milliliter. However, flavonoids have synergistic activity when combined with conventional antibiotics such as ampicillin and gentamicin against vancomycin-resistant enterococcus [8, 45]

- **Gram-Negative Bacteria**

Gram-negative bacteria are generally less susceptible to honey flavonoids and phenolic acids due to the presence of an outer membrane that restricts permeation of hydrophobic compounds. But some flavonoids or flavonoid combinations can help to break this resistance. Opportunistic bacterium Pseudomonas aeruginosa, causing severe infections among immunocompromised people and patients with cystic fibrosis, has variable sensitivity to honey flavonoids [46]. Minimum inhibitory concentration of quercetin against Pseudomonas aeruginosa usually varies between 100 and 300 micrograms per milliliter, and the minimum inhibitory concentrations of gallic acid and caffeic acid are 200-500 micrograms per milliliter. But at lower concentrations, sub-inhibitory doses of flavonoids reduce the biosynthesis of virulence factors such as elastase, pyocyanin, and rhamnolipids, decreasing the pathogenicity of bacteria [47].

Enteropathogenic or uropathogenic Escherichia coli is sensitive to honey flavonoids at minimum inhibitory concentrations varying between 50 and 200 micrograms per milliliter. Quercetin and kaempferol inhibit growth of extended-spectrum beta-lactamase producing Escherichia coli, showing that the mechanism of antibacterial action of honey flavonoids is different from those used by beta-lactams. In the presence of polymyxin B, which destroys Gram-negative cell wall outer membrane, synergistic effects of the drug and flavonoids are observed, and the minimum inhibitory concentration of polymyxin B may be decreased by up to 8 times [48].

Among the most antibiotic-resistant Gram-negative bacteria are strains of carbapenem-resistant Klebsiella pneumoniae. Manuka honey and propolis extract have shown effectiveness against carbapenem-resistant Klebsiella pneumoniae at concentrations ranging from 6% to 12%. The compounds found in these natural products are methylglyoxal and caffeic acid derivatives, which seem to counteract the mechanisms of resistance such as carbapenemase production [49].

- **Fungal Pathogens**

Candida albicans represents the most frequent fungal pathogen in humans, causing diseases, such as mucosal candidiasis and systemic candidiasis, which can be lethal. Flavones and phenolic acids from honey exert antifungal properties by disrupting membranes, blocking ergosterol biosynthesis, and inhibiting yeast hyphal conversion. Chrysin and galangin show inhibition of Candida albicans growth at a minimum inhibitory concentration between 20 and 50 micrograms per milliliter [50]. Additionally, these two flavonoids inhibit biofilm formation and secreted aspartyl protease, which plays an essential role in the virulence of fungi. The combination of chrysin with fluconazole shows synergism for killing fluconazole-resistant strains of Candida albicans, with the minimal inhibitory concentration decreased by 16-32 times [51].

The two acids caffeic and ferulic are capable of inhibiting the growth of Candida albicans with minimum inhibitory concentration at 50-150 microgram per milliliter. The two phenolic acids also decrease the ability of Candida to adhere to epithelial cells and inhibit their ability to form hyphae, which is necessary in order for them to invade tissues. Combination of caffeine acid with amphotericin B leads to additive effects, which can help in lowering doses of this fungicide [50].

The non-albicans varieties of Candida such as Candida glabrata, Candida parapsilosis, and Candida tropicalis tend to be more resistant than Candida albicans, with minimum inhibitory concentration being 2-4 times higher. Nevertheless, combinations of various flavonoids from propolis have been found to inhibit growth of the species, suggesting that different flavonoids used in combination could bypass the relative resistance [52].

- **Activity Against Multidrug-Resistant Pathogens**

The increasing prevalence of pathogens resistant to various types of antibiotics has increased interest in the development of alternative treatments using honey flavonoids and phenolic acids. Honey flavonoids and phenolic acids have been found to have activity against multi-drug resistant pathogens since their mechanism of action differs from that of antibiotics and requires a multi-mutation resistance response for resistance to develop [53]. Honey flavonoids still show activity against methicillin-resistant Staphylococcus aureus bacteria, which are resistant to all beta-lactam antibiotics such as penicillins, cephalosporins, and carbapenems. The minimum inhibitory concentration (MIC) of galangin and chrysin against methicillin-resistant S. aureus is equal to that of methicillin-sensitive strains, meaning that methicillin resistance does not provide cross-resistance to honey flavonoids [8].

Honey flavonoids also inhibit the growth of vancomycin-resistant Enterococcus bacteria, which includes strains resistant to both vancomycin and teicoplanin. Honey flavonoids show MICs of 50 to 200 micrograms per milliliter against vancomycin-resistant Enterococcus bacteria. The addition of quercetin to linezolid or daptomycin provides synergy with antibiotics that are still effective against vancomycin-resistant enterococci [54].

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Carbapenem-resistant Enterobacteriaceae, which have carbapenemase enzymes that break down carbapenem antimicrobials, are sensitive to manuka honey and propolis extracts. Honey, when used in conjunction with the carbapenem antibiotic meropenem, shows a synergistic effect on certain strains of carbapenemase bacteria, implying that the compounds in honey can prevent carbapenemase activity or enhance bacterial membrane permeability to the antibiotic meropenem [55]. Extensively drug-resistant Pseudomonas aeruginosa, which is resistant to three or more classes of antimicrobial drugs, continues to be sensitive to the use of honey flavonoids and colistin, which is a last resort antimicrobial agent. Quercetin, a type of flavonoid, damages the outer membrane of Pseudomonas aeruginosa, allowing better colistin uptake into the bacteria [56].

Role in Wound Healing

- **Anti-Inflammatory Effects**

Persistent inflammation contributes to chronic wounds such as diabetic ulcers, pressure ulcers, and venous leg ulcers, making wound healing difficult. Honey-derived flavonoids and phenolic compounds exhibit strong anti-inflammatory properties through different biological processes that allow them to lower inflammation while retaining the antibacterial properties required for wound healing. Quercetin and kaempferol function by suppressing nuclear factor kappa B signaling pathways, which control inflammatory processes. The two flavonoids suppress the phosphorylation and breakdown of inhibitors of nuclear factor kappa B, the protein responsible for the sequestration of nuclear factor kappa B. Suppression of the migration of nuclear factor kappa B into the nucleus lowers the transcription of inflammatory mediators such as tumor necrosis factor alpha, interleukin-1 beta, and interleukin-6 [57, 58].

Caffeic acid phenethyl ester is known to inhibit the enzymes cyclooxygenase and lipoxygenase, which are involved in the synthesis of inflammatory eicosanoids such as prostaglandins and leukotrienes. This leads to reduced pain, swelling, and tissue injury related to inflammation in the wound site. This is similar to the mechanism of non-steroidal anti-inflammatory drugs; however, the former uses a different mechanism of action that does not produce adverse effects on the stomach. Gallic acid and ferulic acid are potent scavengers of reactive oxygen species that are produced by neutrophils and macrophages in abundance during chronic wounds. Overproduction of these reactive oxygen species damages tissue components such as lipids, proteins, and DNA, thus slowing down the wound healing process [59].

- **Promotion of Tissue Regeneration**

The presence of honey polyphenols like flavonoids and phenolic acids helps induce proliferation and migration of keratinocytes and fibroblasts, respectively. In vitro experiments have demonstrated that quercetin at concentrations of 5 to 20 micromolars increases keratinocyte proliferation by 30 to 50 percent and facilitates the closure of scratches at the rate of 60 to 80 percent. The cellular process takes place due to stimulation of the extracellular signal regulated kinase signaling pathway that aids in cell cycle progression and migration. Another polyphenol that stimulates proliferation of fibroblasts is caffeic acid phenethyl ester, which acts at concentrations of 10 to 50 micromolars. The process involves the upregulation of type I and type III collagen, which are the main components of granulation tissue [60].

Chrysin stimulates angiogenesis, the process of generating new blood vessels, which is necessary for transporting oxygen and nutrients to wounded areas undergoing healing. Chrysin stimulates the growth and development of blood vessel tubes from human umbilical vein endothelial cells at a concentration range between 5 to 20 micromolar. This effect is attributed to increased vascular endothelial growth factor activity mediated by hypoxia-inducible factor 1 alpha [61].

- **Modulation of Immune Response**

Wound healing necessitates an immune system response characterized by elimination of bacteria without causing too much damage to the tissues. Flavonoids and phenolic acids found in honey regulate different processes within the immune system to ensure the maintenance of this balance. The flavonoids increase phagocytosis of macrophages and neutrophils, resulting in more efficient elimination of bacteria. Quercetin-treated macrophages exhibit up-regulated phagocytic receptor expression and increased reactive oxygen species generation that improves bacteria ingestion and killing. But there is an upper limit to this concentration – more than 50 micromoles of quercetin results in inhibition of phagocytosis. Matrix metalloproteinases are known to be decreased by flavonoids. In chronic wounds, there are increased amounts of matrix metalloproteinases that hydrolyse the extracellular matrix proteins, preventing wound healing. Quercetin and kaempferol inhibit the synthesis of matrix metalloproteinases [62].

In addition, they regulate the immune response by preventing the multiplication of T cells and cytokine synthesis. Although this may lower the immune response against bacteria, it also reduces the excessive inflammation typical of chronic wounds. The overall result is positive because wounds treated with honey exhibit decreased inflammation but not elevated infection levels [63].

- **Reduction of Microbial Load in Chronic Wounds**

Chronic wounds are always colonized by bacteria, and high levels of bacteria adversely affect wound healing by causing inflammation, tissue damage, and nutrient and oxygen competition among others. Honey flavonoids and phenolic acids inhibit wound infection levels by utilizing the same antimicrobial mechanisms explained in the earlier part of this paper. Research has found that dressings made from honey can lead to reduction of up to 2 to 4 logs of bacterial levels in chronic wounds within 7 to 14 days of applying the dressings. These results coincide with better wound healing processes such as wound size reduction, enhanced granulation tissue growth, and improved epithelium formation [64], [65]. Honey

flavonoids are especially effective against S. aureus and P. aeruginosa, which are known to be the major bacterial species associated with chronic wounds. The anti-biofilm effects of honey flavonoids are significant when applied to chronic wounds since they are known to contain high amounts of biofilm bacteria that cannot be treated by conventional methods like antibiotics and other defense mechanisms. Biofilm bacteria are dispersed to the planktonic form by honey dressing thus making it easy to kill them [66].

The decrease in bacterial load also results in less bacterial toxin and protease production that may have a negative impact on the wound tissue. Alpha-toxin of S. aureus and elastase of P. aeruginosa destroy extracellular matrix molecules and kill host cells. Honey polyphenols and phenolic compounds inhibit the growth of bacteria and virulence factors production to prevent bacterial tissue destruction [67].

- **Angiogenesis and Collagen Deposition Enhancement**

Angiogenesis and collagen formation are essential aspects of the proliferative stage in wound healing. Blood vessels provide an essential source of oxygen and nutrition for the wounded area, while collagen provides structural support as well as acts as a scaffold on which cells move. Caffeic acid phenethyl ester has proven to be an excellent stimulator of angiogenesis. Animal studies have shown that local administration of caffeic acid phenethyl ester leads to a significant increase in wound vascularity between 50 and 100 percent higher when compared to control wounds [68]. This is due to the stimulation of two angiogenic growth factors: vascular endothelial growth factor and basic fibroblast growth factor. Quercetin acts through collagen accumulation in the wound, as it promotes fibroblast proliferation and collagen production. Moreover, quercetin prevents collagen breakdown by inhibiting matrix metalloproteinase activity. As a result, there is a higher amount of collagen deposited, which makes wound more resistant to tear [69]. Honey is a compound product that possesses a synergistic combination of flavonoids and phenolic acids. Overall, the presence of all these compounds in honey contributes to even better wound-healing properties than individual flavonoids and phenolic acids do [70].

- **Clinical Evidence in Burn and Diabetic Wound Healing**

Honey-based therapies have been tested for their effectiveness in treating wounds, particularly burns and diabetic ulcers. These trials show that the flavonoid and phenolic acid compounds in honey are effective in wound healing in humans. In burns, honey-based wound dressings have been compared with the current standard topical antibiotic treatment for burns, known as silver sulfadiazine. Several clinical trials have shown that honey dressings are superior to silver sulfadiazine in accelerating wound healing by 3-7 days. Honey also appears to be equally effective in preventing wound infections or better than silver sulfadiazine. In treating diabetic foot ulcers, honey dressings have exhibited encouraging results [71]. A meta-analysis of randomized control trials shows that honey dressing treatments resulted in an approximate increase in the proportion of completely healed wounds by 30% compared to control dressings. This effect was more noticeable in non-ischemic ulcers, implying the need for proper blood circulation for honey to facilitate wound healing. Research on individual flavonoids and phenolic acids is less plentiful than that done on honey in general. There have been several studies done on the effect of propolis on wound healing due to the high levels of flavonoids and phenolic acids contained within propolis. Propolis ointments have been shown to be effective in the treatment of pressure ulcers, surgical wounds, and leg ulcers [72, 73].

Pharmaceutical and Biomedical Applications

- **Honey-Based Wound Dressings**

There are commercial wound dressings containing honey that are approved in a number of countries such as Medihoney, Activon Tulle, and Manuka Honey Dressings. These products contain medical grade honey sterilized using gamma radiation that kills bacteria spores while retaining antibacterial activity. The honey can be added to gel dressings, creams, hydrocolloid dressings, and alginate dressings. Medihoney is made from Leptospermum honey that has obtained approval from regulatory authorities, such as the United States Food and Drug Administration, for wound healing purposes [67]. The product has been shown to be effective for treating partial thickness burns, diabetic foot ulcers, venous leg ulcers, pressure ulcers, and wound infections following surgery. This dressing needs to be replaced either once daily or every other day according to the volume of exudates produced from the wound. There are many benefits offered by honey dressings compared to other antimicrobial dressings. They create a moist environment for wound healing, they debride wounds through dissolution of necrotic tissue, they mask the unpleasant smell from wounds, and they do not generate bacterial resistance. Potential drawbacks include frequent dressing changes due to honey dilution, pain when applied on some wounds, and high cost [74].

- **Nanoformulations**

There are some approaches that nanotechnology could use to compensate for some disadvantages of the natural components from honey. In addition to poor stability, low solubility and low bioavailability, nanotech products can be used to deliver these substances to tissues and wounds. Chitosan nanoparticles loaded with quercetin have been recently introduced as topically applied wound dressing. These particles have sizes ranging from 100 nm to 300 nm and deliver quercetin over 24 hours to 72 hours, providing prolonged antibacterial action. At the same time, chitosan itself possesses antimicrobial and wound healing properties. Quercetin-loaded chitosan nanoparticles showed enhanced antibacterial activity against methicillin-resistant

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Staphylococcus aureus compared to free quercetin [75]. Honey and propolis-containing electrospun nanofibers have been proposed as wound dressings due to their large ratio between surface area and volume. In addition, they can serve as mimics of the extracellular matrix promoting cell growth and tissue regeneration. Propolis-containing nanofibers showed antimicrobial properties against wound pathogens. Honey-based or phenolic hydrogels offer a moisturizing condition for healing wounds by releasing antimicrobial agents. Thermoreversible hydrogels with low viscosity at room temperature and gelling at body temperature can be easily applied to complex wound surfaces. Caffeic acid phenethyl ester-loaded hydrogels have been found effective against Pseudomonas aeruginosa biofilm infections and accelerate healing of infected wounds [76].

- **Combination Therapy with Antibiotics**

Synergy between honey flavonoids and phenolic acids and antibiotics could present several benefits. The combined effect could lower the minimum inhibitory concentration of antibiotics, thus reducing doses to lower toxicity and side effects. Antibiotics that have become resistant could regain their susceptibility to antibiotics through synergistic action. Quercetin has been shown to exhibit synergy with various antibiotics in methicillin-resistant Staphylococcus aureus bacteria. Synergy between quercetin and oxacillin lowers the minimum inhibitory concentration of oxacillin from above 256 micrograms per milliliter to 1 to 2 micrograms per milliliter, restoring oxacillin susceptibility. The synergy is attributed to the ability of quercetin to inhibit the bacterial efflux pump responsible for pumping oxacillin out of the methicillin-resistant Staphylococcus aureus bacteria. Caffeic acid phenethyl ester exhibits synergy with colistin in extensively drug-resistant Pseudomonas aeruginosa [77]. Synergy between caffeic acid phenethyl ester and colistin lowers the minimum inhibitory concentration of colistin from 8 to 16 micrograms per milliliter to 0.5 to 1 microgram per milliliter, restoring colistin susceptibility. Galangin acts in synergy with fluconazole against fluconazole-resistant C. albicans. This interaction lowers the minimum inhibitory concentration of fluconazole from >64 μg/ml to 4 to 8 μg/ml, which may permit fluconazole to treat infections where it is ineffective at present. Its action includes the inhibition of efflux pumps involved in resistance to fluconazole [78].

- **Drug Delivery Potential**

The compounds flavonoids and phenolic acids may function as vectors for delivering pharmaceuticals to wounds and sites of infection. Their amphipathic character allows them to associate with hydrophobic and hydrophilic compounds and helps in the enhancement of drug delivery to infected sites. Quercetin is used to design self-emulsifying drug delivery systems which increase the oral bioavailability of poorly water-soluble pharmaceuticals. The same concept can be used for topical application, and quercetin is useful in enhancing the passage of other antimicrobials through the stratum corneum into the infected tissue. Caffeic acid phenethyl ester is incorporated into liposomes for selective targeting to sites of infection. Liposomes are artificial vesicles made up of phospholipids which are able to fuse with the membrane of target cells, allowing the release of their content inside. Caffeic acid phenethyl ester-loaded liposomes exhibit higher antimicrobial action and lower toxicity to host cells than caffeic acid phenethyl ester. Chrysin is linked to gold nanoparticles, which deliver the compound to biofilms. Gold nanoparticles can infiltrate biofilms better than free compounds due to their small particle size and unique surface characteristics. Chrysin-gold nanoparticle complexes were effective against Candida albicans biofilm infections resistant to free chrysin [79, 80].

- **Development of Standardized Medicinal-Grade Honey Products**

The need for the standardization of medicinal honey products is necessary in pharmaceutical applications. This will ensure that each batch of the product consists of similar concentrations of bioactive components to enable dosage and consistent clinical effects. Different methods for standardizing medicinal honey have been suggested. The total phenolics in the samples can be quantified by Folin-Ciocalteu method, and expressed in gallic acid equivalent terms. This method, however, does not differentiate the phenolic compounds based on their biological activity [81]. Another technique that can be used is high-performance liquid chromatography. It allows for quantification of individual components such as flavonoids and phenolic acids. Manuka honey is standardized by its unique manuka factor, which is based on its methylglyoxal concentration, but does not consider contribution from phenolic compounds. Standardized extracts may be another alternative method. Propolis extracts of known flavonoid content have already been commercialized and used in clinical trials. They can be included in formulations of wound dressings, ointments, etc., as extracts of defined composition. The standardization process for these extracts usually includes quantification of individual chrysin, galangin, quercetin and kaempferol concentrations, and expresses total flavonoid content in mg/g. There are different requirements for the regulatory process for medicinal honeys in various countries. In Europe, honey dressings used for healing wounds are subject to medical device regulation. This implies that while their safety and efficacy have to be demonstrated, extensive clinical trials are not required as in the case of pharmaceuticals. In America, honey products are categorized either as medical devices or over-the-counter pharmaceuticals, depending on their use and claims [82, 83].

Challenges and Limitations

- **Variability in Honey Composition**

The makeup of honey differs greatly depending on the type of flower, geographical location, season, weather, soil makeup, bee type, and honey processing post-harvest. The variations of honey pose a major hurdle to its use in pharmaceuticals because different batches of honey can have varying degrees of antimicrobial power and therapeutic effectiveness. The type

of flower used in making the honey is the primary factor in determining the makeup of the honey. Honeys made from monofloral plants exhibit specific phenolic compounds, with some being highly potent antimicrobials while others have little antimicrobial capacity. Manuka honey and jelly bush honey, both from New Zealand and Australia respectively, are known for their high levels of antimicrobial power, but they are not readily available due to their high cost [84]. There are also other types of honey that have moderate levels of antimicrobial activity and are relatively affordable and easily accessible. There are also geographical variations of honey that come from the same type of flowers. Different locations within New Zealand have varying amounts of methylglyoxal and flavonoids in Manuka honey [35].

Seasonal and yearly variations also influence the composition of honey. Honey samples harvested from the same bee farm in different years exhibit wide variations in the amount of phenolics owing to variation in climatic conditions when flowers are blossoming. Conditions like drought stress in plants could increase the production of phenols, whereas favorable conditions could decrease their production. The post-harvesting process like heating, filtering, and storing reduces the amount of phenolics present as well as its antimicrobial properties. Heating of honey above 40 degrees Celsius could break down some phenols, while filtering gets rid of pollen that contains phenolics. High temperatures during storage increase degradation [85].

- **Lack of Standardization in Clinical Formulations**

Lack of standardized clinical formulations is one of the main obstacles to the adoption of honey-based therapies. The problem is that different honey formulations consist of different honey species, different amounts of honey, different excipients, and different delivery methods, complicating the comparison of clinical studies and creation of evidence-based guidelines. In particular, some clinical studies use pure honey, which was directly applied to wounds, while others use dressings that contain various concentrations of honey, usually between 40% and 100%. The kinds of honey utilized include manuka honey, mono-floral and multifloral honeys. As there is no information regarding the phenolic content of different kinds of honey, it is impossible to identify active ingredients [86]. It is necessary to develop reference standards for honey flavonoids and phenolic acids for the purpose of the standardization of honey-based products. There are currently available reference standards for such honey compounds as quercetin, kaempferol, chrysin, galangin, caffeic acid, gallic acid, ferulic acid, and p-coumaric acid, allowing measuring the amounts of these substances in honey. Yet, the concentrations of these compounds required for antibacterial and healing effects are still unknown, as well as the way these compounds interact with each other [87].

- **Stability and Bioavailability Issues**

Flavonoids and phenolic acids are unstable in the presence of light, heat, oxygen, and enzymatic processes. It should be proven that these natural components will remain active in drug preparations during their shelf life. Special accelerated studies at high temperature and humidity provide information on the rate of degradation, which allows determining the expiry date of drug formulations. Quercetin and other flavonoids experience autoxidation in water-containing solutions, resulting in the creation of inactive degradation products. It occurs faster under light, oxidative and alkaline conditions [88]. Protection of the drug against these effects may include antioxidants, dark bottles, and maintaining an adequate pH level. Poor permeability of flavonoids and phenolic acids across the biological membranes and poor solubility restrict the efficiency of their use. For topical drug preparations used to treat wounds, this feature does not matter since the layer of stratum corneum is removed due to damage. In systemic preparations, the oral absorption of flavonoids is relatively poor, accounting for less than five percent of the drug dosage. Drug preparations based on nanotechnology may increase the stability and absorption of flavonoids and phenolic acids. They should be encapsulated into nanoparticles, liposomes, and cyclodextrins [89].

- **Limited Large-Scale Clinical Trials**

There is no large RCT-based evidence supporting the role of honey flavonoids and phenolic acids in wound healing. Future well-designed RCTs are required to provide evidence of efficacy and inform practice. Clinical trials involving honey for wound healing published in the literature usually include between 20 and 100 patients per trial, most often with fewer than 50 patients enrolled in each study. Such sample size is not sufficient to demonstrate even a moderate effect size, as well as to reveal any patient subgroup that may have benefited from or refractory to honey treatment. Larger clinical trials with several hundred patients included in both groups should be performed. Quality of clinical trials investigating honey's impact on wound healing varies considerably. Open label trials may be subjected to bias because practitioners have high expectations of honey efficacy. Blinding is difficult to achieve due to specific physical properties of honey dressings, which differ from conventional dressings in both appearance and smell. Sham honey dressings may be developed [90].

- **Regulatory Barriers in Pharmaceutical Use**

Obtaining regulatory approval for honey-containing drugs encounters various challenges. The natural variation in honey's composition makes it difficult to show consistency in batches, an essential requirement for all pharmaceutical products. The multiplicity of honey's modes of action and its multiple active ingredients make it hard to determine the precise mode of action of honey, a crucial requirement for most pharmaceutical products [86]. Honey products are categorized differently under regulations by different countries and even by different regulatory authorities within

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the same country. In the U.S., the FDA has authorized the use of honey dressings for medical purposes, thus requiring only proof of their effectiveness and safety, but not the rigorous testing required for drug approval. The expenses incurred in the regulatory approval process for a new drug are very high, usually amounting to millions of dollars. It is challenging for the company to recover such expenses in cases where there is no possibility of getting a patent on the product. If there is no patent on the product, any firm can manufacture and distribute the honey product after receiving approval, reducing the profit margin of the company sponsoring the product's approval process [91]. There are honey products that have been approved as medical devices under the Medical Devices Directive within the European Union, where the manufacturer has to demonstrate safety and effectiveness. Although the European process is cheaper compared to the U.S. drug approval process, the products cannot claim therapeutic value [92].

Future Perspectives

- **Isolation and Purification of Active Compounds**

Isolation and purification of individual compounds present in the honey and propolis extracts will allow detailed investigation of their individual properties and standardization of the pharmaceuticals prepared from them. Current chromatographic methods including HPLC and CCC can provide separation of these compounds at very high purities. The ability to work with individual compounds will allow us to determine the MIC for particular pathogens, mechanism of action, and cytotoxicity. All this data could be used to choose potential lead molecules. Currently, quercetin, galangin, and Caffeic acid phenethyl ester are considered as particularly promising leads due to the high level of their antimicrobial activity and safe profile. Isolation of particular molecules will allow to conduct SAR studies that determine key molecular structures that play an important role in antimicrobial activity. SAR data are important to modify naturally occurring compounds for enhanced activity, increased stability, and reduced toxicity. For example, it was determined that for antimicrobial activity, it is necessary to have a pair of hydroxyl groups on B ring of flavonoid molecules with 3',4'-positioning similar to quercetin [93].

- **Synthetic Modification of Flavonoids and Phenolic Acids**

Flavonoids and phenolic acids can be synthetically modified to create analogs with enhanced pharmacological properties. Such modifications have included glycosylation, alkylation, prenylation, and the formation of esters. Glycosylation, which involves adding sugars to the hydroxyl group(s) of flavonoids, typically improves their solubility in water yet decreases their antimicrobial effectiveness. Yet, glycosylated flavonoids could potentially be used as pro-drugs that are converted to active compounds via metabolic enzymes present in bacteria. This would result in selective toxicity of the flavonoid analog against pathogens while sparing human cells. On the other hand, alkylation of flavonoids, or adding an alkyl chain, generally improves their lipophilicity but decreases their water solubility. Derivatives of quercetin with pentyl and hexyl chains have demonstrated greater efficacy against Gram-positive bacteria than quercetin, with decreased minimum inhibitory concentrations 2 to 4 times compared to those for quercetin [45].

Modification through prenylation, which involves adding prenyl groups, is one of nature's modifications in some flavonoids, which increases their antimicrobial potency. Some synthetic derivatives of chrysin and galangin with prenyl groups have been found to exhibit activity against methicillin-resistant S. aureus with minimum inhibitory concentrations of 1 to 2 micrograms/milliliter, which is a 10 to 20-fold improvement over the parent compounds. Another type of derivative that can increase the bioactivity of phenolic acids is their esterification with alcohols. The increased lipophilicity and improved interaction with bacterial membranes are some of the factors that contribute to the increased activity of phenolic esters relative to their parent acids. In the development of other caffeic acid esters using various alcohols, an optimal balance of biological activity and stability could be achieved [26].

- **Nanotechnology-Based Delivery Systems**

A variety of nanotechnological solutions could be considered to enhance the delivery of flavonoids and phenolic acids contained in honey to the affected areas. They may include protection of honey constituents, improved penetrability of those compounds across biological barriers, and provision of sustained release. Polymer-based nanoparticles such as those made of biodegradable poly (lactic-co-glycolic acid) and chitosan exhibit high effectiveness when encapsulating flavonoids. Those nanoparticles vary in size from 100 to 300 nm, which makes them perfect for tissue penetration and prevents rapid elimination. Quercetin-loaded poly (lactic-co-glycolic acid) nanoparticles exhibited sustained release for 7 to 14 days, along with enhanced activity against Staphylococcus aureus biofilm in comparison with free quercetin [94]. Inorganic nanoparticles such as those consisting of silver, gold, and silica could be conjugated with flavonoids to obtain hybrid systems that would exhibit combined antimicrobial properties. For example, gold nanoparticles coated with quercetin exhibit synergetic antimicrobial effects and enable bacteria killing even at the level when individual components lack such ability. Liposomes, which are vesicles made of phospholipids and merge with cell membranes, may allow delivery of flavonoids into bacteria. The activity of liposome-incapsulated caffeic acid phenethyl ester has been demonstrated to be 10 times more active against Pseudomonas aeruginosa; presumably due to better delivery of this compound across the outer membrane of bacteria. Targeted liposomes, with antibodies or lectins that can recognize certain bacteria incorporated in them, may be used specifically for those kinds of bacteria [95]. Electrospun nanofibers loaded with honey or propolis are used as a support for wound healing, with antimicrobial agents released. Such nanofibers are applied topically onto wound areas, and will degrade gradually during several days, thus allowing for the continuous

release of active compounds. Electrospun nanofibers with propolis extracts were proven to accelerate wound healing in mice with diabetes, with wounds fully closing up 5 days earlier compared to non-treated mice. [96]

❖ Genomic and Metabolomic Approaches

In order to gain insight into the biological pathways affected by the action of honey polyphenols, genomic and metabolomic tools have been developed. The use of transcriptomics can provide information on the up-regulation or down-regulation of bacterial genes upon treatment with polyphenols, leading to the identification of the biological pathways targeted by those molecules [97]. Using proteomics, one can identify the bacterial proteins that interact with polyphenols and thus establish whether or not they represent potential drug targets. This has already been achieved using affinity chromatography based on immobilized polyphenols, resulting in the identification of potential targets for further studies. For instance, quercetin was found to interact with the S. aureus NorA efflux pump. Metabolomics analysis could help identify the effects of flavonoids on bacterial metabolism, thereby identifying which metabolic pathways are affected by treatment [45]. Treatment with CAPE in P. aeruginosa leads to accumulation of metabolites in the tricarboxylic acid pathway, indicating inhibition of this pathway. This knowledge would then be used to formulate treatments that will inhibit multiple pathways in combination. Genomics could also be used to establish the reason for susceptibility or resistance of a bacterium to flavonoids. Comparison between genomics data of a susceptible and resistant bacterium will allow us to identify any mutations that could lead to resistance to the flavonoids. Although no clinical cases of resistance against honey or its components have been reported so far, mutation in some efflux pumps and biosynthesis pathways have resulted in resistance through lab selection experiments [98].

❖ Integration into Antimicrobial Stewardship Programs

The use of honey-containing preparations within an antimicrobial stewardship program might decrease the utilization of conventional antibiotics, saving these drugs for use when they are most required. An antimicrobial stewardship program entails optimizing the use of antibiotics to avoid excessive prescribing and slowing down the emergence of resistance. Honey-containing preparations might act as the first-choice therapy in conditions where there is no high risk of systemic infection. By saving antibiotics for infections of high severity only, the chances of developing resistance will be minimized [99]. As honey products are not likely to develop resistance because of their several modes of actions, they are ideal for antimicrobial stewardship. From a cost perspective, honey-containing preparations can be considered under an antimicrobial stewardship program. Although these preparations might be slightly costly compared with conventional dressings initially, they can save money in the long term by promoting faster healing and avoiding further treatments and wound complications. Education regarding the effectiveness of honey products in antimicrobial stewardship program application is key since there are many healthcare practitioners who are unaware of the benefits of using honey for treatment. Guidelines that recommend the use of honey products can go a long way in ensuring the successful use of the products [100].

Conclusion

Honey flavonoids and phenolic acids comprise a fascinating array of phytochemicals with properties of being both antimicrobial and wound-healing-promoting substances. These chemical constituents have a range of modes of antimicrobial action on pathogenic bacteria. These modes include membrane damage of bacteria, suppression of nucleic acid synthesis, protein unfolding, anti-quorum sensing properties, biofilm formation inhibition, and generation of oxidative stress. These chemicals exhibit antimicrobial activity on clinically relevant organisms such as methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus, carbapenem-resistant Enterobacteriaceae, extensively drug-resistant Pseudomonas aeruginosa, and Candida albicans. Hence, honey flavonoids and phenolic acids offer alternative solutions for treating bacterial and fungal infection due to the increasing antibiotic resistance crisis. Apart from their role in fighting off infection, honey flavonoids and phenolic acids act on wound healing through various properties including inflammation reduction, tissue regeneration, immunomodulatory activity, decrease in bacterial load in chronic wounds, and angiogenesis stimulation. There is considerable evidence of the use of honey-containing preparations in burns, diabetes-induced ulcers, pressure ulcers, and surgical wounds, with the same effectiveness compared to conventional treatments.

Several difficulties confront the development of honey-based drugs. These include inconsistency of honey composition, lack of standardized drug formulations used clinically, instability, poor bioavailability, limited large clinical trials, and regulation. Nevertheless, the following developments help to overcome the problems mentioned above. These include methods for isolating and purifying active components of honey, modifications of those compounds synthetically to increase potency, use of honey in nano-formulations, genomic and metabolomic analyses to elucidate the mechanisms underlying honey activity, and incorporation of honey into antimicrobial stewardship programs. The development of antimicrobial resistance as a public health problem is prompting researchers to find natural products that have novel mechanisms of action. Because honey flavonoids and phenolics target multiple pathogens without the potential for development of resistance, these compounds are ideal candidates for incorporation into the range of antimicrobial drugs. Moreover, since such substances have therapeutic

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activities other than killing pathogens, they are ideal candidates for use in treating infected wounds.

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Declarations:

Authors' Contribution:

- **All Authors** Conceptualization, data collection, interpretation, drafting of the manuscript and intellectual revisions
- The authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed

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