Beehive Products as Antibacterial Agents

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Apitherapy is a branch of unconventional medicine that relies on the usage of bee products which consist of honey, pollen, propolis, royal jelly and bee venom (BV). Besides having high nutritional importance and health benefits, honey showed antifungal, antiviral, antiseptic, anticancer, anti-diabetic, anti-inflammatory and cardio protective activities. As for the BV, despite its possible adverse effects like the allergic reactions that might occur after the bee sting, one cannot disregard its various therapeutic effects. BV exerts anticancer, antiviral, antifungal and antibacterial effects, it is also used for the treatment of many neurodegenerative diseases. Regarding propolis, it has the capacity to fight against cancer and many microorganisms. Moreover, pollen possesses antioxidant, anticoagulant and anti-inflammatory properties. Also, royal jelly exhibits several interesting biological activities including antioxidant, anti-aging, antitumor anti-inflammatory, antimicrobial, and neurotrophic activities. Hence, this review aims to highlight one of the most important and commonly shared biological activity of all of the above-mentioned beehive products, which is the antibacterial activity.

Keywords: antibacterial activity ; honeybee products ; honey ; bee venom ; propolis ; royal jelly ; pollen

1. Overview

Honeybees are one of the most marvelous and economically beneficial insects. As pollinators, they play a vital role in every aspect of the ecosystem. Beehive products have been used for thousands of years in many cultures for the treatment of various diseases. Their healing properties have been documented in many religious texts like the Noble Quran and the Holy Bible. Honey, bee venom, propolis, pollen and royal jelly all demonstrated a richness in their bioactive compounds which make them effective against a variety of bacterial strains. Furthermore, many studies showed that honey and bee venom work as powerful antibacterial agents against a wide range of bacteria including life-threatening bacteria. Several reports documented the biological activities of honeybee products but none of them emphasized on the antibacterial activity of all beehive products. Therefore, this review aims to highlight the antibacterial activity of honey, bee venom, propolis, pollen and royal jelly, that are produced by honeybees.

2. Honeybees

Honeybees, also known as the ‘’Golden insect‘’, belong to the genus *Apis,* which is the Latin word for ‘’bee’’. These marvelous social insects live in a well-organized community and belong to the order Hymenoptera and to the family Apidae [1]. Honeybees can be found all around the world and are used for their vital role as pollinators in agriculture, but the main used species for crop pollination is *Apis mellifera* [2]. Since ancient times, honeybee products have been used for medicinal purposes. They have been also cited in many religious books [3]. Egyptians, Romans, Chinese and Persians have all documented for thousands of years the nutritional and medicinal values of bee products [4]. Apitherapy is a branch of unconventional medicine that relies on the usage of bee products which consist of honey, pollen, propolis, royal jelly and bee venom (BV). Besides having high nutritional importance and health benefits, honey showed antifungal, antiviral, antiseptic, anticancer, anti-diabetic, anti-inflammatory and cardio protective activities [5]. As for the BV, despite its possible adverse effects like the allergic reactions that might occur after the bee sting, one cannot disregard its various therapeutic effects. BV exerts anticancer, antiviral, antifungal and antibacterial effects, it is also used for the treatment of many neurodegenerative diseases [6][7][8][9][10]. Regarding propolis, it has the capacity to fight against cancer and many microorganisms [4][11][12]. Moreover, pollen possesses antioxidant, anticoagulant and anti-inflammatory properties [13][14][15]. Also, royal jelly exhibits several interesting biological activities including antioxidant [16], anti-aging [17], antitumor [18][19] anti-inflammatory, antimicrobial [20], and neurotrophic activities [21]. Hence, this review aims to highlight one of the most important and commonly shared biological activity of all of the above-mentioned beehive products, which is the antibacterial activity (Figure 1).

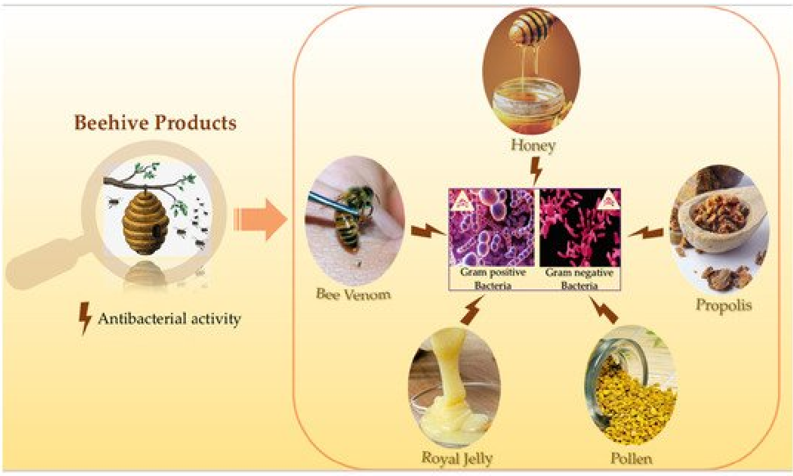

Figure 1. Schematic representation of the antibacterial properties of the beehive products.

3. Antibacterial Activity of Bee Honey

Honey, one of the most well-known and highly valued natural products, is produced by honeybees (*Apis mellifera*) [22]. Since ancient times, honey has been used by mankind for medicinal and nutritional purposes [23].

Honey is a naturally occurring supersaturated sugar solution made up of a complex mixture of carbohydrates (77–86%), with fructose and glucose being the most abundant. It has a low amount of water (around 20%) which gives honey several of its attributes such as high viscosity and high osmotic pressure. It also contains numerous minor but essential components including proteins, enzymes (invertase, catalase, etc…), organic acids (gluconic acid, acetic acid), amino acids, lipids, vitamins, minerals and many others. Furthermore, honey from *Apis mellifera* is characterized by a low pH level ranging between 3.53 and 4.03 [22][23][24][25][26]. Nevertheless, honey composition varies widely depending on several factors like the floral origin, the environmental conditions, as well as the processing procedure it goes through (pasteurization and storage) [27][28][29].

The antibacterial effect of honey is mediated by two mechanisms: a peroxide-dependent and a peroxide-independent pathway. In the hydrogen peroxide (H_2O_2) pathway, H_2O_2 is formed by glucose oxidase, a carbohydrate metabolizing enzyme added to nectar by bees. As indicated by prior studies, the enzymatic oxidation of glucose is responsible for the antimicrobial activity of honey [30][31]. When samples of honey were treated with catalase, an enzyme that breaks down hydrogen peroxide, the bacterial growth was reduced [32][33]. Lastly, honey bacteriostatic DNA degradation was prevented when H_2O_2 was removed by catalase [34].

As for the peroxide independent pathway, the physicochemical properties consisting of high viscosity and high sugar content are the two factors that confer honey its antibacterial effect. In fact, the extraction of moisture from the surrounding environment causes bacterial dehydration by osmotic pressure. In a previous study of infants with gastroenteritis where glucose given in oral rehydration solution was substituted by honey, the recovery time of patients was significantly reduced [35]. This is likely due to the fact that the high sugar content in honey improves electrolyte and water reabsorption in the intestine. Moreover, the low pH level halts microbial growth [36][37]. Methylglyoxal (MGO) is another bioactive compound involved in non-peroxide antibacterial function. It is produced by non-enzymatic conversion of dihydroxyacetone (found in high concentration in the nectar of *Leptospermum scoparium flowers*) [38]. Manuka honey, a honey known to possess non-peroxide antibacterial activity due to MGO, is produced from the Myrtaceae family manuka tree, *L. scoparium* [39][40]. This honey has received a lot of attention from scientists around the world due to the biological properties it has, notably its antioxidant and antibacterial properties. Furthermore, Manuka honey has been used in the treatment of infections such as, surgical wounds, abscesses, traumatic wounds, burns, and ulcers of various origins and has demonstrated its potency in inhibiting human pathogens and prevent the formation of biofilms [41][42][43]. In addition to the bioactive compounds responsible of non-peroxide activity, an antimicrobial peptide present in honey called bee defensin-1 acts against several Gram-positive bacteria such as *Stephalococcus aureus, Bacillus subtilis*, and *Paenibacillus larvae*, the main cause of the ravaging American bee larvae disease [37].

With the emergence and spread of resistant pathogens, the properties of honey could be potentially clinically relevant. Honey demonstrates a wide range of antibacterial activity encompassing both Gram-negative and Gram-positive bacteria. In a previous study, *Escherichia coli* strains with ampicillin-resistance gene (β–lactamase) were found to be sensitive to honey [44]. In addition, Mokaya et al. collected 16 different types of honey from various Kenyan locations. They found that all of the 16 samples prevented *E. coli* growth and had a significant amount of non-peroxide antimicrobial activity [45]. Another study examined two natural honeys against Methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-sensitive enterococci (VSE), and vancomycin-resistant enterococci (VRE) and then compared their antibacterial activity to that of artificial honey. All MRSA strains were resistant to artificial honey but sensitive to both honey types. As for VRE and VSE, both were susceptible to artificial and natural honey, with lower minimum inhibitory concentration (MIC) for natural honey [46]. These findings are in agreement with the study of Katrina et al. where MRSA and VRE were sensitive to the selected honeys and showed that the bacteriostatic effect was H_2O_2 dose-dependent [47].

In addition honey has been discovered to be a powerful inhibitor of *Helicobacter pylori,* causative agent of peptic ulcers [48].

As Manuka honey possesses an exceptional antibacterial activity, Brown et al. tried it against *Staphylococcus pseudintermedius* which cause serious infections in domesticated animals and can be transmitted to their owner. They demonstrated that Manuka honey is not just effective against novel multidrug-resistant *S. pseudintermedius* isolates, but additionally works in tandem with clinically important antibiotics and reduces its virulence [49].

A previous study has assessed the capacity of honey to prevent the adherence of *Salmonella interitidis* to the intestinal epithelial cells in vitro and has demonstrated that honey at dilutions 1:8 diminished the bacterial adherence from 25.6 ± 6.5 (control) to 6.7 ± 3.3 bacteria/epithelial cell [50].

Furthermore, another study found that a 10% concentration of honey could prevent oral bacteria like *Streptococcus mutans* from forming a biofilm, implying that honey could help to minimize oral pathogens in dental plaque [51]. Honey was also effective against biofilms produced by methicillin-susceptible *S. aureus* (MSSA), MRSA, and *Pseudomonas aeruginosa*, with bactericidal rates of 63–82%, 73–63%, and 91–91%, respectively, which were higher than the effect of widely used single antibiotics [52].

In another study, honey demonstrated antibacterial activity against *Burkholderia cepacia,* which is responsible of pulmonary infections especially in patients with cystic fibrosis and chronic granulomatous disease. Twenty strains of *B. cepacia* isolated from the sputum of patients with cystic fibrosis were screened for their susceptibility to eight antibiotics and two types of honey (with and without peroxide activity). All strains were resistant to the antibiotics tested but susceptible to honey at concentrations less than 6% (*v/v*) [53]. In addition, 13 honeys samples from different plant source were tested against *P. aeruginosa* and against *E. coli* at different concentrations (10%, 5%, 2.5%, and 1% *w/v*). All honey samples displayed an inhibitory effect on the growth of *P. aeruginosa* and *E. coli* for concentrations above 2.5%. Only four samples were still effective at a concentration of 2.5%: one against *E. coli* and three against *P. aeruginosa.* No activity was observed at concentrations below 2.5% [54].

The antimicrobial effect of honey has been tested against two Gram-positive organisms: *Streptococcus pyogenes* and *S. aureus*. Four honey samples were taken from beekeepers and tested at various concentrations (undiluted honey, 10 %, 30%, 50% and 70% *w/v*), then compared to standard antibiotics. Honey samples exhibited antibacterial activity with a diameter of the zone of inhibition (ZDI) ranging between 0 and 46 mm for *S. aureus* and between 0 and 44 mm for *S. pyogenes* [55]. Other studies also indicated the antibacterial activity of honey against human pathogens and foodborne pathogens such as *E.coli*, *Klebsiella pneumonia*, *Salmonella* spp., etc… [33][56].

Honey can be utilized to treat a wide range of oral diseases, including periodontal disease which is caused by *Porphyromonas gingivalis*, a Gram-negative bacterium. A previous study has shown that honey helps to prevent periodontal disease by killing anaerobic bacteria [57].

It is worth mentioning that the antibacterial activity of honey differs widely depending on the plant source [29][54]. Also, adulteration, thermal treatment, and long-term storage can all affect honey's antibacterial function [58].

Fuertes et al. have explored the repressive mechanism of honey on the growth of bacteria. They found that when *E. coli* and *S. aureus* were exposed to different honey samples, physiological changes in membrane integrity and polarization occurred. Honey also caused a major metabolic disturbance in *S. aureus* as a primary physiological consequence [59].

Comparing the antibacterial potency of honey to that of antibiotics was also reported. A recent study compared the activity of three honeys with gentamicin against *E. coli* and *P. aeruginosa*. None diluted honey and its 1:2 to 1:6 aqueous dilutions had 100% and 96.4% activity against *P. aeruginosa* and *E. coli,* respectively. Gentamicin, on the other hand, showed lower antibacterial activity when used at concentrations of 8.0 and 4.0 g/mL [60].

Combining honey with other antibacterial compounds increases their inhibitory rate against microorganisms. In fact, when honey was combined with the gentamicin, the killing rate increased to more than 92–93% while the killing rate was around 77% for gentamicin alone and around 45% for honey alone [61]. Another study attempted to combine honey with ethanolic extract of cinnamon bark and found that the mixture had additive activity against acne-causing bacteria [62]. More studies have shown that the combination of honey with other bioactive compounds has increased its antibacterial activity [41][63][64][65].

Concerning clinical trials on honey, Blaser et al. were reported a full recovery in seven consecutive patients with MRSA infected or colonized wounds. Alternatively, antiseptics and antibiotics had previously failed to eradicate infection-related symptoms [66]. Furthermore, another study has demonstrated that consuming honey at least once a week considerably reduced the chance of *H. pylori* infection in a group of 150 dyspeptic patients [67]. Also, in a clinical trial involving 90 patients with infected wounds, non- gamma irradiated honey was associated with gradual decrease of bacterial load over a period of 4 weeks [68].

Studies used different methods to measure the antibacterial activity of honey. The most common methods were agar diffusion assay and serial dilution approach in microtiter plates. The first method, while being effortless and quick in performance, presents many limitations: (1) difficulty in loading a specific volume of the product sample into the agar wells due to the high viscosity of honey, (2) diffusion issues with active components such as defensin-1 and glucose oxidase, which have a large molecular weight across the agar matrix, (3) low reproducibility, (4) difficulty in comparing the results with those of other authors, (5) inability to discriminate between bacteriostatic and bactericidal activity. For the second method, serial dilution assay, the bacteriostatic (MIC) and bactericidal activity (MBC) of examined honey samples can be determined quantitatively using this approach in contrary to that of the first method. The only challenge that this method presents is the preparation of honey output solution [69].

In light of these findings, honey appears to exert wide antibacterial activity against a variety of microorganisms. It has been suggested in the literature that these properties make the development of honey-resistance unlikely and extrapolate that honey may have an important clinical utility in the treatment of antibiotic-resistant bacteria [70][71][72]. Also, the use of honey as an antibacterial agent has no negative side effects for patients, in contrast to antibiotics, and is cost effective [69]. Finally, honey may present some limitations when used as a treatment, like the presence of some toxic substances such as herbicides or pesticides in it, as well as its possible contamination by clostridium endospores. Therefore, it is crucial to follow specific criteria and standards when using honey as a therapeutic agent [23][73]. It is also mandatory to create a sterilizing procedure that is safe for honey proteinaceous antibacterial components (glucose oxidase, bee defensin-1). Gamma-irradiation sterilization procedure appears to be promising [74]. Also, it is important to agree on one standard method to determine the honey's antimicrobial activity, making it easy to compare the activity of the product evaluated in different studies.

4. Antibacterial Activity of Bee Venom

Bee venom (BV) is a widely recognized toxin secreted by female worker bee's poison glands as a protection mechanism [75]. Although it is toxic to predators, BV has been used for many medicinal purposes since Ancient Egypt (4000 BC). In bee venom therapy (BVT), the toxin is applied directly or indirectly into the body for the treatment of certain diseases, such as rheumatism arthritis [76][77][78]. Venoms from different animals or organisms are considered as promising antimicrobial agents that work against several bacteriological pathogenesis [79]. The antimicrobial activity and medical use of BV is due to the presence of bioactive molecules in particular peptides which are the main components and constitute 48–50% of dry BV weight [80]. The *Apis mellifera* venom is an odorless and transparent liquid that is made up of 88% of water and only 0.1 µg of dry venom [78]. The dry venom itself is highly rich in peptides notably melittin, apamin, adolapin, and mast cell degranulating (MCD) peptide, enzymes such as phospholipase A_2 (PLA_2) and hyaluronidas [81][82]. Among these peptides, melittin represents the main biological active compound in bee venom. It represents about 50% of the dry BV [83]. Another major component is phospholipase A_2, being the most toxic bee venom peptide [84]. These two components can act synergically and damage the cell membrane [8]. Melittin possesses a low selectivity to the cell membrane and acts strongly on its lipids via the process of pore formation. This process causes the release of the cell cytoplasmic contents and leads to cell lysis [85]. This mode of action is most likely to be responsible of the antibacterial properties of BV [86]. Several studies have demonstrated the ability of BV to kill both Gram-negative and Gram-positive bacteria [87][88]. Indeed, the antibacterial activity of BV from *Apis mellifera* purebred as well as hybrid was tested against five bacterial strains. The results showed that BV displayed an antibacterial activity against all five bacterial strains. Other studies proved that BV can be used as a complementary antimicrobial agent against pathogenic bacteria even if it is collected by different methods (i.e., from the top of the frames or from under the frames with distance between the bottom board and the brood chamber) [89]. Additionally, the bacterium *Borrelia burgdorferi* is known to be the main cause of Lyme disease. Researchers found that both BV and melittin possess significant effects on all the morphological forms of *B. burgdorferi,* even on antibiotic resistant attached biofilms. However, antibiotics when administrated alone or combined together had limited effects [90]. Food-borne pathogens present a risk to develop antimicrobial resistance and generate biofilms such as *Salmonella*. Sixteen *Salmonella* strains isolated from poultry were tested against apitoxin (Bee venom). In 14 of the 16 tested strains, apitoxin reduced the biofilm formation and destroyed the preformed biofilm by 47%. Also, apitoxin increased the mobility of the bacteria and the MIC varied between 1024–256 µg/mL [7]. Aiming to find an alternative to antibiotics in the poultry industry, broiler chicks were sprayed with BV to evaluate its immunoprophylactic effects against *Salmonella gallinarum*. The results showed that BV increased the antibody production against formalin-killed *S. gallinarum* [91].

Others previous studies focused on the antibacterial effect of bee venom against additional type of bacteria such as *S. aureus* and *E. coli*. The results proved BV to be active in killing both bacterial strains. At 10 × MIC, the regrowth of *E. coli* was not observed at 18 h while For *S. aureus* at 5 × MIC [92][93]. Regarding the MIC for Gram-positive strains, the range was from 200 µg/mL to 8 µg/mL for the most sensitive species *B. subtilis*. Alternatively, Gram-negative strains were found to be more resistant to BV (MIC 60 to >500 µg/mL). This can be explained by the nature of the bacterium cell membrane [87][93]; the outer membrane of Gram-negative bacteria encompasses lipopolysaccharides (LPS) which obstruct the penetration of melittin presented in the BV. Thus, making melittin responsible for the antibacterial activity. While in Gram-positive bacteria, LPS are absent, indicating that melittin can penetrate the cell membrane more easily to form pores and increases the cell permeability through the cytoplasmic membrane [94]. PLA_2, which is also presented in abundance in the crude BV can disrupt the cell membrane of Gram-negative bacteria indirectly by hydrolyzing phospholipids enzymatically. Other works reported the antibacterial activity of the crude BV and its two main biopeptides melittin and PLA_2 against oral pathogen responsible of tooth decay: *Lactobacillus casei, Streptococcus salivarius, S. mutans, Staphylococcus mitis, Staphyslococcus sobrinus, Staphylococcus sanguinis* and *Enterococcus faecali*. Melittin was twice as active as the crude BV against the tested bacteria (4 to 40 µg/mL). As for PLA_2, it was only active against *L. casei* at >400 µg/mL [95][96]. Similarly, melittin showed antimicrobial activity against Staphylococcal strains as well as MRSA strains while PLA_2 did not show any effect on the same strains [97]. Also, it has been demonstrated that certain amino acids and their positions play a crucial role in the antibacterial activity exerted by melittin. For example, the absence of a proline residue in position 14 significantly diminishes the antimicrobial activity of melittin [98]. Likewise, two synthetic melittins, one asparagine-substituted melittin (Mel-N) and the other serine-substituted melittin (Mel-S) were able to penetrate the membrane of *E. coli* but the latter was more efficient [99]. Intriguingly, the antibacterial mechanism of new melittin derived-peptides MDP1 (GIGAVLKVLTTGLPALIKRKRQQ) and MDP2 (GIGAVLKWLPALIKRKRQQ) was evaluated against multidrug resistant strains such as *E. coli, P. aeruginosa* and MRSA. The two derived peptides showed strong antibacterial activity against reference strains of the three multidrug resistant strains compared to the indigenous melittin via membrane alteration and damage [100].

On the other hand, antimicrobial treatments are becoming less efficient by the day due to the increasing number of multi-drug resistant (MDR) bacteria as well as bacterial biofilms which are very hard to treat. Between 317 positive specimens for bacterial growth, 124 were MDR isolate of Gram-negative and Gram-positive bacteria. BV demonstrated a complete growth inhibition percentage (100%) against all tested MDR-isolates with a wide range of MICs and MLCs concentration-ranging between 3.125–50 µg/mL and 6.25–100 µg/mL, respectively against all MDR-GNB and GPB one [101]. The antibacterial activity of BV was tested alone or in combination with antibiotics (ampicillin, gentamicin, penicillin, or vancomycin) on the growth of MRSA strains. A partial synergetic effect was indicated by the index of inhibitory concentration ranging from 0.631 to 1.002 for the combination of BV with ampicillin or penicillin which made the MRSA strains more susceptible to the combination of BV with gentamicin or vancomycin rather than the combination of BV with ampicillin or penicillin [102]. BV was studied for its antibacterial activity against four MDR-*Acinetobacter baumannii* bacterial strains. Results showed an inhibition of the bacterial growth of MDR-*A. baumannii* strains with MIC value 31.25 mg/mL [103]. Melittin combined with doripenem caused a significant decrease in the MIC of MDR- *A. baumannii* strains. The combination of melittin–ceftazidime and melittin–doripenem was administrated to MDR-*P. aeruginosa* strains. This caused a decrease in melittin dosage which led to a decrease in melittin cytotoxicity. Therefore, the combination of melittin–doripenem (for *A. baumannii*) and melittin–doripenem with melittin– ceftazidime (for *P. aeruginosa*) at their MIC dose might serve as a promising therapeutical treatment against MDR bacteria [104].

The investigation of BV in pre-clinical and clinical application is still very slow despite its efficacity when combined with other drugs to treat different types of bacteria. Researchers found that BV and melittin combined with other antibiotics like vancomycin, oxacillin, and amikacin yielded fractional inhibitory concentration (FIC) indices fluctuating between 0.24 and 0.5. Apitoxin and melittin tested against 51 strains of bacteria exhibited strong antibacterial activity against both Gram-negative bacteria (MIC values between 30 and >500 µg/mL) and Gram-positive (MIC values between 10 and 100 µg/mL). Moreover, a strong anti-MRSA and anti-VRE activity was shown by BV and melittin at MIC values of 6–800 µg/mL [87].

When combined with oxacillin, BV and melittin showed an antibacterial activity against MRSA ATCC 3359 [84]. The antibacterial activity of melittin as well as its synergistic effect with *β*-lactam antibiotics against *A. baumannii* was evaluated by means of the broth microdilution method. The MIC value of melittin was 4 µg/mL and results showed inhibition of bacterial growth. Furthermore, FIC indices for combination of melittin with co-amoxiclav, ceftazidime and imipenem showed a synergetic effect [105].

All of the above- mentioned information confirm that BV and its two major compounds melittin and PLA_2 exhibit a very promising antibacterial activity against several pathogenic bacterial strains even against multidrug resistant bacteria. However, one should always keep in mind that these biopeptides are venomous and might act as toxic agents if administrated the wrong way. Melittin is the most toxic component in BV. While being responsible for the majority of the pain associated with bee stings, it only induces a minor allergic reaction. Contrary to PLA_2 which is the most allergenic and immunogenic protein in bee venom [106][107]. Bee envenomation can lead to many clinical manifestations such as local inflammatory reactions, allergic reactions, anaphylactic shocks, and systemic toxic reactions [108]. The ideal treatments against the toxic effect of bee venom are corticosteroids, antihistamine, and intramuscular adrenaline depending on the severity of the bee envenomation [109][110].

Many clinical studies on bee venom have shown promising results. Acne, one of the most common problems among teenagers, is usually treated with antibiotics to kill the causative bacteria but in some cases bacterial resistance can occur [111]. In a clinical study conducted by Han et al., skincare products with or without were tested on 12 subjects suffering from acne. The results showed a 57.7% decrease in ATP measured to evaluate the decrease in skin microbes. Thus, cosmetics and skincare products containing BV can used as a promising therapeutic anti-acne agent [112].

5. Antibacterial Activity of Propolis

Among the many products of honeybees, propolis is considered as one of the most interesting products utilized as the main defensive element and building block in the hives. Propolis is used by bees to fill the cavity in the walls of the hive, it is also used to repair combs and strengthen its thin borders. On the other hand, propolis can mummify intruders that cannot be transported outside thus preventing their decay [113]. Since ancient times, the anti-putrefactive property has been used by humans, notably by Egyptians. The origin of propolis, also known as “bee glue”, had long been a subject of discussion, some thought it came from bees themselves while others thought it originated from plants. Nowadays, the approximate composition of propolis and the factors affecting it became clearer [114]. Propolis is now confirmed to be a bee product made from plants. Bees collect resinous vegetable matter from different parts of the plant such as lipophilic matter on leaves and their buds, latex as well as mucilage [115]. Also, the bees are able to cut the fragments of vegetative tissues in order to extract the products necessary for the production of propolis [116]. Furthermore, propolis is a complex mixture with a variable composition that depends on the geographical region and the plant species used in its production. Surprisingly, having different composition does not indicate different biological activities [12]. Propolis is a resin that can occur in different colors and exhibits a pleasant aromatic resin smell of great value. Propolis is mainly composed of resins, flavonoids, polyphenols, terpenoids, essential oils, and other organics and minerals [117]. The extraction of propolis and its dissolution is needed in order to release its most active ingredients. The extraction process is also required for the later usage of propolis for medicinal purposes. It is also important to mention that solvent types used in extraction can affect the biological activity obtained from propolis. For the antibacterial activity, the abundancy of flavonoids and phenols plays a key role in conferring this bioactivity [118]. Additionally, the presence of many bioactive ingredients and in different concentrations is crucial in preventing the occurrence of bacterial resistance [119]. As for the mechanism of action, propolis can act directly on the microorganism or indirectly by triggering the immune system which results in the activation of the body natural defense mechanism. Studies showed that Gram-negative bacteria are more resistant to propolis than Gram-positive bacteria. This can be explained by two main factors, the first one being the specific structure of the Gram negative bacteria membrane and the second one being the secretion of hydrolytic enzymes which destroy the active ingredients present in propolis [120].

Artepillin C (3, 5-diprenyl-*p*-coumaric acid) is considered as one of the most important phenolic compounds found in propolis. Propolis ethanolic extract showed higher antibacterial activity against MRSA in comparison with hexane extract due to the higher concentration of Artepillin C in ethanolic extract [121]. The antibacterial activity of ethanol extracted propolis and its derivative compounds was investigated against *P. gingivalis*, a bacterium responsible for periodontal diseases. Artepillin C showed a bacteriostatic effect with membrane blebbing [122]. Propolis contains other phenyl derivatives such as 2-dimethyl-8-prenylchromene and 3-prenylcinnamic acid allyl ester. After investigating the chemical constituents and the antibacterial activity of the ethanolic extract of propolis, the results showed a high concentration of *p*-coumaric acid, artepillin-C, drupanin and kaempferide alongside an antibacterial activity against *Listeria monocytogenes, Enterococcus faecalis, S. aureus* and *Staphylococcus saprophyticus* [123]. Also, propolis contains pinocembrin and apigenin. Chilean propolis was subjected to a study and researchers found that the antibacterial activity of pinocembrin and apigenin is higher than that of polyphenols mixture or even chlorhexidine against *S. aureus* [124]. Numerous work reported the presence of antibacterial activity of pinocembrin against different bacterial strains such as *S. mutans, S. sobrinus, S. aureus, E. faecalis, L. monocytogenes, P. aeruginosa* and *K. pneumoniae* [125][126].

Nepalese ethanolic extract of propolis was evaluated for both its antimicrobial activity and its chemical composition. The main components were flavonoid aglycones (mainly neoflavonoids, isoflavonoids) and pterocarpans. Nepalese propolis exhibited its highest antibacterial activity against *H. pylori, S. aureus* and *Shigella flexneri*. When combined with amikacin and tetracycline, the same propolis yielded the strongest effect against *S. aureus* [126]. The ethanolic extract of polish propolis (EEPP) was examined for its antibacterial activity against MRSA and MSSA. The average MIC was 0.54 mg/mL and EEPP was effective against 12 *S. aureus* strains. Also, EEPP combined with 8 antistaphylococcal drugs, provided a stronger antibacterial effect against the tested strains than EEPP alone [127]. The geographical location affects the composition of propolis as well as its antimicrobial activity. 40 ethanol extract of propolis (EEP) collected worldwide were evaluated for their antibacterial activity against *S. aureus* strains. Results showed a moderate activity for Asian and African samples with MICS ranging from 0.0156 to >0.5 mg/mL and 0.0078 to >0.5 mg/mL, respectively with a similar results displayed by samples collected from North and South America and Europe [128]. EEP samples from three different regions of turkey were analyzed. MIC values varied and amounted to 0.018, 0.162, and 0.101 mg/mL and samples showed high antibacterial activity [129].

The number of studies concerning the antibacterial effect of propolis against anaerobic bacteria remains limited. However, it has been presented that propolis is more active on Gram-positive anaerobic bacteria than on Gram-negative ones. Propolis showed antibacterial activity against *Lactobacillus acidophilus, Prevotella oralis, P. gingivalis, Fusobacterium nucleatum, Peptostreptococcus anaerobius, Actinomyces naeslundii* and *Veillonella parvula* [130]. Moreover, research shows an important activity of propolis against *Porphyromona, Fusobacterium, Propionibacterium, Clostridium, Prevotella, Actinomyces* and *Bacteroides* species [131][132].

Propolis displays different antibacterial mechanisms such as inhibition of cell division, collapse of membranes and cell walls of the microbial cytoplasm, decreasing bacterial motility, enzymatic inactivation, bacteriolysis and inhibition of protein synthesis [133]. All the different compounds that constitute the propolis composition are rich in polyphenols which work together with different microbial proteins to form both ionic and hydrogenic bonds. This contributes to the modification of the three-dimensional structure of proteins hence the alteration of their function. These effects have encouraged researchers to combine propolis with antibiotics in order to overcome the problem of bacterial resistance to drugs [134]. A synergy has been observed between EEP, vancomycin and oxacillin; antibiotics that inhibit the synthesis of the bacterial cell wall, against *S. pyogenes*, VRE ATCC 51299 and MRSA NCTC 10442 [135]. Also, synergism was present between EEP and drugs targeting microbial ribosomes (neomycin) but absent with drugs interfering in the biosynthesis of folic acid or DNA (ciprofloxacin and norfloxacin) as well as those inhibiting metabolic pathways (cotrimoxazole) [134]. EEP has proved to be the most effective when combined with antibiotics that interfere with bacterial protein biosynthesis such as tetracycline, linezolid, chloramphenicol, gentamicin, tobramycin and netilmicin against MRSA and MSSA [127].

Propolis is widely used in medicine due to its various bioactivities. The effect of topical propolis extract was tested on facial vulgaris acne where subjects were treated with a topical solution of ethanolic extract of propolis. This solution showed a significant bacteriological activity on *Propionibacterium acnes* and *Staphylococcus epidermidis*, as well as being effective against facial acne [136]. To test the efficiency of propolis in inhibiting dental caries, propolis fluoride was applied to children’s teeth with active dentinal carries surface. Carries were stopped in 99.80% of the cases after just one month of application without causing any black discoloration of the teeth [137]. In another study, researchers treated patients suffering from chronic periodontitis with an irrigation of the selected sites with a hydro alcoholic solution of propolis extract propolis. Results showed that propolis exhibits a considerable activity against chronic periodontitis. This treatment was more effective than the conventional treatment as it significantly decreased the viability of *P. gingivalis* [138].

6. Conclusions

The use of beehive products for medicinal purposes has long been exploited by mankind. Here, the antibacterial properties of honey, bee venom, propolis, pollen and royal jelly have been discussed. Each of these products is characterized by the presence of bioactive compounds which makes the formers powerful inhibitors of pathogenic bacterial strains. Many clinical trials are being conducted to test the safety and effectiveness of beehive products on infected wounds as well as on various bacterial diseases such as gastroenteritis. Studies showed that bee venom exhibits both antibacterial and anti-inflammatory activity and can be used in acne treatment, and propolis was demonstrated to be efficient in both skin and dental treatment. Finally, although bee products are considered as promising and potent antibacterial candidates, future work should emphasize on their possible adverse effects when applied or administrated directly to the human body.

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