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Review

The Role of Bee Products in the Control of Antimicrobial Resistance and Biofilm Formation

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Abstract

The discovery of antibiotics saved many lives. Infections were not as deadly a problem for clinicians as they once were. However, due to inappropriate and excessive use of antibiotics, antibiotic resistance has increased dramatically worldwide. Infectious diseases are becoming more challenging to control, and they cause increased morbidity and mortality. Also, a significant risk to human health is posed by infections associated with biofilms. To combat these drug-resistant microorganisms, several novel and alternative strategies have been identified. Bee products such as honey, bee pollen, propolis, royal jelly, bee venom, bee wax, and bee bread have the potential of being used as antimicrobial or antibiofilm agents in various industrial and medical applications. Although these products have some restrictions such as their varying and complex composition, they possess significant potential in the field of medical practices as viable alternatives to antibiotics. They offer a potential solution to the issue of antibiotic resistance. The objective of this review was to offer a comprehensive analysis and evaluation of strategies based on bee products that are currently employed or have been suggested against antimicrobial resistance.

Keywords: Antimicrobial resistance, Antibiotic alternatives, Biofilms, Bee products

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Introduction

Antibiotics have revolutionized medical care by preventing life-threatening bacterial infections. However, misuse and overuse of antibiotics caused antimicrobial resistance which makes them insufficient or ineffective against bacterial infections. Antibiotic resistance refers to microorganisms' ability to counteract the effects of antimicrobial drugs, rendering them ineffective against bacterial growth [1,2]. Antibiotic misuse is an important issue regarding the development of resistance through two mechanisms: firstly, by suppressing susceptible bacteria and enabling the survival of resistant bacteria, and secondly, by triggering dormant resistance genes within bacteria due to the pressure exerted by antibiotics. The transmission of resistance genes among bacterial strains, influenced by antibiotic usage, can take place within individual hosts as well as between different hosts and communities. Therefore, the judicious administration of antibiotics in agriculture and healthcare is critical to prevent of multidrug-resistant bacterial infections. However, the emergence of antibiotic resistance has hindered progress in the clinical sector and threatened life expectancy, and food safety. The limited availability of new antibiotics due to declining progress and commercialization since the 1990s has compounded the problem [3,4]. This phenomenon poses a silent and dangerous threat to public health, particularly as antibiotics are being increasingly prescribed to treat infections that occur as a result of a primary ones [4,5]. Antibiotic resistance has become a global issue due to the escalating utilization of antibiotics in both medical practices and agriculture. The extensive utilization of antibiotics represents a significant threat to human health and has transformed into an urgent public health emergency. As per the assessment of the World Health Organization (WHO), antimicrobial resistance could lead to ten million deaths by the year 2050 [4,6,7].

Biofilms are conglomerates of bacterial colonies that attach to surfaces and are enveloped by a matrix of extracellular polymeric substances (EPS) manufactured by the cells themselves. These matrices have the capacity to harbor either single-species or multi-species communities of microorganisms, and the physical attributes of the matrix can differ among various bacterial strains. EPS consists of an assortment of biopolymers, encompassing proteins, polysaccharides, lipids, extracellular RNA, and extracellular DNA. They provide structural support and stability to biofilms, facilitate their adhesion to surfaces, safeguard biofilm cells from external forces, and establish a framework for the interconnected, immobilized three-dimensional structure of cells [8-12].

To assess the potential of bee products against antimicrobial resistance and biofilm formation, a descriptive review was conducted. Different bee products (such as propolis, honey, pollen, bee venom, royal jelly, beeswax and bee bread) were critically evaluated on the basis of their effects as documented in experimental studies. ScienceDirect, Web of Science, PubMed, and Google Scholar databases were used to collect bibliographic material. Articles that met the desired selection criteria were screened by evaluating their titles and then their abstracts.

This review aims to comprehensively examine the role of bee products on antimicrobial resistance and biofilm control.

Antimicrobial and Antibiofilm Effects of Bee Products

Honey

Apiculture is the art of rearing 'apis' or bees for the extraction of bee products, especially honey and beeswax. Honey, being high in nutrients, has been consumed worldwide since ancient times. The therapeutic properties of honey can be traced back to ancient times wherein Aristotle (384-322 BC) described honey to be "good as a salve for sore eyes and wounds". Honey is a powerful antioxidant, antimicrobial and antiproliferative agent and has been used traditional since long times [13-15]. It is extremely effective in promoting gastric health and is recommended for peptic ulcers and gastritis [16]. Recently, its anti-inflammatory, antihyperlipidemic, antidiabetic, and anticancer properties have been discovered [17,18]. According to the Codex Alimentarius, honey was described as "the natural sweet substance produced by honey bees from the nectar of plants or secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants" [19]. European Council Directive 2001/110/EC prohibits the addition to this natural product any food additives or other addition other than honey [20]. Honey is a saturated sugar (~80% v/v) solution whose composition depends on a varying range of factors including but not limited to the floral source, type of bee, and environmental and processing factors. These factors contribute to the texture, consistency, odour, color, and other physicochemical properties of honey. Currently, there are more than 300 types of honey, however, the core components of these honey stay more or less the same. A diagrammatic representation of major components in honey, as described by USDA, has been represented in *Fig. 1*. Approximately 20 different types of carbohydrates have been identified in honey with the principal carbohydrates being fructose and glucose. Honey also contains several disaccharides and trisaccharides at concentrations of 5% and 1% respectively [21]. Honeybees regurgitate the pollen of flowers and a small fraction of amino acids and proteins are retained in the honey. However, bee-derived

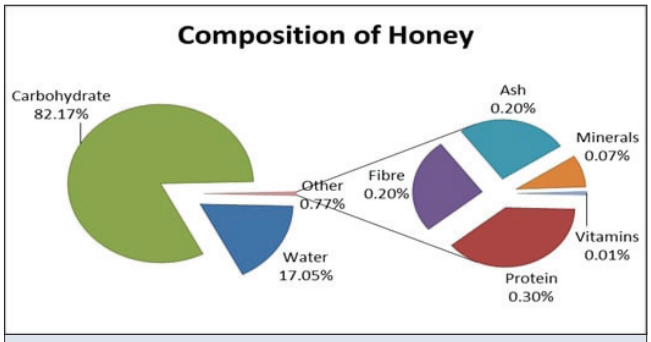

Fig 1. Pie diagram representing the constituents present in honey

and pollen proteins could theoretically be attributed to allergic reactions. Especially glandular proteins that bees produce and pollen from sunflower, ragweed, and sagebrush cause honey allergy. Symptoms of patients allergic to honey including local and systemic reactions. Local symptoms may assume itching in the mouth or gastrointestinal symptoms. Among systemic symptoms are bronchial asthma or generalized urticaria. Sometimes, anaphylaxis reaction is possible. Proline stands out as the most abundant amino acid, accounting for 50% of the total amino acid content in honey. Lipids are used up in the formation of beeswax and hence the amount of lipids in honey is almost negligible (0.002%) [22-24].

Antimicrobial Properties of Honey and Antibiotic Resistance

Honey has been called as supersaturated-sugar syrup containing almost 80% sugar in its total weight. High sugar concentration of honey implies to lower water activity (a_w). The optimum a_w values for bacteria, yeast and moulds are 0.9, 0.7 and 0.8, respectively [25] while the a_w value for honey ranges from 0.5-0.65 [26]. French et al.[27] demonstrated the use of pasture and manuka honey for inhibition of Coagulase-negative *Staphylococcus aureus* growth with MIC values of 3.6±0.7 v/v and 3.4±0.5 v/v respectively. The study showed that the osmotic effect of the sugar content of honey was 5.5 to 11.7 times greater than the antibacterial activity of natural honeys.

Honeybees collect sucrose from flowers and further break it down into glucose and fructose. This glucose is further oxidized and disintegrated into gluconolactone/gluconic acid and H_2O_2 by glucose oxidase enzyme (GOx) secreted by the bee's hypopharyngeal glands. The role of H_2O_2 in the antimicrobial effect of honey has been described by Adcock et al.[28] wherein the activity was diminished when H_2O_2 was decomposed by addition of enzymes such as catalase. Dustmann et al.[29] first demonstrated the antimicrobial activity of honey using a variety of organisms. In the research, it was found that *Pseudomonas*, *Proteus*, *Salmonella* spp., and *Streptococcus* sp. were less affected while more inhibition was observed in *Sarcina lute* and *S. aureus*. Furthermore, it was found that H_2O_2 concentration played an active role in contributing to antimicrobial activity against *Escherichia coli* K-12. A low concentration of H_2O_2 (1-2 mM) was enough to kill bacteria via DNA damage wherein H_2O_2 triggers Fenton reaction thereby leading to DNA strand breaks and generation of active forms of hydroxyl radicals [30]. When honey was added to bacterial cultures along with H_2O_2 supplementation, it was observed that honey reduced MIC of H_2O_2 from 2.5 mM, resulting in DNA damage in the bacterial cultures [31].

Preceding the knowledge of H_2O_2 production and sugar content, it was considered that the antimicrobial activity of honey was attributed to its low pH which ranged from 3.4 to 6.1 depending on several factors during its production [32]. Production of gluconolactone/gluconic acid during glucose breakdown is one of the key factors in its low pH. Most bacteria have an optimum pH at a neutral range and cannot tolerate lower pH. For example, *Salmonella* spp., *E. coli*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes* can tolerate pH up to 4.0, 4.3, 4.4, and 4.5 respectively, and thus honey has been proven beneficial for their inhibition [33,34]. To demonstrate the non-peroxide effect of honey, Tan et al.[35] used catalase along with tualang and manuka honey and demonstrated the bactericidal effect of honey against 13 bacterial isolates.

The flower source predominantly determines polyphenols present in honey. A wide array of flavonoids and phenolic acids have been discovered in honey and act as a biomarker for their authenticity [36]. Several phenolic acids including 4-hydroxybenzoic acid, caffeic acid, vanillic acid, and ferulic acid, and flavonoids such as apigenin, acacetin, kaempferol, chrysin, pinobanksin, naringenin, pinocembrin, and quercetin possessing antimicrobial activity have been discovered in honey [37].

Defensin-1 or def-1 is an antibacterial peptide chain found in honey and royal jelly. Kwakman et al.[38] demonstrated the presence of def-1 in the antimicrobial activity of honey wherein the H_2O_2 activity of honey was neutralized. The study found that 10-20% v/v of honey could effectively inhibit the growth of *Bacillus subtilis*, extended-spectrum ß-lactamase producing vancomycin-resistant *Enterococcus faecium*, *E. coli*, methicillin-resistant *S. aureus*, and ciprofloxacin-resistant *Pseudomonas aeruginosa*.

The presence of Methylglyoxal (MG) in honey is dependent on its storage temperature and flower source. It was first discovered in honey by Weigel et al.[39] in honey prepared by Leptospermum flowers wherein the bees convert the dihydroxyacetone into MG by the nonenzymatic reaction. Honey containing MG at a concentration of 1.1mM could effectively inhibit the growth of *E. coli* and *S. aureus* with a MIC value of 15% v/v [40,41]. The factors that contribute to the antimicrobial effect of honey have been presented in *Fig. 2*.

Fig 2. Factors contributing to the antimicrobial effect of honey

Use of Honey in Biofilm Control

A biofilm may be defined as aggregates or a syntrophic consortium of microbes embedded in a self-produced matrix. Bacteria existing in a biofilm can cause most chronic wound infections as compared to free-living or planktonic bacteria. Biofilms are highly recalcitrant to antibacterial agents and thus lead to a significant delay in wound healing. Combination therapy exhibiting synergistic effects is considered in such cases. Two types of assays are performed *in vitro* to determine the effectiveness of a product against biofilm, namely, biofilm eradication assay and prevention of formation of biofilms [42,43]. The roles of honey in antimicrobial resistance and biofilm formation were presented in *Table 1*.

S. aureus is the most notorious microorganism accounting for most of wound infections [44]. Liu et al.[45] used medical grade manuka honey with four conventional antibiotics to determine its response against *S. aureus* NCTC 8325 biofilms. The study found that rifampicin and fusidic acid showed enhancement in the treatment of established biofilms when combined with honey. Rifampicin prevents adherence of cells to the surface thereby preventing biofilm formation. This, in turn, increases the number of planktonic cells and makes them more susceptible to antibiotics [46]. This could be one of the major reasons for rifampicin exhibiting synergism with honey [45] and other antibiotics such as cefazolin, fusidic acid, gentamicin, etc. [46]. Fusidic acid, on the other hand, works by binding with prokaryotic elongation factor G (EF-G) inhibiting its protein synthesis which eventually results in anomalies and/or inhibition of translocation of peptide and disassembly of ribosomes [47].

Streptococcus pyogenes is one of the other commonly found bacteria forming biofilms on open wounds. It constitutes the normal flora of a healthy individual's nasopharynx and skin and acts opportunistically during infections and injury. *S. pyogenes* binding to fibronectin is inhibited by honey thereby preventing its biofilm formation [48,49]. Sojka et al.[50] showed the presence of def-1 in honeydew and manuka honey by electrophoresis. It was observed that 0.7 and 2.0 µg def-1 per g honey in manuka and honeydew could inhibit the growth of biofilms of *P. aeruginosa, S. aureus*, and *S. agalactiae*.

Table 1. *The role of honey in antimicrobial resistance and biofilm formation*

Honey Types	Targeted Microrganisms	Mode of Action	Efficacy	Main Outcome of Study	References
Pasture honey	Coagulase-negative *S. aureus*	Osmosis causes the dehydration of bacterial cells	MIC: 3.6±0.7 v/v	The increased sugar concentration exerts osmotic pressure on the bacterial cells. This causes dehydration and ultimately cell death	[27]
Manuka honey			MIC: 3.4±0.5 v/v		
Honey + H_2O_2	*E. coli*	DNA damage by Fenton Reaction	MIC 90 value of H_2O_2 decreased from 2.5 mM to 1.25 mM with addition of honey (1:1)	*E. coli* growth is sensitive to the oxidative action of honey H_2O_2	[31]
Tualang and manuka honey	*Stenotrophomonas maltophilia*	Bactericidal effect of honey	MBC: 25% (w/v) for Tualang and 11.25% (w/v) for Manuka	Non peroxide effect of honey was established by the addition of catalase. Similar growth inhibition patterns for most bacteria	[35]
	S. pyogenes		MBC: 25% (w/v) for both		
	Coagulase-negative *Staphylococci; Enterobacter cloacae; Proteus mirabilis,* and *Shigella flexneri*		MBC: >25% (w/v) for both		
Medihoney + Rifampicin	*S. aureus NCTC* 8325 biofilms	Bacteriostatic effect of Rifampicin + antimicrobial activity of both	MIC: Medihoney: Rifampicin = 8 w/v: 0.02 µg/mL	The combination showed strong synergism	[45]
Medihoney + Fusidic acid	*S. aureus NCTC* 8325 biofilms		MIC: Medihoney: Fusidic acid = 8 w/v: 0.04 µg/mL	The combination showed mild synergism	[45]
Manuka honey	Biofilms of *S. aureus, S. agalactiae,* and *P. aeruginosa*	Prevention of bacterial adhesion to the surface and/or early inhibition of bacterial growth	0.7 µg Def-1 in per g honey	Presence of def-1 in manuka and honeydew	[50]
Honey Dew			2.0 µg Def-1 in per g honey		
Manuka honey	*S. pyogenes* MGAS6180	Facilitating cell death and dissociation of cells from biofilms	MIC: 20% (w/v) MBC: 45% (w/v)	Honey could inhibit adherence of *S. pyogenes* MGAS6180 to fibronectin	[49]

Propolis

Propolis, often referred to as "bee glue" is a sticky and resinous substance crafted by bees using plant materials, including leaves, flowers, and bud exudates. Bees transform these natural elements through a combination of their secretions and wax [51]. Honeybees, scientifically known as *Apis mellifera*, produce propolis by gathering resin from evergreen or coniferous trees. They blend this resin with beeswax and their own salivary secretions to create a viscous, dark green substance that serves the purpose of constructing and upkeeping their hives. Since ancient times, people have consumed and utilized propolis as a medication for overall health [52]. Several historical studies have described propolis as a material that may heal wounds, whether used alone or in combination with other medicines. Propolis has lately been widely used as a supplement in beverages to enhance human health and prevent illnesses due to its wide range of natural uses. Propolis is widely used thanks to its antibacterial, antiviral, antioxidant, antiinflammatory, anaesthetic, antimutagenic, antitumoural, antiprotozoal, anti-fungal, antiseptic and antihepatotoxic abilities in addition to its cytotoxic effect [53,54].

Propolis chemical activity is affected by factors such as climatic conditions, geographical location, and the period in which it was harvested [55]. The various types of propolis pose a significant challenge to quality control procedures. To evaluate and quantify phenolic compounds in propolis, the method combining ultra-high-performance liquid chromatography with photodiode array detection (UHPLC-PDA) and electrospray ionization tandem mass spectrometry (ESI-MS/MS) is recommended. This approach is ideal for quality control purposes and for establishing the chemical composition of propolis compounds. The HPLC-PDA-MS technique has demonstrated its effectiveness in evaluating phenolic compounds in propolis, providing a combination of brief retention times and exceptional resolution [56]. Quality represents a fundamental aspect of propolis, significantly influencing the taste, ability to dissolve, preservation longevity of the nectar, as well as its physical characteristics like density, consistency, and potential for crystallization. Propolis is a intricate resinous substance composed of the subsequent components: resins (50%), waxes (30%), pollen (10%), essential oils (5%), and other organic compounds (5%) [57]. Propolis is mainly comprised of a diverse range of constituents, encompassing aromatic acids including ferulic acid, caffeic acid, and cinnamic acid. Also, it contains aromatic compounds vanillin, aromatic esters (cinnamic acid and caffeic acid esters). In addition, in the composion of propolis volatile compounds (e.g. β-eudesmol, nerol, geraniol, and farnesol) are available. Hydrocarbons are also present in propolis including pentacosane, tricosane, eicosane, steroids like stigmasterol, cholinasterol, and fucosterol. Enzymes are also found to be in propolis including amylase. Flavonoids (e.g. pinobanksin, tectochrysin, chrysin, pinocembrin, galangin, kaempferol, and apigenin) and acids (e.g. cerotic acids, melissic acid, and palmitic acid) are also found to be in propolis. Essential oils, encompassing monoterpenes and sesquiterpenes, and micro- and macronutrients such as Al, Ba, Ca, Cl, Fe, Zn, K, Mg, Na, Mn, along with vitamins like B_6, B_2, B_1, C, and E are present in the propolis [53].

Antimicrobial Properties of Propolis and Antibiotic Resistance

As per earlier accounts, a range of naturally existing antibacterial elements within propolis and its derived products have demonstrated efficacy against multiple bacterial strains. They also increase the effectiveness of conventional antibiotics. Chemicals including caffeic acid, flavanol, ester flavonoids, pinocembrin, and galangin are thought to be responsible for their antibacterial properties [58]. These chemicals may also block bacterial RNA polymerase. Propolis has been shown in several tests to be non-toxic and to have no negative effects in either human trials or animal models. Several researchers studied the synergistic antimicrobial capabilities of propolis, and they found that in most of their *in vivo* and *in vitro* tests, there was a significant decrease in bacterial resistance to traditional antibacterial drugs [59,60]. Propolis' antibacterial action should be assessed on two levels. The first is connected to a direct impact on the microorganism, while the second is connected to immune system stimulation, which causes the organism's innate defenses to become active. Nucleic acid synthesis inhibition, adenosine triphosphate (ATP) synthesis, membrane permeability, disturbance of membrane potential, reduced affinity to the development of biofilms and decreased bacterial motility are all effects of propolis that may be deduced from an investigation of its processes [61] *(Fig. 3)*.

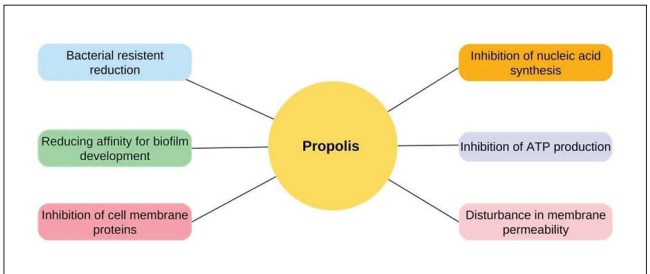

Fig 3. Mode of actions associated with the antibacterial activity of propolis

Afrouzan et al.[49], established the efficacy of poplar propolis against a wide range of bacteria, encompassing both Gram-positive and Gram-negative varieties, including the challenging multidrug-resistant MRSA. Furthermore,

Iranian and Brazilian propolis exhibit effectiveness against Gram-positive bacterial strains, albeit with less impact on Gram-negative counterparts. This disparity in effectiveness can be attributed to the comparatively simpler outer membrane structure of Gram-positive bacteria, rendering them more susceptible to the antibacterial components present in propolis [62]. It has been shown that the outer membrane structure of Gram-positive bacteria accounts for the increased antibacterial activity of propolis against those bacteria. In the cases of MRSA, *S. aureus*, and *S. epidermidis,* quercetin, and its various derivatives showed antibacterial effectiveness. One of the several phenolic mixtures found in propolis is artepillin C, which has effective antibacterial action against MRSA [63]. Similarly, Wojtyczka et al.[64] demonstrated that Polish propolis inhibited the growth of bacteria and altered the formation of biofilms.

Numerous studies have demonstrated that propolis and anti-infection medications work together synergistically. For instance, the combination of Brazilian honey and Brazilian red propolis with chloramphenicol showed synergism against *Salmonella typhi*, while the combination of Brazilian red propolis with fluconazole was beneficial against *Candida* spp. Investigated in Chilean propolis, other flavonoids such as apigenin and pinocembrin showed antibacterial efficacy against *Streptococcus mutans* [51]. The following Gram-negative bacteria, however, were successfully combated by apigenin: *Proteus mirabilis*, *Enterobacter aerogenes*, *Salmonella enterica* serotype Typhimurium, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* [65]. Pinocembrin has also demonstrated antibacterial efficacy against *Listeria monocytogenes*, *Streptococcus sobrinus*, *E. faecalis*, *S. aureus*, *Pseudomonas aeruginosa*, and *Streptococcus mutans* [66].

According to the established research, synergistic anti-bacterial effects were shown when apigenin was added to the β-lactam to treat MRSA and when apigenin was combined with ceftazidime against *Enterobacter cloacae* [67]. Interestingly, cinnamic acid, which has demonstrated substantial activity against several bacteria, *Aeromonas* spp., *Bacillus* spp., *E. coli*, *Enterobacter cloacae*, *L. monocytogenes*, *Micrococcus flavus*, *Mycobacterium* TB, *Salmonella enterica* serotype, *Streptococcus pyogenes*, T*yphimurium*, *Vibrio* spp., and *Yersinia ruckeri* is abundant in propolis [68]. For instance, it is important to note that cinnamic acid inhibits bacterial binary fission, ATPase activity, and the formation of biofilms by breaking the bacterial cell membrane [69].

Use of Propolis in the Biofilm Control

A multi-layered bacterial cluster called a biofilm is enclosed in an extracellular polysaccharide matrix. Persistent infections are aided by biofilms because they are known to boost bacteria's potential to colonize inert objects and protect them from the body's natural defences and antimicrobials. A biofilm is a mucilaginous matrix formed of polymeric extracellular components that is adherent to a surface and contains collections of both living and non-living microorganisms that assemble at the liquid-solid interface. The matrix shields the bacteria from harm by retaining nutrients and restricting access to biocides, oxidants, antibiotics, metallic cations, and poisons. Infections brought on by implanted medical devices like catheters and dental, cardiac, or urological prostheses are therefore greatly aided by biofilms [70].

Biofilms are strongly associated with chronic lung infections, a critical complication in people with cystic fibrosis. These infections are characterized by the presence of drug-resistant biofilms in bronchial mucus, together with regions of high reactive oxygen species levels, primarily due to neutrophil activity [71]. Many antibiofilm chemicals have been found in natural sources against this bacterium, some of which impede bacterial quorum sensing, such as garlic extract and a synthetic derivative of natural furanone. Additional natural antibiofilm compounds encompass ginseng aqueous extract and its component five ursine triterpenes, zingerone, asiatic acid, corosolic acid from *Diospyros dendo*, tannins sourced from *Commiphora leptophloeos, Myracrodruon urundeuva*, and *Anadenanthera colubrina* as well as bacterial products like 3-indolyl acetonitrile. Furthermore, plant extracts have been used to reduce the production of *P. aeruginosa* biofilms [72].

In a comparative examination of the effects of Brazilian red propolis' benzophenone-enriched fraction (BZP-BRP) on strains of *Candida glabrata* resistant to conventional antifungal drugs, Pippi et al.[73] discovered that the fluconazole-resistant bacteria displayed high sensitivity to propolis. Propolis alters gene expression, lowers bacterial viability, and prevents *S. epidermidis* from forming a biofilm, making it susceptible to further antibiotic treatment. It has been shown that Malaysian propolis and chitosan-propolis nanoparticles can inhibit the growth of *Enterococcus faecalis* biofilms [74]. Propolis is effective against *C. albicans* biofilms [75-79]. In situations of persistent infections that are challenging to treat because of the development of biofilm in the wound environment, propolis can minimize biofilm formation and speed up healing processes [80].

Findings indicate that propolis nanoparticles can enhance antibacterial activity and biofilm formation against *E. faecalis*, and when combined with other medications, they may have a synergistic impact that lowers the dosage of each treatment and the amount of time needed to kill germs [81-83]. In a different study, de Mélo Silva et al.[84] also demonstrated that red propolis polymeric nanoparticles

Table 2. *The role of propolis in antibacterial effect and synergestic effect with antibiotics*

Propolis Types	Targeted Organisms	Mode of Action	Efficacy (MIC)	Outcome of Study	References
Propolis ethanolic extract + Ampicillin	*S. typhi*	When compared to ampicillin alone, the combination produced a much larger zone of inhibition	8 µg/mL	Enhanced antibacterial activity	[91]
Propolis ethanolic extract + Cefixime	*S. enteric* in mice	Decreased bacterial load, increased lifespan, corrected hematological parameters, and shielded kidney, spleen, and liver from damage brought on by bacteria	2962 µg/mL	Enhanced antibacterial effect	[61]
Propolis ethanolic extract + Cefoxitin	*S. aureus* and MRSA	Greater inhibition diameter in comparison to each monotherapy	0.39 to 0.78 mg/mL	Enhanced anti-bacterial effect	[64]
Hydroalcoholic propolis extract mixed with carob in a proportion of (60/40, w/w) + Ceftriaxone	*E. coli*	When compared to utilizing ceftriaxone alone, propolis enhanced the effects of ceftriaxone and had a synergistic bactericidal effect	0.125 µg/mL	Synergistic effect	[92]
Hydroethanolic red propolis + Imipenem	*P. aeruginosa* and *S. aureus*	Using imipenem in combination with red propolis collected during the dry season resulted in a considerably decreased MIC value against *P. aeruginosa*; no benefit was shown against *S. aureus*	*P. aeruginosa*:512 µg/mL and *S. aureus*: 64 µg/mL to ≥1024 µg/mL.	*P. aeruginosa*: an enhanced anti-bacterial effect *S. aureus:* no interaction	[93]
Propolis ethanolic extract + Mupirocin	MRSA infected rabbits	Rats' nasal mucous membrane bacteria count and polymorphonuclear leukocyte levels were significantly reduced as compared to the respective monotherapy and combination treatments	-	Enhanced anti-bacterial effect	[94]
Propolis ethanolic extract + Vancomycin	MRSA, *E. faecalis*, *S. pyogenes*, *H. influenza* and *S. pneumonia*	Gram-positive bacteria grow more slowly than gram-negative bacteria due to a powerful synergistic relationship	0.3 to 2.5 mg/mL	Synergistic effect	[95]
Propolis ethanolic extract + Vancomycin	*S. aureus*	The Kirby, Bauer, and E-test methodologies identified synergism	1.5 µg/mL	Synergistic effect	[96]

have the capacity to suppress the growth of *S. aureus* and *Pseudomonas aeruginosa* biofilms. This could be because propolis nanoparticles penetrate the skin more effectively than propolis because of their smaller particle size and higher surface area-to-volume ratio.

According to a study, EEP affects probiotics' *in vitro* viability and capacity to build biofilms in concentration- and strain-dependent ways. In some circumstances, propolis can function as a prebiotic at low concentrations, but at higher concentrations, it may prevent the probiotics from growing in a planktonic state or from creating biofilms [85]. While Brazilian EEPs was extremely active against already-formed biofilms, European EEPs had the highest effect in delaying the creation of biofilms [78]. The Spanish ethanolic propolis extract (SEEP) showed effectiveness against *C. glabrata*. In addition to having antifungal properties, SEEP decreased this emerging opportunistic pathogen's ability to produce biofilms. It presents an intriguing therapeutic approach for the prevention and treatment of biodevice-associated infections due to its anti-biofilm action [86].

According to a different study, *Corynebacterium pseudotuberculosis* strains continue to exhibit great antibiotic sensitivity. There were differences in how the ethanolic and supercritical extracts of green, brown, and red propolis affected the *C. pseudotuberculosis* planktonic isolates. The strongest antibacterial activity among the isolates was demonstrated by the supercritical extract of red propolis and the ethanolic extract of green propolis, both of which were able to prevent the development of biofilm [87]. The ethanolic extract of propolis may inhibit the growth of biofilms and suppress the expression of *EFG1* in *C. albicans* biofilms [88].

To effectively combat *S. mutans*, propolis, essential oil (PEO) inhibits cell viability inside the biofilm, reduces the overall amount of biofilm biomass, and destroys the biofilm structure [89]. When compared to a well-researched antiseptic mouthwash with established outcomes, bee propolis demonstrated promising benefits in suppressing the growth of *S. mutans* produced in a biofilm [90]. Current studies in the literature on the antibacterial effect of propolis and its synergistic effect with antibiotics were reviewed and summarized in *Table 2* [61,64,91-96].

Bee Pollen

When a worker bee travels from one flower to another in search of nectar, pollen grains get attached to the bee's body. This pollen combines with nectar and salivary enzymes; and hardens to form a pellet known as bee pollen. The bees use bee pollen as a source of protein, lipids, micronutrients and minerals [97]. Pollen grains from various plants vary in size between 1.4 to 4 mm and weigh approximately 7.5 to 8 mg. They come in a variety of colours including creamy white, red, yellow, orange, grey, dark brown and green. The wide nutritional and medicinal properties of bee pollen make it a natural superfood. The bee pollen properties differ based on floral

source, geographical origin and seasonal variations [98]. This variation may contribute to the difference in the bee pollen's biological activities and thus its therapeutic effects [99]. Bee pollen may be divided into two groups: monofloral bee pollen and multifloral bee pollen. Bee pollen can be categorized into two primary types: monofloral and multifloral. Monofloral pollen consists of a minimum of 45% pollen grains from a single plant species and displays consistent organoleptic and biochemical characteristics. In contrast, multifloral pollen incorporates pollen grains from various plant sources without any one dominant plant species. The determination of the botanical source of bee pollen is accomplished through palynological analysis. Each individual component of the pollen grains is identified and examined under a microscope [100,101]. Bee pollen is an affordable nutraceutical that has great potential in the food industry [102].

The therapeutic effects of bee pollen are due to the presence of a vast range of secondary plant metabolites, which varies from one plant species to another [99]. Bee pollen is composed of proteins, carbohydrates, lipids, amino acids, enzymes, polyphenols, co-enzymes, minerals and vitamins [102]. According to a systemic review conducted by Thakur and Nanda involving over 100 studies, bee pollen on average contains 13-55% carbohydrates, 10-40% protein and 1-13% lipids [98]. In addition, bee pollen contains vital amino acids such as methionine, phenylalanine, valine, lysine, threonine, tryptophan, histidine, leucine and isoleucine. Lipids are found in the form of essential fatty acids, phytosterols and phospholipids. The total phenol content which includes polyphenols such as catechins, flavonoids (ex. quercetin, kaempferol and isorhamnetin), and phenolic acids make up an average of 30.59mg GAE/g [98,102]. Bee pollen is found to contain minerals such as Mg, Ca, Zn, Fe and Cu and vitamins such as provitamin A, niacin, biotin, thiamine, folic acid and vitamin E [99,103].

Antimicrobial Properties of Bee Pollen and Antibiotic Resistance

Antimicrobial resistance is a serious issue that is currently threatening global public health. Natural products can be used as an alternative method to control antibiotic-resistant pathogens [104]. Till now no microbe has developed resistance against bee pollen and hence it can be used along with traditional antibiotics against resistant microbes [105].

Bee pollen exhibits antimicrobial properties against various bacteria and fungi. Bee pollen from different regions such as Greece, Morocco, Portugal, Spain, Egypt, Turkey, Slovakia, Chile and Slovenia have been studied for its antimicrobial activity [106]. Some of the bacteria against which they have shown activity are Gram-positive bacteria: *S. aureus*, *Bacillus cereus*, *Staphylococcus epidermidis*, *Listeria monocytogenes*, *Clostridium butyricum*, *Streptococcus pyogenes,* and *Clostridium perfringens*, and Gram-negative bacteria: *Salmonella enterica, Campylobacter jejuni,* and *E. coli*. In addition, bee pollen has also been reported for antifungal activity against fungal species including *Candida tropicalis*, *Candida glabrata, Candida krusei*, *Zygosaccharomyces bailii, Aspergillus niger,* and *Aspergillus fumigatus* [106].

The presence of phenolic compounds is credited for the antimicrobial properties found in bee pollen [107]. The antibacterial effects of bee pollen might also involve the enzyme glucose oxidase, which is generated by honeybees [99]. Bee pollen acts against bacteria by breaking down their cytoplasmic membrane which causes the leakage of potassium ions. This triggers the autolysis of the bacterial cell leading to cell death [108]. Several studies have reported that Gram-negative bacteria are less sensitive to bee pollen compared to Gram-positive bacteria. A possible explanation could be that Gram-negative bacteria have a more chemically complex cell wall with a greater lipid content which renders them more resistant to pollen [105]. Antimicrobial resistance is often more pronounced in Gram-negative bacteria, owing to the presence of an outer membrane composed of lipopolysaccharides, which serves as an added layer of protection [109].

A study conducted for antimicrobial activity by Bakour et al.[110] investigated the effect of bee pollen from six different botanical origins *(Centaurium erythraea, Citrus aurantium, Coriandrum sativum, Quercus ilex*, *Punica granatum,* and *Ruta graveolens)* against six strains of human multidrug-resistant pathogens namely, *S. aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumoniae, Acinetobacter baumannii*, *Enterobacter cloacae*. These microbes were tested for antibacterial susceptibility against 28 standard antibiotics and were found to be resistant to some of them. The agar disc diffusion method was used to study the antibacterial activity of the samples and the MIC and minimum bactericidal concentration (MBC) were estimated and summarised in *Table 3*. The findings of the study reported that the ethanolic pollen extracts of *Punica granatum* and *Quercus ilex* showed the best antimicrobial activity followed by *Ruta graveolens*, *Centaurium erythraea*, and *Coriandrum sativum* which showed intermediate activity. *Citrus aurantium* showed no antimicrobial activity. A positive correlation was observed between the antibacterial activity of the pollen extract and its antioxidant content. The difference in MIC and MBC values between different pollen extracts could be due to the variation in their chemical composition and the variability of the cell wall and membrane structure of the bacterial strain [110].

Another study by Pelka et al.[111] reported the antimicrobial resistance activities of bee pollen and bee bread from

different regions of Poland. Authors studied antimicrobial activity prepared extract of bee pollen in comparison to extract of bee bread. Three samples of bee pollen and three samples of bee bread, which were prepared separately showed high anti-staphylococcal potential, were tested against 3 clinical isolates of methicillin-resistant *S. aureus* (MRSA). Beebread showed better activity compared to bee pollen as bee pollen is converted to bee bread by the process of fermentation. This study suggests that the lactic acid bacteria involved in this process produce lactic acid and bacteriocin which are antimicrobial in nature. The lactic acid bacteria cause lipid hydrolysis which produces aliphatic acids which acting as an antimicrobial agent, and this may be the reason for the difference in antimicrobial activity between bee pollen and bee bread. The MIC value for bee pollen ranged between 5 to 10% (v/w) and the MBC value ranged between 5-10% (v/w) *(Table 3)* [111].

The antimicrobial compounds present in bee products work in a synergistic way which might be the reason why microbes are not resistant to bee pollen. Bee products are rich in flavonoids and polyphenols which are known to have the ability to counter bacterial resistance making them potential antimicrobial agents. Bioactive compounds present in bee pollen can be used in the combination with antibiotics to produce a synergistic antimicrobial effect [110]. A study reported that when kaempferol glycosides of plant origin were used along with hydrophilic fluoroquinolones against MRSA, the kaempferol helped to significantly reduce the MICs of the antibiotics used [112]. Another study revealed that the combination of rifampicin, quercetin, and kaempferol acted synergistically to inhibit the β-lactamase enzyme of clinical MRSA [110]. Another study tested the use of apigenin and other flavones along with a penicillin/streptomycin mix against two MRSA strains and coagulase-negative *Staphylococcus* (CNS). The apigenin and the other flavones were unable to improve the antimicrobial activity in this case [113]. The use of polyphenols along with antibiotics helps to increase the efficacy of the antibiotic, lowers its dose and therefore reduces the antibiotics' side effects [114].

Use of Bee Pollen in the Biofilm Control

Biofilm refers to bacterial communities that are encased within an exopolysaccharide matrix and adhere to a surface. It is the cause of many diseases including infective endocarditis, inflammatory bowel disease, impaired wound healing, cystic fibrosis, and pertussis [115].

Schuh et al.[116] studied the exosome-like vesicles (ELVs) present in various bee products for their bacteriostatic, bactericidal, and biofilm-inhibiting effects. These exosome-like vesicles are a part of the hypopharyngeal gland secretions produced by *Apis mellifera*. These ELVs were extracted from different bee products and their minimum biofilm inhibition concentration (MBIC) was estimated using a biofilm-forming strain, *S. aureus*. All the bee product-derived ELVs showed biofilm-inhibitory potential. The MBIC ratio of bee pollen was found to be 10:1 (vesicles: colony forming units). Bee pollen displayed 50% biofilm inhibition at a 1:1 ratio. The ELV fraction of bee pollen was compared with an exosome-depleted fraction. The findings reported that the exosome-depleted fraction showed antibacterial activity at 5% (v/v) concentration whereas the ELV fraction showed antibacterial activity at a lower concentration of 1%. Bee-derived ELVs could be potentially used in wound healing to treat wound-derived infections by preventing biofilm formation and by aiding migratory activity [116].

The ability of bee pollen extract to prevent the adherence of microbes to the inert substratum and cellular substrate (Hep-2 -human epithelioma cells) was studied [117]. The microbial strains used in assessing microbial adherence to an inert surface were standard strains of *S. aureus*, *E. faecalis, E. coli, P. aeruginosa, C. albicans* and clinically isolated strains of *E. cloacae*, *C. glabrata*, *C. famata*, *C. krusei*, *C. guillermondii* and *C. lusitaniae*. The effect of bee pollen in inhibiting microbial attachment to cellular substrates was carried out using *E. faecalis, S. aureus, C. albicans, P. aeruginosa, C. lusitaniae, E. cloacae, C. famata, C. guillermondii, C. glabrata, and C. krusei*. These findings revealed that *S. aureus, P. aeruginosa, and C. glabrata* were the most sensitive strains when it comes to adherence to inert surfaces. According to Ilie et al.[117], when compared to the control, the bee pollen extract exhibited reduced adherence capacity to the cellular surface in the case of the Gram-negative bacteria *E. cloacae and P. aeruginosa*, all yeast (except *C. famata*) and one Gram-positive bacteria *E. faecalis* [117].

Biofilm control is one of the mechanisms by which polyphenols exert antimicrobial properties. This was tested using plant-derived or synthetic compounds. Quercetin glycosides and kaempferol glycosides were found to have biofilm control over yeast and fungi whereas luteolin has antibiofilm properties against *S. aureus and L. monocytogenes* [106]. Plant phenolics prevent biofilm formation by interfering with the bacterial regulatory system such as quorum sensing [118]. It can be concluded from the above studies that the polyphenols present in bee pollen may be responsible for the antibiofilm activity.

Royal Jelly

Royal jelly (RJ) [119], is a creamy substance produced by the hypopharyngeal glands of the young nurse worker bees mainly to mature and maintain the queen bee. It is exclusively served to the queen bee throughout her life, whilst royal jelly is served to other sexually immature females for merely the first 2-3 days [120].

Table 3. *Role of bee pollen in antimicrobial-resistance*

Types of Bee Pollen	Targeted Microorganisms (Resistant Strain)	Mode of Action	Efficacy		Main Outcome of Study	References
			MIC	MBC		
Centaurium erythraea (botanical origin)	*S. aureus*	The presence of Polyphenols helps fight bacterial resistance	Ineffective	Ineffective	Intermediate antimicrobial activity	[110]
	P. aeruginosa		Ineffective	Ineffective		
	A. baumanii		Ineffective	Ineffective		
	E. coli		Ineffective	Ineffective		
	E. cloacae		2.5 mg/mL	2.5 mg/mL		
	K. pneumonia		Ineffective	Ineffective		
Citrus aurantium (botanical origin)	*S. aureus*	The presence of Polyphenols helps fight bacterial resistance	Ineffective	Ineffective	No antimicrobial activity	[110]
	P. aeruginosa		Ineffective	Ineffective		
	A. baumanii		Ineffective	Ineffective		
	E. coli		Ineffective	Ineffective		
	E. cloacae		Ineffective	Ineffective		
	K. pneumonia		Ineffective	Ineffective		
Coriandrum sativum (botanical origin)	*S. aureus*	The presence of Polyphenols helps fight bacterial resistance	2.5 mg/mL	2.5 mg/mL	Intermediate antimicrobial activity	[110]
	P. aeruginosa		Ineffective	Ineffective		
	A. baumanii		2.5 mg/mL	2.5 mg/mL		
	E. coli		Ineffective	Ineffective		
	E. cloacae		2.5 mg/mL	>2.5 mg/mL		
	K. pneumonia		2.5 mg/mL	>2.5 mg/mL		
Punica granatum (botanical origin)	*S. aureus*	The presence of Polyphenols helps fight bacterial resistance	0.62 mg/mL	0.62 mg/mL	Highest antimicrobial activity	[110]
	P. aeruginosa		2.5 mg/mL	2.5 mg/mL		
	A. baumanii		0.31 mg/mL	0.31 mg/mL		
	E. coli		2.5 mg/mL	2.5 mg/mL		
	E. cloacae		2.5 mg/mL	2.5 mg/mL		
	K. pneumonia		2.5 mg/mL	>2.5 mg/mL		
Quercus ilex (botanical origin)	*S. aureus*	The presence of Polyphenols helps fight bacterial resistance	0.62 mg/mL	0.62 mg/mL	Highest antimicrobial activity	[110]
	P. aeruginosa		2.5 mg/mL	2.5 mg/mL		
	A. baumanii		0.31 mg/mL	0.31 mg/mL		
	E. coli		1.25 mg/mL	2.5 mg/mL		
	E. cloacae		1.25 mg/mL	1.25 mg/mL		
	K. pneumonia		2.5 mg/mL	2.5 mg/mL		
Ruta graveolens (botanical origin)	*S. aureus*	The presence of Polyphenols helps fight bacterial resistance	1.25 mg/mL	1.25 mg/mL	Intermediate antimicrobial activity	[110]
	P. aeruginosa		Ineffective	Ineffective		
	A. baumanii		1.25 mg/mL	1.25 mg/mL		
	E. coli		2.5 mg/mL	2.5 mg/mL		
	E. cloacae		Ineffective	Ineffective		
	K. pneumonia		Ineffective	Ineffective		
Czarne, Poland (Geographical origin)	MRSA strain 1, 2,3(clinical isolate)	-	10% (v/w)	10% (v/w)	Lower anti-staphylococcal activity	[111]
Niżna Łąka, Poland (Geographical origin)	MRSA strain 1, 2,3(clinical isolate)	-	5% (v/w)	5% (v/w)	Higher anti-staphylococcal activity	[111]
Modzele, Poland (Geographical origin)	MRSA strain 1, 2,3(clinical isolate)	-	5% (v/w)	5% (v/w)	Higher anti-staphylococcal activity	[111]

MRSA- methicillin-resistant S. aureus; MBC- minimum bactericidal concentration; MIC- minimum inhibitory concentration.

Royal jelly has a complex chemical composition. It consists of water, minerals such as potassium, magnesium, calcium, etc., proteins (about 50% of the dry mass), lipids (primarily short-chain fatty acids including 10-hydroxy-2-decenoic acid), carbohydrates (about 30% of the dry matter), at least 17 amino acids, and vitamins [121]. The chemical makeup, color, and flavor of royal jelly are influenced by the bees' diet. One type of diet comprises solely of the bees' natural food sources, like nectar, pollen, and honey. The second type of diet involves the addition

Table 4. *Antimicrobial Activity of Royal Jelly*

Origins of the Royal Jellys	Targeted Microorganisms	Mean of Inhibition	Effect Same Antimicrobial	References
Chinese royal jelly 15 mg/mL	*Aspergillus niger* *Aspergillus fumigatus* *Candida albicans* *S. aureus* *Bacillis subtilis* *Escherichia coli*	13 to 15 12 to 13 9 to 10 13 to 14 16 to 17 16 to 17	< Clotrimazole < Clotrimazole < Clotrimazole < Penicillin g < Penicillin g < Penicillin g	[124]
Egyptian royal jelly 15 mg/mL	*Aspergillus niger* *Aspergillus fumigatus* *Candida albicans* *S. aureus* *Bacillis subtilis* *Escherichia coli*	20 to 21 24 to 25 19 to 20 23 to 24 24 to 25 10 to 11	= Clotrimazole = Clotrimazole > Clotrimazole = Penicillin g < Penicillin g < Penicillin g	[124]
Southern Córdoba (Argentina) royal jelly 7.1 and 14.5 mg/mL	*S. aureus* *Staphylococcus epidermidis* *Micrococcus luteus* *Streptococcus uberis* *Enterococcus faecalis* *Pseudomonas aeruginosa* *Escherichia coli* *Klebsiella pneumoniae*	15 to 21 13 to 14 15 to 16 11 10 to 12 5 to 6 9 to 10 ----------	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------	[123]
Singapore royal jelly 100 µg/mL	*Aggregatibacter* *actinomycetemcomitans,* *Porphyromonas gingivalis,* *Prevotella intermedia,* *Fusobacterium nucleatum,*	No growth No growth No growth No growth No growth	---------- ---------- ---------- ---------- ----------	[125]
Chinese royal jelly	*S. aureus* *E. coli* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Pseudomonas aeruginosa* *Methicillin* resistant *S. aureus* *Salmonella typhimurium* *Salmonella paratyphi* *Proteus vulgaris* *Enterobacter aerogenes*	1 to 1.5 1 to 1.5 0.5 to 1 1 to 1.5 1 to 1.5 1 to 1.5 2 to 2.5 1 to 1.5 1 to 1.5 1 to 1.5	= Penicillin Significant difference with Penicillin Significant difference with Penicillin Significant difference with Penicillin Significant difference with Penicillin Significant difference with Penicillin Significant difference with Penicillin Significant difference with Penicillin Significant difference with Penicillin Significant difference with Penicillin	[126]
Jordanian royal jelly	*S. aureus* *E. coli* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Pseudomonas aeruginosa* *Methicillin* resistant *S. aureus* *Salmonella typhimurium* *Salmonella paratyphi* *Proteus vulgaris* *Enterobacter aerogenes*	1.75 to 2 2 to 2.5 2.40 to 2.75 1.40 to 1.75 1.5 to 1.75 0.75 to 1 0.75 to 1 1.5 to 1.75 1.4 to 1.5 1.5 to 1.75	= Penicillin Significant difference with Penicillin Significant difference with Penicillin Significant difference with Penicillin Significant difference with Penicillin Significant difference with Penicillin Significant difference with Penicillin Significant difference with Penicillin Significant difference with Penicillin Significant difference with Penicillin	[126]

of other nutrients, such as proteins and carbohydrates, to their natural food [122].

Antimicrobial Properties of Royal Jelly and Antibiotic Resistance

The results obtained in the study of García et al.[123] showed that 2 RJ specimens, acquired from different regions of Argentina, both inhibited the growth of Gram-negative and Gram-positive bacteria strains capable of infecting cutaneous wounds *Staphylococcus epidermidis*, *S. aureus*, *Micrococcus luteus, Streptococcus uberis, Enterococcus faecalis, Pseudomonas aeruginosa, S. aureus, E. coli,* and *Klebsiella pneumoniae* being the most sensitive strain of either RJ sample tested. Moselhy et al.[124] indicated that RJ obtained from various regions in Egypt and China possesed bacteriostatic effects on Gram-positive and Gram-negative bacteria at different levels. The effect of royal jelly on the growth of periodontopathic bacteria were examined in the study conducted by Coutinho et al.[125] and the results suggested that RJ samples were active against the growth of the tested periodontal pathogens. Al-Abbadi [126] showed that the effects of royal jelly acquired from Jordan and China against bacteria and fungi pathogenic to humans. The results of this study indicated that both RJ types prepared in different ways were effective against Gram-positive and/or Gram-negative bacteria *(Table 4)*.

Royal jelly's ability to combat microbes can be attributed to specific elements, including Major royal jelly proteins (MRJPs 2-5, 7), antimicrobial peptides (Jelleines I, II, and III, as well as royalisin), and fatty acids like 10-HAD [127,128]. The antibacterial activity of MRJPs against Gram-negative *E. coli* depends on their interaction with bacterial cell walls. Furthermore, several studies have shown the wide scope of antibacterial effects of MRJP4 and MRJP2 against fungi, yeasts and both Gram-positive and negative

bacteria. The proteins act as antimicrobial peptide (AMP)-like proteins because they can attach to the cell walls of bacteria and deconstruct its structure [129]. On the other hand antibacterial peptides are also positively charged which allows them to collapse the cell membrane through interacting with its anionic phospholipids [130]. Jelleines I, II and III, royalisin and 10-HDA seem to be effective against Gram-positive and negative bacteria with the latter two being also effective against fungi [127]. determined that both intramolecular disulfide linkages and the presence of 11 amino acids at the C-terminus in royalisin are crucial for its antimicrobial efficacy [131]. Moselhy et al.[124] showed that while the antimicrobial capabilities of royal jelly are evident, there doesn't seem to be any one definite cause, suggesting that the effect may be of a combined or synergistic nature [124].

In antoher study Mierzejewski et al.[132] examined the antimicrobial effects of three honeybee products including honey, royal jelly and propolis in comparison to three antibiotics including kanamycin, penicillin and tetracycline on bacteria strains *Bacillus cereus*, *Staphylococcus epidermidis*, *E. coli,* and *S. aureus***.** The results showed that kanamycin and tetracycline were the most effective antimicrobial agents in general; nonetheless, royal jelly was more effective against *Staphylococcus epidermidis*, and *E. coli*. The study concluded that honey, propolis, and royal jelly contain antibacterial components that can be used as standard first-line treatments for mild bacterial infections. The study also suggested that commercial products containing these components can be manufactured to increase effectiveness, which makes them also potentially preventative against infectious diseases [132]. Another study compared the antimicrobial activity of royal jelly with gentamicin, doxycycline, and the combination of the two antibiotics against *E. coli*. This study concluded that RJ shows significant activity against *E. coli* growth [133].

Use of Royal Jelly in Biofilm Control

A study was conducted both *in vitro* and *in vivo* using a rat model to investigate the effect of a 50% concentration of Royal Jelly (RJ). The results showed a highly statistically significant reduction in the adherence of MRSA bacteria in all samples tested ($P<0.01$) [134].

According to Shuch et al.[116] study, bee-derived exosome-like vesicles (ELVs) exhibit the capacity to impede the formation of biofilms in *S. aureus* strains in a laboratory setting. This finding indicates a potential role for ELVs in the prevention and management of wound infections. Notably, when the ratio of royal jelly was less than 1 vesicle per viable bacterial cell, it led to the inhibition of *S. aureus* growth and a significant reduction in biofilm formation, approximately by half [116]. In another study, it was showed that RJ extracellular vesicles (EVs) incorporated into collagen gels significantly reduced biofilm formation [135].

In a different research investigation, it was observed that royal jelly concentrations at 25% or higher effectively suppress bacterial growth. However, when the concentrations were subinhibitory, they were found to promote pyocyanin production and enhance biofilm formation in *P. aeruginosa* [136].

A recent study concluded that Sub-MICs of 10-HDA could be effective in inhibiting biofilm formation and eliminating the mature biofilms of *S. aureus*, as verified by significant reductions in biofilm biomass and cell viability. Furthermore, the biofilm structure was noticeably damaged after treatment with 10-HAD [137].

Bee Venom

Bee venom (BV), also called apitoxin, is an acidic transparent and odorless liquid secreted by bees [21]. BV is synthesized by bee workers and queens and used for the defence of the colony or of the individual [22,138]. BV discovery was attributed to ancient Egyptian. It had been used as a therapeutic product starting from the second century BC. BV was first used in Eastern Asia [20,21].

BV is a mixture of several active compounds. It is a complex mixture of polypeptides with melittin being the major constituents (40-60% of dry weight) [22]. Additional peptides like apamin, adolapin, and mast cell degranulating peptide can be found within BV. Phospholipase A2 ranks as the predominant enzyme in BV, closely followed by hyaluronidase. Other molecules are also present in BV composition such as: sugars, minerals, amino acids and catecholamines [23,24]. Volatiles compounds are also among BV components. However, due to their volatile properties they are lost easily during collection [19].

Bee venom is using in an alternative medicine for the treatment of some diseases, such as rheumatism arthritis, Parkinson's disease (PD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS) [139].

Nonetheless, the necessity for standardizing it as a reliable and safe medicinal product is imperative. In this regard, the method for apitoxin standardization proposed in Korea appears to be a valuable approach. This method involves the purification of bee venom, followed by the application of a stepped-gradient open column (ODS-A; 120 Å, 150 mesh). Consequently, this process results in an increased yield of melittin while simultaneously eliminating allergenic proteins. The purified bee venom is analyzed by HPLC, and concentration of mellitin is examined. Finally, bee venom is diluted to required concentration and proper dose of apitoxin may be applied using injection water in pharmacopuncture [140].

Antimicrobial Properties of Bee Venom and Antibiotic Resistance

Bee venom has been largely investigated for its antimicrobial properties and many research papers and reviews have been published in the last recent years. BV was found to inhibit several bacterial strains including pathogenic bacteria *(Table 5)* [141-145].

Maitip et. al.[145] investigated the antimicrobial properties of crude BV of four bee species in Thailand: *A. cerena*, *A. meliffera*, *A. florea* and *A. dorsata* and compared its effectiveness with synthetic melittins derived from the four studied bee species. All BV and synthetized melittins were more active against Gram-positive bacteria. BV showed lower MIC values regarding all tested bacterial strains with the lowest values for *A. cerena* crude BV compared to melittin. Melittin inhibited Gram-positive bacteria in the following order: *S. epidermidis* (MIC=12.5-50 µg/mL) > *M. luteus* (MIC=25-50 µg/mL) > *S. aureus* (MIC=50-200 µg/mL) > MRSA (MIC=100-400 µg/mL) > *B. subtilis* (MIC >400 µg/mL). Differences between the studied BV was related to differences in their chemical composition [145].

The effectiveness of the tested peptide was pointed to be influenced by their seize, sequence, charge, structure, hydrophobicity and amphipathicity [146]. *A. florea* and *A. dorsata* melittins exhibited a higher activity when compared to the extracted BV. For *A. cerena* and *A. meliffera*, results of BV and melittins were similar [145]. Alia et al.[147] also reported an interesting effect of melittin on *L. monocytogenes* (ATCC 19111) and *S. aureus* (ATCC 11632) with MIC value of of 12.5 µg/mL and 25 µg/mL respectively. While melittin were less active on the tested Gram-negative bacteria *S. enterica* and *Y. kristensenii* with MIC values of of 100 µg/mL and 200 µg/mL respectively.

The emergence of antimicrobial resistance stands out as a highly concerning issue within the domain of public health. The spread at which bacteria develop resistance far exceeds the ability to treat it or at least to stop this evolution. The return to nature for research for new antimicrobial molecules is a key point in favor of bee products. Many investigations focusing on strains of increasing resistance have proved the effectiveness of BV and its synthetized or extracted polypeptides [148]. BV and melittin showed very promising results on resistant bacterial strains.

Table 5. *BV antibacterial properties*

Bee Venom and Its Active Constituents	Targeted Microorganisms	Mode of Action (If Available)	Efficacy (e.g. IC50)	Main Outcome of Study	References
Natural and commercial apitoxin, melittin and phospholipase A_2	*S. salivarius* (ATCC 25975), *S. sanguinis* (ATCC 10556), *S. sobrinus* (ATCC 33478), *S. mutans* ATCC 25175), *S. mitis* (ATCC 49452), *L.. casei* ATCC 11578), *E. faecalis* (ATCC 4082)	ND	20-40 µg/mL (apitoxin) 2-40 µg/mL (melittin) >400 µg/mL (phospholipase A_2) 6-80 µg/mL (melittin + phospholipase A_2)	Melittin was found to be the most active component apitoxin melittin, were found to be very effective against oral pathogens.	[141]
Egyptian BV sac Apitox (Apitonoc services, CANADA) Vacsera (Egyptian vaccine and serum organization)	*S. aureus, S. pyogens, K. pneumonia, E. coli, P. aeruginosa*	ND	1.00-3.80 mg/mL	BV inhibits the Survival and the growth of and of the tested. In consequence, it can be used ascomplementary antimicrobial agent	[142]
BV, melittin (synthetized and purified)	*S. aureus* ISP4790 and MU50 (clinical isolates), MRSA (USA300 (LAC), Newman, MW2, MRSA1, MRSA2), *S. agalactiae* CNCTC 10/84, *S. epidermidis* RP62a, *S. pneumonia* TIGR4, *S. gordonii* M99 and *S. bovis* NEM760	ND	1.56-12.5 µg/mL	BV had negative effects when used as an anti-MRSA therapy. Melittin may have a therapeutic potential for the management of MRSA infections.	[143]
Anatolian BV	*E. faecalis* (ATCC 29212), *L. monocytogenes* (ATCC 11994), *S. pyogenes S. aureus* (ATCC 25923 and MRSA), , *B. subtilis* (ATCC 6633 and clinical strain, *B. cereus* (702 Roma), *M. smegmatis* (ATCC 607), *E. coli* (ATCC 25922), *K. pneumoniae* subsp. *pneumoniae* (ATCC, 18883), *Y. pseudotuberculosis* (ATCC 911), *Vibrio* sp. (Clinic strain), *A. hydrophila* (ATCC 7966), *A. sobria* (ATCC 43979), *P. aeruginosa* (ATCC 27853)	Synergetic effect of melittin and PLA2 (mecanism of action not clarified)	3.06-50.00 µg/mL	BV is an interesting alternative source of antimicrobial compounds, including resistant microorganisms. *In vivo* research are required to evaluate BV safety and effectiveness for future therapeutic uses.	[144]
Crude venom *from A. mellifera, A. cerana, A. dorsata*, and *A. florea* (Thailand) and melittin	*S. aureus, MRSA* *S. epidermidis, B. Subtilus, M. luteus,* *K. pneumonia,* *E. coli, S. thyphimurium*	ND	16.7-7.2 ≥400.0±0.0 µg/mL	*A. cerana* crude venom and melittin were most effective in against Gram-positive bacteria and MRSA. Crude venom is more effective than melittin. Both substances might be potential sources of antimicrobial agents against Gram-positive and antibiotic-resistant bacteria.	[145]

ND: not determined

They were reported to be active on methicillin-resistant *S. aureus* [141,144,145] and *B. burdorferi* [149]. Melittin was also reported to inhibit other antibiotic-resistant bacterial strains. Melittin was tested on 20 isolates of *A. baumannii*. MIC and MCB values were between 0.5 and 16 µg/mL for MIC and 0.5 and 32 µg/mL for MBC [150]. Gopal et al.[151] demonstrated the effectiveness of melittin against 32 isolates of antibiotic-resistant bacteria including *E. coli*, *S. thyphimurium, P. aeruginosa* and *S. aureus* suggesting the potential use of melittin to treat microbial infection. Khozani et al.[152] investigated the antibacterial activity of melittin against 33 *P. aeruginosa* strains isolated from patients with burns (third degree) and compared its effect to conventional antibiotic namely colistin, ceftazidime and doripenem. *In vitro* results indicated that melittin was the most active. Then, authors conducted an *in vivo* study using topical application in animal model such as mouse with infected burn and confirmed the results obtained *in vitro*.

S. aureus represents a significant pathogenic agent responsible for infections in both community and healthcare settings. The rise of MRSA has presented a substantial challenge to healthcare systems worldwide [153,154]. Choi et al.[143] examined *in vitro* the effect of BV and melittin on MRSA and sensitive *S. aureus*. In addition, other Gram-positive bacteria were also tested. *In vivo* experiments were performed on infected mice with *S. aureus*. MRSA tested strains were more sensitive to BV with MIC values ranging between 0.78 and 3.12 µg/mL. While *S. aureus* sensitive strains were less active and exhibited a higher MIC values (3.13-12.5 µg/mL). However, the i.p injection of BV into mice infected with HSA300 strain indicated that the use of 1.25 or 2.5 µg/kg BV at the time of infection did not show any protective effect. The same results were reported with the injection of BV one hour before the induction of infection. All mice died in the treated group. BV treatment seems to enhance the caused bacteremia. Moreover, no significant difference was observed between the control and the BV treated group in an induced *staphylococcal* skin infection model. Treatments were applied daily for 10 days. Melittin was administered alone and in combination with PLA2 at various concentrations. No significant effect was observed on the MRSA cell death. Purified and synthetized melittin at 5 mg/kg injected into infected mice one hour after bacterial injection did not kill all the treated mice and 50% survived.

Several mechanisms have been suggested to explain melittin antibacterial properties. One of the proposed mechanisms is related to alpha helical form and its effect on the surface membrane of the cells. This form is involved in the membrane disintegration caused by the insertion of the alpha helical form into the surface membrane causing the formation of pores affecting membrane permeability and leading to osmotic cytolysis [155,156]. According to several authors, melittin antimicrobial effect is similar to other antimicrobial peptides. Because its amphipathicity, melittin can integrate into the phospholipide bilayer when low concentrations are used. However, in higher concentration, melittin become homodimerized and can form pores, disrupt phospholipid and release Ca^{2+} [157-159].

B. burgdorferi is a spirochetal bacterial causing lime disease. Several factors can cause the transformation of *B. burgdorferi* in a defensive form. Unfortunately, the defensive forms were reported to possess a high resistance to the currently used treatment [160]. Melittin effects on the surface components of spirochetal organisms include the increasing bleeding of surface membrane and the modification of the outer envelope integrity. However, the DNA is not damaged, and analysis showed intact DNA. Melittin act also on *B. burgdorferi* motility at the beginning of the treatment [149,161].

In addition to melittin, BV antimicrobial activity was also attributed to PLA2. Both components act by producing pores in bacteria membrane causing their damage then their lyse [139,162,163]. The two compounds may form a complex by hetero-oligomerization, which indicate that the toxic effect may result on an enhanced activity due to the synergism effect of the two BV toxins. The formed complex is responsible of the rapid lyse of the bacteria. The combination of melittin to PLA2 can enhance its ability to bind to the outer membrane. This binding enables melittin oligomerization and pore formation on the bacterial membrane [164]. The described binding was explained by several authors [165,166] as a result of an electrostatic attraction between melittin basic amino acid residues and phosphate group of a key constituent the cell membrane, phosphatidylcholine.

Use of Bee Venom in the Biofilm Control

Antimicrobial peptides are a promising strategy to combat resistant strains [167, 168]. The development of antibiotic resistance within biofilms primarily results from the existence of dormant populations residing within the established film. Standard antibiotics cannot act actively on biofilm because they are effective on actively growing population. Therefore, the dormant population is not eliminated [169]. Antimicrobial peptides such as melittin can act on both active and dormant populations. They cause membrane disruption followed by the death of both populations. Recently, antimicrobial peptides were pointed to act using other mechanisms affecting protein synthesis and/or nucleic acid [170].

BV was evaluated for its antibacterial and antibiofilm effect on multidrug resistant bacteria (MDR) isolated from clinical specimens. Results indicated that MDR

Gram-positive bacteria were more sensitive to BV. BV sub-MICs values reduced biofilm formation of *S. aureus*, vancomycin resistant *S. aureus*, *S. hemolyticus*, *E. faecalis* and *P. aeruginosa* with reduction varying from 63.8% to 92% [171].

In the last recent years, biofilm was described for *Borrelia*. The aggregate forms were found to be highly resistant both *in vivo* of *in vitro* studies [172,173]. Sacarros et al.[161] investigated the effect of *A. meliffera* BV and melittin on the biofilm (surface attached and floating-aggregates) caused by *B. burgdorferi* and compared it to cefoperazone, doxycycline and daptomycin used alone or in combination. The tested antibiotics were recently reported to be effective against persistent forms. The treatment by cefoperazone, daptomycin and antibiotic combination was found to reduce viable and persister *spirochetes*. While doxycycline was not active on persisted forms. BV antibiofilm effect was tested at 100, 400 and 800 µg/mL. While melittin was tested at 50, 200 and 400 µg/mL. Stationary cultures were incubated for 7 days. Finally, viability was assessed through the utilization of the SYBR Green I/PI assay and direct counting methods. It was observed that BV led to a marked reduction in the number of viable cells across all tested concentrations when compared to daptomycin and the negative control. After 7 days incubation, BV above 400 µg/mL was found to be as effective as cefoperazone and the tested combination of antibiotics. Melittin significantly reduced *spirochetes* at all tested concentrations compared to doxycycline and negative control. Persistent forms were significantly reduced by melittin, which was more effective than BV and all the tested antibiotics alone or in combination [161].

BV and melittin were studied against planktonic and biofilm states of *S. aureus* methicillin-resistant MRSA [174]. Melittin was more effective compared to BV with MIC and MCB values of 6.7 and 26 µg/mL respectively. BV and melittin demonstrated a bactericidal synergism with oxacillin. BV and melittin did not affect MRSA enterotoxin production or release. They seem to cause cell distortion and disintegration associated to cytoplasm loss of content.

Melittin effect on the formation and viability of bacteria within biofilms was evaluated by several authors [151,175-177]. Melittin was effective against several strains such as *P. aeruginosa (P. aeruginosa* ATCC15442, *P. aeruginosa* ATCC27853, clinical strain and MDR *P. aeruginosa), E. coli* ATCC8739 and clinical strain, *K. pneumonia* and *A. baumannii*. Melittin was able to decrease biofilm formation in a dose and time-dependent manner [151,152,175-177]. In addition, melittin was more active compared to chloramphenicol, ampicillin and levofloxacin [151].

Beeswax and Beebread

Beeswax is a honeycomb raw material and a bee product that has an extensive usage area. It is a product used in many sectors such as coating agents in the food sector, health sector, cosmetics, soaps and creams, textiles, ornaments production, paint, and paper industry [178]. Beeswax is a functional natural substance preferred by manufacturers in recent years due to its rich nutrient content. Currently, beeswax is used in the food industry as a brightener, flavor retainer, and coating agent, in chewing gum, confectionery, chocolate, snacks, nuts, and fruit and vegetable products [179]. Regarding the studies on extending the shelf life of foods and investigating the antimicrobial resistance of beeswax, it has been seen that there are still insufficient numbers of studies using beeswax. These studies are shown in *Table 6* and *Table 7*.

Bee bread plays a critical role in human nutrition and is a valuable source of polyunsaturated fatty acids, which the human body cannot produce naturally. The ratio of chemical and nutritional components of bee bread; contains 5.91% water, 7.79% total lipid content, approximately 20% complete protein, 24-35% total carbohydrates, and various vitamins, minerals, and enzymes [180]. Bee bread is one of the bee products with high nutritional content. It is produced by anaerobic fermentation from bee pollen by means of several *Lactobacillus* spp. It possesses water, carbohydrates, free amino acids, proteins, lipids, fatty acids, vitamins, and bioactive compounds. Bee bread serves as the primary source of nutrition for both queen bees and young bees. The young bees that emerge from the pupa is fed with bee bread for the first 5 days [181,182]. Bee bread is a functional natural ingredient that has been preferred by producers recently with its rich nutritional content and bioactive properties. However, when we look at the studies on extending the shelf life of foods, it has been seen that there are very few studies using bee bread [183]. These studies were given in *Table 6* and *Table 7*.

Bee bread is created through the collection of pollen by bees, which they then process by adding honey and enzymes before storing it in honeycombs [184] *(Fig. 4)*.

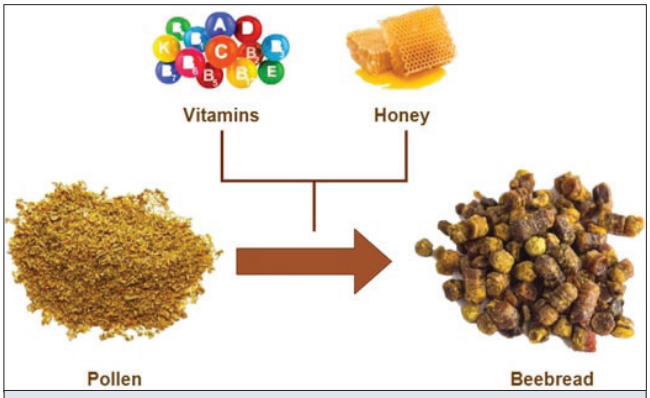

Fig 4. Bee bread production

Antimicrobial Properties of Beeswax, Bee Bread and Antibiotic Resistance

Ochoa et al.[185] evaluated the physicochemical and antimicrobial effects of microemulsified wax-based edible films. Beeswax was applied to the modified starch films as a 1% microemulsion, creating homogeneity in the edible films, and no change in thickness and opacity. The water vapor permeability, elasticity, and tensile strength exhibited a decline. In edible films treated with 1% wax, the activity of the combination of natural antimicrobials *(Rhizopus stolonifer*, *Salmonella saintpaul*, *Botrytis cinerea*, and *Colletotrichum gloeosporioides)* was inhibited. In the data obtained, it has been seen that beeswax can be used as a coating to increase the shelf life of fresh products without being felt by the consumers. In edible films, it offered a homogeneous surface, less water vapor permeability, and improved mechanical properties without a thickness or opacity that could negatively affect the preference for coated foods.

Meindrawan et al.[186] conducted an investigation to evaluate the extension of the shelf life of salak fruit *(Salacca zalacca)* using an edible film coating composed of glucomannan-wax-chitosan polymer. This edible coating, which consisted of glucomannan-wax-chitosan, led to a 10% reduction in the activity of *E. coli* and *S. aureus* compared to the control group. Additionally, it effectively curbed the rate of water vapor transfer and decreased the weight loss of salak fruit by 27% when compared to the control group. Salak fruit treated with glucomannan-wax-chitosan did not show any mold growth for 3 days at room temperature. Judging by the results in this test, the glucomannan-wax-chitosan edible coating successfully demonstrated antimicrobial activity on the lowest processed salak fruit, suggesting that similar results could probably be obtained from other fruits as well.

Disayathanoowat et al.[187] studied both bacterial and fungal viability in bee bread collected in the hive of two different honeybees, *Apis cerana*, and *Apis mellifera* in China. While a pH decrease occurred in the bee bread collected in the pack, it was noted in the data that while the bacterial population decreased, the fungal population did not change. In a concise time, there was a serious decrease in the number of bacteria in the bee bread accumulated in the hive. However, the amount of *Acinetobacter* was high. In the amount of fungus, Cladosporium genus was found in a large amount in bee bread. In addition, *Cladosporium* and various other filamentous fungi that survived in bee bread stored in the hive stimulated honeybees to conserve pollen by releasing organic acids. In this study, the microbial interactions of two honeybees in the food source were examined. It was determined that bee bread showed an antimicrobial resistance against bacteria but could not show an effect against fungi.

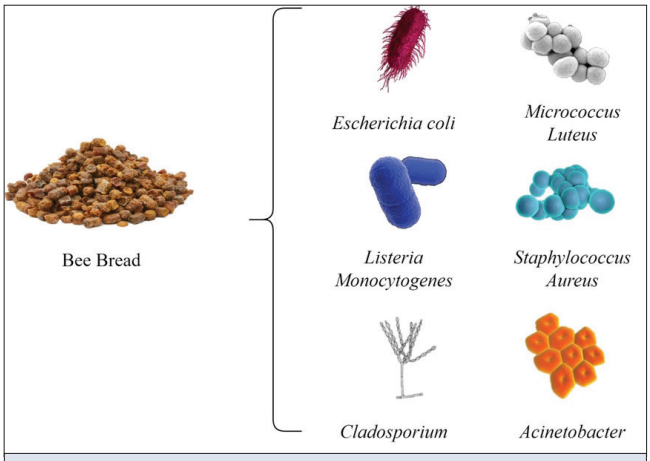

Fig 5. Antimicrobial properties of bee bread against some pathogens

In the study conducted by Kaya et al.[181], extracts of bee bread were prepared using three different solvents: methanol, distilled water, and a mixture of methanol and pure water. These extracts were then tested for their antibacterial effects against *L. monocytogenes, E. coli, M. luteus*, and *S. aureus*. The results indicated that all bee bread samples dissolved in methanol displayed effectiveness against *E. coli*. Similarly, bee bread samples dissolved in methanol and a methanol: pure water mixture were found to be effective against *L. monocytogenes*, except for two samples from the methanol-dissolved group, which did not show efficacy against *M. luteus.* It was established that all other samples, as well as those of bee bread dissolved in methanol, exhibited antibacterial activity against *S. aureus (Fig. 5)*.

In the study by Kowalski and Makarewicz [188] the functional properties of honey enriched with bee bread and propolis were investigated. The effects of enriched honey on total phenolic content, growth of microorganisms, and antioxidant activity were observed. It has been observed that honey enriched with bee products has antibacterial solid activity and exhibits strong antioxidant properties, especially against *E. coli*, but not all tested honey shows the same effect against *Micrococcus luteus*. It was stated that bee bread has the most important effect on antioxidant activity and the addition of 1% propolis has antibacterial activity. Studies have shown that fortifying honey with both bee bread and propolis is highly beneficial for its antioxidant and antimicrobial properties. Consequently, bee bread and propolis have the potential to serve as natural dietary supplements with robust antibacterial activity, particularly against *E. coli*, and high antioxidant properties.

Use of Beeswax and Bee Bread in Biofilm Control

Consumption of foodstuffs shortly after their production is generally not possible. Therefore, food should be packaged to preserve its quality and nutritional value

Table 6. *Antibacterial properties of the beeswax and bee bread*

Beeswax/Bee Bread Type	Applied Product	Targeted Microbe (Fungal/Bacterial)	Outcomes	Reference
Beeswax	Modified corn starches	*R. stolonifer, C. gloeosporioides, B. cinerea,* and *S. Saintpaul*	It has been observed that beeswax can be used as a coating	[185]
Glucomannan, chitosan, and beeswax	*Salacca zalacca*	*S. aureus, Escherichia coli*	Successfully demonstrated antimicrobial activity on beeswax-treated salak fruit	[186]
Apis mellifera and *Apis cerana* bee breads	-	*Acinetobacter, Cladosporium, Oxalis sp.* and *Coreopsis sp.*	It was determined that bee bread showed antimicrobial resistance against bacteria, but it was not effective against fungi	[187]
Bee Bread	-	*E.coli, L.monocytogenes, M.luteus,* and *S.aureus*	It was determined that bee bread showed antibacterial activity against all the factors mentioned	[181]
Bee Bread and Propolis	Honey	*E.coli* and *M.luteus*	Studies have shown that enriching bee bread and propolis with its antioxidant and antimicrobial properties is beneficial for honey	[188]

throughout its shelf life [189-191]. The most critical tasks of edible films and coatings; keeping oxygen, carbon dioxide, and lipid transfer in balance, delaying the loss of taste and aroma, and preserving antioxidants, antimicrobial substances, pigments, ions, and vitamins that inhibit browning reactions in the food, while prolonging the quality and shelf life [72].

Hromiš et al.[192] tested the antibacterial properties of chitosan and the antimicrobial effects of combining beeswax with cumin essential oil were examined, using *E. coli* and *S. aureus* as the targeted bacteria. The introduction of essential oil and beeswax led to alterations in the visual and sensory attributes of the pure chitosan film. The application of wax to the chitosan film expanded its coverage area by diminishing its susceptibility to ambient humidity, resistance to swelling at different pH levels, and water solubility. Moreover, the inclusion of wax into the chitosan film also resulted in a reduction in the water vapor transmission rate. In the group with the highest wax content, there was a noteworthy 7-fold reduction in water vapor permeability. These modified films demonstrated successful antimicrobial and antioxidant properties, which are highly advantageous characteristics for packaging applications.

Oliveira et al.[193] aimed to create biopolymer films that offer the best water vapor transmission rate with a biopolymeric coating hydrophobized with wax for postharvest storage of guavas. Biopolymeric coatings are highly efficient in maintaining the chemical and sensory qualities of fruits and vegetables, as they play a crucial part in preserving a multitude of nutrients during storage. In this context, beeswax was incorporated into the polymeric matrix as a hydrophobic agent at different proportions relative to the dry weight of the biopolymer. Among the tested biofilm variations, the one containing 10% wax yielded the most favorable outcomes in terms of the water vapor transmission rate. It was also very effective in delaying the loss of chlorophyll. An increase of 80% in elasticity values and a decrease of 15% in solubility indicated that its resistance to adverse environmental conditions increased. In the physicochemical analysis, the application of beeswax minimized weight loss and gave the fruits sufficient ripening opportunity for 15 days. Sensory analyzes performed at the end of the storage period of coated and uncoated guavas showed that fruits stored with wax-treated films achieved greater acceptability. Looking at all these data shows that the potential of the wax applied coating is quite high.

Hromiš et al.[194] beeswax in various proportions, together with chitosan, was applied to the collagen casings used in the production of sausages. FTIR spectra, Film thickness, and water vapor barrier properties were measured. As a result of the addition of beeswax at various rates to the chitosan layer, the film thickness increased to 112 µm in the sheath with 5 g of beeswax, 225 µm with 25 g of beeswax, and 83 µm in the collagen sheath in the control group. The thickness of the films resulting from the beeswax added in various proportions increased by approximately 25% to 63%. The water vapor barrier performance improved in parallel with the increasing amount of beeswax in the chitosan layer. It was measured as 130.71 g/m^2/24 h in the 5 g beeswax applied to film, 66.96 g/m^2/24 h in the 25 g beeswax applied to film, and 290.64 g/m^2/24 h in the control group. Beeswax application has shown that the laminated collagen-chitosan film has a significant potential to increase the water vapor barrier performance. With the beeswax application, the water vapor permeability rate decreased by up to 77%.

Wultańska et al.[195] study tested the antibacterial and antibiotic activity of bee bread against *Clostridioides*. Biofilm was cultured in titration plates. The MIC values of bee bread for *Clostridioides* were adjusted as 50 mg/L, 100 mg/L, and 200 mg/L respectively. Bee bread affected biofilm formation at 200 mg/L and 100 mg/L concentrations. At the same time, bee bread increased the adhesion of *Clostridioides*. Bee bread did not affect biomass formation. In the data obtained, it was observed that bee bread was active against the *Clostridioides* strains.

Table 7. *Beeswax and bee bread anti-biofilm properties*

Beeswax/bee bread type	Applied product	Targeted effect	Outcomes	References
Chitosan, caraway essential oil and beeswax	Petri dishes	Antimicrobial activity against *E. coli* and *S. aureus*	The combined use of chitosan, essential oil, and beeswax gave positive results in the use of biofilm	[192]
Brasil beeswax	'Paluma' variety guava	Creating biopolymer films that offer the best water vapor transmission rate by applying wax	A positive effect was obtained on the water vapor transmission rate by using beeswax	[193]
Chitosan, caraway essential oil and beeswax	Sausage coating	Film thickness and water vapor barrier properties were determined	Positive results were obtained. There was a decrease in water vapor transmission	[194]
Bee bread (a combination of flower pollen, honey, royal jelly, and bee enzymes)	Biofilm plates	*Clostridioides*	Bee bread was found to be active against *Clostridioides* strains	[195]

Conclusion and Future Perspectives

As a result of this review, it is seen that bee products have a significant potential for biofilm control along with their antimicrobial properties. Bee products such as propolis, royal jelly, and honey contain antimicrobial compounds that control pathogenic microorganisms. In addition, these products can prevent biofilm formation. Biofilm is a structure formed due to microorganisms attaching to surfaces and creating a film. Biofilm formation can increase antimicrobial resistance and make infections more difficult to treat. However, bee products can inhibit biofilm formation and therefore help control microorganisms.

In summary, bee products represent a significant alternative approach for addressing antimicrobial resistance and managing biofilms. These products can play a crucial role in controlling pathogenic microorganisms and mitigating resistance. Nevertheless, additional research is warranted to ascertain their efficacy and determine appropriate dosages. Notably, bee products exhibit fewer adverse effects and are environmentally sustainable due to their natural origins. Thus, the utilization of bee products in the battle against antimicrobial resistance stands to offer substantial benefits for human and animal health alike.

Highlight Keypoints

· Bee products can offer natural alternatives to control drug-resistant infections.

· Compounds obtained from bee products have the potential to inhibit biofilm formation in bacterial infections.

· Natural bee products can serve as a resource to support innovative treatments to combat antimicrobial resistance.

· More research is needed to explore the full health potential of bee products.

· One Health approach, involving collaboration between human and veterinary medicine, can optimize the use of bee products in combating biofilm-related challenges.

Declarations

Competing Interests: There is no conflict of interest.

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