A review of selected bee products as potential anti-bacterial, anti-fungal, and anti-viral agents

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Abstract:

Antimicrobial resistance (AMR) is one of the greatest medical challenges the world faces. It was recently estimated that by 2050, AMR will account for 10 million extra deaths annually with additional economic costs in the region of $100 trillion. In order to combat this, novel antimicrobial agents with a broad spectrum of activity are required. Bee products, such as; honey, propolis, defensins, royal jelly, bee pollen and venom have been used to treat infectious diseases for several centuries, although they were largely disregarded by Western medicine during the antibiotic era. There has since been a resurgence in interest in their antimicrobial properties, especially due to their reported activity against multi-drug resistant pathogens displaying high levels of AMR. This paper reviews the literature surrounding the use of honey, propolis, honey bee, defensins, royal jelly, bee pollen and bee venom as antimicrobial agents. The antimicrobial properties of honey are well documented and the identification of individual components that demonstrate activity in different honeys has begun alongside the elucidation of the mode of action for some types of honey. Within propolis research the focus has centered on the antimicrobial activity of extracts of propolis, where both antibacterial and antifungal activity has been recorded. Bee defensins have been isolated from some honeys, and have demonstrated a good activity against a wide range of pathogens. As some antimicrobial peptides are already used clinically these bee defensins could be considered for future clinical use. The research into the potential for bee pollen and bee venom to be used as antimicrobial agents is at an earlier stage and although there are some studies showing promising activity, more evidence of their activity and safety would need to be established before they could be taken forward as a clinical antimicrobial option. This review has highlighted the antimicrobial activity of each of these products and their potential future research directions.

Keywords:
Honey, propolis, bee venom, defensins, antimicrobial, antimicrobial resistance, bee products, royal jelly

1. Introduction

Natural compounds of both plant and animal origin have traditionally been used in a medicinal context due to their broad-spectrum of therapeutic activity, including; anti-bacterial, fungal, and viral activity, as well as anti-inflammatory and immunomodulatory effects [1-8]. In recent years the interest in natural products as a potential source of novel antimicrobial agents has grown, due to a concomitant decline in the number of effective antibiotics that are available and the ever increasing emergence of antibiotic resistance within pathogenic bacteria [9-13]. This effect has been compounded by a decline in the manufacture of new antimicrobial agents by traditional pharmaceutical companies [14-16], and both the over and misuse of the available antimicrobial agents [17-19]. Together these factors have led to a situation whereby bacteria have evolved various resistance mechanisms to conventional antibiotics and in some cases become multi drug resistant (MDR) or pan resistant [20-25].

The rise of antimicrobial resistance (AMR) as outlined above is a significant global problem, currently accounting for approximately 700,000 deaths annually, and predicted to lead to 10 million deaths annually by 2050 if no action is taken to find alternative ways of combating MDR pathogens [16,26]. In addition to the increased morbidity and mortality of patients caused by AMR there is also a large financial burden causing an estimated cost of between $70,000 and $100,000 per person [27]. It is likely that the cost of AMR is higher than the estimated figures, as there will also be an impact on routine operations, such as joint replacements, which require prophylaxis in order to stop secondary infections [27-29]. These factors combined illustrate the need for novel antimicrobial agents, which can be used to bolster the lineup of currently available therapeutics as part of a multidisciplinary strategy for reducing patient morbidity and mortality rates.

Within the wide range of natural products that are currently being investigated for their novel antimicrobial activity, there has been a renewed interest in elucidating the antimicrobial activity of apitherapeutics (bee products). There is a growing body of evidence that suggests bee products such as honey, propolis, bee venom (BV) and honey bee defensins could have a role to play in mitigating the effect of AMR, by providing an alternative source of antimicrobial activity, which could be used to tackle infection alone or enhance the activity of current antimicrobial agents [30-37]. This review will consider a range of bee products and evaluate the evidence available for their potential use as antimicrobial agents.

2.1 Antimicrobial properties of Honey.

For over two millennia, the medicinal properties of honey have been known to many historic civilisations (such as the ancient Greeks, Romans, Egyptians, and Chinese), however much of this knowledge was based on anecdotal evidence rather than designed scientific experimentation [38]. It wasn't until late in the 19th century when the first scientific publication showing the antimicrobial efficacy of honey was published by Van Ketal [39]. Since this publication, bar a momentary pause at the beginning of the antibiotic-era, interest in honey as an antimicrobial agent has increased. As stated previously, this resurgence is in part due to the emergence of MDR pathogens [40], but also due to the natural qualities of honey and the breadth and depth of components from which it is composed.

Honey is a complex solution with three distinct "fractions"; a sugar fraction, water fraction, and a highly variable fraction that contains a range of amino acids, antioxidants, enzymes, flavonoids, phenolic acids, minerals, and vitamins. Both the sugar and water fractions are highly conserved between different honey types [41], conferring a basic level of antimicrobial activity through a high osmotic potential and its acidic attenuation [42]. In many studies looking into the antimicrobial effects of honey, an "artificial honey" formulated from these two fractions can be used as a control. In most instances, the artificial honey is found to possess significantly reduced antimicrobial activity than that of honey containing the variable fraction [43].

The largest variation in honey composition, which alters the smell, taste, aroma, and ultimately antimicrobial activity, occurs (unsurprisingly) within the variable fraction. This fraction is dependent on both plant and bee-derived products, which in turn are subject to

different environmental, geographic, temporal, and phyletic variables [44,45]. It is because of the highly variable nature of summative components, that there are 100s of different honey types, each having varying degrees of antibacterial efficacy, with some variability between batches of the same honey. A range of studies (summarised in [46]) have shown >50 bacterial species to be inhibited by these different honey types, with some studies highlighting the anti-viral and anti-fungal properties of honey [47-51]. Determining which of the compounds within the variable fraction contributes to the bulk antimicrobial activity of each honey is very difficult, due to the potential for complex interactions between any of the 200 compounds that may be present within the honey [52].

Some of the most promising compounds which are currently being researched are bee-defensin 1, hydrogen peroxide, leptosperin, and methylglyoxal [53,54]. The former two can be found in many different honeys and not associated with a specific type, whilst the latter two are commonly associated specifically with manuka honey, a honey typically from New Zealand and Southern Australia, which has received increased research interest due to its heightened antimicrobial activity. Many studies have shown manuka honey to be capable of inhibiting >50 different bacterial species [55]. Due to the exceptional activity of manuka honey, its potential mechanism(s) of action against two problematic pathogens (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) have been identified. Two distinct mechanisms of antimicrobial action have been revealed [33,56], however the components within the honey that elicit this mechanistic effect have yet to be elucidated. A broader effect against *Escherichia coli* was investigated by Blair and colleagues [57], identifying various regulatory changes in the presence of honey; however the components within honey and their corresponding effects are yet to be fully identified. For an in depth review on some of the mechanistic effects of honey, see [58].

Whilst many honeys have exceptional antimicrobial activity in their own right, some researchers have found the effects to be antibiotic enhancing. Two recent studies have shown the sensitisation of methicillin resistant *S. aureus* (MRSA) to antibiotics (such as oxacillin, tetracycline, and mupirocin), following combined therapies with honey [34,37]. These effects are not limited to a single species, with other studies observing a multitude of antimicrobial enhancing effects against other pathogens, such as *P. aeruginosa*, *K. pneumonia*, and *E. coli* [59,60]. The ability of honey to not only work concurrently with antibiotics, but to enhance their effects, is of great clinical significance as it has the potential to alleviate some of the problems associated with AMR and chronic infection with MDR pathogens.

To date, much of the work determining the antimicrobial efficacy of honey relates specifically to its topical application; however some studies have diverted from this trend. A recent study by Jenkins and colleagues [59] have identified manuka honey as a potential therapeutic for the inhibition of pathogens associated with cystic fibrosis lung infections. Further to this, Daglia and colleagues [61] have shown the ability of some key antimicrobial components within manuka honey to resist simulated gastroduodenal digestion. In light of the ever growing body of evidence for honey as an antimicrobial agent, its efficacy against these pathogens is not disputed, however if honey is to be used for other applications (such as lung and gastrointestinal infections as suggested by the examples above), effective formulation and application strategies need to be identified, so as to ensure the safe application and an obtainable inhibitory concentration at the site of infection.

2.2 Antimicrobial properties of Propolis

Propolis is a resinous substance used by bees for structural repairs [62] that has been widely used within traditional medicines, with recent studies showing its potential for use in mainstream medicine [63]. Like honey its composition is highly variable due to bees foraging from tree resins which are present in their local area [63]. This make propolis a good health indicator for the local ecosystem [64], however this variability makes the use of propolis in medicine problematic. For medicinal use, a constant and quantifiable level of biological activity is required, however to the best of our knowledge there is no standardized medical grade propolis. This is in contrast to honey products which are available in many countries at a medicinal grade. In light of this, researchers have instead focused on bioactive compounds that have been extracted from

propolis via a variety of chemical extraction techniques. Many groups report that the antimicrobial activity of propolis varies depending on when and where the samples were collected, with a positive correlation between the flavonoid content of samples and their antibacterial activity [65-67]. Conversely, a study by Sforcin and colleagues suggests that variability of the components which make up propolis, and their respective concentrations, have no correlation with the overall antimicrobial activity [68]. Therefore the overall composition of propolis should not be used as an indicator of its antimicrobial potential.

Recently there has been much interest in the antibacterial properties of South American propolis. Samples of a Brazilian propolis were compared with a Bulgarian propolis with regards to their antimicrobial activity and potential synergy with antibiotics. Both propolis samples demonstrated inhibitory efficacy against *Salmonella* Typhi and *S. aureus* at concentrations of <10% and 0.5% v/v respectively, with synergistic effects when combined with commonly used antibiotics [69]. The mechanism of action for the two propolis samples differed however, with only the Brazilian propolis showing bactericidal activity [69]. Brazilian propolis has now been classified according to it physicochemical characteristics [70]. Green Brazilian propolis has been shown to have some antimicrobial activity against various oral pathogens, such as; *Streptococcus mutans, Streptococcus sanguinis* and *Porphyromonas gingivalis*. The same study established that there was no cytotoxicity to mammalian cells at concentrations required to inhibit bacterial cells (2000 µg/ml) [71]. An investigation of red Brazilian propolis, which is produced by bees foraging a red resin produced by the *Dalbergia ecastophyllum* tree, also identified antimicrobial activity against *S. aureus*, although this activity was variable and dependent on the season of collection [72]. In addition to the well-studied antimicrobial activity, Brazilian propolis has confirmed antifungal activity, with a minimum inhibitory concentration of <5% v/v against *C. albicans* and *C. tropicalis* [73].

Other Southern American propolis samples, such as those from Chile, show promise as an antibacterial compound, particularly against Gram positive *Streptococcus sp*. An *in vitro* test of 20 Chilean propolis samples against *S. mutans* and *S. sobrinus* showed a good level of activity against the pathogens [65]. Interestingly, variability in antimicrobial activity was observed between Chilean propolis samples, with a clear north/south geographic divide, the latter having increased antimicrobial activity over the former [65]. Polyphenol rich extracts of Chilean propolis have also been shown to have activity against *S. mutans*, down-regulating expression of the surface proteins GtfB, GtfC, GtfD and SpaP, thereby inhibiting the bacterium's ability to attach to surfaces and form biofilms [74]. The phenolic composition of the propolis has been shown to be important in this activity, with propolis samples containing higher polyphenol concentrations also providing a higher level of inhibitory and bactericidal activity against *S. mutans* [67].

As with other bee products, some investigators have chosen to chemically separate propolis and extract components of interest. Although ethanolic extracts of Turkish propolis showed promising levels of antifungal activity against various fungal pathogens such as *C. albicans, C. glabrata, Trichosporon* sp. and *Rhodotorula* sp., once again the antifungal activity of the propolis samples varied depending on their source [75]. Extracts collected from the Eastern Anatolia region of Turkey showed antimicrobial activity against *E. coli, P. aeruginosa* and *S. aureus* and antifungal activity against *C. albicans* [76]. *In vivo* studies have also demonstrated that ethanolic extracts of propolis are able to successfully treat *S. aureu*s keratitis in rabbits, and enhance the activity of ciprofloxacin to treat this infection [77].

2.3 Antimicrobial properties of Bee Defensin

Bees, along with other insects do not have a lymphocyte based immune system [78], relying instead on a range of antimicrobial peptides (AMPs) and barrier immunity to protect them from infection [79,80]. These small, cystine rich cationic peptides are expressed in several tissue types in response to various pathogenic challenges [79]. In honey bee colonies these challenges include bacteria such as; *Paenibacillus larvae larvae* [81], the fungal pathogen *Nosema ceranae* and parasites such as

Crithidia mellificae [82]. Some researchers have found the expression of AMPs to vary between different colonies with increased expression directly correlating to a reduction in microbial disease within the colony. High levels of AMP expression have also been shown to have a fitness cost, leading to a reduction in larvae production [83]. It should also be noted that although much of the research into AMPs has been carried out in Western honey bee populations, it has been show that Asian honey bee populations also carry very similar AMPs, with similar levels of antimicrobial activity [84].

Bee AMPs also show activity against human and animal pathogens and this has been explored in detail, using both recombinant forms of various AMPs [85,86] and purified extracts from bees themselves [78]. AMPs have increased activity against Gram positive bacteria [81] with both bactericidal or bacteriostatic effects observed and efforts to utilise them as an antibiotic have begun [87,88]. To date six different AMPs have been identified in honeybees; hymenptaecin [78], defensin 1 and the closely related royalisin [89,90], defensin 2 [91], abaecin [92] and apidaecin [93]. All of the AMPs discovered to date have demonstrable *in vitro* antimicrobial activity against a wide range of pathogens, but it is the defensins which are most widely found AMPs in bee products.

Defensins are short chain polypeptides containing an alpha helix and two parallel β sheets which are cross linked [89]. They can be found in non-manuka honeys and royal jelly. Defensin 1 and Royalisn, a defensin found exclusively within royal jelly, are very closely related and expressed by the same gene within the bee, however they undergo different post-transcriptional and translational modifications [91]. Defensin 2 is closely related, both genetically and structurally to defensin 1, however it is expressed by a different gene [91]. Some of the antimicrobial activity of Revamil® honey, a honey which is produced by bees foraging on limited plant sources in order to control its content, is attributable to the presence of Defenisn 1 within the honey [54]. Neutralisation of defensin 1 leads to a reduction in the antimicrobial activity of the honey. It should be noted however, that the inactivation of defensing-1 does not completely negate the antimicrobial activity of the honey, highlighting the multifactorial nature of honey, as described above [54]. In contrast to these findings in Revamil® honey, it has been shown that Defenisn 1 does not contribute to the antimicrobial activity of manuka honey [94]. Recent work has shown that this is not due to differences in defensin expression levels in the colonies foraging on the different plants, but rather the high levels of MGO found within manuka honey [95]. MGO has an ability to react with lysine and arginine residues within proteins, including defensin, leading to their glycosylation and subsequent inactivation [96]. MGO levels in manuka honey have been shown to increase as the honey matures [97] and it is this increase that leads to the inactivation of the defensin proteins which are secreted by the bees into the original honey.

2.4 Antimicrobial properties of Royal Jelly

Royal jelly is a secretion produced in the hypopharingeal and mandibular glands of honey bees [98]. As with many bee products, royal jelly is very variable in its composition, with its bioactive potential affected by both seasonality and geographical diversity [99,100]. It is produced by worker bees as a source of nutrition for larvae less than three days old and queen bees throughout their lives, it contains complex combinations of pheromones which control the honey bee colony hierarchy [101]. Royal jelly is a complex mixture of proteins, carbohydrates, fatty acids, sugars, lipids and vitamins [102]. Like honey, it contains several known antimicrobial compounds and several studies have shown that royal jelly, and its extracts have antimicrobial activity against a wide range of bacterial and fungal sp. [103]. Assessment of the antimicrobial potential of Bulgarian royal jellies showed that some samples were active at concentrations of 5% v/v against the enteropathogen *Aeromonas hydrophilia* [104] and MRSA [105]. Similarly Algerian royal jelly was shown to have inhibitory efficacy against *P. aeruginosa*, and that this activity could be further enhanced by combining royal jelly with honey [106]. It is interesting to note that although the AMP royalisin is only reported to have activity against Gram positive bacteria, non-extracted samples of royal jelly have reported activity against Gram negative bacteria [107]. Similarly, Bíliková and colleagues [103] reported that royal jelly showed a strong antifungal activity against *Botrytis cinerea*,

however extracted royalisin was only active against the fungi at concentrations of over 27 µg/ml. Taken together these results suggest that, as with many honeys, it is the interaction of various antimicrobial compounds within royal jelly which gives it such potent antimicrobial activity.

It should be noted that many investigators do not work directly with the royal jelly, preferring instead to chemically isolate fractions which contain several substances, some of which may have antimicrobial activity, or extract individual active components from the royal jelly. The extraction process has not only a high level of waste, with one group reporting the production of 180 mg from an initial 30 g sample of royal jelly [103], but the exact extraction method chosen dictates which compounds will be obtained. Recently there has been an effort to standardise the extraction process and classify royal jelly [102,108] and work has been carried out to show the effect, if any, that processing may have on the activity of royal jelly. A recent study has shown that lyophilisation, which allows storage and further processing of the royal jelly, did not alter its antimicrobial activity against *S. aureus, S. epidermidis, S. pneumoniae, E.coli, K. pneumoniae, Proteus mirabilis, S. enteritidis* or *P. aeruginosa* [107].

The majority of the dry mass of royal jelly is protein, more than 80% of which are identified as belonging to the major royal jelly proteins (MRJP) family, a member of which is MRJP1 [109]. The precursor of MRJP1 is also responsible for the production of the antimicrobial jellein peptides [110]. Fontana and colleagues [111] reported the discovery of 4 jellein peptides following separation of royal jelly. Further investigation showed that of the four jellein peptides identified, three showed antimicrobial activity against a panel of Gram positive and negative bacterial isolates and a yeast, although antimicrobial activity was reduced in one of the jelleins [111]. Further investigation of the three active jelleins confirmed the activity of jellein 1 against *S. aureus, Listeria monocytogenes, Salmonella* Enterica and *E. coli*, but found no activity against the bacterial isolates tested for the other two jelleins [112].

A study of the remaining protein components of royal jelly identified 20 other proteins, including one termed; royalisin [113]. Royalisin is a 55 kDa disulphide rich protein made of 51 amino acids. As with other defensin proteins it contains an amphipathic α helix, a carboxyl terminal tail which is aminated and antiparallel β strands, which are cross-linked by six cystine residues forming disulphide linkages [114]. Although there are small differences between the structure of royalisn in Western and Asian honey bee populations, both have been shown to have antimicrobial activity [86]. Recently is has been possible to express a recombinant form of royalisin within *E. coli*, in both its original and a modified form, which contains a truncated C terminal and no disulphide linkages of the β strands. Although the modified royalisin maintained some of its antimicrobial activity it was much less active that the intact form [114]. When the antimicrobial activity of Asian honey bee royalisin expressed within *E. coli* was assessed against a panel of Gram positive and negative bacterial isolates as well as fungal pathogens, it was only active against certain Gram positives species tested, including *S. aureus, Bacillus subtilis and Micrococcus luteus* [85,86]. Further investigation of the expressed proteins indicated that they acted on the cell walls of *B. subtilis* increasing cell surface hydrophobicity [86]. It is interesting to note that although a similar mechanism of antimicrobial action was reported by [114], these authors found that their recombinant royalisin protein was active against both Gram positive and negative bacteria. This finding is unusual since other authors working with defensin proteins, and in particular royalisin, typically only report activity against Gram positive species [85,86,90]. It is possible that the mechanism of expression and modification within the *E. coli* could account for these differences, indeed reductions in antimicrobial activity have been reported where royalisin [114] or jellein [112] peptides were modified. Further structural analysis and comparison of the proteins expressed by different groups is required to confirm this hypothesis.

Royal jelly also contains fatty acids, the most common of which is 10-hydroxy-2-decenoic acid (10-HDA) [115]. As with other royal jelly components, 10-HDA has been shown to have a range of bio-activities, including; antitumor activity [116], neurogenesis [117], anti-rheumatoid arthritis activity [118] and modulation of diabetes [119]. 10-HDA also exhibits potent

antimicrobial activity against the Gram positive dental pathogen *S. mutans*. Furthermore, it was found that 10-HDA was able to modulate biofilm formation within *S. mutans* by reducing expression of two glucosyltransferases (gtfB and gtfC), which in turn led to a decrease in its attachment to embryonal carcinoma cells [120].

2.5 Antimicrobial properties of Bee Pollen

Bee pollen is composed of plant pollen combined with nectar or the salivary secretions of bees. Therefore it is similar to other bee products in that it is composed of a wide range of secondary plant metabolites such as: thiamine, tocopherol, biotin, niacin, folic acid, polyphenols, carotenoid pigments, phytosterols and enzymes [121,122] and has been used as a component of human medicine for thousands of years [123]. Research groups have outlined several potential bioactive roles for bee pollen and its components, including; antioxidant [124], immunomodulatory [125] cardioprotective [126], and antimicrobial activities [127,128].

There are several studies which report variability in the antimicrobial activity of bee pollen, attributing this to the geographical and botanical source of the pollen, which in turn will influence the phytochemical composition [121,129-133]. As a result of this variation, there are thought to be over 250 biologically active compounds within pollen [134-136]. The majority of work into the antibacterial potential of pollen has been carried out on chemically extracted of pollen (using either ethanol or methanol) and then tested *in vitro.* As with propolis extracts, the method by which the pollen extracts are made may well impact on the content and thus the activity of the extract that is then tested.

The antibacterial activity reported from bee pollen extracts is thought to be linked to the presence of polyphenols (3-5%) and phenolic acids (0.19%) within the pollen, depending on origin [134,136-138]. Several studies have shown that the antibacterial activity of pollen is linked to the level of phenolic compounds, and in some studies have identified individual components responsible for this activity such as; kaempferol 2-O-rhamnoside, quercetin 3-O-glucoside, isorhamnetin 3-O-xylosyl (1-6) glucoside and 7-O-methylherbacetin3-O-xylosyl-8-O-galactoside [134,135,139]. Research has shown that the activity of polyphenols within pollen is likely to disrupt bacterial metabolism and therefore viability by several mechanisms, including; forming complexes within bacterial cell walls, inhibiting electron flow within the electron transport chain, inhibiting DNA gyrase and blocking ion channels [140,141]. More specifically, high quercetin and kampferol levels seen in some bee pollen extracts are suggested as particular flavonoids that could be responsible for the activity described above [139].

The antimicrobial activity of bee pollen *in vitro* has been established against a wide range of both antibiotic sensitive and antibiotic resistant bacteria and fungi[142-150]. Studies have demonstrated activity of methanol and ethanol extractions of pollen against pathogens such as *S. aureus, Bacillus cereus, P. aeruginosa, E. coli, C. albicans* and *Aspergillus fumigatus* among others[142-150]. The range of activity seen suggests a potential for a role for bee pollen as an antimicrobial agent against microbes of medical relevance . However in contrast to the activity seen in the studies described above, two studies by Ozcan [138,151] which assessed the antimicrobial activity of pollen extracts at 0.002, 2.5, 2 and 5 % against tested against a range of bacteria and fungi including; *E. coli, S. aureus, S.* Typhimurium, *Candida arugosa*, *Alternaria alternate*, *Fusarium oxysporium*, and reported that the microbial viability was not affected. This lack of antimicrobial activity is in direct contrast to the results presented above and highlights the inherent variability of this natural product which may be problematic when used medicinally. The differences could be attributed to the low concentration of pollen extract used in Ozcan's studies, or as with both honey and propolis the variations seen could be due to the differences in geographical and floral sources of the pollen tested. As many of the studies cited above use either methanol or ethanol extraction methods before testing the pollen, it could be suggested that using individual components extracted and identified as having antimicrobial activity might give more reproducible results. As the current information stands to warrant using pollen as a clinical antimicrobial agent there would need to be more extensive *in vitro* studies prior to randomized clinical trials.

Currently there are commercially available pollens, with a study by Pascoal [149] confirming the antimicrobial activity of these pollens against a range of microbes *in vitro*. It is important to note that any pollen or pollen extract that was to be utilised primarily for clinical antimicrobial use, rather than as a food product would need to undergo sterilization, as a study by Nogueira [152] showed, commercially available pollens can contain aerobic mesophiles, molds and yeasts.

2.6 Antimicrobial effects of Bee Venom.

Apitoxin, or Bee venom (BV) as it is more commonly known, is another apitherapy product that has received increased interest throughout the last century. Much of the research into the medicinal effects of BV has focused on the treatment and relief of various chronic diseases, unearthing many anti-inflammatory, anti-mutagenic, anti-nociceptive, and anti-cancer effects (For a review see [153,154]). Studies examining the potential of BV in the treatment of infectious diseases are quite limited, however in light of the impending antimicrobial resistance (AMR) crisis [40] the antimicrobial effects are beginning to be elucidated.

BV is a colourless liquid composed of various amino acids, peptides, pheromones, phospholipids, proteins, sugars and minerals [155]. It is evident from numerous studies that the biochemical profile of BV can vary in a similar manner to that of honey, affected by bee species, season, and geographical region [156-159]. The overall activity of BV is better suited to the inhibition of Gram positive bacteria as opposed to Gram negative species, however some activity is still retained against Gram negative bacteria [160]. An interesting observation made by Han and colleagues [161] identified the antimicrobial activity of BV to be pH-independent with comparable inhibitory efficacy at a range of different pH levels (2-11).

The primary bee venom component (BVC), in terms of both dry weight (~50% w/w) and biological activity, is the antimicrobial peptide; melittin [158]. This 26-amino acid residue has exceptional non-selective lytic activity, capable of inhibiting both eukaryotic and prokaryotic cells [162]. For a prospective antimicrobial agent this might appear to be counter intuitive, however the dose required for bacteriolytic activity is much lower than that required to elicit a cytolytic effect for eukaryotic cells [155,163].

The secondary BVC, in terms of both dry weight (~10% w/w) and more importantly biological activity, is phospholipase A2. This hydrolase is capable of cleaving phospholipids and altering their membrane association. There are significantly fewer studies on phospholipase A2 derived from BV than its more common counterpart, however antimicrobial effects have been demonstrated against both Gram positive and negative bacteria [164]. Despite this, a recent study by Leandro and colleagues [165] showed the inhibitory effects of phospholipase A2 to be less than melittin. In addition to this, the combination of the two BVCs did not appear to interact effectively, concluding that the majority of activity was due to the presence of melittin. This is in direct contrast to initial observations by Mollay and colleagues [166,167] whereby melittin was found to enhance the efficacy of phospholipase A2 Interestingly, a recent *in vivo* study showed melittin to be more effective than BV at reducing bacterial load in a surface wound, whilst concurrently enhancing would healing [168].

In recent years, some researchers have taken to assessing the antibiotic-enhancing effects of BV (or singular components), specifically against multi-drug resistant bacteria for which the number of effective treatment regimes in operation may be diminishing. Han and colleagues [161] have shown antibiotic enhancing effects of BV (as a whole) against MRSA. Conversely Dosler and colleagues [169] have identified antibiotic enhancing effects of melittin (alone) against *E. coli* and *K. pneumonia*, with only inhibitory effects observed against *P. aeruginosa*, most likely due to the innate resistance mechanisms of this organism. A more all-encompassing study by Al-Ani and colleagues [170] showed that BV and its main component; melittin, inhibited over 50 different strains of both Gram positive and negative organisms, including strains with increased AMR.

The additive and synergistic effects observed between BV/BVCs is interesting from a therapeutic perspective. Due to the high cytotoxic effects associated with elevated doses of BV/BVCs, reduced doses would be preferable. By combining the two, we may be able to reinvigorate ailing antibiotics whilst also reducing potential

side effects, both of which would be welcomed in clinical practice. Importantly, whilst there is potential for BV and BVCs as antimicrobial agents, it is essential that prospective patients are tested for potential allergies to apitherapy-products prior to treatment, so as to avoid potential life-threatening side effects

3. Conclusion

The research presented within this review demonstrates that bee products including; honey, propolis, honey bee peptides, royal jelly, bee pollen and bee venom show great promise as antimicrobial agents against a wide range of microbial pathogens. All the bee products reviewed have a broad spectrum of reported activity against both Gram positive and negative bacterial species and several products also show promising activity against a range of fungal species of medical relevance.

One of the problems highlighted in this review is that many of the studies report varying levels of antimicrobial activity due to the inherent variability, and poorly defined chemical nature of these products.

Many natural products show similar variances in composition and activity, however if products are to be considered for use in modern medical applications they must have a consistent and specific level of activity. This has already been achieved with products like medical grade manuka honey and work is now beginning to classify propolis and its activity. It would be beneficial if other apitherapeutics were to also undergo this process. Once antimicrobial activity, and the extraction methods used to release the antimicrobial fractions are standardized and classified it will be possible to make direct comparisons of products and their relative activity. Similarly, standardized and sterile products are favoured for use in *in vivo* and in-patient studies, which is the natural next step for many of the products reviewed here.

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