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

Helen Louise Brown*, Aled Edward Lloyd Roberts*, Rose Cooper, Rowena Eleri Jenkins

*Both authors contributed equally

Department of Biomedical Sciences, Cardiff Metropolitan University, Cardiff, Wales, UK

hbrown@cardiffmet.ac.uk, aeroberts@cardiffmet.ac.uk,rcooper@cardiffmet.ac.uk, rojenkins@cardiffmet.ac.uk

Antimicrobial resistance (AMR) is one of the greatest medical challenges the world faces. It was estimated recently 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, including; 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. In this paper we review the current scientific literature of honey, propolis, honey bee, defensins, royal jelly, bee pollen and bee venom. We highlight the antimicrobial activity each of these products has displayed and potential future research directions.

Keywords:

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

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.

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.

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 Chillean 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].

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

199 that Asian honey bee populations also carry very similar AMPs, with similar levels of antimicrobial
200 activity [84].
201
202 Bee AMPs also show activity against human and animal pathogens and this has been
203 explored in detail, using both recombinant forms of various AMPs [85,86] and purified extracts from
204 bees themselves [78]. AMPs have increased activity against Gram positive bacteria [81] with both
205 bactericidal or bacteriostatic effects observed and efforts to utilise them as an antibiotic have begun
206 [87,88]. To date six different AMPs have been identified in honeybees; hymenptaecin [78], defensin 1
207 and the closely related royalisin [89,90], defensin 2 [91], abaecin [92] and apidaecin [93]. All of the AMPs
208 discovered to date have demonstrable *in vitro* antimicrobial activity against a wide range of
209 pathogens, but it is the defensins which are most widely found AMPs in bee products.
210
211 Defensins are short chain polypeptides containing an alpha helix and two parallel β sheets
212 which are cross linked [89]. They can be found in non-maukua honeys and royal jelly. Defensin 1 and
213 Royalisn, a defensin found exclusively within royal jelly, are very closely related and expressed by
214 the same gene within the bee, however they undergo different post-transcriptional and translational
215 modifications [91]. Defensin 2 is closely related, both genetically and structurally to defensin 1,
216 however it is expressed by a different gene [91]. Some of the antimicrobial activity of Revamil®
217 honey, a honey which is produced by bees foraging on limited plant sources in order to control its
218 content, is attributable to the presence of Defenisn 1 within the honey [54]. Neutralisation of defensin
219 1 leads to a reduction in the antimicrobial activity of the honey. It should be noted however, that the
220 inactivation of defensing-1 does not completely negate the antimicrobial activity of the honey,
221 highlighting the multifactorial nature of honey, as described above [54]. In contrast to these findings
222 in Revamil® honey, it has been shown that Defenisn 1 does not contribute to the antimicrobial
223 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.

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 its 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].

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 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.

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,

373 however in light of the impending antimicrobial resistance (AMR) crisis [40] the antimicrobial effects
374 are beginning to be elucidated.

375

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

384

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

391

392 The secondary BVC, in terms of both dry weight (~10% w/w) and more importantly biological
393 activity, is phospholipase A2. This hydrolase is capable of cleaving phospholipids and altering their
394 membrane association. There are significantly fewer studies on phospholipase A2 derived from BV
395 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 collegues [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 collegues [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

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.

References:

1 Atiba, A. *et al.* Aloe vera oral administration accelerates acute radiation-delayed wound healing by stimulating transforming growth factor-beta and fibroblast growth factor

production. *The American Journal of Surgery* **201**, 809-818, doi:10.1016/j.amjsurg.2010.06.017 (2011).
2 Abu-Al-Basal, M. A. Healing potential of *Rosmarinus officinalis* L. on full-thickness excision cutaneous wounds in alloxan-induced-diabetic BALB/c mice. *Journal of Ethnopharmacology* **131**, 443-450 (2010).
3 Zhao, L., La, V. D. & Grenier, D. Antibacterial, antiadherence, antiprotease, and anti-inflammatory activities of various tea extracts: potential benefits for periodontal diseases. *Journal of medicinal food* **16**, 428-436 (2013).
4 Harris, J. C., Cottrell, S. L., Plummer, S. & Lloyd, D. Antimicrobial properties of *Allium sativum* (garlic). *Applied Microbiology and Biotechnology* **57**, 282-286 (2001).
5 Saurav, K. *et al.* In search of alternative antibiotic drugs: Quorum-quenching activity in sponges and their bacterial isolates. *Frontiers in Microbiology* **7**, 416, doi:10.3389/fmicb.2016.00416 (2016).
6 Cazander, G. *et al.* Maggot excretions affect the human complement system. *Wound Repair and Regeneration* **20**, 879-886, doi:10.1111/j.1524-475X.2012.00850.x (2012).
7 Mansourian, A., Boojarpour, N., Ashnagar, S., Momen Beitollahi, J. & Shamshiri, A. R. The comparative study of antifungal activity of *Syzygium aromaticum*, *Punica granatum* and nystatin on *Candida albicans*; an *in vitro* study. *Journal of Medical Mycology* **24**, e163-168, doi:10.1016/j.mycmed.2014.07.001 (2014).
8 Hashemipour, M. A., Tavakolineghad, Z., Arabzadeh, S. A., Iranmanesh, Z. & Nassab, S. A. Antiviral activities of honey, royal jelly, and Acyclovir against HSV-1. *Wounds : a compendium of clinical research and practice* **26**, 47-54 (2014).
9 McGrath, L. J., Becker-Dreps, S., Pate, V. & Brookhart, M. A. Trends in antibiotic treatment of acute otitis media and treatment failure in children, 2000-2011. *PLoS One* **8**, e81210, doi:10.1371/journal.pone.0081210
PONE-D-13-29268 [pii] (2013).
10 Martin, D. R., Heffernan, H. M. & Davies, H. G. Methicillin-resistant *Staphylococcus aureus*: an increasing threat in New Zealand hospitals. *New Zealand Medical Journal* **102**, 367-369 (1989).
11 Kam, K. M. *et al.* Emergence of multiple-antibiotic-resistant *Streptococcus pneumoniae* in Hong Kong. *Antimicrobial Agents and Chemotherapy* **39**, 2667-2670 (1995).
12 Filius, P. M. & Gyssens, I. C. Impact of increasing antimicrobial resistance on wound management. *American Journal of Clinical Dermatology* **3**, 1-7, doi:030101 [pii] (2002).
13 Hwang, A. Y. & Gums, J. G. The emergence and evolution of antimicrobial resistance: Impact on a global scale. *Bioorganic & Medicinal Chemistry* doi:S0968-0896(16)30261-9 [pii]
10.1016/j.bmc.2016.04.027 (2016).
14 Fischbach, M. A. & Walsh, C. T. Antibiotics for Emerging Pathogens. *Science* **325**, 1089-1093, doi:10.1126/science.1176667 (2009).
15 Bragginton, E. C. & Piddock, L. J. UK and European Union public and charitable funding from 2008 to 2013 for bacteriology and antibiotic research in the UK: an observational study. *Lancet Infect Dis* **14**, 857-868, doi:S1473-3099(14)70825-4 [pii]
10.1016/S1473-3099(14)70825-4 (2014).
16 O'Neill, A. J. Tackling a global health crisis:initial steps. 1-21 (London, 2015).
17 Huttner, A. *et al.* Antimicrobial resistance: a global view from the 2013 World Healthcare-Associated Infections Forum. *Antimicrobial Resistance and Infection Control* **2**, 31, doi:10.1186/2047-2994-2-31
2047-2994-2-31 [pii] (2013).

18 Marc, C. *et al.* Inappropriate prescription of antibiotics in pediatric practice: Analysis of the prescriptions in primary care. *Journal of Child Health Care*, doi:1367493516643421 [pii]
10.1177/1367493516643421 (2016).
19 Lima, S. I. *et al.* Rationality of antimicrobial prescriptions in community pharmacy users. *PLoS One* **10**, e0141615, doi:10.1371/journal.pone.0141615
PONE-D-15-30906 [pii] (2015).
20 Falagas, M. E. *et al.* Outcome of infections due to pandrug-resistant (PDR) Gram-negative bacteria. *BMC Infectious Diseases* **5**, 24, doi:1471-2334-5-24 [pii]
10.1186/1471-2334-5-24 (2005).
21 Bathoorn, E. *et al.* Emergence of pan-resistance in KPC-2 carbapenemase-producing *Klebsiella pneumoniae* in Crete, Greece: a close call. *Journal of Antimicrobial Chemotherapy* **71**, 1207-1212, doi:dkv467 [pii]
10.1093/jac/dkv467 (2016).
22 Vilacoba, E. *et al.* Widespread dispersion of the resistance element tet(B)::ISCR2 in XDR *Acinetobacter baumannii* isolates. *Epidemiology & Infection* **144**, 1574-1578, doi:S0950268815002897 [pii]
10.1017/S0950268815002897 (2016).
23 Weterings, V. *et al.* An outbreak of colistin-resistant *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* in the Netherlands (July to December 2013), with inter-institutional spread. *European Journal of Clinical Microbiology & Infectious Diseases* **34**, 1647-1655, doi:10.1007/s10096-015-2401-2
10.1007/s10096-015-2401-2 [pii] (2015).
24 Iyamba, J. M., Wambale, J. M., Lukukula, C. M. & za Balega Takaisi-Kikuni, N. High prevalence of methicillin resistant staphylococci strains isolated from surgical site infections in Kinshasa. *Pan African Medical Journal* **18**, 322, doi:10.11604/pamj.2014.18.322.4440
PAMJ-18-322 [pii] (2014).
25 Kulkova, N., Babalova, M., Sokolova, J. & Krcmery, V. First report of New Delhi metallo-beta-lactamase-1-producing strains in Slovakia. *Microbial Drug Resistance* **21**, 117-120, doi:10.1089/mdr.2013.0162 (2015).
26 O'Neill, A. J. Reveiw on antimicrobial resistance, tackling drug resistant infections globally. Infection prevention, control and surveillence: limiting the development and spread of drug resistance 1-32 (London, 2016).
27 Smith, R. & Coast, J. The true cost of antimicrobial resistance. *BMJ* **346**, f1493 (2013).
28 Pfeffer, I. *et al.* Prevalence and risk factors for carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae among patients prior to bowel surgery. *Diagnostic Microbiology and Infectious Disease* doi:S0732-8893(16)30078-5 [pii]
10.1016/j.diagmicrobio.2016.04.002 (2016).
29 Getzlaf, M. A. *et al.* Multi-disciplinary antimicrobial strategies for improving orthopaedic implants to prevent prosthetic joint infections in hip and knee. *Journal of Orthopaedic Research* **34**, 177-186, doi:10.1002/jor.23068 (2016).
30 Zhang, H. *et al.* Melittin restores PTEN expression by down-regulating HDAC2 in human hepatocelluar carcinoma HepG2 cells. *PLoS One* **9**, e95520, doi:10.1371/journal.pone.0095520
PONE-D-14-04193 [pii] (2014).
31 Maddocks, S. E., Jenkins, R. E., Rowlands, R. S., Purdy, K. J. & Cooper, R. A. Manuka honey inhibits adhesion and invasion of medically important wound bacteria *in vitro*. *Future Microbiology* **8**, 1523-1536, doi:10.2217/fmb.13.126 (2013).

32 Tonks, A. J. *et al.* Honey stimulates inflammatory cytokine production from monocytes. *Cytokine* **21**, 242-247, doi:S1043466603000929 [pii] (2003).
33 Jenkins, R., Burton, N. & Cooper, R. Manuka honey inhibits cell division in methicillin-resistant *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* **66**, 2536-2542, doi:dkr340 [pii]
10.1093/jac/dkr340 (2011).
34 Jenkins, R. & Cooper, R. Improving antibiotic activity against wound pathogens with manuka honey *in vitro*. *PLoS One* **7**, e45600, doi:10.1371/journal.pone.0045600 (2012).
35 Roberts, A. E., Maddocks, S. E. & Cooper, R. A. Manuka honey is bactericidal against *Pseudomonas aeruginosa* and results in differential expression of oprF and algD. *Microbiology* **158**, 3005-3013, doi:mic.0.062794-0 [pii]
10.1099/mic.0.062794-0 (2012).
36 Toreti, V. C., Sato, H. H., Pastore, G. M. & Park, Y. K. Recent progress of propolis for its biological and chemical compositions and its botanical origin. *Evidence-Based Complementary and Alternative Medicine* **2013**, 697390, doi:10.1155/2013/697390 (2013).
37 Müller, P. *et al.* Synergism between Medihoney and Rifampicin against Methicillin-Resistant *Staphylococcus aureus* (MRSA). *PLoS One* **8**, e57679, doi:10.1371/journal.pone.0057679 (2013).
38 Zumla, A. & Lulat, A. Honey--a remedy rediscovered. *Journal of the Royal Society of Medicine* **82**, 384-385 (1989).
39 Dustman. J, H. Antibacterial effects of honey. *Apiacta* **1** (1979).
40 Brogan, D. M. & Mossialos, E. A critical analysis of the review on antimicrobial resistance report and the infectious disease financing facility. *Global Health* **12**, 8, doi:10.1186/s12992-016-0147-y
10.1186/s12992-016-0147-y [pii] (2016).
41 Alvarez-Suarez, J., Gasparrini, M., Forbes-Hernández, T., Mazzoni, L. & Giampieri, F. The composition and biological activity of honey: A focus on manuka honey. *Foods* **3**, 420 (2014).
42 Olaitan, P. B., Adeleke, O. E. & Ola, I. O. Honey: a reservoir for microorganisms and an inhibitory agent for microbes. *African Health Sciences* **7**, 159-165 (2007).
43 Cooper, R. A., Molan, P. C. & Harding, K. G. The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. *Journal of Applied Microbiology* **93**, 857-863, doi:1761 [pii] (2002).
44 Danforth, B. N., Sipes, S., Fang, J. & Brady, S. G. The history of early bee diversification based on five genes plus morphology. *Proceedings of the National Academy of Sciences* **103**, 15118-15123, doi:10.1073/pnas.0604033103 (2006).
45 Michez, D., Patiny, S., Rasmont, P., Timmermann, K. & Vereecken, N. J. Phylogeny and host-plant evolution in Melittidae s.l. (Hymenoptera: Apoidea). *Apidologie* **39**, 146-162, doi:10.1051/apido:2007048 (2008).
46 Blair, S. E. in *Honey in modren wound management* eds R. Cooper, P. C. Molan, & R. White) Ch. 3, 21-47 (Wounds UK publishing, 2009).
47 Al-Waili, N. S. Topical honey application vs. acyclovir for the treatment of recurrent herpes simplex lesions. *Medical Science Monitor* **10**, MT94-98 (2004).
48 Feas, X. & Estevinho, M. L. A survey of the in vitro antifungal activity of heather (*Erica sp*.) organic honey. *Journal of medicinal food* **14**, 1284-1288, doi:10.1089/jmf.2010.0211 (2011).

49 Irish, J., Carter, D. A., Shokohi, T. & Blair, S. E. Honey has an antifungal effect against *Candida* species. *Medical Mycology* **44**, 289-291, doi:10.1080/13693780500417037 (2006).
50 Shahzad, A. & Cohrs, R. J. *In vitro* antiviral activity of honey against varicella zoster virus (VZV): A translational medicine study for potential remedy for shingles. *Translational Research in Biomedicine* **3**, doi:10.3823/434 (2012).
51 Watanabe, K., Rahmasari, R., Matsunaga, A., Haruyama, T. & Kobayashi, N. Anti-influenza viral effects of honey *in vitro*: Potent high activity of manuka honey. *Archives of Medical Research* **45**, 359-365, doi:http://dx.doi.org/10.1016/j.arcmed.2014.05.006 (2014).
52 Eteraf-Oskouei, T. & Najafi, M. Traditional and modern uses of natural honey in human diseases: a review. *Iranian Journal of Basic Medical Sciences* **16**, 731-742 (2013).
53 Kato, Y. *et al.* Identification of a novel glycoside, leptosin, as a chemical marker of manuka honey. *Journal of Agricultural and Food Chemistry* **60**, 3418-3423, doi:10.1021/jf300068w (2012).
54 Kwakman, P. H. *et al.* How honey kills bacteria. *FASEB Journal* **24**, 2576-2582, doi:fj.09-150789 [pii]
10.1096/fj.09-150789 (2010).
55 Carter, D. A. *et al.* Therapeutic Manuka Honey: No Longer So Alternative. *Frontiers in Microbiology* **7**, 569, doi:10.3389/fmicb.2016.00569 (2016).
56 Maddocks, S. E. & Jenkins, R. E. Honey: a sweet solution to the growing problem of antimicrobial resistance? *Future Microbiology* **8**, 1419-1429, doi:10.2217/fmb.13.105 (2013).
57 Blair, S. E., Cokcetin, N. N., Harry, E. J. & Carter, D. A. The unusual antibacterial activity of medical-grade Leptospermum honey: antibacterial spectrum, resistance and transcriptome analysis. *European Journal of Clinical Microbiology & Infectious Diseases* **28**, 1199-1208, doi:10.1007/s10096-009-0763-z (2009).
58 Roberts, A., Brown, H. L. & Jenkins, R. On the antibacterial effects of manuka honey: mechanistic insights. *Research and Reports in Biology* **6**, 215-224 (2015).
59 Jenkins, R., Wootton, M., Howe, R. & Cooper, R. A demonstration of the susceptibility of clinical isolates obtained from cystic fibrosis patients to manuka honey. *Archives of Microbiology* **197**, 597-601, doi:10.1007/s00203-015-1091-6 (2015).
60 Khalil, A. T. *et al.* Synergistic antibacterial effect of honey and Herba Ocimi Basilici against some bacterial pathogens. *Journal of Traditional Chinese Medicine* **33**, 810-814 (2013).
61 Daglia, M., Ferrari, D., Collina, S. & Curti, V. Influence of in vitro simulated gastroduodenal digestion on methylglyoxal concentration of Manuka (*Lectospermum scoparium*) honey. *Journal of Agricultural and Food Chemistry* **61**, 2140-2145, doi:10.1021/jf304299d (2013).
62 Pietta, P. G., Gardana, C. & Pietta, A. M. Analytical methods for quality control of propolis. *Fitoterapia* **73 Suppl 1**, S7-20, doi:S0367326X02001867 [pii] (2002).
63 Burdock, G. A. Review of the biological properties and toxicity of bee propolis (propolis). *Food and Chemical Toxicology* **36**, 347-363, doi:S0278-6915(97)00145-2 [pii] (1998).
64 Sforcin, J. M. & Bankova, V. Propolis: is there a potential for the development of new drugs? *Journal of Ethnopharmacology* **133**, 253-260, doi:S0378-8741(10)00735-X [pii]
10.1016/j.jep.2010.10.032 (2011).
65 Barrientos, L. *et al.* Chemical and botanical characterization of Chilean propolis and biological activity on cariogenic bacteria *Streptococcus mutans* and *Streptococcus sobrinus*. *Brazilian Journal of Microbiology* **44**, 577-585, doi:10.1590/S1517-83822013000200038

bjm-44-577 [pii] (2013).
66 Gonsales, G., Orsi, R., Fernandes Júnior, A., Rodrigues, P. & Funari, S. Antibacterial activity of propolis collected in different regions of Brazil. *Journal of Venomous Animals and Toxins Including Tropical Diseases* **12**, 276-284 (2006).
67 Veloz, J. J. *et al.* Antibiofilm activity of Chilean propolis on *Streptococcus mutans* is influenced by the year of collection. *BioMed Research International* **2015**, 291351, doi:10.1155/2015/291351 (2015).
68 Sforcin, J. M., Fernandes, A., Jr., Lopes, C. A., Bankova, V. & Funari, S. R. Seasonal effect on Brazilian propolis antibacterial activity. *Journal of Ethnopharmacology* **73**, 243-249, doi:S0378-8741(00)00320-2 [pii] (2000).
69 Orsi, R. d. O., Sforcin, J. M., Funari, S. R. C., Fernandes Junior, A. & Bankova, V. Synergistic effect of propolis and antibiotics on the *Salmonella* Typhi. *Brazilian Journal of Microbiology* **37**, 108-112 (2006).
70 Park, Y. K., Alencar, S. M. & Aguiar, C. L. Botanical origin and chemical composition of Brazilian propolis. *Journal of Agricultural and Food Chemistry* **50**, 2502-2506 (2002).
71 Oda, H. *et al.* Effect of Brazilian green propolis on oral pathogens and human periodontal fibroblasts. *Journal of Oral Biosciences* **58**, 50-54, doi:http://dx.doi.org/10.1016/j.job.2015.11.001 (2016).
72 Daugsch, A., Moraes, C. S., Fort, P. & Park, Y. K. Brazilian red propolis--chemical composition and botanical origin. *Evidence-Based Complementary and Alternative Medicine* **5**, 435-441, doi:nem057 [pii]
10.1093/ecam/nem057 (2008).
73 SFORCIN, J. M., Fernandes Júnior, A., Lopes, C., Funari, S. & Bankova, V. Seasonal effect of Brazilian propolis on *Candida albicans* and *Candida tropicalis*. *Journal of Venomous Animals and Toxins* **7**, 139-144 (2001).
74 Veloz, J. J. *et al.* Polyphenol-rich extract from propolis reduces the expression and activity of *Streptococcus mutans* glucosyltransferases at subinhibitory concentrations. *BioMed Research International* **2016**, 4302706, doi:10.1155/2016/4302706 (2016).
75 Silici, S. & Kutluca, S. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. *Journal of Ethnopharmacology* **99**, 69-73, doi:S0378-8741(05)00123-6 [pii]
10.1016/j.jep.2005.01.046 (2005).
76 Silici, S., Koc, N. A., Ayangil, D. & Cankaya, S. Antifungal activities of propolis collected by different races of honeybees against yeasts isolated from patients with superficial mycoses. *Journal of Pharmacological Sciences* **99**, 39-44, doi:JST.JSTAGE/jphs/FPE05002X [pii] (2005).
77 Oksuz, H., Duran, N., Tamer, C., Cetin, M. & Silici, S. Effect of propolis in the treatment of experimental *Staphylococcus aureus* keratitis in rabbits. *Ophthalmic Research* **37**, 328-334, doi:87943 [pii]
10.1159/000087943 (2005).
78 Casteels, P., Ampe, C., Jacobs, F. & Tempst, P. Functional and chemical characterization of Hymenoptaecin, an antibacterial polypeptide that is infection-inducible in the honeybee (*Apis mellifera*). *Journal of Biological Chemistry* **268**, 7044-7054 (1993).
79 Jefferson, J. M., Dolstad, H. A., Sivalingam, M. D. & Snow, J. W. Barrier immune effectors are maintained during transition from nurse to forager in the honey bee. *PLoS One* **8**, e54097, doi:10.1371/journal.pone.0054097
PONE-D-12-03945 [pii] (2013).
80 Evans, J. D. *et al.* Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Molecular Biology* **15**, 645-656, doi:IMB682 [pii]

10.1111/j.1365-2583.2006.00682.x (2006).
81 Bachanová, K., Klaudiny, J., Kopernicky, J. & Simúth, J. Identification of honeybee peptide active against *Paenibacillus larvae* larvae through bacterial growth-inhibition assay on polyacrylamide gel. *Apidologie* **33**, 259-269 (2002).
82 Schwarz, R. S. & Evans, J. D. Single and mixed-species trypanosome and microsporidia infections elicit distinct, ephemeral cellular and humoral immune responses in honey bees. *Developmental & Comparative Immunology* **40**, 300-310, doi:S0145-305X(13)00077-3 [pii]
10.1016/j.dci.2013.03.010 (2013).
83 Evans, J. D. & Pettis, J. S. Colony-level impacts of immune responsiveness in honey bees, *Apis mellifera*. *Evolution* **59**, 2270-2274 (2005).
84 Xu, P., Shi, M. & Chen, X. X. Antimicrobial peptide evolution in the Asiatic honey bee *Apis cerana*. *PLoS One* **4**, e4239, doi:10.1371/journal.pone.0004239 (2009).
85 Shen, Y., Stojicic, S. & Haapasalo, M. Bacterial viability in starved and revitalized biofilms: comparison of viability staining and direct culture. *Journal of Endodontics* **36**, 1820-1823, doi:S0099-2399(10)00687-4 [pii] 10.1016/j.joen.2010.08.029 (2010).
86 Shen, L. *et al.* Mechanism of action of recombinant acc-royalisin from royal jelly of Asian honeybee against gram-positive bacteria. *PLoS One* **7**, e47194, doi:10.1371/journal.pone.0047194
PONE-D-12-09648 [pii] (2012).
87 Hancock, R. E. W. & Patrzykat, A. Clinical Development of Cationic Antimicrobial Peptides: From Natural to Novel Antibiotics. *Current Drug Targets - Infectious Disorders* **2**, 79-83, doi:10.2174/1568005024605855 (2002).
88 Miyasaki, K. T. & Lehrer, R. I. β-sheet antibiotic peptides as potential dental therapeutics. *International Journal of Antimicrobial Agents* **9**, 269-280 (1998).
89 Casteels-Josson, K., Zhang, W., Capaci, T., Casteels, P. & Tempst, P. Acute transcriptional response of the honeybee peptide-antibiotics gene repertoire and required post-translational conversion of the precursor structures. *Journal of Biological Chemistry* **269**, 28569-28575 (1994).
90 Fujiwara, S. *et al.* A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. *Journal of Biological Chemistry* **265**, 11333-11337 (1990).
91 Klaudiny, J., Albert, S., Bachanova, K., Kopernicky, J. & Simuth, J. Two structurally different defensin genes, one of them encoding a novel defensin isoform, are expressed in honeybee Apis mellifera. *Insect Biochemistry and Molecular Biology* **35**, 11-22, doi:S0965-1748(04)00160-2 [pii]
10.1016/j.ibmb.2004.09.007 (2005).
92 Casteels, P. *et al.* Isolation and characterization of abaecin, a major antibacterial response peptide in the honeybee (*Apis mellifera*). *European Journal of Biochemistry* **187**, 381-386 (1990).
93 Casteels, P., Ampe, C., Jacobs, F., Vaeck, M. & Tempst, P. Apidaecins: antibacterial peptides from honeybees. *The EMBO Journal* **8**, 2387-2391 (1989).
94 Kwakman, P. H. S. *et al.* Medical-grade honey enriched with antimicrobial peptides has enhanced activity against antibiotic-resistant pathogens. *European Journal of Clinical Microbiology & Infectious Diseases* **30**, 251-257, doi:10.1007/s10096-010-1077-x (2011).
95 Majtan, J. *et al.* Methylglyoxal-induced modifications of significant honeybee proteinous components in manuka honey: Possible therapeutic implications. *Fitoterapia* **83**, 671-677, doi:S0367-326X(12)00051-2 [pii]

10.1016/j.fitote.2012.02.002 (2012).
96 Poulsen, M. W. *et al.* Advanced glycation endproducts in food and their effects on health. *Food and Chemical Toxicology* **60**, 10-37, doi:10.1016/j.fct.2013.06.052 (2013).
97 Adams, C. J., Manley-Harris, M. & Molan, P. C. The origin of methylglyoxal in New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydrate Research* **344**, 1050-1053, doi:http://dx.doi.org/10.1016/j.carres.2009.03.020 (2009).
98 Cao, L. F., Zheng, H. Q., Pirk, C. W., Hu, F. L. & Xu, Z. W. High Royal Jelly-Producing Honeybees (*Apis mellifera ligustica*) (Hymenoptera: Apidae) in China. *Journal of economic entomology*, doi:10.1093/jee/tow013 (2016).
99 Zheng, H.-Q., Hu, F.-L. & Dietemann, V. Changes in composition of royal jelly harvested at different times: consequences for quality standards. *Apidologie* **42**, 39-47, doi:10.1051/apido/2010033 (2011).
100 Wongchai, V. & Ratanavalachai, T. Seasonal variation of chemical composition of royal jelly produced in Thailand. *Thammasat International Journal of Science and Technology* **7**, 1 - 8 (2002).
101 Kodai, T., Umebayashi, K., Nakatani, T., Ishiyama, K. & Noda, N. Compositions of royal jelly II. Organic acid glycosides and sterols of the royal jelly of honeybees (*Apis mellifera*). *Chemical and Pharmaceutical Bulletin* **55**, 1528-1531, doi:JST.JSTAGE/cpb/55.1528 [pii] (2007).
102 Kanelis, D. *et al.* A suggestion for royal jelly specifications. *Archives of Industrial Hygiene and Toxicology* **66**, 275-284, doi:10.1515/aiht-2015-66-2651 (2015).
103 Bíliková, K., Wu, G. & Šimúth, J. Isolation of a peptide fraction from honeybee royal jelly as a potential antifoulbrood factor. *Apidologie* **32**, 275-283 (2001).
104 Stratev, D., Vashin, I., Balkanska, R. & Dinkov, D. Antibacterial activity of royal jelly and rape honey against *Aeromonas hydrophila* (ATCC 7965). *Journal of Food and Health Science* **1**, 67-74, doi:10.3153/JFHS15006 (2015).
105 Dinkov, D., Stratev, D., Balkanska, R. & Sergelidis, D. Antibacterial activity of royal jelly and rape honey agaist methicillin resistant *Staphylococcus aureus* strains. *Journal of Food and Health Science* **2**, 67-73, doi:10.3153/JFHS16007 (2016).
106 Boukraa, L. Additive activity of royal jelly and honey against *Pseudomonas aeruginosa*. *Alternative Medicine Review* **13**, 330-333 (2008).
107 Nascimento, A. P. *et al.* The lyophilization process maintains the chemical and biological characteristics of royal jelly. *Evidence-Based Complementary and Alternative Medicine* **2015**, 825068, doi:10.1155/2015/825068 (2015).
108 Sabatini, A. G., Marcazzan, G. L., Caboni, M. F., Bogdanov, S. & Almeida-Muradian, L. Quality and standardisation of royal jelly. *Journal of ApiProduct and ApiMedical Science* **1**, 1-6 (2009).
109 Schmitzova, J. *et al.* A family of major royal jelly proteins of the honeybee *Apis mellifera* L. *Cellular and molecular life sciences : CMLS* **54**, 1020-1030 (1998).
110 Kimura, Y. *et al.* Structural features of N-glycans linked to royal jelly glycoproteins: structures of high-mannose type, hybrid type, and biantennary type glycans. *Bioscience, Biotechnology and Biochemistry* **64**, 2109-2120 (2000).
111 Fontana, R. *et al.* Jelleines: a family of antimicrobial peptides from the Royal Jelly of honeybees (*Apis mellifera*). *Peptides* **25**, 919-928, doi:10.1016/j.peptides.2004.03.016 (2004).
112 Romanelli, A. *et al.* Peptides from royal jelly: studies on the antimicrobial activity of jelleins, jelleins analogs and synergy with temporins. *Journal of Peptide Science* **17**, 348-352, doi:10.1002/psc.1316 (2011).

113 Schonleben, S., Sickmann, A., Mueller, M. J. & Reinders, J. Proteome analysis of *Apis mellifera* royal jelly. *Analytical and Bioanalytical Chemistry* **389**, 1087-1093, doi:10.1007/s00216-007-1498-2 (2007).
114 Bilikova, K., Huang, S. C., Lin, I. P., Simuth, J. & Peng, C. C. Structure and antimicrobial activity relationship of royalisin, an antimicrobial peptide from royal jelly of *Apis mellifera*. *Peptides* **68**, 190-196, doi:S0196-9781(15)00059-5 [pii] 10.1016/j.peptides.2015.03.001 (2015).
115 Shen, L. *et al.* Expression of Acc-royalisin gene from royal jelly of Chinese honeybee in *Escherichia coli* and its antibacterial activity. *Journal of Agricultural and Food Chemistry* **58**, 2266-2273 (2010).
116 Townsend, G. F. *et al.* Studies on the *in vitro* antitumor activity of fatty acids I. 10-hydroxy-2-decenoic acid from royal jelly. *Cancer research* **20**, 503-510 (1960).
117 Hattori, N., Nomoto, H., Fukumitsu, H., Mishima, S. & Furukawa, S. Royal jelly and its unique fatty acid, 10-hydroxy-trans-2-decenoic acid, promote neurogenesis by neural stem/progenitor cells *in vitro*. *Biomedical Research* **28**, 261-266 (2007).
118 Wang, J. *et al.* 10-Hydroxy-2-decenoic acid inhibiting the proliferation of fibroblast-like synoviocytes by PI3K–AKT pathway. *International Immunopharmacology* **28**, 97-104 (2015).
119 Takikawa, M. *et al.* 10‐Hydroxy‐2‐decenoic acid, a unique medium‐chain fatty acid, activates 5'‐AMP‐activated protein kinase in L6 myotubes and mice. *Molecular Nutrition & Food Research* **57**, 1794-1802 (2013).
120 Yousefi, B. *et al.* Hydroxy decenoic acid down regulates *gtfB* and *gtfC* expression and prevents *Streptococcus mutans* adherence to the cell surfaces. *Annals of clinical microbiology and antimicrobials* **11**, 21, doi:1476-0711-11-21 [pii] 10.1186/1476-0711-11-21 (2012).
121 Morais, M., Moreira, L., Feas, X. & Estevinho, L. M. Honeybee-collected pollen from five Portuguese Natural Parks: palynological origin, phenolic content, antioxidant properties and antimicrobial activity. *Food and Chemical Toxicology* **49**, 1096-1101, doi:S0278-6915(11)00029-9 [pii] 10.1016/j.fct.2011.01.020 (2011).
122 Almeida-Muradian, L., Pamplona, L. C., Coimbra, S. l. & Barth, O. M. Chemical composition and botanical evaluation of dried bee pollen pellets. *Journal of food composition and analysis* **18**, 105-111 (2005).
123 Hansen, M. *The healing power of pollen and other products form the beehive, propolis, royal jelly and honey*. (Thorsons, 1979).
124 Mărghitaş, L. A. *et al.* In vitro antioxidant capacity of honeybee-collected pollen of selected floral origin harvested from Romania. *Food Chemistry* **115**, 878-883 (2009).
125 Gebara, E. C., Lima, L. A. & Mayer, M. Propolis antimicrobial activity against periodontopathic bacteria. *Brazilian Journal of Microbiology* **33**, 365-369 (2002).
126 Cook, N. & Samman, S. Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. *The Journal of Nutritional Biochemistry* **7**, 66-76 (1996).
127 Garcia, M., Pérez-Arquillue, C., Juan, T., Juan, M. & Herrera, A. Note. Pollen analysis and antibacterial activity of Spanish honeys. *Food Science and Technology International* **7**, 155-158 (2001).
128 Proestos, C., Chorianopoulos, N., Nychas, G. J. & Komaitis, M. RP-HPLC analysis of the phenolic compounds of plant extracts. investigation of their antioxidant capacity and antimicrobial activity. *Journal of Agricultural and Food Chemistry* **53**, 1190-1195, doi:10.1021/jf040083t (2005).

129 Almaraz-Abarca, N. *et al.* Variability of antioxidant activity among honeybee-collected pollen of different botanical origin. *Interciencia* **29**, 574-578 (2004).
130 Balch, P. A. *Prescription for Nutritional Healing: The A-to-Z Guide to Supplements*. (Avery, 2002).
131 Carpes, S. T., Begnini, R., Alencar, S. M. d. & Masson, M. L. Study of preparations of bee pollen extracts, antioxidant and antibacterial activity. *Ciência e Agrotecnologia* **31**, 1818-1825 (2007).
132 Broadhurts, C. L. Bee product: medicine from the hive. *Nutrition science news* **4**, 366-368 (1999).
133 Freire, K. R. *et al.* Palynological origin, phenolic content, and antioxidant properties of honeybee-collected pollen from Bahia, Brazil. *Molecules (Basel, Switzerland)* **17**, 1652-1664, doi:10.3390/molecules17021652 (2012).
134 Campos, M. G. *et al.* Pollen composition and standardisation of analytical methods. *Journal of Apicultural Research* **47**, 154-161 (2008).
135 Campos, M., Frigerio, C., Lopes, J. & Bogdanov, S. What is the future of Bee-Pollen. *Journal of ApiProduct and ApiMedical Science* **2**, 131-144 (2010).
136 Rzepecka-Stojko, A. *et al.* Polyphenols from bee pollen: Structure, absorption, metabolism and biological activity. *Molecules (Basel, Switzerland)* **20**, 21732-21749, doi:molecules201219800 [pii]
10.3390/molecules201219800 (2015).
137 Baltrušaitytė, V., Venskutonis, P. R. & Čeksterytė, V. Antibacterial activtiy of honey and beebread of different origin agaist *Staphylococcus aureus* and *S. epidemidis*. *Food Technology and Biotechnology* **45**, 201-208 (2007).
138 Erkmen, O. & Ozcan, M. M. Antimicrobial effects of Turkish propolis, pollen, and laurel on spoilage and pathogenic food-related microorganisms. *Journal of medicinal food* **11**, 587-592, doi:10.1089/jmf.2007.0038 (2008).
139 Graikou, K. *et al.* Chemical analysis of Greek pollen - Antioxidant, antimicrobial and proteasome activation properties. *Chemistry Central Journal* **5**, 33-33, doi:10.1186/1752-153x-5-33 (2011).
140 Nakamura, K. *et al.* Bactericidal activity and mechanism of photoirradiated polyphenols against Gram-Positive and -Negative Bacteria. *Journal of Agricultural and Food Chemistry* **63**, 7707-7713, doi:10.1021/jf5058588 (2015).
141 Cushnie, T. T. & Lamb, A. J. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents* **26**, 343-356 (2005).
142 Tichy, J. & Novak, J. Detection of antimicrobials in bee products with activity against viridans streptococci. *The Journal of Alternative and Complementary Medicine* **6**, 383-389 (2000).
143 Abouda, Z., Zerdani, I., Kalalou, I., Faid, M. & Ahami, M. The antibacterial activity of Moroccan bee bread and bee-pollen (fresh and dried) against pathogenic bacteria. *Research Journal of Microbiology* **6**, 376 (2011).
144 Kačániová, M. *et al.* Antimicrobial activity of bee collected pollen against Clostridia. *Lucrari Stiintifice : Zootehnie si Biotehnologii* **47**, 362-365 (2014).
145 Kacániová, M. *et al.* The antimicrobial activity of honey, bee pollen loads and beeswax from Slovakia. *Archives of Biological Sciences* **64**, 927-934 (2012).
146 Mohdaly, A. A. A., Mahmoud, A. A., Roby, M. H. H., Smetanska, I. & Ramadan, M. F. Phenolic extract from propolis and bee pollen: Composition, antioxidant and antibacterial activities. *Journal of Food Biochemistry* **39**, 538-547, doi:10.1111/jfbc.12160 (2015).

147 MĂRGĂOAN, R. A. *et al.* Antimicrobial activity of bee pollen ethanolic and methanolic extracts on *Staphylococcus aureus* bacterial strain. *Bulletin of the University of Agricultural Sciences and Veterinary Medicine* **72**, 78-80 (2015).
148 Khider, M., Elbanna, K., Mahmoud, A. & Owayss, A. A. Egyptian honeybee pollen as antimicrobial, antioxidant agents, and dietary food supplements. *Food Science and Biotechnology* **22**, 1-9 (2013).
149 Pascoal, A., Rodrigues, S., Teixeira, A., Feás, X. & Estevinho, L. M. Biological activities of commercial bee pollens: Antimicrobial, antimutagenic, antioxidant and anti-inflammatory. *Food and Chemical Toxicology* **63**, 233-239 (2014).
150 Koc, A. N. *et al.* Antifungal activity of the honeybee products against *Candida spp*. and *Trichosporon spp. Journal of medicinal food* **14**, 128-134, doi:10.1089/jmf.2009.0296 (2011).
151 Özcan, M., Ünver, A., Ceylan, D. A. & Yetisir, R. Inhibitory effect of pollen and propolis extracts. *Food/Nahrung* **48**, 188-194 (2004).
152 Nogueira, C., Iglesias, A., Feás, X. & Estevinho, L. M. Commercial bee pollen with different geographical origins: A comprehensive approach. *International Journal of Molecular Sciences* **13**, 11173-11187, doi:10.3390/ijms130911173 (2012).
153 Bogdanov, S. Bee venom: composition, health, medicine: a review. *Peptides* **1** (2012).
154 Lee, G. & Bae, H. Bee venom phospholipase A2: Yesterday's enemy becomes today's friend. *Toxins* **8**, 48 (2016).
155 Ali, M. Studies on bee venom and its medical uses. *International Journal of Advancements in Research & Technology,* **1**, 1-15 (2012).
156 Schumacher, M. J., Schmidt, J. O., Egen, N. B. & Dillon, K. A. Biochemical variability of venoms from individual European and Africanized honeybees (*Apis mellifera*). *Journal of Allergy and Clinical Immunology* **90**, 59-65 (1992).
157 Ferreira Junior, R. S. *et al.* Africanized honey bee (*Apis mellifera*) venom profiling: Seasonal variation of melittin and phospholipase A(2) levels. *Toxicon : official journal of the International Society on Toxinology* **56**, 355-362, doi:10.1016/j.toxicon.2010.03.023 (2010).
158 Banks, B. E. C. & Shipolini, R. A. in *Venoms of the Hymenoptera: Biochemical, Pharmacological and Behavioural Aspects* (ed T. Piek) (Academic Press, 1986).
159 Danneels, E., Van Vaerenbergh, M., Debyser, G., Devreese, B. & de Graaf, D. Honeybee venom proteome profile of Queens and winter bees as determined by a mass spectrometric approach. *Toxins* **7**, 4468 (2015).
160 Fennell, J. F., Shipman, W. H. & Cole, L. J. Antibacterial action of melittin, a polypeptide from bee venom. *Experimental Biology and Medicine* **127**, 707-710 (1968).
161 Han, S. M. *et al.* Antibacterial activity and antibiotic-enhancing effects of honeybee venom against methicillin-resistant *Staphylococcus aureus*. *Molecules (Basel, Switzerland)* **21**, 79, doi:10.3390/molecules21010079 (2016).
162 Gauldie, J., Hanson, J. M., Rumjanek, F. D., Shipolini, R. A. & Vernon, C. A. The peptide components of bee venom. *European Journal of Biochemistry* **61**, 369-376 (1976).
163 Benton, A. W. & Morse, R. A. Venom toxicity and proteins of the genus *Apis*. *Journal of Apicultural Research* **7**, 113-118 (1968).
164 Boutrin, M. C., Foster, H. A. & Pentreath, V. W. The effects of bee (*Apis mellifera*) venom phospholipase A2 on *Trypanosoma brucei* brucei and Enterobacteria. *Experimental parasitology* **119**, 246-251, doi:10.1016/j.exppara.2008.02.002 (2008).
165 Leandro, L. F. *et al.* Antimicrobial activity of apitoxin, melittin and phospholipase A(2) of honey bee (*Apis mellifera*) venom against oral pathogens. *Anais da Academia Brasileira de Ciencias* **87**, 147-155, doi:10.1590/0001-3765201520130511 (2015).

166 Mollay, C. & Kreil, G. Enhancement of bee venom phospholipase A2 activity by melittin, direct lytic factor from cobra venom and polymyxin B. *FEBS Letters* **46**, 141-144, doi:http://dx.doi.org/10.1016/0014-5793(74)80354-6 (1974).
167 Mollay, C., Kreil, G. & Berger, H. Action of phospholipases on the cytoplasmic membrane of *Escherichia coli*. Stimulation by melittin. *Biochimica et Biophysica Acta - Biomembranes* **426**, 317-324, doi:http://dx.doi.org/10.1016/0005-2736(76)90340-0 (1976).
168 Choi, J. H. *et al.* Melittin, a honeybee venom derived antimicrobial peptide, may target methicillin resistant *Staphylococcus aureus*. *Molecular Medicine Reports* **12**, 6483-6490 (2015).
169 Dosler, S., Karaaslan, E. & Alev Gerceker, A. Antibacterial and anti-biofilm activities of melittin and colistin, alone and in combination with antibiotics against Gram-negative bacteria. *Journal of Chemotherapy*, 1973947815Y0000000004, doi:10.1179/1973947815y.0000000004 (2015).
170 Al-Ani, I., Zimmermann, S., Reichling, J. & Wink, M. Pharmacological synergism of bee venom and melittin with antibiotics and plant secondary metabolites against multi-drug resistant microbial pathogens. *Phytomedicine* **22**, 245-255, doi:10.1016/j.phymed.2014.11.019 (2015).