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Review Article

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A Review on the Antimicrobial Activity of Propolis and its Synergy with Other Antimicrobial Compounds

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Abstract

The alarming increase in the development of drug resistant microbes and the decrease in the efficacy of numerous antibiotics have led to the rise in patient mortality and morbidity affecting various fields of medicine. To combat this development and or the improvement of drugs are paramount. Natural products have long been utilized in the treatment of diseases showing broad spectrum of activity against a wide range of pathogens. In this study, the potential application of propolis to combat drug resistant microbes is reviewed, highlighting the presence and diversity of bioactive compounds with focus on its antimicrobial activity against a number of drug resistant microbes and its synergistic properties with other antimicrobial compounds. It is hoped that this study would enable researchers to continue to analyse propolis compounds and its interactions with other antimicrobial compounds which ultimately paves the way for new drug development.

Keywords: Propolis, Synergy, Antimicrobial Compounds, Drug Resistance Microbes

Introduction

The successful treatment of microbial infections over the past years has predominantly been through the use of various antibiotics that target specific bacterial proteins, DNA, RNA, and cell wall synthesis [1]. However, in recent years, there has been an alarming increase in the number of antibiotic resistance microbes (ARM), such as carbapenem-resistant *Enterobacteriaceae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* listed as critical for new antibiotic development by the World Health Organization (WHO) 2017, Methicillin-resistant *Staphylococcus aureus* (MRSA), ampicillin-resistant *Haemophilus influenzae* and fluoroquinolone-resistant *Shigella* spp [2, 3]. This increase has led to rise in patient mortality and morbidity; and without the development of new drugs or methods to combat this issues, many fields of medicine will be severely affected [4]. One such approach is to develop new drugs from natural products due to the wide range of structurally diverse compounds that can be obtained [5, 6].

Natural products have been a source of medicinal agents since ancient times with numerous pharmaceuticals formulated based on the compounds derived from natural sources [7]. They include: reserpine from *Rauwolfia serpentina*; vinblastine and vincristine from *Catharanthus roseus*; and artemisinin from *Artemisia annua* [8]. Furthermore, various compounds extracted from plants were shown to be effective against a wide range of microbes including, thionins extracted from *Triticum aestivum* shown to be toxic to yeast, fungi, and bacteria and snakins isolated from potatoes shown to be effective against fungi and bacteria at concentrations below 10 μM [9].

In addition to analyzing natural compounds derived from plants, the utilization of animal derived products to treat various illnesses is becoming increasing popular [10]. Commonly studied animal derived products are those obtained from honeybees. Honeybees products such as honey, bee venom, propolis and royal jelly have long been utilized by ancient cultures such as the Greeks, Egyptians, Romans and Chineses, who employed these products in the treatment of wounds and intestinal diseases [11, 12]. When subjected to various laboratory studies, these products were shown to exhibit anti-inflammatory, anti-bacterial, anti-fungal, anti-viral and antioxidant activities; in addition to inhibiting tumor cell growth, metastasis and inducing apoptosis of cancer cells [11].

Aside from the development of new drug, an alternative approach is combination therapy; where two or more drugs are combined to directly target ARM [4].

Studies have shown that the synergistic combination of two or more compounds can significantly reduce toxicity and adverse side effects associated with high intake of a single drug and as a result, combination therapy is seen as potential method to treating ARM [13].

Due to the increasing incidence of drug resistant microbes (DRM) and the urgent request for new methods and compounds to combat

this issue. The remainder of this paper would analyse the use of a natural product namely propolis in combination therapy as a possible solution to DRM [14].

Propolis - An Introduction

Propolis (bee glue) is a natural resinous substance produced by both honeybees particularly *Apies mellifera* and stingless bees [15, 16]. It is a heterogeneous mixture of many substances collected from various plants and metabolized by the bees to repair the beehive and protect the colony from diseases [10, 16]. The word propolis is derived from the Greek words pro and polis meaning defense of the city, hence, its use by the bees primarily as a sealant, protectant against invaders and maintenance of temperature and moisture [17, 18].

Application

Since ancient times, propolis have been used extensively by various civilisations; the Egyptians utilized its anti-putrefactive properties in embalming their dead, Greek and Roman physicians used it to treat wounds and mouth related diseases while the Incas used it as an antipyretic agent [19]. Nowadays, it is employed as a remedy for lowering blood pressure and cholesterol levels, preventing tooth decay, and sold as an ingredient in cosmetics, ointments, lotions and beverages [17, 20].

Literature indicates that propolis possess a wide range of biological properties including antiviral, cytotoxic, antioxidant, anti-inflammatory, antitumor, anti diabetic anti browning and antimicrobial abilities; with numerous publications focusing on its antimicrobial activity and its ability to enhance the efficacy of other antimicrobial compounds. However, its biological activity greatly depends on its chemical composition, bee species, and flora biodiversity surrounding the hives, climate, and geographical area [17, 19-21].

Chemical Composition

Due to propolis being a natural product, various factors such as the beehive, geographical area, seasons and plant source plays a major role in the variability observed in its chemical composition [22]. However, in general raw propolis consists of plant remnant gathered by bees mixed with beeswax and sugar with the addition of various vitamins like vitamin A, B, C, as well as some minerals like zinc, cobalt, potassium and calcium [23, 24]. In addition, enzymes such as succinic dehydrogenase, glucose-6-phosphate dehydrogenase and acid phosphatase enzyme were observed in propolis extract [25].

Constituent Profile

To date, over 300 compounds have been identified with significant variation in the constituent's profile of propolis from various regions [19]. Compounds like pinocembrin, galangin, chrysin, phenethyl ester of caffeic acid, pinobanksin and its 3- O-acetate are commonly found in all propolis with *Acacia spp*, *Azadirachta indica, Mangifera indica*, *Populus nigra*, *Populus suave lens* and *Populus tremula* being the most frequent plant source for bees [26, 27].

In propolis samples obtained from Ethiopia, gas chromatography-mass spectrophotometer (GC-MS) indicated a high concentration of triterpenoids compounds including α-, β-amyrins and amyryl acetates [28, 29]. Whereas, Uzel et al., identified flavonoid compounds such as pinocembrin, naringenin, galangin and chrysin across four different Anatolian propolis samples. Using gas liquid chromatography/mass spectrophotometer(GC-MS) coupled with high performance liquid chromatography (HPLC) Al-Ani et al., concluded that an ethanol extract of propolis from Czech Republic, Ireland and Germany contained high concentrations of caffeic acid, galangin, pinocembrin and chrysin [30].

As flavonoid and phenolic compounds were the main constituents identified in propolis obtained from temperate regions, Huang et al., identified poplar trees mainly *Populus nigra* L. and *Populus Alba* L to be the main source of plant material for bees in this region. Further analysis performed by Greenaway, Scaysbrook and Whatley, (1990) using GC-MS to analyze propolis obtained from Bulgarian and Hungarian regions, confirmed that the choice of plant for bees was predominantly *Populus nigra*. Although, poplar species seems to be the dominant plant source for bees in temperate regions, it is worth noting that other researchers have identified the presence of *Betula, Salix, Alnus* and Aesculus plant exudates in propolis obtained from this region [31].

In contrast, bud exudates from *Baccharis dracunculifolia*, *Hyptis divaricata* and *Poplar* tree were identified in Brazilian propolis [32]. This is due to the diverse climates which ranges from tropical to subtropical and equatorial, in addition to the varying species of honey and stingless bees who mix plant resins with wax (cerumen) and clay (geopropolis) [33]. Brazilian propolis are categorised into 13 groups based on their physicochemical characteristics [32, 34]. Machado et al., analysed and compared yellow, green, red and brown propolis from Brazil with yellow propolis obtained from Cuba; the results indicated the absence of phenolic compounds in yellow propolis with high concentration of triterpenes. Further analysis performed by Alencar et al., using GC-MS identified seven new compounds in Brazilian red propolis four of which were is flavones including homopterocarpin, medicarpin and 4′,7-dimethoxy-2′-isoflavonol. Also, phenolic compounds such as vestitol, neovestitol, and medicarpin were found in red propolis from Brazil [35, 36].

By contrast, propolis obtained from pacific regions like Okinawa Island and mainland Japan contained prenylflavonoids not observed in temperate and tropical regions [36]. While research performed by Raghukumar et al., on propolis samples from The Solomon Island led to the isolation of prenylflavonones including propolin H, G, and D and C This led to the conclusion that the plant source for pacific propolis is *Macaranga tanarius* [36, 37].

Method Used to Extract Bioactive Compounds from Propolis

Apart from the geographical area and plant source, the type of extraction methods used to extract propolis may give rise to the variation in propolis constituent profile. Various methods are used to obtain propolis extracts they include maceration, ultrasound (UE) and microwave assisted extraction (MAE) using ethanol as the solvent [38]. Figure 1 below gives an example of a standard method used to obtain dry purified propolis extract.

As of recent, studies are focusing on the use of supercritical fluid extraction (SFE) as an alternate method (Machado et al., 2016) due to several distinctive properties of SFE such as, short extraction time which produces extracts with minimum organic solvent residues [39, 40]. In addition, information on the extraction process and mechanism can be obtained and used to optimise the extraction process [41].

Paviani et al, compared SFE methods using water and ethanol as the solvents on the total polyphenolic content of Green Brazilian propolis; it was concluded that an ethanol extract of propolis had a higher concentration of polyphenols with a higher concentration of flavonoids obtained from SFE. Similar result was observed by Machado et al., who indicated ethanol to be the best solvent for extracting propolis compounds with a higher concentration of Artepillin C and p-coumaric acid obtained from SFE [39, 42].

In spite of this, the use of ethanol extract of propolis is rarely encountered in the treatment of certain diseases including ophthalmology and pediatrics [43]. Only a few papers have analysed the constituent profile of propolis using other solvents with their results indicating ethanol to be a better solvent for the extraction of propolis compounds with greater antimicrobial activity.

Antimicrobial Activity of Propolis

With the variation observed in propolis constituent profile, one might imagine that the biological properties of propolis would vary from region to region. Nonetheless, research has proven that the biological properties of propolis are relatively similar across the different regions even if different constituent profiles are observed [44].

Kujumgiev et al., investigated the antimicrobial, antifungal and antiviral properties of propolis from different geographical regions, their results indicated similarities in activity against the tested fungal and Gram-positive bacterial strains despite their different chemical profile [45]. Similarly Runyoro, Ngassapa and Kamugisha, (2017), examined two propolis sample from Iringa and Tabora regions of Tanzania, it was discovered that an ethanol extract of the two sample were effective against *Staphylococcus aureus* (*S.aureus*), Streptococcus faecalis (*S.faecalis*), *Pseudomonas aeruginosa* (*P.aeruginosa*), *Proteus vulgaris*, *Salmonella typhi* (*S.typhi*) and *Escherichia coli* (*E.coli*) [20, 46]. However *P.aeruginosa* showed more resistant to Tabora propolis at a tested concentration of 0.42-1.67mg/ml whereas, Iringa propolis was less active on S.aureus, S.typhi, *P.aeruginosa* and *S.faecalis* at concentrations ranging from 1.67-6.67mg/ml [20].

As propolis extracts are commonly added as an ingredient in toothpaste, mouth rinses and lozenges with proven efficacy against oral pathogens, an *in vitro* experiment using ethanol extracts of propolis against *Streptococcus mutans* and *Lactobacillus* spp indicated a significant reduction in *Lactobacillus* spp with a minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 9.01mg/ml and 5.91mg/ml respectively [47]. Similar result was obtained *in vivo* using rats as test subjects, were a significant reduction in dental caries was observed in rats fed with propolis [48]. As both studies revealed similar results both *in vivo* and *in vitro* their finding could be usefully in predicting propolis efficacy *in vivo* based on *in vitro* studies.

When compared to other natural products the antimicrobial efficacy of propolis is often superior, Koo et al., compared the antimicrobial activity of *Arnica montana* with propolis using agar diffusion method, they deemed propolis as a more effective compound as water soluble glucan formation and cell adherence were significantly inhibited with *Actinomyces* spp being more susceptible. Furthermore, the antimicrobial activity of propolis in an emulsion system was analysed with results indicating propolis as an effective agent in inhibiting *Bacillus cereus* spores in comparison to lysozyme [46, 49].

Mode of Action

It is well documented that propolis is more effective against Gram+ showing moderate activity against Gram^{+} bacteria using methods such as disk diffusion and dilution methods, the difference in activity may be related to the different structure of the corresponding organisms; as Gram+ contain thick peptidoglycan layer with echoic acid while Gram_ contains a thin peptidoglycan layer with a thick coat of plasma membrane surrounding the cell [50, 51].

Various researchers have suggested the possible mechanism by which propolis exhibits its antimicrobial activity; they include inhibition of cell division and protein synthesis, disorganization of the cytoplasm, cytoplasm membrane and cell wall causing partial bacteriolysis; indicating structural damage as the mode of action [30, 51, 52].

The wide variety of mechanism employed by propolis to inhibit the growth of microbes has sparked the interest of various researchers to study the synergistic effects of propolis with other antimicrobial compounds [30].

Propolis Synergistic Interactions

The molecular targets of current antibiotics are cell wall synthesis, protein synthesis, RNA synthesis, DNA replication, cell wall and membrane stability [1]. Pathogens acquire resistance through; 1) Direct inactivation of the antibiotics, 2) Target site modification, 3) Removal of antibiotics from the cell with the help of ABC transporters [53]. It is suggested that combination of various drugs with different target sites can be useful in combating (DRM) [13].

The combining of different drugs leads to 3 different effects including synergistic, antagonistic and additive [54]. Synergy is defined as the minimum concentration that causes an effect and is consequently lower than the effective dose of the individual compounds while additive combination results in no difference in compound efficacy [55]. In contrast, antagonist combination inhibits the activity of each compound resulting in no observable effect [55]. Different methods are used to analyse and interpret synergistic interactions between different combinations with a relative degree of agreement between each method, they include: 1) Isobologram, 2) Time kill assay, 3) checkerboard and 4) E-test [55].

With numerous studies demonstrating propolis wide-spectrum antibacterial activity, several researchers have started to combine propolis with various compounds exhibiting antimicrobial activity in order to treat DRM. On analysis of these studies it was discovered that majority focused on the combination of propolis with various antibiotics (Table 1) with limited studies on propolis interaction with natural products (Table 2).

Synergism between Propolis and Antibiotics

Various researchers have commented on the possible mechanism of propolis interactions with different antibiotics; indicating that propolis enhances the efficacy of antibiotics acting against cell wall synthesis, targeting microbial ribosomes [57]. But not with those inhibiting metabolic pathways and synthesizing folic acids and DNA [20].

Based on the results from table 1, propolis was shown to synergistically enhance the efficacy of a wide variety of antibiotics with different propolis samples showing varying potential to

potentiate antibiotic activity [58]. This was evident in the study performed by Mantovani et al., where Brazilian propolis from the south was better at enhancing the efficacy of the entire tested antibiotic except chloramphenicol, while Brazilian propolis from the southeast did not enhance the efficacy of 4/7 of the tested antibiotics including chloramphenicol [59]. A more recent study on Brazilian propolis combination with chloramphenicol indicated a bacteriostatic effect on the tested microbe showing weak synergy [57]. On the other hand, Bulgarian propolis combination with chloramphenicol had a bactericidal effect indicating strong synergy [57]. It is thought, that the variation observed between the results might be related to the different constituent profile.

In spite of the varying propolis-antibiotic synergy observed in propolis from different regions, the interest in propolis derived products are continuously growing [16]. Where researchers are not only focusing on propolis synergy with antibiotics but also studying its interaction with other naturally derived antimicrobial compounds (Table 2).

Table 1: Synergistic Effects of Propolis and Different Antibiotics on Various Microbes

Propolis	Antibiotic	Microbe species	Method	Synergy	Reference
European	vancomycin, Oxacillin Levofloxacin	*Streptococcus pyogenes* *Streptococcus pneumoniae* *Haemophilus influenzae* *MRSA Enterococcus faecalis*	CB & Time kill assay	+ + + + +	Al-Ani et al., 2018
Indian	cefixime	*Salmonella enterica Typhimurium*	CB & Time kill assay	+	Kalia, Kumar & Harjai 2017
Indian	cefixime	*Salmonella enterica Typhimurium*	*in vivo*	+	Kalia, Kumar & Harjai 2016
Poland	Fluconazole Voriconazole	candida albicans	disk diffusion, time kill assay CB	+	Gucwa et al., 2018
Serbia	Gentamicin	staphylococcus aureus, candida albicans Klebsiella pneumoniae,	disk diffusion	+ + +	Stepanovic et al 2003.
Brazil (southeast)	cephalothin, oxacillin, vancomycin, chloramphenicol netilmicin, clindamycin tetracycline	coagulase-negative staphylococcus	E-test	+ + + + - - -	Mantovani et al., 2008.
Brazil (south)	cephalothin, oxacillin, vancomycin, clindamycin Netilmicin, chloramphenicol	coagulase-negative staphylococcus	E-test	+ + + + + -	Mantovani et al., 2008.
Durango - Mexico	ciprofloxacin	MRSA	isobologram	-	Guzman & Cruz 2017
- indicates no synergy +indicates synergy.					

Synergism Between Propolis and Natural Products

Table 2: synergistic effects of Propolis and different natural products on various microbes

Propolis	Antibiotic	Microbe species	Method	Synergy	Reference
Durango-Mexico	Garlic	*MRSA*	isobologram	+ *	Guzman & Cruz 2017
Durango-Mexico	Oregano	*MRSA*	isobologram	+	Guzman & Cruz 2017
Egypt	Honey (Saudi Arabia)	*Staphylococcus Aureus* *scherichia Coli* *Candida Albicans*	broth microdilution	+ + +	Al-Waili et al., 2012
Saudi Arabia	Honey (Saudi Arabia)	*Staphylococcus Aureus* *Escherichia Coli* *Candida Albicans*	broth microdilution	++ ++ ++	Al-Waili et al., 2012
Azarian	Honey	*Staphylococcus Aureus* *Escherichia Coli* *Staphylococcus epidermidis* *Streptococcus mutans* *Enterococcus faecalis*	broth microdilution & one way ANOVA	-** + + + +	Eslami et al., 2016
Brazil	Bacteriocins from *Lactobacillus plantarum* ST8SH	*Listeria monocytogenes strains*	broth microdilution	+	Todorov et al., 2017

* high concentration resulted in antagonistic interactions.
+ indicates synergy
-**Additive (no significant increase or decrease in the MIC)
++ indicates stronger synergy.

Natural products are known to possess a wide variety of bioactive compounds which have and can contribute to the development of new drugs and enhance the efficacy of other drugs [56]. For instance, the essential oil of citrus lemon and *Cinnamomum zeylanicum* when combined with amikacin against multidrug-resistant *Acinetobacter* spp reduced the MIC of amikacin from 625µg/mL to 312.5µg/mL and 39.0625µg/mL respectively [60]. Furthermore, in table 2 garlic and oregano were shown to potentiate the efficacy of propolis against MRSA at low concentration [61]. Many researchers have attributed the successful combination of these products to the presence of various diversified bioactive compounds, which interact with each other in a way that limits the development of resistance in the microbes [61].

In spite of this knowledge, there is a scarcity in the number of studies analyzing the possible synergy between propolis and other natural compounds and those that have analysed its synergy focused mainly on its interaction with honey and less on plants. This may be as a result of the challenges involved in identifying compounds that have synergistic interactions with low risk of harmful drug-drug interaction that are effective against DRM [62]. Despite these challenges, it is suggested that as the interest in propolis increases, more researchers should focus on the possible interactions of propolis with natural's products namely plants and focus on addressing the issues relating to the unpredictable pharmacokinetics of multi-drug components [62].

Future Perspective of Propolis

In light of the various biological properties of propolis and its synergistic capabilities with a wide range of compounds, various researchers are analyzing its potential application in different industries, including the food and agriculture industry.

Food Industry

As a result of propolis antimicrobial efficacy on a wide variety of pathogens, a number of studies have been published on the development of active packaging formulated, using propolis-chitosan films and polylactic acid films with ethanolic extracts of propolis as active agents to extend the shelf-life of products and as an alternative to non-biodegradable packaging [63, 64]. In both experiments, the researchers noted on the bactericidal effects of the films on microbes and the durability of the films.

Agriculture Industry

Corresponding to the ban by various countries on the use of antibiotics in animal food, the possible use of propolis in animal feed was investigated and it was shown to increase the performance of hens and broilers with minimal effects on the intestinal flora of the animals [65]. In addition, its efficacy as a pesticide for the elimination of common plant pathogens was investigated [66]. The result indicated that propolis combined with chitosan polymers was effective at inhibiting the germination of the rust fungus (*Hemileia vastatrix*) while propolis alone inhibited 54.4% of the fungus growth at a low concentration of 0.2mg/ml [66]. Although research into this area is still at the development stage, the results obtained seem promising.

Discussion

As a result of the alarming increase in the development of DRM and the decrease in the efficacy of various antibiotics(WHO) 2017, the search for and development of new drug is becoming more paramount. Two approaches were proposed in this study, one being the utilization of natural product and the other being the use of combination therapy [4]. It is well documented that natural product possesses a wide range of bioactive compounds which decreases the development of resistance in microbes [61]. However, combination therapy is more widely applied due to the reduced dosage intake and toxicity.

In this study, the prospect of using propolis in combination therapy was discussed and it was shown that all propolis samples regardless of their geographical origin displayed antimicrobial proficiency ranging

from bacteriostatic to bactericidal, with greater activity observed in Gram positive bacteria than Gram negative [4, 50]. Furthermore, the combination of propolis with other natural antimicrobial compounds was shown to display synergistic effects resulting in low doses of both compounds with greater efficacy. However, there are certain limitations to propolis uptake, such as the extreme variability in its constituent profiles which leads to varying biological activity and varying synergy with antimicrobial compounds, the limited knowledge on the pharmacokinetics of multi-drug components and the negative effects of drug-drug interactions [67-70].

In conclusion, it is suggested that in light of the increasing DRM, the effective combination of propolis and other antimicrobial compounds could aid in the treatment of ARM diseases and pave the way for new drug development. This can be achieved through the increased research into propolis synergism and its mode of action with antimicrobial compounds namely plants, the development of standards for the chemical composition of different propolis from different regions and the study of the pharmacokinetics of multi-drug components and the negative effects.

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