Propolis Composition and Applications in Medicine and Health

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Propolis Composition and Applications in Medicine and Health

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Abstract— Propolis is a promising natural product that have been widely studied and investigated for health and medical benefits. It appears as a sticky, greenish-brown compound found on the trees and evergreens formed by the sap, bee discharges and bees wax. Propolis is used to line the beehives. The high content of flavonoids in propolis has contributed tremendously to the traditional use of propolis for many illnesses. The variation in chemical and biological composition is due to the type of propolis and locations of the source. Various methods have been used for propolis optimization based on its chemical and biological properties. The side effects of chemical drugs and toxicity is one of the reasons a natural product like propolis is gaining popularity to treat various medical conditions. Propolis has been investigated for its anti-bacterial, anti-fungal, anti-tumor, antidiabetic, and as a treatment for allergy, bronchial asthma and gastric disorders. The promising results from these research areas have paved the way to characterize propolis and elucidate the pathways to explain its mechanism of action. This review will highlight propolis production, its chemical and biological composition and its main biological functions; with a special focus on propolis as a wound healing agent.

Keywords: Propolis, antibacterial, antitumor, antifungal.

1. Introduction

The management of honey bees is known as apiculture or beekeeping [1], which started as a modern initiative in 1970s. The impact of bee products such as honey, pollen, bee bread, royal jelly and propolis on health have been well documented. It is available as food products, supplements or medicine. Bee products are added to increase the nutritional value of food products [2] or improve the immune system [3]; most likely attributed to the flavonoid contents [4]. The use of functional food is 6very important due to its positive impact on human health [2,5].

1.1 Propolis

The word propolis is derived from Greek 'pro' which means 'the entrance of' and 'polis' which means 'city' [5], aptly describing this bee product which has the function to guard the entrance to the beehives. Propolis is a sticky dark-coloured resinous material produced by bees to line the beehives from the inside. The composition of propolis is very different between sources and this is depending on the accessible types of plants to the bees [7]. So far, the chemical composition is affected by plant source, geographical area and propolis harvest season [6]. The bee species is also an important factor in determining the chemical and biological profile of propolis [7].

Physically, propolis appears from yellow-green to brown in colour, depending on its source and age [8]. Bees produce propolis by collecting resin from buds, skins, or other parts of the plant. Resins are then mixed with the bees' saliva and enzymes to become new resins; an elastic mixture of wax and flower pollen, which forms the propolis [9]. Propolis is a part of the bees' barrier mechanism against invaders. The defence process starts by sealing the internal walls, holes and cracks inside the beehives [10]. During adverse weather conditions, propolis is used to line the cavities, thus reducing the size of the entrance to the beehives. The propolis that is produced by stingless bee Trigona thoracica is known as "lebah kelulut" in Malaysia. It has many medical and health benefits on the immune system, possess anti-bacterial activity [11], anti-inflammatory [12] and anti-oxidant effects [13]. Stingless bees produce high amount of propolis and low quantity of honey in comparison to other bees [14].

Propolis consists of resin (50%), wax (30%), oils (10%), pollen (5%) and additional phenolic compounds like flavonoids [15]. Propolis also contains amino acids, glucose, Vitamin A, B, C, D and E and minerals and butyric acid [16]. Other than Trigona spp., propolis may be produced by other bees like Apis spp. [17]. However, production of propolis is through mixing of saliva and food substances like pollen, amino acids, vitamins and biofalvanoids [18]. The physiochemical components are different between propolis types based on the producing bee. For instance, Malaysian propolis from Genitrigona thoracica has low moisture and this criterion is very useful for storage because it minimizes the chance of bacterial, yeast and fungi. Ash value is used to evaluate the quality and purity of the crude sample, for Genitrigona Thoracica propolis the ash value is high (4.11-5.99%). This percentage indicates the presence of impurities like carbonate, oxalate and silicate. Protein content is 2.17-3.87% [7].

1.2 Propolis Composition

Chemical composition of propolis greatly affects its biological activities [19]. Bees utilize multiple natural vegetations for propolis production which causes variations in its chemical compounds, based on the specificity of local flora, plant sources, geographical area and collection season [6, 16]. The biological functions of propolis are closely related to the presence of active compounds like flavonoids, phenolic acid and terpenoids. Studies on propolis around the world suggested that propolis might compose of at least 300 constituents [20]. Nazir et al. studied the chemical constituents of Geniotrigona thoracica (MGT) propolis collected from Kota Bharu, Kelantan, located at the north-eastern region of Malaysia [21]. Ethanolic extract of propolis (EEP) was analyzed by gas chromatography–mass spectrometry (GCMS). Through library searching on Wiley 275 and NIST 02 mass spectral databases, 30 compounds were found in "kelulut" propolis for the first time out of 48 compounds which had been identified. The main compounds were phenolic and terpenoids which have good anti-inflammatory effects [22]. Steroids came in third in the form of cyclohexanol, which has been shown to have hyperglycaemic effect [23].

Propolis components differ based on the bee species and site of collection. A study showed the profile of methanolic extract propolis from two main Malaysian stingless bee species Heterotrigona itama (MHI) and (MGT) was investigated by looking at the physiochemical, phytochemical, total phenolic content profiling and biological activity [7]. Although propolis is collected from the same collection center and the hives are close together, a marked difference on physiochemical content had been demonstrated such as differences in moisture, fat, crude, fibre, protein and ash content. A study from Cameroon showed that the moisture content is higher in MHI compared to MGT extracts. Lower moisture content makes MGT stronger in preventing bacterial, fungal and yeast growth among storage period. Terpenoids, flavonoids and essential oils are common in both extracts, but steroid, saponin and coumarin are only present in MHI. Thin layer chromatography analysis showed chemical composition of propolis extract from MHI has more antioxidant activity than MGT. This landmark study suggests that applications of stingless bee propolis in medical and health need to be tailored to the chemical and biological profiles of the bee species [24].

2. Applications in Medicine and Health

Biological functions of propolis is strongly related to its benefits in health and medical conditions. Propolis has multiple and varied biological functions such as antimicrobial [25], antidiabetic [26-28], anticancer [29, 30], anti-inflammatory activities [31, 32], antiulcer [33], antioxidant [34] and antitumor properties. These diverse effects promote propolis as a key player in wound healing [35].

2.1 Antibacterial activity

The phenolic compounds and flavonoids are playing an important role in the antibacterial activity of propolis. Propolis stimulates the immune system to act against microorganisms and it may have direct effect on the microbes [37]. Working on the assumption that different propolis would have different potency and antimicrobial characteristics, mixing two different propolis samples showed a better antimicrobial and wound healing activity (39). Minimum inhibitory concentration assessment was measured and compared with the effect of individual propolis to treat wound healing in rabbits. A propolis extracts from Babil collected during spring was compared to propolis from Dhiala area during summer, and tested against Escherichia coli, Staphylococcus aureus, and Candida albicans. This study concluded that propolis from different geographical areas gave different and improved wound healing rate and antimicrobial activity, thus giving a new standpoint to therapeutic and commercialization strategies [38].

On the forefront of tissue-engineering technology, antimicrobial efficiency of propolis was studied by mixing propolis extract with polyvinylalcohol (PVA) polymer solutions, then electrospun onto polypropylene non-woven fabric. In vitro experiments showed that antimicrobial efficiency of extract-containing nanocomposite samples was better than control. Nanocomposite fabrics with propolis extract provided antimicrobial effect against gram positive bacteria (S. aureus) but not to gram negative bacteria (A. baumannii and P. aeruginosa). This indicated that electrospun PVA/propolis extract nanocomposites were effective in infected wound by gram positive bacteria [39]. Turkish propolis extracted in Azmir, Turkey, showed antibacterial effect against Gram positive (Staphylococcus aureus, Bacillus cerius) and Gram-negative bacteria (Pseudomonas aeruginosa and Salmonella enteritidis). This study proved that the extraction materials have different level of antibacterial activity, whereby the ethanolic extract propolis was more effective than acetone extract [40].

2.2 Antifungal activity

Antifungal activity of propolis extract from Trigona spp and Apis millifera was weakly demonstrated in denture stomatitis samples [41]. The antifungal effect of propolis was supported by another study against yeast isolated from blood culture [42]. Siquera, et al. evaluated the antifungal activities of red propolis alcoholic extract against candida species isolated from chronic peridontitis patients. Fluconazole treatment of Candida parapsilosis was used as a control for evaluating the fungicidal and fungistatic activity of propolis. All Candida species were susceptible to red propolis alcoholic extracts except five samples of C. albicans, one sample of C. tropicalis and one of C. glabrata. They suggested that propolis is potential to treat diseases affected by the above Candida species [43].

Sariguzel et al. demonstrated a significant antifungal activity of propolis samples collected from Kayseri, Turkey, against yeast isolated from blood cultures (43). Yeast from blood cultures was isolated from intensive care patients. Toxic effect of antifungals is one of the main setbacks in the current treatment process. It was demonstrated that the sensitivity of propolis against yeast is comparable with fluconazole and itraconazole.

2.3 Anticancer

Propolis can induce apoptosis in cancer cells which is demonstrated by cell cycle arrest. The main components involved in this process are caffeic acid phenethyl ester (CAPE) and chrysin. The induction of apoptosis is through many proteins activation like Bax, P53, P21, P38 MAPK, and release of cytochrome C which leads to caspase cascade activation [29]. Egyptian propolis was also shown to demonstrate anti-cancer effects [44]. In an in vivo Ehrlich ascites carcinoma (EAC) experimental mice model, Egyptian propolis was characterized and showed high content of flavonoids and dihydroflavanol and total phenolics. The expression of Bax, caspase-3 and cytochrome C was elevated, and Bcl-2 expression was downregulated, resulting in reduced tumour volume. The renal toxicity parameters were improved by EEP in EAC-bearing mice treated with methotroxade. To test the DNA damage inhibitory effect of propolis, a study was conducted in Azmir, Turkey. Increasing the concentration of propolis improved its protective effect against hydroxyl radical-mediated DNA damage [40], which might be due to propolis antioxidant activity [45].

2.4 Dentistry

Propolis as a natural product which possess antibacterial, antifungal and anti-inflammatory functions, has encouraging success for oral conditions [46]. Addition of propolis in toothpaste has shown a distinctive anti-bacterial effect in prevention of gingivitis due to oral plaques [47]. The effect of Egyptian propolis in mouthwash in children with high risk of developing caries has been tested. Propolis mouthwash reduced the bacterial count significantly and reduced plaque accumulation after 3 weeks of use [48]. Propolis as an active component showed its potential in maintaining oral cavity hygiene. Propolis improved gingival health as propolis toothpaste had shown reduction in gingival inflammation [49].

2.5 Antidiabetic

Various studies have explored the role of propolis as an antidiabetic agent. Kang et al. in 2010 recommended that propolis may be a potential antidiabetic agent for the treatment of insulin-insensitive diabetes. In an in vitro study using liver hepatocellular carcinoma cells (HepG2 cells), propolis reduced the expression of the enzyme glucose-6-phosphatase (G6Pase) by autophosphorylation process [50]. In a streptozotocin-induced type 1 diabetic rats model, EEP demonstrated antidiabetic as well as lipid lowering effect; which could be attributed to its strong antioxidant activity based on the results of pancreatic lipid peroxidation and superoxide dismutase level [51].

2.6 Bronchial asthma

Propolis and its products have been traditionally used to treat respiratory problems for centuries. In an immunised ovalbumin BALB/c murine asthma model, propolis hydroalcoholic extract from Scaptotrigona aff. postica stingless bee by gavage showed reduction in the cell number in the bonchoalveolar fluid as a result of reduced migration of inflammatory cells to alveolar space [52]. Histological evidence of reduced peribronchovascular inflammation and polyphormonuclear cells may pave the way for propolis to be an alternative therapeutic agent for bronchial asthma.

2.7 Gastric disorders

Helicobacter pylori is a major cause of peptic ulcer disease and gastric cancer, which can severely affect morbidity and mortality. The concern for emerging antibiotic resistance to H. pylori prompts researchers to explore propolis as an agent to speed up ulcer healing, prevent recurrence and eradicate the infection. Antiurease activity of 15 different EEP was studied in H. pylori strain J99 which revealed significant inhibition of the enzyme activity [53]. Food containing propolis was also shown to have a positive effect on the intestinal barrier function through the activation of AMP-activated protein kinase (AMPK) and extracellular-signal-regulated kinase (ERK) signaling pathways [54].

3. Propolis for Wound Healing

Wound healing in the skin involves a complex process which happens as the skin structures undergo repair after an injury. Briefly, the tissue goes through four overlapping phases which are hemostasis, inflammatory, cellular proliferation and tissue remodeling. Several clinical trials involving propolis have been conducted to evaluate its effects on wound healing in humans and animals. Propolis applications include several methods and indications:

3.1 Wound dressing materials

The physical properties of propolis make it advantageous for closing of wound. A study in 2015 evaluated the role of propolis in cutaneous wound healing in adult mongrel dogs. Cutaneous wound healing involves inflammatory cells, fibroblasts, keratinocytes and endothelial cells. Propolis paste was demonstrated to improve cutaneous wound healing process in dogs in a time-dependent manner. The acceleration of wound healing is believed due to the immunomodulatory, antimicrobial, antioxidant, analgesic and anti-inflammatory effects of propolis [35]. A comparative study in Turkey in 2005 between propolis and silver sulfadiazine in the treatment of burns in Wester Albino rats found that topical application of propolis is effective in controlling the infection and produced a clean granulation tissue [55]. In another clinical study in rats between three treatment group (propolis, silver sulfadiazine (SSD) and cold cream as control), histologic evidence showed propolis group was less inflamed compared to SSD. A mixture of propolis cream treatment also showed significant improvement in wound healing [56].

In an in vitro comparative test between Malaysian and Brazilian red propolis on human fibroblastic cell line CRL-7522, Malaysian and Brazilian propolis showed excellent fibroblasts proliferation and migration in a dose-dependent manner [57]. For dental application, propolis was demonstrated to show anti-inflammatory properties by direct effect on the fibroblasts from periodontal ligament and dental pulp [58]. This finding signified that propolis was effective as intracanal treatment to replace a more toxic agent like calcium hydroxide. A randomized controlled study by Perkins and his colleagues showed that propolis accelerated post tonsillectomy wound, evident by reduction in pain and cessation of bleeding. When propolis entered a wound site with open epithelium, it deeply infiltrates into the tissue and stimulates cell regeneration and proliferation. It was proposed that the flavonoid in propolis prevents cicatrization, increases prostaglandin concentration, inhibits lipid peroxidation, and decreases reactive oxygen species. These processes would eventually facilitate epithelial regeneration and scar formation [60].

Another wound dressing application was studied in a streptozotocin-induced diabetic animal model using cellulose based film loaded with vitamin C and/or propolis. This novel eco-friendly film represented a new therapeutic approach to accelerate diabetic wound healing. Extracted cellulose (Cel) from rice husk using a facile protocol was blended with polyvinyl alcohol) (PVA) to form films by casting method. Vitamin C and/or propolis was encapsulated in the Cel-PVA matrix. The films improved wound healing in diabetic mice without altering the pathological condition [61]. It was proposed that propolis in Cel-PVA matrix reduces H-bonds density and help in drug release and liquid uptake which acted favorably in wound healing process. The effects of a single application of propolis in complicated foot ulcer and poor wound healing in diabetic patients were evaluated by rate of epithelial closure, wound morphometry, cellular infiltrate, and blood vessel branching density [62]. Increased rate of reepithelization and accelerated wound healing in the propolis group was significantly demonstrated compared to control group.

Propolis treatment showed normalization of neutrophilia caused by persistent inflammation in diabetic rats. This effect was attributed to antioxidant activity of propolis by inhibiting myeloperoxidase activity in the dermis. Neutrophil and macrophage infiltration in the wound also paved the way for early tissue remodeling. The clean granulation tissue formation was significant in the propolis treatment group [63]. This was a landmark study in animals or humans showing that propolis improved wound healing in diabetic subjects [62].

3.2 Drug delivery agents for wound healing

Addition of propolis in the fabrication of scaffolds is a potential drug delivery system. In a study comparing diclofenac and propolis on gelatin scaffolds, propolis was shown to inhibit bacteria strongly due to its phenolic compounds. Propolis showed an acceptable drug release due to the presence of many hydrogen bonds and hydrophobic interactions between gelatin and the propolis compounds such as capric acid, azulene, naphthalene, and others. There are strong intermolecular bonds formed between the compounds which makes this system potential for site specific sustained release of drug [64]. There were a few studies investigating the antimicrobial effects of propolis in combination with biological scaffolds. Oliviera et al. combined propolis with hydrogel as a 3D complex. In this study they used PVA which is non-toxic and showed high mechanical strength and high swelling capacity. PVA in combination with 15% propolis gel acted as a good antibacterial agent against Staphylococcus aureus. PVA–propolis samples acted as barriers to microbial penetration [65].

To encounter a highly morbid incidence of pressure ulcers, the use of biocompatible and biodegradable polyhydroxybutirate and chitosan was evaluated for tissue regeneration and drug delivery [66, 67]. The aim was to produce a porous scaffold which has the capacity to absorb moisture and delivering propolis in ulcer treatment. A synthesized electrospun mats of propolis and silver nitrate also showed that propolis displayed excellent antimicrobial activity, emphasizing the role of propolis as a drug delivery system [68]. A recent study aimed to explore a wound healing function of propolis. They established a scratch wound healing model using human immortalized keratinocytes and they found that propolis increased the wound healing in keratinocytes. They explored the reason of this positive effect and they found that propolis generated reactive oxygen species (ROS) which diffuse through the plasma membrane via aquaporin 3 (AQP3) leading to intracellular response modulation. Based on previous studies they found that AQP3 can mediate the H2O2 transportation in different tissues.

A recent study highlighted the use of a hybrid membrane from natural rubber with propolis extract to study wound healing in a rat burn model. The hydrophilic and porous nature of the rubber-propolis membrane was shown to be effective in the treatment of second degree burns. Hydrophilicity is a polymer property which displays the ability to absorb exudates, and porosity prevents trapping of air bubbles beneath the membrane [69]. Another in vivo study in New Zealand White rabbits compared the wound healing process by using calcium hydroxide paste and Thai propolis as pulp capping agent after pulpotomy (71). The pulp capping agent makes the dentinal tubules in the dentin bridge of the teeth to have a linear arrangement than in the group of calcium hydroxide paste. Clinically, there was no significant difference in wound healing between propolis and calcium hydroxide paste. However, histological evidence proved that propolis promote mineralization process and angiogenesis [70].

4. Conclusion and Future Directions

There are various methods to optimize the use of propolis in health and medicine by utilizing its biological and chemical properties. Characterization of the chemical constituents to enhance the therapeutic values of a stingless bee propolis is a research area which needs more investigations. Inconsistent composition and biological activity of propolis due to seasonal variation and location are among its limitations. As a way forward, optimization of propolis composition, standardization, and further investigations into the mechanisms by which propolis exert its biological effects are potential research areas. Advancement of research in this area including animal and human clinical trials which will pave the way for more innovative treatment strategies; especially in complicated wound healing and skin regeneration.

5. Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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