Chemical composition and pharmacological aspects of Malaysian stingless bee propolis: An up-to-date systematic review

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Received November 20, 2023; Accepted February 28, 2024

DOI: 10.3892/br.2024.1887

Abstract. Propolis is a sticky substance produced by stingless bees for construction and defence of their hive. It has notable anti-inflammatory, antioxidant, antibacterial, anti-fungal, anti-hyperglycemic, and wound healing effects. The present review summarised and examined the phytochemical properties, mode of action and current research prospects of Malaysian propolis. A database search using Google Scholar, Web of Science and ScienceDirect generated 780 references; 30 relevant articles were included in the present review, of which 23 were *in vitro* studies and 7 were *in vivo* or animal studies. Propolis demonstrated antioxidant, antibacterial, antifungal, anti-inflammatory and anti-hyperglycemic properties, indicating potential as a wound healing agent. Despite favourable findings, due to the scarcity of studies in the literature, more in-depth research and clinical validation on the synergistic effects, efficacy and optimum dosage of propolis are needed.

Introduction

Honey and stingless bees (*Apidae Meliponini*), called 'lukut' in Philippines, and 'damar' in India (1), generate propolis, a naturally occurring resinous substance, by gathering exudates and materials from plant parts, including flower buds, tree bark and leaf buds, and combining them with beeswax and enzymes (2-4). The active constituents of propolis are determined by the local flora (5). Bees use propolis to build and maintain their hive to seal holes and fissures and smooth the internal walls because of its waxy structure and mechanical characteristics. Propolis has been an essential part of apitherapy. More recently, it has also been used as a food additive or supplement in alternative and traditional medicine (6). Natural products produced by stingless bees, such as propolis, honey, beehives and pollen exert pharmacological effects and used as traditional medicine by many Asian cultures (4). Propolis exerts antibacterial, antioxidant, antiseptic, anti-inflammatory, antifungal, hepatoprotective, and immunomodulatory effects (7). The present systematic review aimed to analyse the possible usefulness of propolis as a preventive and therapeutic method based on *in vitro* and *in vivo* research.

Materials and methods

Google Scholar (8), Science Direct (7), and Web of Science (WoS) (6), were searched for studies assessing and reporting the biological activities of Malaysian propolis. The search strategy was 'propolis' AND 'Malaysian' for Science Direct and WoS. For Google Scholar, the 'Advance Search' option was used to discover papers that contained the words 'propolis, Malaysian,' 'Malaysian,' or 'Malaysia,' but not 'systematic review, meta-analysis, or review.' Studies on the biological activities of Malaysian propolis conducted *in vitro* and *in vivo* were included. PRISMA 2020 flow diagram for new systematic reviews which included searchers of databases and registers was used (7). Only full-text studies published in English between January 2012 and June, 2023 were included. Review papers and research that included meta-analyses were not included in. The present review did not include any studies on non-Malaysian propolis. Two authors completed the data extraction, which included the main author, year, bee species, location, propolis preparation, study type and biological activities.

Results and Discussion

Database search. WOS is considered the global leading platform for scientific citation search and analytical information (9). To ascertain whether Google Scholar can be utilized as a reliable source of scientific information and data for scientific evaluation, a previous study (10), reviewed

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Key words: propolis, pharmacological, antioxidant, anti-inflammatory, antibacterial, anti-hyperglycemia, wound healing

91 comparative articles from 2005 to 2016 that compared Google Scholar with various databases, particularly WOS, and revealed that Google Scholar is a powerful database of scholarly literature, having broadened its scope over the years. PubMed is the most commonly searched database for systematic reviews (11). However, Pubmed resulted in a very small number of articles (n=19) in the present preliminary search and most of the articles were identical to with the results of Google Scholar, ScienceDirect and Web of Science. Similar to PubMed Single Citation Matcher, the ScienceDirect advanced search function allows user to search by author, title, volume, issue, and page (12). Therefore, the present review utilized three databases for literature search, namely Google Scholar, ScienceDirect and Web of Science.

In total, 780 records were identified from the first primary database search, of which 777 remained after duplicates were eliminated (Fig. 1). After the 777 titles were filtered, 746 were removed (irrelevant article title); 31 selected for further inspection and the full texts were then obtained. Then, 30 entries were chosen from a full-text review to be included in the review, and one was omitted (non-English language article). Of these, 23 studies were conducted *in vitro* and seven were conducted *in vivo* or on animals (Table I).

Phytochemicals properties of propolis. Generally, propolis is rich in various bioactive compounds, such as fatty, aliphatic and aromatic acids, flavonoids, terpenoids, sugars, alcohol and esters. The chemical composition of propolis is affected by its geographical location as well its botanical origin as the resin from different plant species may contain various compounds (13). In Malaysia, 78 stingless bee species have been discovered, including *Geniotrigona thoracica*, *Heterotrigona itama*, *Tetrigona apicalis* and *Tetragonilla atripes* (14). *H. itama* is the most common stingless bee species in Malaysia (15). *H. itama* favours *Averrhoa carambola* and *Antigonon leptopus* because both flowers produce notable amounts of nectar, and their morphology is compatible with *H. itama* tongue morphology (16). Hydrocarbons and oxygenated sesquiterpenes derivatives, such as β-caryophyllene, copaene, cyclohexane, 1H-cycloprop[e]azulen-7-ol and β-caryophyllene oxide, are detected in *T. apicalis* propolis from Malaysia by gas chromatography-mass spectrometry (GC-MS) analysis. *T. apicalis* propolis also contains triterpenoids, such as α-amyrin and β-amyrin (17). The GC-MS chromatographic analysis of *G. thoracica* propolis from Malaysia demonstrates presence of phenol, benzoic acid, trimethylsilyl ester, hydroginkgol, resorcinol, Δ-cadinene, nootkatone, β-amyrenol, friedelany-al, cycloeucalenol and myristic, palmitic linoleic and octadecanoic acid (18). A recent study by Syed Salleh *et al* (19) revealed the prevalence of some classes of compounds in *T. apicalis*, *T. binghami* and *Homotrigona fimbriata* propolis from Malaysia. The identified components included phenolic (Gallic acid, Vanillin, p-coumaric acid, Quercetin, Pinocembrin Artepillin C methyl ester, Naringenin, Catechin, Epicatechin), terpenoids (mangiferolic acid, Cycloartenol, Ambonic acid, Mangiferonic acid, Ambolic acid, amyrin), Prenylated benzophenones (7-epi-nemorosone, Xanthochymol, Guttiferone C, Gambogenone and Aristophenone A), carboxylic acids, sugar alcohols, hydrocarbon, aldehydes and amino acids when analysed using GC-MS. Therefore, Malaysian propolis is considered as terpenoid-type propolis. The chemical structures of bioactive compounds in propolis are shown in Fig. 2.

Pharmacological activity and mechanism of action of propolis

Cytotoxic activity. Cytotoxicity refers to the capacity of a molecule or compound to result in cellular damage, which may involve damage to specific cell structures or the essential functions that keep cells alive, such as cell division, survival and normal physiology (20). Extracts of *H. itama* propolis from different locations possess low to moderate cytotoxic effects against HeLa cells with half-maximal inhibitory concentration (IC_{50}) value ranging from 14 to 60 μg/μl (21). Propolis extract induces apoptosis in HeLa cells in a dose-dependent manner. Cytotoxic activities of propolis extract are also species-dependent. Propolis produced by *H. itama*, *G. thoracica*, *L. terminate* and T. *apicalis* have been extracted and evaluated for their cytotoxicity against three cancer cell lines (22). *H. itama* propolis extract demonstrates the highest cytotoxic activity against MDA-MB-231, SK-UT-1 and HeLa cells, with IC_{50} values of 5, 4 and 8 μg/ml, respectively (22). It is proposed that the capacity of terpenoid compounds to impede proliferation, induce apoptosis and inhibit metastasis makes them useful against tumours and inflammation (22). Another study demonstrated that propolis extract from *T. apicalis* exerts cytotoxic activity against breast cancer cell lines in a dose- and time-dependent manner (17). IC_{50} values of *T. apicalis* propolis extract were reduced with longer incubation time in MCF7 cells. However, in MCF 10A cells, longer incubation time increased the IC_{50} values of the propolis extract (17). MCF7 is an Estrogen Receptor, Progesterone Receptor (PR)-positive, and Human Epidermal Growth Factor Receptor 2 (HER2)-negative breast cancer cell line while MCF 10A is originally non-tumorigenic cells. The effects of propolis on cell proliferation of MCF7 and MCF10A were cell type-dependant, thus the activity is reduced in MCF7 cells and increased in MCF 10A cells. At incubation up to 72 h, *T. apicalis* propolis extract demonstrated selectivity, with a high selectivity index (SI) of 2.20 (17). SI is an important indicator to evaluate the toxicity of a compound or extract against normal cells, and to predict their therapeutic potential on cancer cells (23,24). FITC Annexin V with flow cytometry is one of the most powerful tools for quantitative determination of the percentage of cells that are actively undergoing apoptosis within a population (25). A previous study utilized this method to evaluate apoptosis induction by *T. apicalis* propolis extract in MCF7 cells (26). At IC_{50} of 32.70 μg/ml and 72 h incubation with MCF7 cells, the percentage of apoptosis induction by propolis extract in viable, early and late apoptotic and necrotic or dead cells corresponded to 48.39±2.06, 14.02±0.98, 35.25±1.16 and 2.34±0.14%, respectively (26). It was suggested that the antioxidant properties of propolis extract are partly responsible for apoptosis induction in cancer cells (26). Apart from cytotoxicity study in mammalian cells, there was also a study that tested *H. itama* propolis extracts using the Brine shrimp lethality test; extracts showed a low level of toxicity (15).

Antimicrobial activity. An antimicrobial substance generally eliminates or prevents the growth of bacteria. Antimicrobial substances can be microbiostatic, which prevents microbial development, antibacterial, which fights

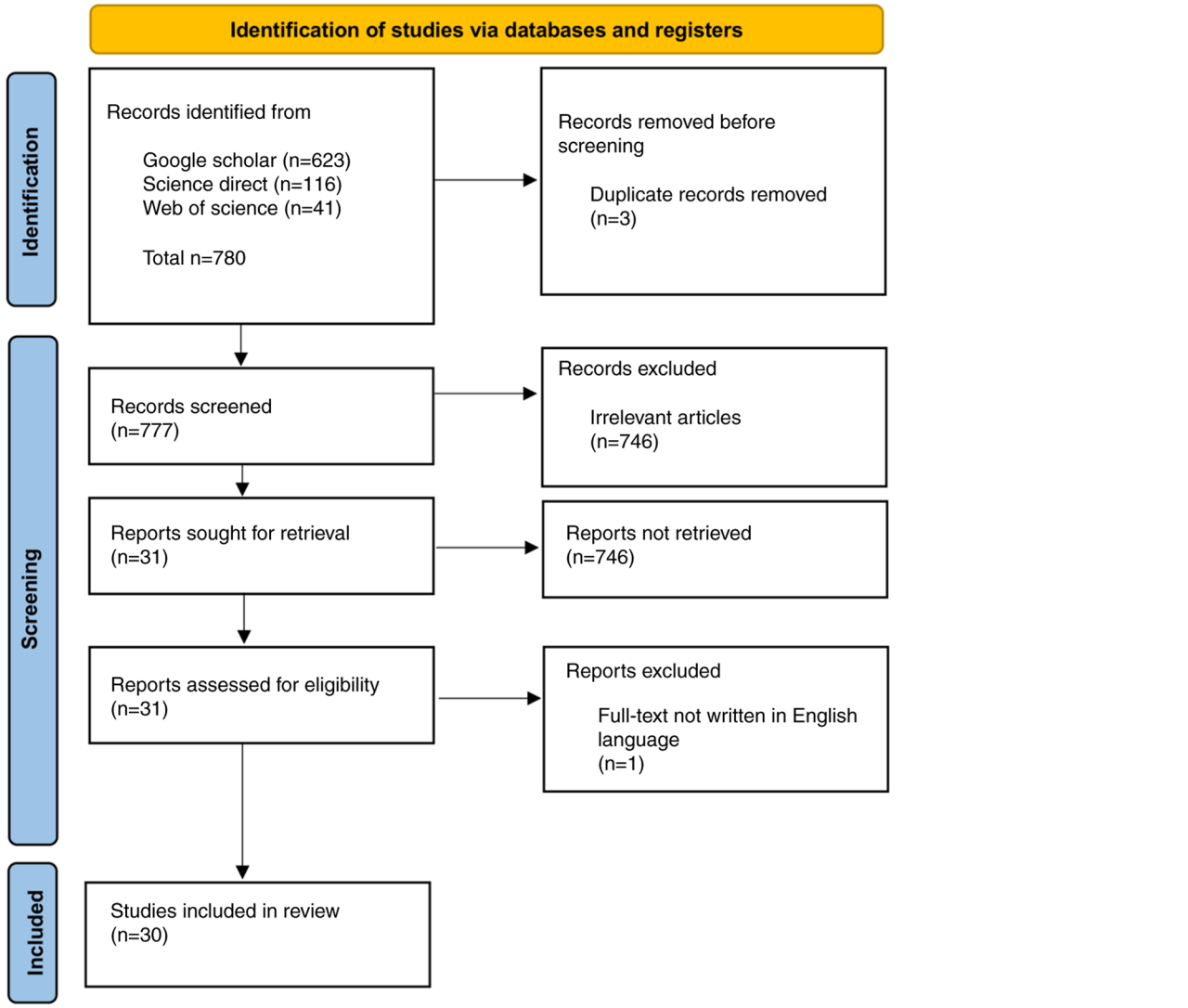

Figure 1. Flowchart of identification and selection of study articles.

bacteria, or antifungal, which fights fungi (27). Propolis extract from *Trigona spp*. exerts antifungal activities against oral *Candida albicans*, *C. tropicalis* and *C. glabrata* with minimal inhibitory concentration (MIC) of 500 mg/ml (28). Limited studies have reported the antifungal activities of propolis extracts (28,29). However, there are numerous of studies that evaluated their antibacterial effects (30,31).

A study utilized ethanol and water as solvents to extract *T. thoracica* propolis, then evaluated the antimicrobial activity of the extracts against *Staphylococcus aureus* using broth microdilution method (31). Ethanolic extract of the propolis exhibited the strongest antimicrobial effect due to its higher content of phenolic compounds, such as quercetin (31). At 1 mg/ml, *H. itama* propolis methanolic extract demonstrated the highest antimicrobial activities against *S. aureus* and *E. coli*, both with an inhibition zone of 10 mm, in comparison with hexane extract and ethyl acetate extract (<10 mm) (15).

Antibacterial activity of *H. itama* propolis and *G. thoracica* propolis extracts has been evaluated against Gram-positive bacteria, such as *S. aureus*, *Bacillus subtilis*, *Enterococcus faecalis* and *Listeria monocytogen*, as well as Gram-negative bacteria, such as *Acinetobacter baumannii*, *Salmonella typhi* and *E. coli* (30). It was found that the extract from *H. itama* propolis demonstrates better inhibition against the strains (*S. aureus*, *B. subtilis*, *E. faecalis* and *L. monocytogen*, *A. baumannii*, *S. thyphi* and *E. coli*) with an inhibition zone of 6-14 mm compared with *G. thoracica* propolis extract (6-7 mm) (29). Both propolis extracts exhibited greater inhibitory effect against *S. aureus* (Gram-positive) than *E. coli* and *S. thyphi* (Gram-negative) (30). The aforementioned study suggested that the antibacterial activities of propolis were species-dependent and affected by the polar phenolic compounds of the extracts (30). These results were supported by another study that reported better antibacterial activity shown

Table I. *In vitro* and *in vivo* biological activity of Malaysian propolis.

First author/s, year	Bee species	Location	Propolis extract	Study type	Biological activity	(Refs.)
Chew *et al*, 2014	N/A	N/A	2.50% ethanol	*In vitro*	Increases stem cell proliferation	(32)
Jacob *et al*, 2015	*Trigona spp*	Gurun, Kedah	80.00% ethanol	*In vitro*	Enhance the Wound healing activity against normal human fibroblast cell line CRL-7522	(33)
Ibrahim *et al*, 2015	*Heterotrigona itama; Geniotrigona thoracica*	Besut, Terengganu	100.00% methanol	*In vitro*	Possess antibacterial activity	(34)
Akhir *et al*, 2017	*Heterotrigona itama*	Parit Botak, Johor	100.00% hexane and 70.00% ethanol	*In vitro*	*Heterotrigona itama* propolis from southern Malaysia have antimicrobial and antioxidant activity	(35)
Yusoff *et al*, 2016	*Trigona spp*	N/A	100.00% water	*In vitro*	Weaker antifungal activity	(29)
Rosli *et al*, 2016	*Trigona apicalis*	N/A	100.00% Ethanol	*In vitro*	Possess high antioxidant activity with higher phenolic and flavonoids contents	(18)
Usman *et al*, 2016	N/A	Kota Bharu, Kelantan	100.00% Water and ethanol	*In vitro*	Exhibited higher antioxidant activity	(20)
Ahmed *et al*, 2017	*Tetratrigona spp*	Kuband Kerian, Kelantan	70.00% ethanol	*In vivo*	Direct cytotoxicradical-scavenging activity, which provides cardioprotective action against ISO-induced oxidative stress.	(15)
Azemin *et al*, 2017	*Heterotrigona itama*	Terengganu	100.00% Ethanol	*In vitro*	Unprocessed propolis has considerably higher antioxidant activity, regardless of extraction method	(36)
Ong *et al*, 2017	N/A	Pahang	100.00% Ethanol and ethyl acetate	*In vitro*	Chitosan-propolis nano formulation might be considered a possible anti-biofilm agent in fighting infections	(37)
Salim *et al*, 2018	*Geniotrigona horacica*	Kuala Kangsar, Perak	80% ethanol	*In vitro*	Active components in propolis contribute to its antioxidant properties	(16)
Lim *et al*, 2022	*Heterotrigona itama*	Besut, Dungun, Terengganu; Tanah Merah, Gua Musang, Kelantan	95% ethanol	*In vitro*	Propolis and metformin combination in reducing histological features of diabetic cardiomyopathy	(38)
Nna *et al*, 2018	*Heterotrigona itama*	Kelantan	70% ethanol	*In vivo*	Reduces hepatic lesion and has a synergistic protective effect	(39)

Table I. Continued.

First author/s, year	Bee species	Location	Propolis extract	Study type	Biological activity	(Refs.)
Usman *et al*, 2018	*Heterotrigona itama*	Kelantan	70% ethanol	*In vivo*	Improvement in pregnancy outcomes and placental oxidative stress	(31)
Asem *et al*, 2020	N/A	Kuala Kangsar, Perak	80% ethanol	*In vitro*	Demonstrated positive antioxidant activity	(40)
Annisava *et al*, 2019	*Heterotrigona itama*	Kelantan; Terengganu	100.00% Ethanol	*In vitro*	Contains the highest total phenolic, flavonoid content and antioxidant activity.	(41)
Nafi *et al*, 2019	*Heterotrigona itama*, *Geniotrigona thoracica*, *Lepidotrigona terminate; Tretrigona apicalis*.	N/A	95% ethanol	*In vitro*	*Heterotrigona itama* possessed the highest antioxidant activity compared to other species	(42)
Badiazaman *et al*, 2019	*Geniotrigona thoracica*	Besut; Dungun, Terengganu; Tanah Merah, Kelantan; Gua Musang, Kelantan	100.00% Methanol	*In vitro*	Possessed the highest total flavonoid content and antioxidant activity	(13)
Yusop *et al*, 2019	*Trigona itama*	Beladin, Sarawak	100.00% Hexane, ethyl acetate and methanol	*In vitro*	Antioxidant and antibacterial effects against both gram-positive and gram-negative bacteria	(30)
Nna *et al*, 2019	*Heterotrigona itama*	Kelantan	70% ethanol	*In vivo*	Reduced testicular oxidative stress, inflammation, and apoptosis in diabetic rats	(43)
Mohamed *et al*, 2020	*Tetrigona apicalis*	Tanjung Malim, Perak	80% ethanol	*In vitro*	Demonstrated antioxidant activity and suppressed the proliferation of MCF7 cells	(28)
Mohd Suib *et al*, 2021	*Geniotrigona thoracica*	Kuala Kangsar, Perak	80% ethanol	*In vitro*	Inhibition of the formation of THP-1 derived macrophage foam cells	(26)
Zainal *et al*, 2022	*Tetrigona apicalis*	Kuantan, Pahang	100.00% Water and 70 and 80% ethanol	*In vitro*	Exhibited strong antioxidant activity	(44)
Syed Salleh *et al*, 2021	*Tetrigona apicalis, Tetrigona binghami; Homotrigona fimbriata*	Selangor	100.00% Water	*In vitro*	Demonstrated greater antioxidant potential, with higher phenolic and flavonoid levels	(19)
Maroof *et al*, 2023	*Geniotrigona thoracica*	Negeri Sembilan	100.00% Ethanol	*In vitro*	Increased antioxidant activity and antibacterial efficacy against gram-positive bacteria	(22)

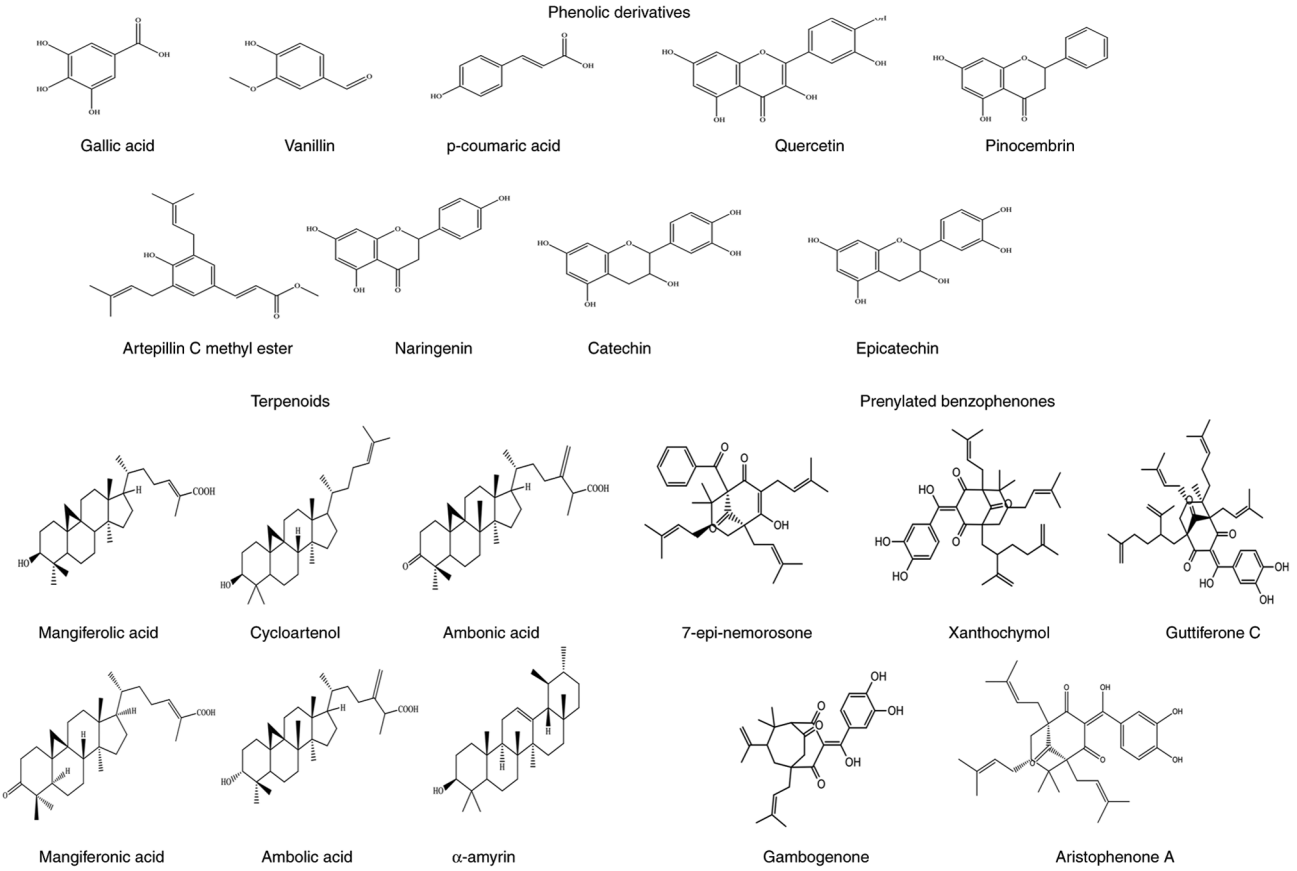

Figure 2. Representative chemical compounds founds in Malaysian propolis.

by propolis extract against Gram-positive bacteria (*B. cereus* and *S. aureus*) in comparison with Gram-negative bacteria (*E. coli* and *Salmonella*) (45). Extracts of *Acacia mangium* and *Garcinia mangostana* propolis demonstrated promising antibacterial effects against *S. aureus* with an inhibition zone of 20.00±0.1 and 24.00±0.52 mm, respectively, compared with erythromycin (24.80±0.72 mm) (35). However, there are no antibacterial activities shown on Gram-negative bacteria (*E. coli* and *P. aeruginosa)* by both propolis extracts (35). It is suggested that the lower susceptibility of Gram-negative bacteria may be due to lipopolysaccharides of the outer membrane that hinder the penetration of propolis antibacterial components into bacterial cells (35).

Antioxidant activity. Oxidative stress is an imbalance between the production and accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and the capacity to neutralize and eliminate them. Moderate concentrations of ROS and RNS are key for many physiological processes within the human body. Key endogenous antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). SOD converts superoxide anion to H_2O_2, a substrate for CAT and GSH-Px (46). When reacting with GSH, CAT metabolizes H_2O_2 in water and oxygen while GSH-Px lowers H_2O_2 and organic hydroperoxide levels (46). Several studies have investigated the antioxidant activity of propolis in *in vitro* and animal models (32,47,48). Propolis from UniSZA Apiary, Besut (BST-1) has the highest total phenolic and flavonoid content (TPC and TFC, respectively) and antioxidant activity (35). BST-1 extract with the highest phenolic content had higher antioxidant activity than the other localities (35). Another study compared ethanolic extract of propolis samples was produced from three different stingless bee species, *T. apicalis*, *H. itama* and *G. thoracica*, collected from bee farms in Perak, Malaysia (33). Among the species tested, *G. thoracica* had the highest antioxidant activity, with IC_{50} values of 206.27 for 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 64.98 mg/ml for 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay, respectively. This was consistent with another study on *A. mangium* and *G. mangostana*-derived propolis (35). The is no significant difference in the ABTS+ scavenging effect (*A. mangium*, 0.05±0.00; *G. mangostana*, 0.05±0.00 mg/ml) and metal chelating activity (*A. mangium*, 51.44±4.99; *G. mangostana*, 52.12±1.61 mg/ml) between the two propolis samples. However, SOD enzyme-like activity is considerably higher in *G. mangostana* propolis (0.17±0.01 mg/ml) compared with *A. mangium* (0.26±0.00 mg/ml) (35). The ethanolic extracts from propolis produced by *H. itama* in Terengganu, Malaysia, have the strongest antioxidant activity with an IC_{50} of 30 μg/ml and the highest percentage of inhibition with 85.69%, followed by *G. thoracica* with an IC_{50} of 40 μg/ml and 82.22% inhibition at 150 μg/ml concentrations. The *H. itama* scavenging activity is comparable to quercetin and trolox, with IC_{50} values of 10 and 9 μg/ml, respectively. On the other hand, *Lepidotrigona terminate* has poor antioxidant activity with an IC_{50} of 128 μg/ml and an inhibition percentage of 80.47% at a dosage of 500 μg/ml. *T. apicalis* demonstrates antioxidant activity with IC_{50} values >500 μg/ml. The differences in antioxidant activity may be attributed to the phenolic,

flavonoid or other components of propolis extracts, which have been associated with antioxidant capabilities (22). This finding was consistent with that of *H. itama* propolis from Besut, which had the best antioxidant activity with the lowest IC_{50} values (10.000±2.623), followed by Dungun (84.000±2.623) and Gua Musang (151.000±2.623 μg/ml) (21). The aforementioned experiment demonstrated that propolis from Besut had the highest antioxidant activity while propolis from Tanah Merah had the lowest antioxidant activity of DPPH scavenging radicals.

Soft propolis of *H. itama* (found inside the beehive) contains more phytochemicals, specifically flavonoids, phenols and terpenoids, than hard propolis (which forms part of the wall of the hive) (47). In the DPPH assay, the propolis samples demonstrate a dose-dependent increase in radical scavenging activity, with soft propolis *H. itama* exhibiting a significant effect (IC_{50}, 79.90±11.75) compared with hard propolis *H. itama* (180.00±16.67 μg/ml) (47). In addition, at a higher concentration of 1,000 g/ml, soft propolis *H. itama* demonstrated stronger H_2O_2 scavenging activity (53.94±1.88) than hard propolis *H. itama* (43.34±0.51%) (47).

The impact of processing and extraction methods on chemical profiles and antioxidant activity of propolis has been studied. In comparison with processed propolis (maceration, sonication, maceration-sonication), unprocessed propolis has more potent antioxidant activity with the lowest IC_{50} value. For the processed sample, raw propolis was heated at 1 h at 37˚C (42). Meanwhile, the unprocessed sample was retrieved fresh from the hives (21). Another study reported the antioxidant activity of *G. thoracica* propolis from different locations in Terengganu, Malaysia. It was demonstrated that propolis from Besut has the lowest IC_{50} value of 53 μg/ml, whereas propolis from Dungun had the highest IC_{50} value of 190 μg/ml and propolis from Lundang was inactive. The stronger the radical scavenging activity, the lower the IC_{50} value (22). Thus, propolis from Besut exerts the strongest antioxidant and radical scavenging properties. These differences could be attributed to changes in the chemical composition of the propolis extracts (49). In addition, the hexane extract of *H. itama* propolis from Johor, Malaysia, exhibits the highest Ferric Reducing Antioxidant Power value of 6.64 mM Ferrous Equivalent/g). These findings demonstrate that propolis is a potent natural antioxidative agent (45). In another study, ethanolic extract of *T. apicalis* inhibited ABTS+ radical with an IC_{50} of 1.68 mg/ml while the positive control (Trolox) used as a standard reference compound had a lower IC_{50} of 0.31 mg/ml (17). Another study demonstrated that the ethanolic extract of *G. thoracica* propolis in Perak, Malaysia has an IC_{50} value of 48.3±0.2 μg/ml using DPPH assay (50).

Furthermore, antioxidant properties of *T. apicalis* propolis extract are dose-dependent, with IC_{50} value for DPPH test of 4.27 mg/ml (51). The antioxidant properties of propolis extract are regulated primarily by its phenolic and flavonoid content. A similar pattern has been identified in which TPC and TFC concentrations were related to the antioxidant activity of *T. apicalis* propolis extract (14). For all extraction solvents, a significantly high correlation between antioxidant activity and TPC and TFC has been detected using maceration and ultrasound-assisted extraction. Furthermore, propolis extracted with 70% ethanol gave the highest extraction yield and had significantly higher radical scavenging activity, TPC and TFC than water extract of propolis (52). In addition, methanol (IC_{50}, 17.18 μg/ml) extract has the highest percentage of antioxidants compared with hexane (32.10), ethyl acetate (21.05) and ascorbic acid (30.63) (52).

In vivo, pre-treatment with propolis significantly improved SOD, GRx, GPx, and GST enzyme levels in rats (47). The effects of propolis supplementation on antioxidant levels and its mode of action in the aorta of diabetic rats have been examined; the propolis-treated group showed lower SOD/(CAT + GPx-1) ratios than the control group, indicating that the propolis has an antioxidative capability in avoiding hydrogen peroxide accumulation (53). In another animal study, propolis treatment in diabetic rats led to a significant decrease in the antioxidant status of pancreatic tissue. Specifically, the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GR), and catalase (CAT) were notably reduced compared to the control group (47).

Anti-inflammatory activity. Inflammation contributes significantly to the development of cardiovascular illness and other comorbidities, such as hypertension, hypercholesterolemia, type 2 diabetes, chronic renal disease and obesity (34). Another study aimed to investigate the anti-inflammatory effect and possible mechanisms of propolis in Sprague Dawley rats models. *In vivo*, proliferating cell nuclear antigen and IL-10 increased while malondialdehyde, NF-κB, TNF-α, IL-1 and cleaved caspase-3 decreased significantly in the propolis-treated diabetic groups compared with the diabetic control group (48). It has been reported that the elevated IL-17 levels are associated with better outcomes in patients with myocardial infarction (MI) caused by atherosclerosis (37). *In vitro* study also confirmed anti-inflammatory effects; cytokine secretion of TNF-α and IL-1b in supernatant of treated THP-1-derived macrophages was measured using ELISA. TNF-α and IL-1b secretion levels were significantly reduced in THP-1-derived macrophages treated with both oxidized Low-Density Lipoprotein (oxLDL) and ethanolic extract of propolis compared with THP-1-derived macrophages treated with only oxLDL at 6, 24, and 48 h. Ethanol extract of ≤200 ug/ml was used to avoid toxic effect on THP-1 derived macrophages cells. This finding indicates that ethanol extract inhibited the release of both of these pro-inflammatory cytokines (41).

Anti-hyperglycemia and MI activity. Type 2 diabetes mellitus (T2DM) is a type of diabetes characterized by high blood glucose levels, insulin resistance and a poorer insulin-stimulated response in the presence of high blood glucose levels compared with other forms of diabetes (39). In a rat study where diabetes was induced using intraperitoneal streptozotocin (60 mg/kg), the effects of propolis extract were investigated. The rats were administered either propolis alone (300 mg/kg/day), metformin alone (standard diabetes medication), or a combination of both (DM + M + P). The study aimed to assess their impact on blood sugar levels. The results showed significant improvements in glycemic control. Specifically, the fasting blood glucose (FBG) levels were reduced to 8.9 (2.7) mM, 11.9 (0.5) mM, and 5.6 (0.8) mM in the propolis-treated, metformin-treated, and combined treatment groups, respectively. By contrast, the FBG value in the

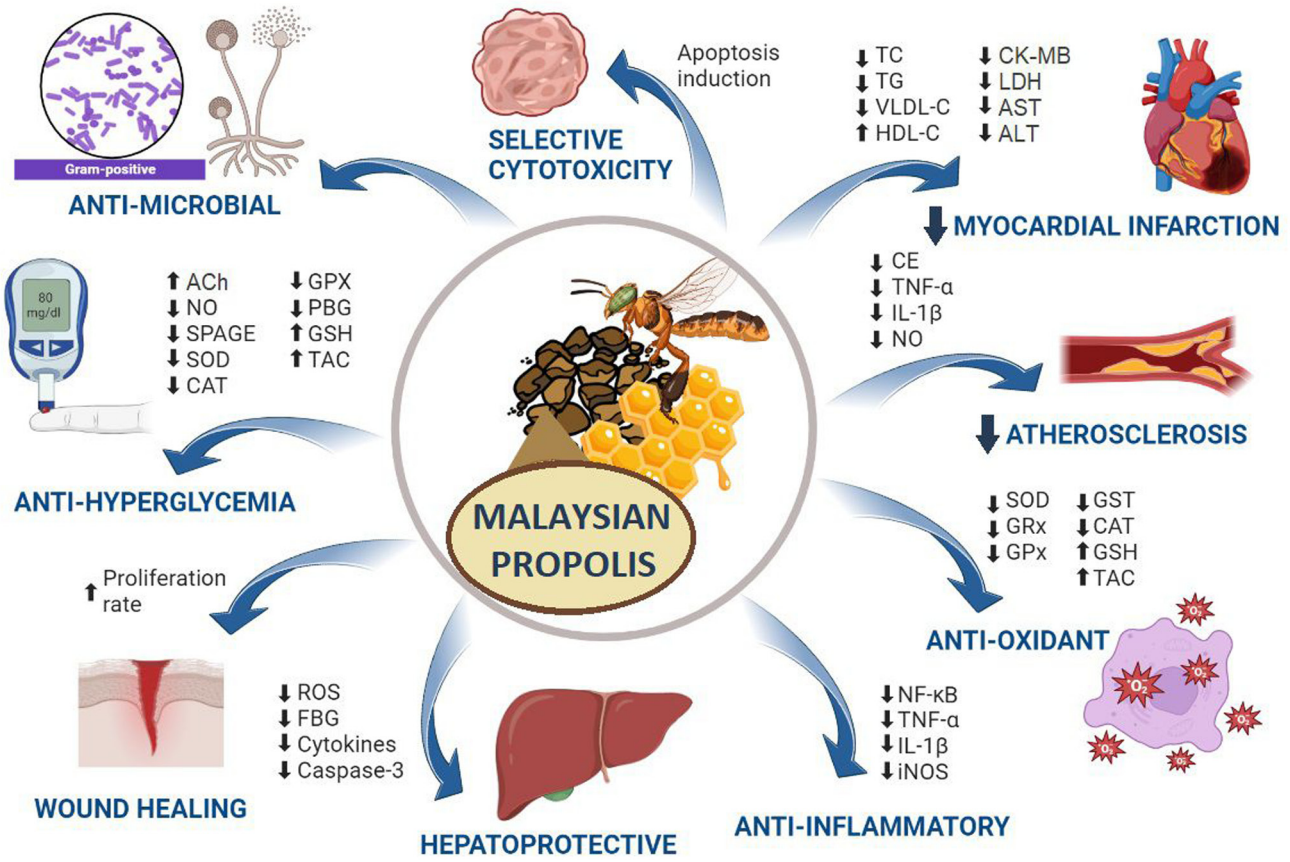

Figure 3. Summary of the proposed mechanism of action of Malaysian propolis in pharmacological activities such as antimicrobial, anti-hyperglycemia, wound healing, hepatoprotective, anti-inflammatory, antioxidant, atherosclerosis, myocardial infarction and cytotoxicity. ACh, acetylcholine; iNOS, inducible nitric oxide synthase; SPAGE, spatial gene enhancement; SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; PBG, postprandial blood glucose; GSH, glutathione; TAC, total antioxidant capacity; ROS, reactive oxygen species; FBG, fasting blood glucose; TC, total cholesterol; TG, triglyceride; VLDL-C, very low-density lipoprotein; HDL-C, high density lipoprotein; CK-MB, creatine kinase-myocardial band; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CE, carcinoembryonic antigen; GRx, glutaredoxin; GPx, glutathione peroxidase.

diabetic group was substantially higher at 27.0 (5.8) mM (32). Combination treatment of metformin and propolis results in the highest FBG improvement in comparison with metformin or propolis treatment alone. In the aforementioned study, all treatment arms significantly improved acetylcholine-induced relaxation compared with the DM group. Another study examined the effects of single oral dose of metformin, soft propolis *H. itama* methanol extract (MP) and their combination on the blood glucose levels of fasting rats over 9 h. MP had no discernible impact on blood sugar levels of normal rats compared with the control (47). Furthermore, the aforementioned study also reported that the most potent inhibitor of α-glucosidase is soft *H. itama* with lower IC_{50} value (1.23±0.32 mg/ml) than acarbose (1.48±0.13 mg/ml) (positive control). Therefore, one of the mechanisms used by soft *H. itama* to lower blood sugar levels may involve restricting the digestion of ingested carbohydrates to prevent glucose absorption. A previous study examined the impact of propolis, both individually and in combination with insulin treatment, on the maternal condition, pregnancy outcomes, and placental oxidative stress in streptozotocin-induced diabetic rats (29). The final FBG in the propolis-treated diabetic rat group was comparable to the insulin-treated diabetic rat group, indicating that propolis and insulin are equally effective in producing an antihyperglycemic effect (29). Furthermore, the antihyperglycemic effect was more pronounced in the combined group (propolis + insulin)-treated diabetic rats compared with the propolis- and the insulin-treated diabetic rat group groups, indicating that propolis in combination with insulin produced a more significant antihyperglycemic effect than propolis or insulin monotherapy (29). This suggests propolis may protect against DM-induced poor pregnancy outcomes and placental oxidative stress, with more significant effects when supplemented with insulin.

MI, also known as a heart attack, is caused by disruption in the delivery of blood to heart tissue. Necrosis of the myocardium occurs because of coronary artery blockage. The primary cause of myocardium necrosis after MI is an imbalance between coronary blood supply and myocardial demand (47). Pre-treatment with propolis significantly decreases levels of creatine Kinase-MB, Lactate Dehydrogenase, Aspartate Aminotransferase (AST), and Alanine Aminotransferase (diagnostic markers of MI in rats), Total Cholesterol (TC), Triglyceride and Very Low-Density Lipoprotein Cholesterol while increasing the level of High-Density lipoprotein Cholesterol. Furthermore, compared with control group, rats receiving prior propolis treatment exhibit substantially decreased serum cardiac troponin levels (47). Receptor for advanced glycation end products (RAGE) exists as a full-length receptor that is attached to the cell membrane.

The soluble(s)RAGE isoform is produced by either alternate splicing of the pre-mRNA (endogenous secretory RAGE; esRAGE) or shedding of RAGE by sheddase (cleaved RAGE; cRAGE). The cRAGE portion is large while the esRAGE portion is minor. sRAGE (cRAGE and esRAGE) binds and sequesters RAGE ligands or competes with RAGE binding, shielding the cell from the damaging effects of AGE-RAGE signalling. Serum sRAGE levels were observed to be greater or lower in people with T2DM (43). Another study reported that propolis-treated diabetic rats have higher heart/serum esRAGE levels than diabetic control groups, indicating a higher concentration of protective decoy receptor esRAGE in the heart compared with the serum. Because esRAGE binds to excess AGE and eliminates it, the AGE/esRAGE ratio may be used as a biomarker in diabetic cardiomyopathy. The combination of propolis and metformin results in significant cardiac AGE/esRAGE ratio alterations, implying a synergistic impact in preventing diabetic cardiomyopathy. The cardioprotective activity of propolis requires more research into whether propolis directly stimulates esRAGE formation or indirectly increases esRAGE by lowering AGE, as in hyperglycemia improvement (40). Furthermore, combination of propolis and metformin has synergistic cardioprotective activity in the heart, as demonstrated by lower cardiac AGE/esRAGE ratio (40).

Wound healing activity. Wound healing is a dynamic system involving constant cell-cell and cell-matrix interactions in a succession of overlapping phases, including haemostasis (blood coagulation cascade), inflammatory, proliferative, and remodelling (48). Several mediators and cell types regulate this system, including platelets, inflammatory cells, fibroblasts, keratinocytes, cytokines, growth factors and matrix metalloproteinases (48). Propolis at low concentrations (0.005, 0.125, 0.250 and 0.500 mg/ml) maintains or increases stem cell proliferation considerably. Propolis is bioactive and biocompatible at optimal concentrations and may be used to boost stem cell proliferation in culture media (44). In a proliferation assay using propolis, the average number of cells increased, peaking at 500 μg/ml after 48 h and then falling significantly with 1,000 μg/ml (30). This concentration-dependent pattern is the mechanism by which propolis influences the proliferation of fibroblast cells. In addition, propolis at concentrations of 1, 10 and 250 μg/ml results in significantly faster wound closure than controls, but the other concentrations have no notable effect. Nonetheless, only the 250 μg/ml concentration demonstrated a significantly higher migration rate than the control at 30 h. Therefore, propolis showed a generally positive effect on both assays compared with the control, and it followed a concentration-dependent curve, with 250 μg/ml being the most optimal concentration for cell migration and 500 μg/ml for cell proliferation. Doses >500 μg/ml may have toxic effect on proliferation of fibroblast cells. The proposed modes of action of propolis on pharmacological activities are illustrated in Fig. 3. Moreover, to have a better understanding of safe dosages and beneficial effects, it is crucial that future research assess the toxicity effect of propolis.

In summary, the present review provided insight into the therapeutic potential of Malaysian propolis based on *in vitro* and *in vivo* studies. Apart from antioxidant activity, propolis exhibits antimicrobial, proliferative, anti-inflammatory, anti-hyperglycaemia, hepatoprotective, wound healing effects and prevents MI and atherosclerosis. Nonetheless, this review only summarised propolis activities based on the limited number of studies available. In the future, more extensive research and clinical studies, as well as meta-analyses, are required.

Acknowledgements

The authors acknowledge the financial and technical support for this Fundamental Research Grant Scheme (FRGS) project provided by the Ministry of Higher Education of Malaysia (MOHE).

Funding

The present study was funded by the Fundamental Research Grant Scheme (FRGS) provided by the Ministry of Higher Education of Malaysia (MOHE) (grant no. FRGS/1/2022/STG02/UNISZA/02/1/).

Availability of data and materials

Not applicable.

Authors' contributions

NAJ, NAM and KSM contributed to the conception of the study and critically reviewed the article. NAJ wrote and edited the manuscript and constructed figures. NAJ, NAM and AAMB contributed to the data acquisition and analysis and drafted the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Hamzah SA, Zawawi N, Sabri S, Amir Hamzah AS and Sabri S: A review on the association of bacteria with stingless bees. Sains Malays 49: 1853-1863, 2020.
2. Syahariza ZA and Kee LS: Antioxidant activity of stingless bee propolis using different extraction method. Int J Eng Adv Res 4: 1-15, 2022.
3. Ibrahim N, Niza NFSM, Rodi MM, Zakaria AJ, Ismail Z and Mohd KS: Chemical and biological analyses of Malaysian stingless bee propolis extracts. Malays J Anal Sci 20: 413-422, 2016.
4. Guler HI, Tatar G, Yildiz O, Belduz AO and Kolayli S: Investigation of potential inhibitor properties of ethanolic propolis extracts against ACE-II receptors for COVID-19 treatment by molecular docking study. Arch Microbiol 203: 3557-3564, 2021.

5. Abdullah NA, Ja'afar F, Yasin HM, Taha H, Petalcorin MIR, Mamit MH, Kusrini E and Usman A: Physicochemical analyses, antioxidant, antibacterial, and toxicity of propolis particles produced by stingless bee *Heterotrigona itama* found in Brunei Darussalam. Heliyon 5: e02476, 2019.
6. Abd Mutalib N, Syed Mohamad SA, Jusril NA, Hasbullah NI, Mohd Amin MCI and Ismail NH: Lactic acid bacteria (LAB) and neuroprotection, what is new? An up-to-date systematic review. Pharmaceuticals (Basel) 16: 712, 2023.
7. Justesen T, Freyberg J and Schultz ANØ: Database selection and data gathering methods in systematic reviews of qualitative research regarding diabetes mellitus-an explorative study. BMC Med Res Methodol 21: 94, 2021.
8. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, *et al*: The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ 372: n71, 2021.
9. Li K, Rollins J and Yan E: Web of Science use in published research and review papers 1997-2017: A selective, dynamic, cross-domain, content-based analysis. Scientometrics 115: 1-20, 2018.
10. Halevi G, Moed H and Bar-Ilan J: Suitability of google scholar as a source of scientific information and as a source of data for scientific evaluation-review of the literature. J Informetr 11: 823-834, 2017.
11. Colvin J: Electronic resources reviews. Music Ref Serv Q 13: 122-124, 2010.
12. Bankova V, Popova M and Trusheva B: The phytochemistry of the honeybee. Phytochemistry 155: 1-11, 2018.
13. Badiazaman AAM, Zin NBM, Annisava AR, Nafi NEM and Mohd KS: Phytochemical screening and antioxidant properties of stingless bee *Geniotrigona thoracica* propolis. Malays J Fundam Appl Sci 15: 330-335, 2019.
14. Sari DM, Anwar E and Arifianti AE: Antioxidant and tyrosinase inhibitor activities of ethanol extracts of brown seaweed (Turbinaria conoides) as lightening ingredient. Pharmacogn J 11: 379-382, 2019.
15. Ahmed R, Tanvir EM, Hossen MS, Afroz R, Ahmmed I, Rumpa NE, Paul S, Gan SH, Sulaiman SA and Khalil MI: Antioxidant properties and cardioprotective mechanism of Malaysian propolis in rats. Evid Based Complement Alternat Med 2017: 5370545, 2017.
16. Salim NHM, Omar EA, Omar WAW and Mohamed R: Chemical constituents and antioxidant activity of ethanolic extract of propolis from Malaysian stingless bee *Geniotrigona thoracica* species. Res J Pharm Biol Chem Sci 9: 646-651, 2018.
17. Nna VU, Abu Bakar AB, Md Lazin MRML and Mohamed M: Antioxidant, anti-inflammatory and synergistic anti-hyperglycemic effects of Malaysian propolis and metformin in streptozotocin-induced diabetic rats. Food Chem Toxicol 120: 305-320, 2018.
18. Rosli NL, Roslan H, Omar EA, Mokhtar N, Hapit NHA and Asem N: Phytochemical analysis and antioxidant activities of Trigona Apicalis propolis extract. AIP Conf Proc 1791: 020018, 2016.
19. Syed Salleh SNA, Mohd Hanapiah NA, Ahmad H, Wan Johari WL, Osman NH and Mamat MR: Determination of total phenolics, flavonoids, and antioxidant activity and GC-MS analysis of Malaysian stingless bee propolis water extracts. Scientifica (Cairo) 2021: 3789351, 2021.
20. Usman UZ, Bakar ABA and Mohamed M: Phytochemical screening and comparison of antioxidant activity of water and ethanol extract propolis from Malaysia. Int J Pharm Pharm Sci 8: 413-415, 2016.
21. Mohamed WAS, Ismail NZ, Muhamad M, Omar EA, Abdul Samad N, Ooi JP and Mohamad S: Q-TOF LC-MS compounds evaluation of propolis extract derived from Malaysian stingless bees, *Tetrigona apicalis*, and their bioactivities in breast cancer cell, MCF7. Saudi J Biol Sci 29: 103403, 2022.
22. Maroof K, Chen KF, Lee RF, Goh BH, Mahendra CK, Siow LF and Gan SH: A preliminary study on phenolics, antioxidant and antibacterial activities of *Acacia mangium* and *Garcinia mangostana* propolis collected by *Geniotrigona thoracica*. Food Chem Adv 2: 100255, 2023.
23. Nagaraju P, Reddy PN, Padmaja P and Ugale VG: Synthesis, antiproliferative activity and molecular docking studies of novel benzo[a]pyrano-[2,3-c]phenazine derivatives. Chemical Data Collections 30: 100541, 2020.
24. Pereira CPM, Souza ACR, Vasconcelos AR, Prado PS and Name JJ: Antioxidant and anti-inflammatory mechanisms of action of astaxanthin in cardiovascular diseases (Review). Int J Mol Med 47: 37-48, 2021.
25. Balica G, Vostinaru O, Stefanescu C, Mogosan C, Iaru I, Cristina A and Pop CE: Potential role of propolis in the prevention and treatment of metabolic diseases. Plants (Basel) 10: 883, 2021.
26. Mohd Suib MS, Wan Omar WA, Omar EA and Mohamed R: Ethanolic extract of propolis from the Malaysian stingless bee *Geniotrigona thoracica* inhibits formation of THP-1 derived macrophage foam cells. J Apic Res 60: 478-490, 2021.
27. Mandal BK: Scopes of green synthesized metal and metal oxide nanomaterials in antimicrobial therapy. In: Nanobiomaterials in Antimicrobial Therapy. Grumezescu AM (ed). Vol. 6. William Andrew Publishing, Norwich, NY, pp313-341, 2016.
28. Mohamed WAS, Ismail NZ, Omar EA, Abdul Samad N, Adam SK and Mohamad S: GC-MS evaluation, antioxidant content, and cytotoxic activity of propolis extract from peninsular malaysian stingless bees, *Tetrigona apicalis*. Evid Based Complement Alternat Med 2020: 8895262, 2020.
29. Yusoff NYN, Mohamad S, Abdullah HN and Rahman NA: Antifungal activity of Malaysian honey and propolis extracts against pathogens implicated in denture stomatitis. AIP Conf Proc 1791: 020006, 2016.
30. Yusop SATW, Sukairi AH, Sabri WMAW and Asaruddin MR: Antioxidant, antimicrobial and cytotoxicity activities of propolis from Beladin, Sarawak stingless bees Trigona itama extract. Mater Today Proc 19: 1752-1760, 2019.
31. Usman UZ, Bakar ABA and Mohamed M: Propolis improves pregnancy outcomes and placental oxidative stress status in streptozotocin-induced diabetic rats. BMC Complement Altern Med 18: 324, 2018.
32. Chew Shi Fung HM, Hashim SNM, Htun AT and Ahmad A: Proliferative effect of Malaysian propolis on stem cells from human exfoliated deciduous teeth: An in vitro study. Methodology, 2014.
33. Jacob A, Parolia A, Pau A and Davamani Amalraj F: The effects of Malaysian propolis and Brazilian red propolis on connective tissue fibroblasts in the wound healing process. BMC Complement Altern Med 15: 294, 2015.
34. Ibrahim N, Zakaria AJ, Ismail Z and Mohd KS: Antibacterial and phenolic content of propolis produced by two malaysian stingless bees, *Heterotrigona itama* and *Geniotrigona thoracica*. Int J Pharmacogn Phytochem Res 8: 156-161, 2016.
35. Akhir RAM, Bakar MFA and Sanusi SB: Antioxidant and antimicrobial activity of stingless bee bread and propolis extracts. AIP Conf Proc 1891: 020090, 2017.
36. Azemin A, Md-Zin NB, Mohd-Rodi MM, Kim-Chee AS, Zakaria AJ and Mohd KS: Application of metabolite profiling and antioxidant activity in assessing the quality of processed and unprocessed stingless bee's propolis. J Fund Appl Sci 9: 637-660, 2017.
37. Ong TH, Chitra E, Ramamurthy S, Siddalingam RP, Yuen KH, Ambu SP and Davamani F: Chitosan-propolis nanoparticle formulation demonstrates anti-bacterial activity against Enterococcus faecalis biofilms. PLoS One 12: e0174888, 2017.
38. Lim OZ, Yeoh BS, Omar N, Mohamed M, Zin AAM and Ahmad R: Stingless bee propolis, metformin, and their combination alleviate diabetic cardiomyopathy. Braz J Pharm Sci 58: e19652, 2022.
39. Nna VU, Bakar ABA and Mohamed M: Malaysian propolis, metformin and their combination, exert hepatoprotective effect in streptozotocin-induced diabetic rats. Life Sci 211: 40-50, 2018.
40. Asem N, Abdul Gapar NA, Abd Hapit NH and Omar EA: Correlation between total phenolic and flavonoid contents with antioxidant activity of Malaysian stingless bee propolis extract. J Apic Res 59: 437-442, 2020.
41. Annisava AR, Mohd KS, Nafi NEM, Khadar ASA, Zin NBM, Pauzi N, Badiazaman AAM and Zakaria AJ: Chemical profiling and antioxidant activity of Malaysian stingless bee propolis from ten different locations. Biosci Res 16: 91-104, 2019.
42. Nafi NEM, Zin NBM, Pauzi N, Khadar ASA, Anisava AR, Badiazaman AAM and Mohd KS: Cytotoxicity, antioxidant and phytochemical screening of propolis extracts from four different Malaysian stingless bee species. Malays J Fundam Appl Sci 15: 307-312, 2019.
43. Nna VU, Abu Bakar AB, Ahmad A, Eleazu CO and Mohamed M: Oxidative stress, NF-κB-mediated inflammation and apoptosis in the testes of streptozotocin-induced diabetic rats: Combined protective effects of Malaysian propolis and metformin. Antioxidants (Basel) 8: 465, 2019.

44. Zainal WNHW, Azian NAAM, Albar SS and Rusli AS: Effects of extraction method, solvent and time on the bioactive compounds and antioxidant activity of *Tetrigona apicalis* Malaysian propolis. J Apic Res 61: 264-270, 2022.
45. Basari N, Ramli SN, Abdul-Mutalid NA, Shaipulah NFM and Hashim NA: Flowers morphology and nectar concentration determine the preferred food source of stingless bee, *Heterotrigona itama*. J Asia Pac Entomol 24: 232-236, 2021.
46. Ragavi R, Adole PS, Vinod KV and Pillai AA: Altered expression of a disintegrin and metalloproteinase 10 in peripheral blood mononuclear cells in type 2 diabetes mellitus patients with the acute coronary syndrome: A pilot study. Endocrine 77: 461-468, 2022.
47. Mohd-Yazid NA, Zin NBM, Pauzi N and Mohd KS: Preliminary evaluation of antioxidant and cytotoxic activity of ethanolic extract of stingless bees propolis from different localities. J Agrobiotech 9: 132-141, 2018.
48. Ustuner O, Anlas C, Bakirel T, Ustun-Alkan F, Diren Sigirci B, Ak S, Akpulat HA, Donmez C and Koca-Caliskan U: In vitro evaluation of antioxidant, anti-inflammatory, antimicrobial and wound healing potential of *Thymus sipyleus* Boiss. subsp. *rosulans* (borbas) *Jalas*. Molecules 24: 3353, 2019.
49. Kong YT, Abdullah V, Mokhtar SU and Kutty RV: Preliminary studies on extraction of propolis using vitamin E d-α-tocopheryl polyethylene glycol succinate (vitamin E TPGS) and compare their antimicrobial activities. Malays Journal of Microbiol 16: 346-352, 2020.
50. Logue SE, Elgendy M and Martin SJ: Expression, purification and use of recombinant annexin V for the detection of apoptotic cells. Nature protocols 4: 1383-1395, 2009.
51. Misra TN, Singh RS, Upadhyay J and Srivastava R: Chemical constituents of Vernonia cinerea. Isolation and structure elucidation of a new pentacyclic triterpenoid. J Nat Prod 47: 865-867, 1984.
52. León-Mejía G, Guevara AM, Moreno OF and Cruz CU: Cytotoxicity as a fundamental response to xenobiotics. In: Cytotoxicity-New Insights into Toxic Assessment. doi: 10.5772/intechopen.96239. Accessed Feb 17, 2021.
53. Yeoh BS, Omar N, Mohammad M, Mokhtar SS and Ahmad R: Antioxidative propolis from stingless bees (*Heterotrigona itama*) preserves endothelium-dependent aortic relaxation of diabetic rats: The role of nitric oxide and cyclic guanosine monophosphate. Braz J Pharm Sci 57: e19187, 2021.