Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

Antioxidant and Anti-acne Activities of Stingless Bee Honey and Propolis Extract

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Article info

Article history:
Received : 11 March 2024
Revised : 9 April 2024
Accepted : 11 April 2024

Keywords:
Stingless bee honey, Antioxidant, Propolis, Anti-acne

Abstract

Stingless bees form a large group of bees that lack a sting and are found in tropical and sub-tropical areas. Honey and propolis are major products that are produced by the stingless bee. This study aimed to investigate honey and propolis extracts, leading us to isolate bioactive compounds for their antioxidant and anti-acne properties. The four different stingless bee species (*Geniotrigona thoracica*, *Heterotrigona itama*, *Tetragonula pagdeni*, and *Lepidotrigona terminate*), collected from a community enterprise area (southern Thailand). The raw propolis was extracted via maceration with 20% ethanol. The present study aimed to assess the concentration of phenolics and flavonoids using Folin-Ciocalteu method, Aluminum Chloride Colorimetry method and DPPH respectively, as well as the antibacterial against bacteria *Propionibacterium acne* (DMST 14917) using the agar well diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined by the in vitro method. The findings indicate that *G. thoracica* honey has the highest content of total phenolic (30.28±0.51 mgGAE/g), flavonoids (59.77±0.05 mgRE/g) and DPPH (1.251±0.27 mg/mL), while the phenolic (25.34±0.06 mgGAE/g), flavonoids (44.33±0.41 mgRE/g) and DPPH (2.210±0.28 mg/mL) of *H. itama* propolis extract are higher than those of the other extracts.The antibacterial activity of the honey against *P. acne* was categorized as highly strong inhibitory, while propolis extract was classified as moderate inhibitory. Additionally, *G. thoracica* honey exhibits the highest antibacterial activity against *P. acnes* at 37.74±1.5 mm, MIC (62.5 µg/mL) and MBC (125 µg/mL), while *H. itama* propolis extract was 14.43±2.7 mm, MIC (125 µg/mL) and MBC (250 µg/mL). These results indicated that stingless bee honey and propolis extracts contain valuable quantities of phenolic and flavonoid compounds, which serve as natural anti-oxidants and exhibit notable anti-microbial activity. The results suggest that stingless bee products hold commercial potential as a natural source of bioactive ingredients to be formulated into cosmetic agents.

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Introduction

Stingless bees (SB) are named after their atrophied sting, subfamily Meliponinae, family Apidae and order Hymenoptera, however they are also referred to as indigenous bees. Without a sting, SB defend their nests by biting or applying their opponents' skin with plant resin, entering body cavities, or hiding in the hair (Barbiéri & Francoy, 2020). Unlike honeybees, which primarily or solely build their nests using wax, stingless bees typically utilize plant gums, resins, or even earth to construct their nests. They also have short harvesting distances when searching for food and honeycomb-free colonies. They are normally found in the warm and humid environment of tropical and sub-tropical forest areas (Nates-Parra, 2001). In Thailand, there are more than 32 species and approximately 500 species have been reported in Southeast Asia (Fletcher et al., 2020). Southern Thailand is the primary source of stingless bees since it includes a variety of natural substances that are bioactive (Klakasikorn et al., 2005). SBs are a great value as main pollinators of wild flowering and cultivated plants, thus playing a fundamental role in the maintenance of biodiversity. Moreover, it produces unique and high nutrition honey for economic crops (Rao et al., 2016). Meliponiculture is the practice of keeping stingless bees, where beekeepers nurture, propagate and utilize colonies of these bees to profit from harvested honey, pollen, cerumen and propolis, which serve as sources of bioactive compounds (Syafrizal et al., 2020). Bees gather and alter plant nectars from diverse vegetation and natural surroundings, incorporating specific organic substances such as saliva secretions from abdominal glands and enzymes from cephalic glands. Honey produced by stingless bees (SBH) exhibits a fluid texture and limited crystallization ability. It is stored and allowed to mature within colonies, resulting in distinctive taste, sweetness, unusual acidity levels and medicinal properties (Ávila et al., 2018). The main nutritional content of honey is a variety of protein and carbohydrate compounds such as amino acids, sucrose, glucose, fructose, carbohydrate dextrins and other substances, including trace amounts of enzymes, vitamins and minerals (Biluca et al., 2020). The biological activities and chemical composition of honey are variable based on bee species and the flora at the site of bee collection. Honey is noted as having antimicrobial, antiparasitic, anti-inflammatory, immunosuppressive, antimutagenic and anticancer activities. Honey has also been shown to be rich in phenolic compounds and flavonoids, generally known as antioxidant compounds (Gül & Pehlivan., 2018). The antioxidant capacity of natural honey products is considered one of their most significant attributes, given its effectiveness in combating diseases and aging. Honey has traditionally been utilized by the ancients to heal numerous diseases, in addition to being used as a taste enhancer (Rao et al., 2016).

Besides honey, stingless bees also produce propolis (SBP), often constructing their nests using collected plant resins. Propolis is a sticky substance formed from a combination of bee saliva's β-glycosidase enzyme, plant bud exudates and beeswax. Typically, propolis consists of 50% resinous substances, 30% wax, 10% essential oils, 5% pollen and 5% minor constituents such as impurities, amino acids, soil and deceased bees (Salleh et al., 2021). The composition of propolis varies depending on the bee species and the resins collected from different plant sources. Propolis plays a crucial role in hive construction, providing waterproofing and inhibiting fungi, bacteria and viruses that could harm the bees' habitat. Resinous hives are a very complex mixture, containing over 300 chemicals, including phenolic compounds, flavonoids, terpenes and several specific antioxidant and antibacterial compounds (Bachevski et al., 2020). The differences in chemical composition greatly influence the biological activity of propolis, which can be harnessed in sectors such as pharmaceuticals and health due to its antimicrobial, antifungal, antiviral, anticancer, antidiabetic, antioxidant and other beneficial properties (Kraikongjit et al., 2018).

The honey and propolis produced by stingless bees are still natural because they are obtained directly from the forest and used for a wide variety of purposes. They were declared to have potency as natural antibiotics and reported to possess various pharmacological properties (Ávila et al., 2018). Recently, the cosmeceutical potential of honey as an antiacne, skin moisturizing and anti-hypertrophic scar agent has been studied. Skin acne is caused by the accumulation of nonlipid-soluble sebum, which is metabolized by *Propionicbacterium acne* into fatty acids that cause inflammation in the sebaceous glands. Furthermore, the high sebum content found in sebaceous follicles provides a nutrient-rich and anaerobic environment conducive to the growth of bacteria and the formation of biofilm (Soleymani et al., 2020). The antioxidants present in honey contribute to its anti-inflammatory properties, which can potentially reduce inflammation during the formation of acne. SBH is particularly notable for its

high levels of antioxidants and anti-inflammatory properties, with its polyphenolic compound content being approximately ten times greater than that of other types of honey (Mustafa et al., 2018).

In Thailand, the evaluation of honey and propolis extracts of *Geniotrigona thoracica, Heterotrigona itama, Tetragonula pagdeni* (Schwarz) and *Lepidotrigona terminata* (Smith) has not been reported as anti-acne. All of the species that are widely cultivated by communities in Thailand. Therefore, in this study, we aimed to examine the honey and propolis extracts, which led us to isolate active compounds for antioxidant and anti-acne from stingless bees for medicine and cosmetics applications from a community enterprise in southern Thailand.

Materials and methods

1. Bacterial preparation

Propionibacterium acnes (DMST 14917) was obtained and well identified from the Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, Thailand. They were maintained in gelatin culture. Pure bacterial cultures were initially regenerated into liquid medium (Trypticase Soy Broth, TSB, DIFCO®) for 18-24 hr at 37°C, under CO_2 conditions until their growth stage reached a log-phase. The cultures were then centrifuged at 1000xg for 10 min. The supernatant was removed, while the pellet was washed three times using a phosphate-buffered saline (PBS) solution. For the challenge test, the bacteria suspensions were diluted with sterilized distilled water and standardized with a spectrophotometer at 600 nm to an optical density (OD) of 0.1, corresponding to 10^7 CFU/mL (Djakaria et al., 2020).

2. Preparation of honeys and propolis extracts

In the investigation, the whole stingless bee honey (SBH) and propolis samples used in the study of *Geniotrigona thoracica, Heterotrigona itama, Tetragonula pagdeni* (Schwarz) and *Lepidotrigona terminata* (Smith) were collected in October 2023 from the Ban Lamphu community enterprise area, Tumbon Lamphu, Mueang Narathiwat, Narathiwat Province, Thailand, by scraping the propolis toppings stingless bee hives and kept in dark places at 0°C to prevent photodegradation prior to use (Fig.1). Asst. Prof. Matintarangson identified and confirmed the stingless bee species at the Laboratory of Bioproducts Innovation Department, Faculty of Science and Technology, Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathum Thani, Thailand. The pH of that SBH slurry was measured using a pH meter and the amount of total soluble solids (°Brix) was determined using a refractometer at 37°C (Kamal et al., 2019).

The raw stingless bee propolis collected was manually cut into small pieces and ground into powder. Approximately 100 g of powdered propolis was macerated with 300 mL of 20% ethyl alcohol in a

Fig. 1 Images of the raw propolis (top) and crude ethanolic extract of propolis (bottom) of *G. thoracica* (1) *H. itama* (2) *T. pagdeni* (3) *L. terminata* (4) used in present study

maceration vessel at room temperature for 7 days while stirring occasionally. Next, the macerate was filtered using Whatman No.1 filter paper and the supernatant was concentrated by a rotary evaporator at 40°C until no residue solvent was left to obtain propolis extract powder (Fig. 1). The concentrated propolis extracts were then further dried in an oven at 40°C and the dried ethanolic extract of stingless bee propolis (SBP) was maintained at 4°C until further analysis (Lim et al., 2023). The yield (%) of the extraction was evaluated by comparing the dry weight of the extract with the initial weight of propolis (Aboulghazi et al., 2022). The honey and ethanolic propolis extracts from maceration were further tested for antioxidant properties. The extracts and honeys were dissolved in 10% dimethyl sulphoxide (DMSO) to achieve a final concentration of 100 mg/mL. They were then stored at 5°C in labelled sterile screwcap bottles for their antibacterial efficacy.

3. Total phenolic content

The total phenolic content (TPC) was determined by the Folin–Ciocalteu method with some modification (Hernández Zarate et al., 2018). A 20 g of sample was diluted with 99.99% ethanol. Then, 100 µL of diluted sample in 8.4 mL of distilled water was mixed with 500 µl of freshly prepared diluted Folin–Ciocalteu reagent (0.2 N). After 1 min, 1 mL of 20% (w/v) sodium carbonate was added. Mixtures were incubated at room temperature for 2 hr in the dark. The absorbance at 760 nm was measured by a UV-VIS spectrophotometer. The total phenolic content was determined using the calibration curve of gallic acid and expressed as gallic acid equivalents (GAE), measured in milligrams per gram of the sample (mg GAE/g). Each determination was performed in triplicate.

4. Total flavonoid content

The colorimetric aluminum chloride method was used to determine the total flavonoid content (TFC), following the procedure reported by Mokhtar et al. (2019). A total of 10 mg of the sample was weighed and diluted with 80% ethanol. Then, 1 mL of the samples was put in a 10 mL volumetric flask containing 4 mL of distilled water and mixed with 0.3 ml of 5% $NaNO_2$ solutions. After 6 min, 0.3 mL of a 10% $AlCl_3.6H_2O$ solution was added. $Al(NO_3)_3$ was added to the flask for another 6-min reaction. After an additional 6 min, 2 mL of 1 M NaOH was added. The reaction solution was thoroughly mixed and allowed to stand for 15 min. The absorbance was then measured at 510 nm using a UV-VIS spectrophotometer. Qualification was performed using the Rutin as the standard and the results were represented as milligrams of rutin equivalent (mgRE) per gram of the sample (mgRE/g). All determinations were conducted in triplicate.

5. Antioxidant assay

A DPPH (2,2-diphenyl-1-picryl-hydrazyl) assay was performed as described in Wattanuruk et al. (2020). Briefly, 0.02 g of sample was diluted with 40 mL of 99.99% ethanol. A range of concentrations of the sample, including 31.25, 62.50, 125, 250 and 500 mg/mL, were prepared. In brief, each sample (1 mL) was mixed with 2 mL of a 0.1 mmol/L DPPH solution and left to react in darkness for 30 min before measuring the absorbance at 517 nm. The radical scavenging activity was determined using the formula: % Inhibition = [(AB - AA)/AB] × 100, where AA represents the absorbance of the tested sample solution and AB represents the absorbance of the blank sample.

The sample concentration providing 50% effective concentration (EC_{50}) was calculated from the graph plotting inhibition percentage against sample concentration.

6. Antibacterial activity assay

The antibacterial activities for the SBH and EEP were determined by the standard agar well diffusion method with slight modification (Wavinya et al., 2021). In this experiment, the microorganisms used were sensitive standard anti-acne bacteria of *P.acnes* (DMST 14917). The medium utilized was Trypticase Soy Agar (TSA). Twenty-milliliter aliquots of sterile medium were added to petri dishes and left to harden. Inoculum containing 10^7 CFU/mL of *P. acnes* suspension was swabbed on the surface of TSA plates, subsequently wells of 6 mm diameter were punched using a sterile cork borer into the agar medium and filled with 50 µL (1000 µg/mL) (w/v) of SBH and SBP extract and allowed to diffuse at room temperature for 2 hr. The plates were then incubated in an upright position at 37°C for 24 hr under CO_2 conditions. Wells containing the same volume of DMSO (10%) served as negative controls and oxytetracycline served as positive controls at a concentration of 10 µg/well. Following incubation, the widths of the growth inhibition zones were determined in millimeters (mm). Three replicates of each extract were performed against each test organism and the average values were noted. The data were presented as mean±standard deviation. Based on the zone of inhibition, it could be classified into five categories: no inhibitory (<6 mm), mild inhibitory (6-10 mm), moderate inhibitory (10-15 mm),

strong inhibitory (15-20 mm) and highly strong inhibitory (>20 mm) (Elgayyar et al., 2001).

7. Bacteriostatic and bactericidal activity

The bacteriostatic, or MIC, which is commonly defined as the lowest concentration of an antimicrobial agent that prevents the growth of a microorganism after a specified incubation period, was determined using the tube dilution method as described by Wavinya et al. (2021) with some modifications. Concisely, 12 sterile test tubes were arranged in the rack and the stocks of SBH and SBP extract samples, each at a concentration of 1000 µg/mL (w/v), were resuspended in 10% DMSO to generate serial twofold dilutions ranging from 0.97 to 500 µg/mL. This resulted in final concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.95 and 0.97 µg/mL (w/v) for each sample. To initiate the procedure, 1 mL of freshly prepared TSB was added to each tube and sterilized before being allowed to cool. Subsequently, 1 mL of the sample solution was added to the first test tube (designated as the sample control tube), followed by test tube 2, using sterile micropipettes and tips. Two fold serial dilution was achieved by transferring 1 mL from test tube 2 to test tube 3 with separate sterile micropipettes and tips, followed by vortexing for homogenization. After thorough mixing, 1 mL of the mixture was transferred from test tube 3 to test tube 4 using a different sterile micropipette and tips. This process was repeated until a dilution of 0.97 was achieved in the 11^{th} test tube. Subsequently, 1 mL was obtained from test tube 11 and discarded. Following this, 1 mL of suspended bacterial suspension (1×10^5 CFU/mL) was inoculated into test tubes 2-12, followed by incubation at 37°C for 24 hr under CO_2 conditions. Following the incubation period, the smallest concentration of SBH and SBP that inhibited the growth of the bacterial isolates (*P. acnes*) in the test tubes was determined through visual inspection for the absence or presence of growth. The concentration with the least turbidity was identified and recorded as the MIC value. Two test tubes, including the samples with no bacteria and the bacteria with no samples, were used as the negative control and the positive control, respectively. All samples were tested in triplicate. To evaluate the minimum bactericidal concentration (MBC), the tubes with no visible growth (clear solution) were collected from each tube using an inoculation loop and re-subcultured on TSA. Following further incubation, the lowest concentration at which no visible bacterial growth was observed was recorded as the MBC value. All assays were conducted in triplicate.

8. Statistical analysis

All treatments and measurements were conducted in triplicate and the results are presented as the mean ± standard deviation. The data was analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple range tests with a p-value < 0.05.

Results and discussion

1. The characteristics of stingless bee honey and propolis

According to previous reports, there are around 32 species of stingless bees found in Thailand's eastern and southern forests. However, only four species are commercially nurtured for pollinating and economic: *Geniotrigona thoracica, Heterotrigona itama, Tetragonula pagdeni* and *Lepidotrigona terminate*, which are widely cultivated by communities in Thailand. The characteristics of SBH are amber-brown, sour taste, sweetness and high moisture content. The general physical characteristics of propolis are dark brown, hard and brittle. In the study, the determination of pH and total soluble solid (TSS) of SBH was done and the result revealed that, the investigated honey was acidic (pH 3.27–3.76) and TSS in the examined SBH samples ranged between 65.20 and 75.12 °Brix (Data are the means of four samples collected from each species). Organic acids such as gluconic acid, lactic acid, acetic acid and citric acid, are also found in honey (Shamsudin et al., 2019). Furthermore, its principal constituents are fructose and glucose, which constitute around 65% of sugars, with a very small proportion of hydroxymethylfurfural (Fatima et al., 2018; Mustafa et al., 2018). The propolis samples underwent extraction using the maceration technique, which is the primary method for isolating and recovering compounds from ethanolic propolis extract samples. This phenomenon would promote the penetration of the solvent into the sample to release compounds into the solvent. The extended maceration period of 7 days likely facilitated the diffusion of compounds from the resinous raw propolis, leading to the breakdown of its polymeric structure (Xu et al., 2017).

The results of the extraction yield of ethanolic propolis extracts of *G. thoracica, T. pagdeni, H. Itama* and *L. terminata* were 15.67, 14.41, 15.59 and 13.45%, respectively. In this research, a percentage of ethanol (20%) was usually used as the solvent system to extract bioactive compounds from propolis by the maceration method, as the extract obtained a dark brown powder

with a distinctive odor. The solvent system with a higher water composition was selected due to propolis primarily containing phenolic acids and hydrolyzable tannins, which are water-soluble. Water is used to soften the structure of raw propolis, while ethanol serves as a suitable solvent for extracting phenolic compounds. Previous studies have indicated that ethanol is an effective solvent for polyphenol extraction (Do et al., 2014).

2. Total phenolic content, total flavonoid content and antioxidant activity of SBH and SBP extract

Table 1 shows the total phenolic content, flavonoids and antioxidant activities of honey and propolis samples. TPC was determined by using the Folin-Ciocalteu reagent as the gallic acid equivalent. Phenolic compounds are secondary metabolites found in plants, typically produced in response to plant injury or stress and can be found in plant exudates. Phenolics are a diverse group of compounds, primarily tannins, flavonoids and phenolic acids. As shown in Table 1, the SBH of *G. thoracica, H. itama, T. pagdeni* and *L. terminata* showed TPCs of 30.28±0.51, 29.76±0.28, 26.40±0.22 and 27.63±0.41 mgGAE/g, respectively. The SBP extract showed to have the TPC 25.38±0.04, 21.34±0.06, 22.28±0.22 and 23.24±0.12 mgGAE/g, respectively. Moreover, it was found that the high total phenolic content observed was from SBH, while the low total phenolic content recorded was from the propolis extract. Different stingless bee species, bee plant preferences and extraction solvents utilized all contribute to differences in the total phenolic values of the honey and propolis samples (Abdullah et al., 2019).

Table 1 Total phenolic content, total flavonoid content and antioxidant activity of SBH and SBP extract

Sample	Total phenolic content (mgGAE/g)		Total flavonoid content (mgRE/g)		Antioxidant activity (EC_{50}) (mg/mL)	
	SBH	EPP	SBH	EPP	SBH	EPP
G. thoracica	30.28±0.51	21.38±0.04	59.77±0.05	42.77±0.05	1.251±0.27	2.231±0.07
H. itama	29.76±0.28	25.34±0.06	56.33±0.41	44.33±0.41	1.360±0.21	2.210±0.28
T. pagdeni	26.40±0.22	22.28±0.22	55.44±0.42	42.41±0.42	1.968±0.11	2.318±0.12
L. terminata	27.63±0.41	23.24±0.12	55.29±0.32	40.29±0.32	1.874±0.03	2.724±0.14
BHT					0.128	

Remark: No significant difference detected for both TPC and TFC since $p > 0.05$

The flavonoid contents were assessed by means of the $AlCl_3$ method as rutin equivalent. As shown in Table 1, The total flavonoid content of 59.77±0.05 mgRE/g, 56.33±0.41 mgRE/g, 55.44±0.42 mgRE/g and 55.29±0.32 mgRE/g belonged to the SBH of *G. thoracica, H. itama, T. pagdeni,* and *L. terminate* respectively. The total flavonoid content of 42.77±0.05 mgRE/g, 44.33±0.41 mgRE/g 42.44±0.42 mgRE/g and 40.29±0.32 mgRE/g belonged to SBP extract of *G. thoracica, H. itama, T. pagdeni* and *L. terminate,* respectively. Honeys with darker colors tend to exhibit stronger antioxidant activities, attributed to various pigment compounds like flavonoids, carotenoids and phenolic compounds, which are known for their antioxidant properties (Kek et al., 2014). SBH possesses significant potential in acne treatment due to its high levels of flavonoids and phenolic compounds, which help reduce inflammation and irritation during acne formation. Compared to other honey varieties, SBH contains higher concentrations of these beneficial compounds, making it a potent antioxidant (Mustafa et al., 2018).

To date, researchers have identified over 300 compounds in propolis, primarily consisting of polyphenols, particularly flavonoids and ester groups (Anjum et al., 2019). The specific active compounds in propolis vary depending on the plants surrounding the beehive. Furthermore, the composition, bioactive compounds and physiochemical properties of honey can differ significantly based on factors such as the botanical source, geographical location, climatic conditions, soil type, beekeepers' practices and storage methods during commercial production. Additionally, the chemical composition of propolis from stingless bee species is influenced by various factors, including the geographical location of the propolis, the species of bees, seasonal variations, the availability of plants and flora at the collection sites and the bees' preferences for floral and resin sources (Mohammad et al., 2020).

As can be seen in Table 1, the most commonly used test to verify the antioxidant activity corresponds to the use of the DPPH radical. BHT was used as a positive control. The antioxidant activities of honey and propolis were examined by scavenging of free radical 2,2-diphenyl-1-picrylhydrazyl. The antioxidant activities were compared with the known artificial standard antioxidants (BHT). The antioxidant activity (EC_{50}) by DPPH assay of *G. thoracica, H. itama, T. Pagdeni* and *L. terminata* shows results of 1.251±0.27, 1.360±0.21, 1.968±0.11 and 1.874±0.03 mg/mL, respectively. The propolis extract, DPPH was 2.231±0.07, 2.510±0.28, 2.318±0.12 and 2.724±0.14 mg/mL, respectively. A positive control, BHT, was 0.128 mg/mL. The SBH extracts had higher total phenolic and total flavonoid content than the SBP extracts, which increased their

antioxidant activity. This is probably due to conditions that play a role in extraction. The type of tree that provides the sap, the climate zone (at the time of sample collection), temperature, the location and the circumstances surrounding the beekeeping can all have an impact on the compound composition of propolis (Martysiak-Żurowska & Wenta, 2012). The plants surrounding the beehive influence the active compounds in propolis (Costa et al., 2020). The bee species, propolis collecting site, extraction solvents, plant resinous source and chemical compositions are some of the factors that may affect the antioxidant activity of the SBH and SBP extracts (Mohammed et al., 2020). Propolis has the capability to be an effective natural antioxidant source that may be used in modern times because of its bioactive components' ability to effectively scavenge free radicals (Pazin et al., 2017). Antioxidants are crucial as the human body's defense mechanism against free radicals, hence research on safe, consumable antioxidant activity of honey and propolis is now relevant. The quantity of wax in propolis and the season or month of collection, however, can have an impact on the substance's antioxidant properties (Araújo et al., 2016). A strong positive correlation was found between phenolics, flavonoids and DPPH, indicating that, in addition to total phenolic content, flavonoids are good indicators of the antioxidant potential of honey and propolis. Honey's color intensity shows the presence of pigments such as flavonoids and carotenoids, which are well-known to contribute to its antioxidant activity (Moniruzzaman et al., 2013). When compared to honey generated by *Apis dorsata*, research on the SB (*Trigona* sp.) showed that the honey produced had a greater level of phenolics, flavonoids and overall antioxidant activity (Ranneh et al., 2018). SBH has a high concentration of phenolic and flavonoid components, which make it an excellent antioxidant and potential acne therapy. Additionally, because of the antioxidants, it has anti-inflammatory qualities that may lessen inflammation during the formation of acne (Zulkifli & Hadi., 2023).

3. Antibacterial activity assay

The agar well diffusion technique was utilized to ascertain the antibacterial activity. *P. acne.* was the bacterium chosen for this experiment. Following that, oxytetracycline at a concentration of 1000 µg/ml was then used as a positive control. Oxytetracycline is a natural bacteriostatic tetracycline compound produced by *Streptomyces* species. The usage of oxytetracycline was justified by its broad spectrum of antibacterial activity against gram-positive bacteria through the inhibition of translation and subsequent protein synthesis (Mog et al., 2020). The results of the inhibition of these bacteria are shown in Table 2 and 3.

Table 2 Antibacterial activity in agar diffusion assay, MIC and MBC of SBH

Sample	Inhibition zone of *P. acne* (mm)	MIC (µg/mL)	MBC (µg/mL)	Inhibition categorized
G. thoracica	37.74±1.5	62.5	125	highly strong inhibitory
H. itama	34.70±2.1	62.5	125	highly strong inhibitory
T. pagdeni	34.08±1.5	62.5	125	highly strong inhibitory
L. terminata	33.52±1.8	125	250	highly strong inhibitory

Table 3 Antibacterial activity in agar diffusion assay, MIC and MBC of SBP extracts

Sample	Inhibition zone of *P. acne* (mm)	MIC (µg/mL)	MBC (µg/mL)	Inhibition categorized
G. thoracica	11.51±2.1	125	250	moderate inhibitory
H. itama	14.43±2.7	125	250	moderate inhibitory
T. pagdeni	12.52±1.5	125	250	moderate inhibitory
L. terminata	11.09±1.8	125	250	moderate inhibitory

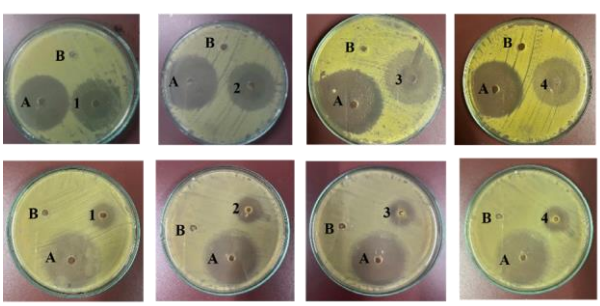

Fig. 2 The inhibition zone (mm) of SBH (top) and SBP extracts (bottom) against *P. acne* of *G. thoracica* (1), H. itama (2), *T. pagdeni* (3) and *L. terminata* (4) (A) represent the positive control, oxytetracycline (B) represent the negative control, 10% v/v DMSO

In this study, we tested SBH and SBP extracts for antimicrobial activity against bacterial infections affecting skin. The results of the antimicrobial screening by agar diffusion assay are showed in Table 2 and 3. The results indicated that the SBH and SBP extracts from stingless bees at concentrations of 1000 µg/mL showed varying degrees of growth inhibition of the test microorganisms. Antimicrobial activity using *P. acnes* shows inhibition honey of *G. thoracica*, *H. itama*, *T. pagdeni* and *L. terminate*, with the inhibition zone being 37.74±1.5, 34.70±2.1, 34.08±1.5 and 33.52±1.8mm, respectively and the inhibition zones being 11.51±2.1, 14.43±2.7, 12.52±1.5 and 11.09±1.8 mm. belong to SBP

extracts of *G. thoracica*, *H. itama*, *T. pagdeni* and *L. terminata*, respectively. The positive control for oxytetracycline averaged 44.40±1.5 mm at 1000 µg/well (Fig. 2). The outcomes of the oxytetracycline inhibitory and positive controls did not differ significantly. In contrast to the SBP extract, which displayed a moderate inhibitory category, the antibacterial activity of the SBH against bacteria demonstrated a very strong inhibitory category.

4. Bacteriostatic and bactericidal activity

The effectiveness of the antimicrobial compounds in the SBH and SBP extracts was assessed using the MIC and MBC assays. In diagnostic laboratories, MIC and MBC determination is essential because it confirms a microorganism's resistance to an antimicrobial agent and monitors the effectiveness of new antimicrobial agents. The MIC and MBC values of the extract against all tested pathogenic bacteria are summarized in Table 2 and 3 with values ranging between 62.5 and 250 µg/mL. There is a wide range of MIC and MBC values that are dependent on the different SBH and SBP extract samples and stingless bee strains.

Honey's ability to kill bacteria is mostly due to its acidity, high osmolarity and hydrogen peroxide concentration, all of which are unfavorable to the growth of *P. acne* (Albaridi, 2019). According to Lusby et al. (2005), the amount of hydrogen peroxide in honey affects its antibacterial effectiveness. Since hydrogen peroxide is one of the reactive oxygen species (ROS), this hydrogen peroxide will enhance the production of cytokines for the inflammatory response to destroy the bacteria. As a result, it acts against bacteria by dissolving their cell structures. Since *P. acnes* prefers neutral or slightly alkaline environments (pH 6.0 to 7.0), the honey's acidic pH will make it difficult for the bacteria to proliferate (Minden-Birkenmaier & Bowlin, 2018). Therefore, honey has a greater power to prevent the growth of germs at lower pH values (Fatima et al., 2018). In this research, the investigated honey had a pH value of pH 3.27–3.76. In the research of Muhammad & Sarbon. (2021), Tualang honey (pH= 4.13±0.02) and Acacia honey (pH= 4.20±0.02) were contrasted with this research. Hence, this may deduce that SBH possesses potent antimicrobial activity since acidity contributes to its properties. Its low pH level and high sugar content limit the development of germs, while its high viscosity acts as a barrier inhibiting infection (Lusby et al., 2005). Honey generally refers to a supersaturated sugar solution with a low water content. As a result, the high sugar content in honey will cause bacterial cells to exhibit a large osmotic gradient, causing the water within the cells to flow out through the osmosis process. Due to the bacterial cells becoming dehydrated, the bacteria are unable to develop and multiply in the hypertonic sugar solution (Almasaudi, 2020). In our studies, the results showed that TSS in the examined SBH samples ranged between 65.20 and 75.12 °Brix. Additionally, honey has osmotic and glucose oxidase properties, especially when polyphenols and defensin-1 are present. These attributes help honey's bactericidal effects (Santos-Buelga & González-Paramás, 2017).

According to Chancao (2009; 2013), honey containing antibacterial activity was generated by Thai stingless bees. Honey has antibacterial and antioxidant qualities that may help prevent eye conditions, including glaucoma and cataracts (Rao et al., 2016) It has been shown by several researches that honey has the ability to suppress gram-positive and gram-negative bacteria (Nasir et al., 2010). In another study, propolis was found to be a resource for bioactive chemical isolation. The recognition of phenolic chemicals is astounding since they not only inhibit the growth of bacteria and some types of disease, but also serve as antioxidants (Bachevski et al., 2020). Aside from the pathogen examined, other variables, including soil type, climate and maturation time, also contribute to the difference. These include bee species, honey concentration and type, floral and entomological origin in different seasons and pathogen testing (Alvarez-Suarez et al., 2018). Additionally, SBH shows potential as an antibacterial substitute for treating infection and inflammation (Ávila et al., 2018). Even though this bee has some biological functions, including 3'–O–methyldiplacone, nymphaeol A and 5,7,3′, 4′–tetrahydroxy–6–geranyl flavonol. These compounds have potent cosmetic properties, especially as anti-acne by showing antioxidant, anti-inflammatory and anti-acne properties (Arung et al., 2023).

Conclusion

Considering the findings of this study, the greater phenolic and flavonoid content boosted the antibacterial and antioxidant capabilities of honey and the ethanolic propolis extract. The SBH had better antioxidant activity and antibacterial activity than the SBP extract. The antibacterial activity of SBH against bacteria was a highly strong inhibitory, whereas SBP extract was a moderate inhibitory when compared to the positive control (oxytetracycline). At a concentration of 1000

µg/mL, the honey of *G. thoracica* had better inhibition of *P. acne* bacteria (37.74±1.5 mm) compared to other species. Further investigation into the efficiency of honey/propolis in various cosmetic formulations has been shown to be useful in the treatment of acne.

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