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Effect of Solvent Type on the Antibacterial Activity of *Trigona laeviceps* Propolis Extract

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ABSTRACT

Propolis is a resinous compound which is mixed with saliva, wax, and other metabolic products of bees. One of the benefits of propolis is as an antimicrobial agent. The objective of this research was to determine antibacterial activity Trigona laeviceps bees propolis originating from Bogor West Java extracted with various solvents (water, ethanol, hexane). The tested bacteria consist of 2 Gram negative and 5 Gram positive bacteria. The raw propolis were characterized for gum, tannin, flavonoid, alkaloid, saponin, total phenol content, and proximate composition. Antibacterial activity was tested using the disc diffusion method. The data were analyzed using the ANOVA test and further tested with the Duncan test at a significance level of 5%. The results showed that the largest component of the sampel was lipid (65.47%). Raw propolis contains phenol (3.08 mg/g), tannin (34.45 mg/g), and flavonoid (21.88 mgQCE/g). The highest yield of propolis extract was obtained with hexane solvent (51.03%), followed by ethanol (18.17%), and water (15.58%). All propolis extracts did not have an inhibition zone against Gram-negative bacteria, but did for Gram-positive bacteria. The propolis extract using ethanol showed the highest diameter inhibition zone (16.92 mm). The finding of this study may be utilized to improve Trigona laeviceps propolis quality in Bogor.

1. INTRODUCTION

Propolis is a resin compound mixed with saliva, wax and other metabolic products of bees (Sforcin, 2016; Freitas *et al*., 2019). Based on SNI 8490:2018, raw propolis is a material in the form of a plastic solid, colored and has a distinctive aroma produced by bees which comes from the sap of various plants that are selected, collected and processed biologically, and contains resin, wax and other compounds. Liquid propolis is a liquid material that comes from the extraction process of raw propolis, contains propolis extract plus common liquid fillers (water, polyethylene glycol, propylene glycol and glycerol), has a distinctive color, aroma and taste and is usually used as a raw material for natural medicines, health supplements, food and cosmetics (BSN, 2018). Propolis has long been known for its many health benefits. Propolis and propolis extract are antiseptic, anti-inflammatory, antioxidant, antibacterial, antifungal, antiulcer, anticancer and immunomodulatory (Gonsales *et al*., 2006; Pasupuleti *et al*., 2017).

The bioactive components of propolis vary depending on the plant source consumed by the bees. The components generally contained in propolis are wax, resin, balm, essential oils, as well as primary and secondary metabolites from smoked plants such as amino acids, vitamins, phenols, terpenoids and alkaloids (Zulhendri *et al*., 2022; Huang *et al*., 2014; Salatino & Salatino, 2021). Propolis ethanol extract is reported to have antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*). This antibacterial activity is correlated with the flavonoid content of propolis contained in it (Gonsales *et al*., 2006). Research by Al-Ani *et al*. 2018 shows that propolis has moderate levels of antibacterial activity in inhibiting Gram-positive and Gram-negative bacteria and has antifungal activity.

Indonesia has approximately 200 million hectares of forest with the potential to produce 2,243 tons of propolis from *Trigona* spp. bees every four months, or 6,729 tons per year. The uniqueness of *Trigona* spp. bees is that they are easy to breed and more resistant to disease compared to *Apis mellifera* bees. The propolis produced has more diverse phytochemical components because its color and flavor are also more diverse, and it has a higher yield of 3 kg per colony per year compared to Apis bees, which produce 20-30 g of propolis per colony per year (Mulyati *et al*., 2020; Syafrizal *et al*., 2014). *Trigona laeviceps* is a stingless bee species found on the island of Java, especially in West Java (Purwanto & Trianto, 2021). This type of bee is capable of producing propolis. Morphologically, *T. laeviceps* has a body length of 3.57 mm and a tongue length of 1.11 mm (Ramli *et al*., 2023). This bee has a small head covered with short hairs. It has large, reddish compound eyes and large, black ocelli. The thorax of this bee has finely punctuated mesoscutum, small, black, and completely covered with yellowish seta, the scutellum is completely black and covered with yellowish seta at the posterior end (Purwanto & Trianto, 2021). It has a flabellum in the mouth that is in the shape of a hairy spoon with a shape resembling a parallelogram. *Sensilla chaetica* at the end of the flabellum of the *T. laeviceps* bee consists of one strand, in contrast to the stinging honey bee which has a branched flabellum. This causes *T. laeviceps* to be less selective in their food sources compared to stinging bees (Ramli *et al*., 2023).

Several studies have reported the bioactive components of propolis. Research by Chewchinda & Vongsak (2019) shows that propolis from *T. laeviceps* Smith from Thailand contains the components α-Mangostin and γ-Mangostin which are phenolic compounds in the xanthones group. Another study was conducted by (Sanpa *et al*., 2015) regarding the propolis content of *T. laeviceps* from Thailand. Apart from the phenolic components of the xanthone group such as α-Mangostin, γ-Mangostin, Mangostanin, Garcinone B, Gartanin and 8-Deoxygartanin, propolis from Thailand also contains the triterpenoid compound Dipterocarpol and the lignan Methyl pinoresinol, which have antibacterial activity against *Bacillus cereus*, *Listeria monocytogenes*, *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Echerichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Serratia marcescens*.

Several studies in Indonesia have reported the antibacterial activity of *Trigona* spp. propolis. For example, Amanda *et al*. (2019) demonstrated that ethanolic extracts of *Trigona thorasica* propolis exhibited antibacterial activity against *Porphyromonas gingivalis*. Saleng & Sari (2016) reported inhibitory effects of ethanolic extracts of Trigona incisa propolis against *Klebsiella pneumoniae* and *Staphylococcus aureus*. In addition, Erlianda *et al*. (2017) showed that flavonoid concentrates from *Trigona* spp. propolis inhibited the ATPase activity of *Streptococcus* mutans. These findings indicate the antibacterial potential of *Trigona* propolis; however, differences in extraction solvents may significantly influence the composition and bioactivity of the resulting extracts. To date, limited information is available regarding the antibacterial activity of *T. laeviceps* propolis in Indonesia extracted using solvents with different polarities. Therefore, this study aims to evaluate the antibacterial activity of *T. laeviceps* propolis from Bogor, West Java, extracted with water (polar), ethanol (semi-polar), and hexane (non-polar). It is expected that the results of this research will be useful for increasing the selling value of the *Trigona* laevicep bee propolis industry from Bogor.

2. RESEARCH MATERIALS AND METHODS

2.1. Material

The material used in this research is raw propolis from *Trigona laeviceps* bees originating from Bogor, West Java. The chemicals used for analysis were n hexane (Supelco, Germany), distilled water, ethanol 96%, ether (Supelco, Germany), n-butanol, suspension of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 19659), *Listeria monocytogenes* (ATCC 7644), *Salmonella typhimurium* (ATCC 14028), *Bacillus cereus* (ATCC 10876), *Streptococcus mutans* (ATCC 31987) obtained from LDITP IPB and IPB Culture Collection, Mueller Hinton Broth (HiMedia, USA), Mueller Hinton Agar (HiMedia, USA), Gram dye (crystal violet dye, safranin dye), reagents (Mayer, Dragendorff, Wagner), chloramphenicol, and dimethyl sulfoxide (DMSO) (Supelco, Germany).

2.2. Characterization of Raw Propolis

Characterization of raw propolis included analysis of wax, gum, and total phenols based on SNI-8490-2018 (BSN, 2018), proximate analysis was based on SNI 01-2891-1992 (BSN, 1992) including water content, ash content, protein content, fat content, and carbohydrate content. Apart from that, other phytochemical compounds were also measured

including tannin, flavonoid, alkaloid, and saponin levels (Sutomo *et al*., 2016; Zahra *et al*., 2021).

2.3. Experimental Design

This study employed a completely randomized design (CRD). In this study, raw propolis was extracted with three solvents with different polarities, namely water, which is polar, ethanol, which is semi-polar, and hexane, which is non-polar. Water and ethanol solvents were chosen because antibacterial compounds generally dissolve in water and alcohol (Wagh, 2013). Meanwhile, hexane solvent was chosen because the highest content of propolis is fat and several fatty acids have antibacterial capabilities (Murhadi 2009; Agustini 2017). Apart from that, hexane is also able to dissolve terpenoid compounds which also have antibacterial capabilities (Agustini 2017; Dasor *et al*., 2021). Meanwhile, antibacterial activity was evaluated using a factorial design consisting of solvent type and extract concentration. The solvent factor comprised three levels (water, ethanol, and hexane), while the extract concentration factor comprised four levels (negative control, 10%, 30%, and positive control).

2.3.1. Raw Propolis Extraction

Extraction was carried out using the ultrasonic method using an Ultrasonic tool (Branson series 3510, USA). The treatment in this research was extraction of propolis samples with 3 types of solvents, namely hexane, ethanol, and water. A 35 gram propolis sample was added with solvent at a ratio of 1:10 and covered with aluminum foil (Sanpa *et al*., 2015). Next, samples with water and ethanol solvents were extracted using ultrasonics for 20 minutes, temperature 20 °C, and frequency 50 Hz, while samples with hexane solvents were extracted using a stirring hot plate (Thermolyne Cimarec 2 SP46925, USA) at a speed of 900 rpm for 4 hours (Zahra *et al*., 2021). Next, the extraction results are filtered with filter paper using a vacuum filter. The residue was extracted again 2 times. The filtered filtrate was then evaporated using a rotary evaporator (BUCHI, Switzerland) at a water bath temperature of 40 °C (hexane extract), 60 °C (ethanol extract), and 80 °C (water extract) to obtain a solid extract which was collected and blown through with nitrogen gas to ensure the solvent had completely evaporated and stored at -20 °C for the next test process. Samples were analyzed in two repetitions. The yield of propolis extract was calculated as the following (Sayuti, 2017):

$$\text{Propolis Yield (w/w)} = \frac{dry\ extract\ weight\ (g)}{initial\ sample\ weight\ (g)} \times 100\% \quad (1)$$

2.3.2. Antibacterial Activity

a. Test preparation

Culture confirmation. The culture confirmation stage is carried out using the Gram staining method. The Gram staining technique begins by cleaning the glass object using 95% alcohol. The glass object is heated by passing it over a Bunsen flame, then waiting until it cools slightly. The bacterial isolate was taken with a tube aseptically and smeared thinly on the surface of the glass object. The specimen was fixed by passing it over a Bunsen flame three times. Crystal violet dye was dropped onto the object glass until it covered the entire preparation. The specimen was left for 30-60 s at room temperature and then washed gently with distilled water for 5 s. The object glass that looks blue is dripped with Lugol's solution, left for 1-2 min at room temperature, then washed in running water for 5 s. The next stage is decolorization by dripping with 95% alcohol. The preparation was rinsed with water for five seconds to stop the decolorization activity. The next step is to drip the object glass with safranin dye and leave it for 1 min, then rinse it gently with water for 5 s and dry it in the air. After that, it was observed under a microscope to see the bacterial response to the dye. If the bacteria look purple, this indicates that the bacteria are Gram positive bacteria. Meanwhile, if the bacteria appear red, this indicates that the bacteria are Gram negative bacteria (Rahmatullah *et al*., 2021).

Regeneration and Bacterial Concentration. The test bacterial culture was grown at 35 °C for 24 h. Bacterial inoculum is made by diluting the liquid culture medium so that it meets the 0.5 McFarland turbidity standard, which is equivalent to approximately 1.5 $\times 10^8$ CFU/mL (Ibrahim *et al*., 2016).

b. Antibacterial Activity Test

The suspension of the tested microbes was spread over solid media in a 100 mm diameter petri dish with a media thickness of 4 mm. The culture is taken with a sterile swab rubbed on the surface of the agar by wiping in one

direction, then rotated 120° and scratched again, and rotated again 120° and scratched again. Discs measuring 6 mm in diameter were impregnated with 20 µl samples of propolis extract each with various solvents (10% and 30%) that had been extracted as well as chloramphenicol and DMSO (Kusumaningrum *et al*. 2023). Discs impregnated with samples and controls were placed on the surface of the cup using sterile tweezers. Disc placement was carried out within 15 min after inoculation. The positive control used was the antibiotic chloramphenicol and DMSO as a negative control. The plates containing the samples and controls were incubated at 37 °C for 24 h (Gonsales, 2006). The diameter of the inhibition zone is measured in mm. Antibacterial activity is seen based on the clear zone produced after the incubation period (Saleng *et al*., 2016). Testing was carried out in triplicates.

2.4. Data Analysis

Statistical analysis was performed using analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) at a significance level of 0.05. Yield data were analyzed using IBM SPSS Statistics version 26 with duplicate measurements, while antibacterial activity data were analyzed in triplicate using the same software.

3. RESULTS AND DISCUSSION

3.1. Characteristics of Raw Propolis

The physicochemical characteristics of raw *Trigona laeviceps* propolis from Bogor, West Java, are presented in Table 1. The composition of the raw propolis was classified into major constituents and phytochemical compounds. The major constituents included wax, gum, moisture, ash, lipid, protein, and carbohydrates, while the phytochemical compounds comprised phenols, tannins, flavonoids, and alkaloids. Qualitative analysis confirmed the presence of wax and gum in the raw propolis.

3.1.1. Macronutrient and Micronutrient Components

According to the results of the proximate test, it is known that the main content of raw *T. laviceps* bee propolis from Bogor is fat, namely 65.47%, followed by carbohydrates at 23.91%. In raw *T. laviceps* bee propolis from Bogor, water, ash and protein contents were also found, namely 4.95%, 1.32% and 2.49% respectively. Anjum *et al*. (2019) stated that propolis contains 50% resin and vegetable balsam, 30% wax, 5% pollen, and 10% essential and aromatic compounds. The difference in percentage of propolis is influenced by harvest time and regional origin. Anjum *et al*. (2019) also stated that propolis contains fatty acid compounds (C7-C18), amino acids such as alanine, arginine, asparagine, cystine, cysteine, valine, tyrosine, serine and others, as well as sugars such as *d*-ribofuranose, *d*-fructose,

Table 1. Characteristics of *T. laeviceps* raw propolis from Bogor, West Java

No	Parameter	Results
1	Candle	Positive
2	Gum	Positive
3	Water content	4.95%
4	Ash Content	1.32%
5	Fat Content	65.47%
6	Protein Content	2.49%
7	Carbohydrate Levels	23.91%
8	Total Phenol	3.08 mg/g
9	Tannin	
	Qualitative	Positive
	Quantitative	34.45 mg/g
10	Flavonoids	
	Qualitative	Positive
	Quantitative	21.88 mgQCE/g
11	Alkaloids	
	Mayer	Negative
	Wagner	Negative
	Dragendorf	Positive
12	Saponin	2.98 %

d-glucositol, *d*-gulose, talose, sucrose, *d*-glucose. Apart from that, minerals such as Sr, Ba, Cd, Sn, Pb, Ti, Fe, K, Zn, Cu, Mg, Na, and others are also found in propolis (Anjum *et al*., 2019; Pasupuleti *et al*., 2017).

3.1.2. Phytochemical Components

According to the qualitative phytochemical test results in Table 1, it is known that raw *Trigona* laviceps bee propolis from Bogor was detected to contain tannins, flavonoids and alkaloids. These results are in accordance with research by Khairunnisa *et al*. (2020) which shows the qualitative test results of propolis extracted with ethanol, methanol and water containing alkaloid, flavonoid, phenolic and tannin compounds. Research by Huang *et al*. (2014) also showed that there are flavonoids, saponins and tannins in propolis originating from various regions.

Based on the results of quantitative phytochemical tests, the total phenol content was 3.08 mg/g, tannins were 34.45 mg/g, and flavonoids were 21.88 mg/g. Mohamed *et al*.'s research (2022) showed that propolis extract from the stingless bee Tetrigona apicalis in Malaysia contained 31.99 mgGAE/g total phenols and 66.40 mgQCE/g flavonoids. Mayworm *et al*. (2014) research with propolis samples from Brazil showed a tannin content of 0.7-4.1%.

a. Alkaloids. In the qualitative alkaloid test with Mayer's and Wagner's reagents, negative results were obtained, while in the test with Dragendorf's reagent, positive results were obtained. A sample is declared to contain alkaloids if two of the three reagents show positive results (Zahra *et al*., 2021). So based on the results obtained, *Trigona laeviceps* propolis from Bogor does not contain alkaloids. This is likely because alkaloids are much more abundant in the roots, whereas bees collect plant resin and sticky exudates from bark or plant leaf buds to produce propolis, so alkaloids are not contained in propolis (Ebiloma *et al*., 2020).

b. Flavonoids. Flavonoids are the main phytochemical components in propolis. Flavonoids play a significant role in the biological activity of propolis. The broad biological properties of flavonoids include antibacterial, antiviral, and anti-inflammatory. Flavonoids in propolis can be categorized based on their chemical structure, namely flavones, flavonols, flavanones, flavanonols, kalskon, dihydrokalskon, isoflavones, isodihydroflavones, flavans, isoflavans, and neoflavonoids (Huang *et al*., 2014). The flavonoids in propolis are kaempferol, catechin, and apigenin (Anjum *et al*., 2019). Falcao *et al*. (2013) stated that propolis derived from *Apis mellifera* bees from Portugal is rich in flavonoids such as kaempferol-dimethylester. Anjum *et al*. (2019) also stated that propolis contains flavonoids and phenolic compounds. The flavonoids in propolis are kaempferol, catechin, apigenin, isosativan, 20-hydroxy-7,40-dimethoxyisoflavan, liquiritigenin, isoliquiritigenin, formononetin, vestitol, neovestitol, medikarpin, 7-o-neovestitol, pinobanksin, pinocembrin, chrysin, astrapterocarpan, 3,8-dihydroxy-9-methoxy-pterocarpan, broussonin B, 8-prenylnaringenin, and gerontosanton H (Huang *et al*., 2014; Ebiloma *et al*., 2020).

c. Phenolic Compounds. Other phytochemical components found in propolis are phenolic compounds which are not flavonoids. Huang *et al*. (2014) reported the presence of phenolic compounds in the form of cinnamic acid, *p*-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, and their derivatives in propolis from Brazil. Prenylated cinnamic acid is a chemical compound that has good antibacterial activity among other phenolic components found in propolis from Brazil. In propolis from Kenya, another phenolic compound was found, namely Stilbene. Stilbenes were identified into two geranilstilbenes, namely schweinfurthin A and schweinfurthin B.

d. Saponin. Saponins are a group of compounds consisting of glycosides which are polar and terpenoids which are non-polar. Previous research showed that propolis extract from Makassar contains the compounds nigakilactone I and picrasin D which are included in the saponin class of compounds (Mulyati *et al*., 2020).

e. Tannin. Tannins are polyphenols that are able to bind proteins and form complex bonds. Tannins have antimicrobial abilities. Tannins consist of two classes, namely hydrolyzable tannins (gallates and/or ellagates) which are found in limited quantities in plant angiosperms and proanthocyanidins (condensed tannins) which are found in many plants (Bernays *et al*., 1989; Mayworm *et al*., 2014). Research with propolis samples from Brazil showed that all samples contained proanthocyanidin type tannins (Mayworm *et al*., 2014).

3.2. Propolis Yield

Based on the yield analysis, the highest yield was obtained for propolis extract with hexane solvent, namely 51.05%, then continued with propolis extract with ethanol solvent, namely 18.17%, and the smallest was the extract with water

solvent, namely 15.58%. Based on ANOVA analysis, the average yield value of the three solvents was significantly different (p = 0.000). The results indicate an inverse relationship between solvent polarity and extraction yield. This is in accordance with the results of proximate analysis which shows that fat is the main content in raw propolis (65.47%). Ethanol and water contain hydroxyl groups, which are capable of forming hydrogen bonds with phytochemical compounds. Meanwhile, hexane is included in the hydrocarbon group and has difficulty forming hydrogen bonds. Hexane is a suitable solvent for extracting non-polar components such as fats and other types of lipids (Srikacha & Ratananikom, 2020). Propolis extract with ethanol solvent had a smaller yield than research by Zahra *et al.* (2021) with *Trigona* sp propolis samples from North Lombok, namely producing a yield of 21.63%. The yield of *Trigona laeviceps* bee propolis extract from Bogor, West Java can be seen in Figure 1.

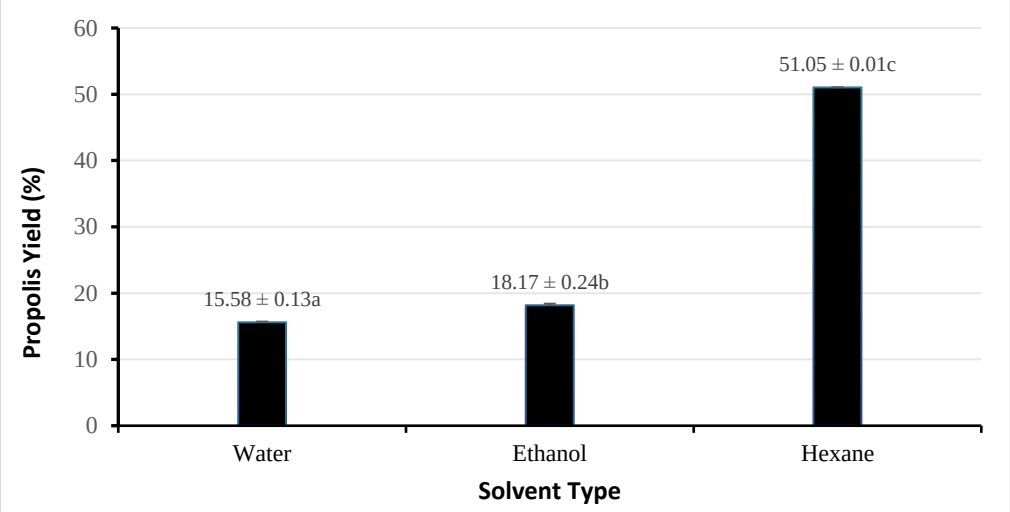

Figure 1. Propolis yield of *Trigona laeviceps* extract from Bogor using various solvents

3.3. Antibacterial Activity of *Trigona laeviceps* Bee Propolis Extract from Bogor

The antibacterial activity of *Trigona laeviceps* bee propolis extract from Bogor, West Java was tested on seven bacterial strains which were divided into five Gram-positive bacteria and two Gram-negative bacteria. Gram positive bacteria consist of *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Listeria monocytogenes*, and *Streptococcus mutans*. Meanwhile, Gram negative bacteria are *Escherichia coli* and *Salmonella typhimurium*. Based on the analysis results in Table 2, it was found that the propolis extract from the three solvents did not have an inhibition zone against all Gram-negative bacteria, namely *Escherichia coli* and *Salmonella typhimurium*. Meanwhile, in Gram-positive bacteria, namely *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Listeria monocytogenes*, and *Streptococcus mutans*, inhibition zones were found with varying values between concentrations and between solvents. These results are in accordance with the research results of Anjum *et al.* (2019), namely that propolis extracted with ethanol is more effective at inhibiting Gram-positive bacteria than Gram-negative bacteria. This is caused by the multi-layered and very complex structure of the cell wall of Gram-negative bacteria (cytoplasmic membrane, peptidoglycan, perillary space, lipoproteins and lipopolysaccharides) thereby reducing the ability of antibacterial substances to enter the cell (Ramadhani *et al*., 2021).

According to the different types of extraction solvents, it was found that propolis extract with ethanol solvent had the highest inhibition zone compared to the other two solvents at the same concentration level. Propolis extracted with water has the lowest zone of inhibition when compared to the two extracts with ethanol and hexane solvents. Samples of propolis extract with water did not have an inhibition zone against the growth of *S. aureus* and *S. mutans* at concentrations of 10% and 30% (Table 2). Samples of propolis extracted with water inhibited *L. monocytogenes* at a concentration of 30%, but no inhibition at a concentration of 10%. In samples of propolis extracted using water, the inhibition zone for *B. cereus* was highest at a concentration of 10% with an inhibition zone of 9.47 mm, while for *B.*

subtilis and *L. monocytogenes* the inhibition zone was largest at a sample concentration of 30%.In samples extracted with ethanol, 10% extract concentration had the highest zone of inhibition against *S. aureus*, *B. cereus*, and *B. subtilis*, namely 13.47, 14.72, and 16, 16 mm, respectively. Meanwhile, for *L. monocytogenes* and *S. mutans* bacteria, the highest inhibition zone was at a concentration of 30%, namely 16.92 mm and 16.72 mm. In samples extracted with hexane, 30% extract concentration had the highest inhibition zone, namely 14.37 mm for *S. aureus* bacteria, 17.19 mm for *B. cereus*; 19.37 mm in *B. subtilis*, 17.15 mm in *L. monocytogenes*, and 15.27 mm in *S. mutans*.

Statistically, the extract with ethanol solvent had antibacterial activity against *S. aureus* ($p = 0.000$), *B. subtilis* ($p = 0.000$), and *S. mutans* ($p = 0.000$) which was significantly better than the extract with water and hexane solvents. In the bacteria *S. aureus* ($p = 0.064$) and *B. subtilis* ($p = 0.143$), the zone of inhibition of the extract with ethanol solvent was not significantly different from the positive contract (chloramphenicol). In *B. cereus* bacteria, the antibacterial activity of the extract with ethanol solvent was the highest at a concentration of 10% and was significantly different ($p = 0.000$) from the extract with 30% concentration of ethanol solvent, the extract with water solvent, and the extract with hexane solvent. For *L. monocytogenes* bacteria, the antibacterial activity of the extract with ethanol solvent was the highest at a concentration of 30% and was significantly different ($p = 0.000$) from the extract with 10% concentration of ethanol solvent, the extract with water solvent, and the extract with hexane solvent.

If we look at the antibacterial activity in this study, ethanol is the most suitable solvent for extracting *Trigona laeviceps* bee propolis from Bogor, West Java. This is due to the polarity of the solvent. The presence of hydroxyl groups in ethanol makes this solvent capable of forming hydrogen bonds with phytochemical compounds which generally have antibacterial capabilities. Meanwhile, hexane is included in the hydrocarbon group and is quite difficult to form hydrogen bonds. However, the low antibacterial activity in samples with water as a solvent indicates that antibacterial activity does not go hand in hand with increasing polarity (Srikacha & Ratananikom, 2020). Wigmore *et al*. (2016) and Seleshe & Kang (2019) stated that water solvent extracts generally contain a mixture of non-active components such as carbohydrates, organic acids, proteins and minerals. The antibacterial activity of sample extracts with ethanol is related to the presence of phytochemical compounds such as tannins, flavonoids, saponins and phenolic compounds which are found in crude propolis samples. These components have the ability to act as antimicrobials by damaging microbial cell membranes, weakening cellular mechanisms, controlling biofilm formation, and inhibiting capsule production (Srikacha & Ratananikom, 2020).

Table 2. Inhibition Zone of *Trigona laeviceps* Bee Propolis Extract from Bogor, West Java

No	Bacteria	Inhibition Zona (mm)											
		Water				Ethanol				Hexane			
		K (-)	10%	30%	K (+)	K (-)	10%	30%	K (+)	K (-)	10%	30%	K (+)
1	*S. aureus*	-^{a}	7.31^{c}	4.57^{b}	13.49^{d}	-^{a}	13.47^{d}	13.35^{d}	16.08^{d}	-^{a}	8.37^{c}	9.56^{c}	14.31^{d}
2	*B. cereus*	-^{a}	9.47^{c}	7.99^{b}	16.82^{g}	-^{a}	14.72^{f}	11.76^{e}	16.86^{g}	-^{a}	9.88^{cd}	11.19^{de}	16.52^{g}
3	*B. subtilis*	-^{a}	2.78^{a}	9.17^{b}	14.84^{d}	-^{a}	16.16^{d}	15.34^{d}	15.16^{d}	-^{a}	10.29^{bc}	13.37^{cd}	16.22^{d}
4	*L.monocytogenes*	-^{a}	-^{a}	10.43^{c}	16.44^{e}	-^{a}	14.62^{d}	16.92^{e}	16.59^{e}	-^{a}	8.69^{b}	11.15^{c}	17.07^{e}
5	*S. mutans*	-^{a}	-^{a}	-^{a}	25.70^{d}	-^{a}	15.25^{c}	16.72^{c}	24.21^{d}	-^{a}	3.75^{a}	9.27^{b}	24.78^{d}
6	*E. coli*	-^{a}	-^{a}	-^{a}	17.71^{b}	-^{a}	-^{a}	-^{a}	19.38^{b}	-^{a}	-^{a}	-^{a}	17.81^{b}
7	*S. Typhimurium*	-^{a}	-^{a}	-^{a}	24.09^{bc}	-^{a}	-^{a}	-^{a}	23.40^{b}	-^{a}	-^{a}	-^{a}	24.55^{c}

Note: K(-): negative control, K(+): positive control. Mean values on the same row with different notations indicate statistically significant ($p \leq 0.05$)

3.4. Antibacterial Components in *Trigona laeviceps* Propolis

Based on the data in Table 2, we can conclude that *Trigona laeviceps* propolis from Bogor, West Java, which is extracted with water, ethanol and hexane, has antibacterial potential. The compounds in *Trigona laeviceps* propolis from Bogor that have antibacterial potential are:

a. Phenolic Compounds

In previous research (Mulyati *et al*., 2020), quercetin was identified in samples of propolis extract using ethanol solvent from Lampung. Quercetin is a phenolic compound and is reported to have antibacterial ability against both Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *S. Enteritidis*). According to Anjum *et al*. (2019), propolis has a significant effect against bacteria such as *Enterococcus* spp., *Escherichia coli*, and

Staphylococcus aureus. Propolis extracted with ethanol is more effective against Gram-positive bacteria than Gram-negative bacteria. Propolis shows antibacterial activity against several aerobic bacteria such as *B. cereus*, *B. subtilis*, *Enterococcus* faecalis, *Micrococcus luteus*, *Nocardia asteroids*, *Rhodococcus equi*, *Staphylococcus auricularis*, *S. epidermidis*, *S. capitis*, *S. haemolyticus*, *S. warnerii*, *S. mutans*, *S. hominis*, *Streptococcus cricetus*, *St. faecalis*, *St. pyogenes*, *St. pneumoniae*, *St. sobrinus* and *St. viridians*. Propolis acts as a bactericidal agent by stopping bacterial cell division, destroying cell walls and bacterial cytoplasm. The inhibitory action of propolis against bacteria is an interaction between phenolic compounds and flavonoid compounds, namely galangin pinosembrin, acetin, chrysine and catechin (Anjum *et al*., 2019; Tirtasari *et al*., 2024).

b. Flavonoids

Sanpa *et al*. (2015) found eight compounds found in *Trigona laeviceps* bee propolis from Thailand. These compounds are α-mangostin, mangostanin, 8-deoxygartanin, gartanin, dipterocarpol, Γ-mangostin, garcinone, and methylpinoresinol. The compounds α-mangostin and garcinone are the most active compounds as antibacterials compared to the other six compounds. The compounds α-mangostin and garcinone are included in the xanthone class of flavonoid compounds.

c. Tannin

Propolis is also reported to contain tannin compounds. Mayworm *et al*. (2014) stated that propolis samples from Brazil contained proanthocyanidin type tannins. Based on the results of this research, proanthocyanidin has the ability to inhibit the growth of pathogenic bacteria that cause periodontitis. Proanthocyanidins have a highly hydroxylated structure consisting of flavan-3-ol, forming an oligomeric structure with a variable number of units (two or more). Mostly, Flavan-3-ols are Catechins (C), Epicatechins (EC), or substituted derivatives linked via C4–C8 or C6 bonds (B-type). According to the number of hydroxyl substitutions in ring B, Proanthocyanidins can be categorized as Propelargonidin (one hydroxyl substitution), Procyanidin (two hydroxyl substitutions), and Prodelphinidin (three hydroxyl substitutions) (Nawrot-Hadzik *et al*., 2021).

d. Fatty acid

Propolis also contains C7-C18 fatty acid compounds (Anjum *et al*., 2019). In the results in table 2, it is known that the propolis sample from Bogor which was extracted with hexane solvent also has antibacterial activity. According to Murhadi (2009), fatty acids such as myristoleic acid (14:1), palmitoleic acid (16:1), linolenic acid (18:3), capric acid (10:0), lauric acid (12:0), and myristic acid (14:0) have antibacterial activity against *S. aureus* bacteria but fatty acids from plants do not have an inhibitory effect on Gram-negative bacteria, except those containing low carbon chain fatty acids (< C8) mainly in the form of monoacylglycerol. The most active saturated fatty acid as an antibacterial compound is lauric acid (12:0), while for monounsaturated fatty acids and poly/plural unsaturated fatty acids, respectively, they are palmitoleic acid (16:1) and linolenic acid (18:3). The location and number of double bonds in C12-C22 fatty acids influence antibacterial activity more than fatty acids with a number of C atoms less than 12. The geometric configuration of the fatty acid structure that is active as an antimicrobial is the cis form, while the trans isomeric form is inactive. In addition, fatty acids in the form of polyzole esters can increase antibacterial activity, whereas in the form of monohydric alcohol esters they actually inactivate the antibacterial properties. According to Lam *et al*., (2016), saturated fatty acids such as oleic acid and palmitic acid can disrupt bacterial cell membranes and can cause cell death. Antibacterial effectiveness is influenced by chain length and saturation level. Previous research reported that palmitic acid showed inhibitory activity against various bacterial strains (Charlet *et al*., 2022).

4. CONCLUSION

The main component of raw *Trigona laeviceps* bee propolis from Bogor is fat with a content of 65.47%, followed by carbohydrates at 23.91%. The highest yield of propolis extract was obtained with hexane solvent (51.03%), followed by ethanol (18.17%), and water (15.58%). The results of the antibacterial activity analysis showed that all extracts did not have an inhibition zone against Gram-negative bacteria, namely *E. coli* and *S. Typhimurium*. Meanwhile, in Gram-positive bacteria (*S. aureus*, *B. cereus*, *B. subtilis*, *L. monocytogenes*, and *S. mutans*) there is an inhibition zone with the largest inhibition zone in samples of propolis extract with ethanol solvent. This shows that the active antibacterial

components in propolis are semi-polar. In future research, analysis needs to be carried out to determine the specific antibacterial components of raw *T. laeviceps* bee propolis from Bogor. These findings indicate that ethanol is the most effective solvent to extract antibacterial compounds from *T. laeviceps* propolis. Further research can be focused on food preservation applications.

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AUTHOR CONTRIBUTION STATEMENT

Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
INA	✓	✓			✓		✓		✓		✓		✓	✓
DRA	✓	✓		✓		✓	✓	✓		✓	✓	✓		
UH	✓	✓		✓		✓		✓		✓	✓	✓		
NDY	✓	✓	✓	✓		✓		✓		✓	✓	✓		

C: Conceptualization
M: Methodology
So: Software
Va: Validation
Fo: Formal Analysis
I: Investigation
D: Data Curation
R: Resources
O: Writing - Original Draft
E: Writing - Review & Editing
Vi: Visualization
Su: Supervision
Fu: Funding Acquisition
P: Project Administration

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