Physicochemical Analyses and Antibacterial Potential of Propolis by Stingless Bee (*Homotrigona apicalis*) Found in East Kalimantan, Indonesia

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Abstract: This study examined the antibacterial potential of *Homotrigona apicalis* propolis extract against *Staphylococcus epidermidis* and *Propionibacterium acnes*, which are bacteria found on human skin that can cause opportunistic infections. The extract was obtained through the collection, processing, extraction, and fractionation of fresh herb samples. The antibacterial activity of the methanol extract, n-hexane fraction, ethylacetate fraction and residual fraction, at concentrations of 2.5%, 5%, and 10% was evaluated using the disc diffusion method, with clindamycin 0,1% and DMSO 1% as positive and negative controls respectively. Statistical analysis was performed using ANOVA. The results revealed that both the methanol extracts and the ethyl acetate fraction of *Homotrigona apicalis* propolis showed significant inhibitory effects on bacterial growth. The greatest antibacterial activity was observed at the concentration ethyl acetate fraction of 10%, with inhibition zones of 13.27 mm for *Staphylococcus epidermidis* and 12.86 mm for *Propionibacterium acnes*. Importantly, *Staphylococcus epidermidis* exhibited higher susceptibility to the propolis extract and fraction compared to *Propionibacterium acnes*. These findings suggest that *Homotrigona apicalis* propolis extract has the potential to be used as an active ingredient in cosmetics targeting acne-causing bacteria, specifically *Staphylococcus epidermidis* and *Propionibacterium acnes*. The extract demonstrated efficacy against these pathogens suggests its potential as an alternative to conventional antibiotics.

1 INTRODUCTION

Staphylococcus epidermidis and *Propionibacterium acnes* are known as commensal bacteria in human skin which can change into opportunistic (Nakase et al. 2014; Chessa et al. 2015). *Staphylococcus epidermidis* covered various parts of the skin, while *P. acnes* resides mainly at pilosebaceous skin follicles. This microbial interplay mediated through molecules involved in intercellular competition or communication, may have an impact on a fine balanced skin ecosystem. Disturbed balance or dysbiosis significantly impacts skin health and might initiate or contribute to events that lead to skin disorders. One such disorder is acne vulgaris, a multifactorial disease of pilosebaceous units of the skin that affects adolescents (Christensen et al. 2016).

Propionibacterium acnes related significantly to the initial stage of acne causes increasing lipogenesis originating in sebaceous glands. It induces inflammation and pustules on the skin (Neves et al. 2015; Blaskovich et al. 2019). Meanwhile, *S. epidermidis* could act as opportunistic when it enters the bloodstream (Nakase et al. 2014; Tabri 2019). Skin clinic acne treatment usually uses antibiotics that could overcome inflammation and kill such bacteria as tetracycline, erythromycin, doxycycline, and clindamycin (Nakatsuji et al. 2009; Doğ an et al.

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2017). However, these drugs' side effects are identified as irritation and allergy and long-term consumption of antibiotics causes resistance, organ damage, and immune hypersensitivity (Adawiyah et al. 2010; Tan et al. 2018; Dikicier 2019). The entire side effect leads researchers to discover and develop new sources for antimicrobial agents of natural products, e.g. medicinal plants (Abdallah 2011). Sadeek & Abdallah (2019), some phytochemical compounds extracted from medicinal plants showed effective antibacterial potential against multi-drug-resistant pathogens and these compounds could be exploited as antibacterial drugs.

Antibiotic resistance is the changing sensitivity of microorganisms due to antibiotics, therefore, higher concentrations are needed to inhibit the growth of resistant bacteria compared to susceptible strains (Galderm, 2014). A significant factor that contributes to increasing resistance is the irrational consumption of antibiotics and antibiotics (Walsh*et al*, 2016). Related to this fact other alternatives are sought to treat infections using natural ingredients (Utami*et al*, 2021). The natural ingredient mostly found as an antibacterial is Propolis from *the* stingless bee *Homotrigona apicalis.*

The bee *Homotrigona apicalis* is a species of stingless honey-producing bee part of the Meliponidae family and it has no sting and is small in size (Francoy *et al*., 2019). These insects make nests in tree holes, wall cracks, and bamboo cavities, a source of food for bees in the form of pollen, nectar, and resin, naturally. Bees are herbivorous animals (Achyani and Wicandra, 2019) and Bee hive *homotrigone* useful benefit for the health of the human body and can produce honey, royal jelly, *bee pollen,* and propolis (Syafrizal*et al*, 2016). This natural bee product is known to have antibacterial, antifungal, anticancer, anti-inflammatory, and anti-asthmatic benefits (Campos et al., 2015; Lopez et al., 2019; Farias et al., 2014).

It is described that propolis is one of the substances produced by bees and consists of a mixture of bee saliva and plant exudates that they collect (Mardiah, 2017). Propolis is trusted as a natural ingredient, empirically and relatively safe completed plenty of benefits (Lutpiatina, 2015). The common benefits of propolis are as a medicine or supplement, mouthwash, anti-inflammatory, disease therapy, and accelerating wound healing. Based on Rosyidi *et al* (2018), it has plenty of chemical compounds and varies depending on the environment surrounding the bee farm, therefore, plenty of differences in compounds in propolis in Indonesia are found. Propolis consists of amino acids, terpenoids, and polyphenols (phenolic acids, esters, and flavonoids), in general (Pujirahayu *et al*, 2014) and Flavonoids are one of the significantly important ingredients in propolis which have antioxidant, anticancer, anti-inflammatory, anti-allergic, antiviral and antibacterial effects (Rismawati et al, 2017; Alexandra, 2018; Hermalinda, 2019).

Lutpiatina (2015) found that propolis has antibacterial properties, the inhibitory zone of the ethanol extract of kelutut bee propolis (*Trigona sp)* originating from the South Kalimantan area at concentrations of 20%, 40%, 60%, 80% and 100% against *Staphylococcus aureus* is 6.4 mm; 10mm; 12.6 mm; 14.4mm; 16.4mm, meanwhile, Gusmawarni *et al*, (2021) concluded that the ethanol extract of bee propolis (*Trigona sp*) originating from Pekanbaru area has inhibitory zone activity against bacterial growth *Streptococcus mutans* at concentration of 40%, 60% and 80% respectively 8 mm, 9.3 mm and 11 mm. Methanol extract of propolis shows the highest inhibitory rate at concentrations of 750 µg/mL (6 mm) and 1000 µg/mL (10 mm) against *E. Coli* and *Staphylococcus aureus* were compared by hexane and ethyl acetate extracts (Yusop *et al*, 2018). Empirical research by Mayangsari (2013) found that Lawang propolis extract against *Fusobacterium nucleatum* obtained MIC (Minimum Inhibitory Concentration) of 1.48% and MBC (Minimum Bactericidal Concentration) of 1.54%.

Based on the significant benefits of propolis and easy bee farm *Homotrigona apicalis*, this study implementing bee propolis *Homotrigona apicalis* by breeders at Palaran District, City of Samarinda, East Kalimantan to identify antibacterial characteristics of propolis methanol extract through bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis***.**

2 METHOD

2.1 Instruments and Materials

This study implemented glassware (*pyrex*), blender, vortex*, hotplate* (*Ceran*®), petri dishes, spirit lamps, autoclaves, incubators, *magnetic ironer*, micropipette, analytical balance, caliper, water bath, *laminator airflow cabinet* (LAF), maserator, *rotary evaporator*, *spectrophotometer*, knife, cutting board. Meanwhile, materials contributes for this research are methanol, n-hexane, ethyl-acetate, bee propolis *Homotrigona apicalis*, filter paper, sterile cotton, distilled water, Muller Hinton Agar (MHA) media,

Nutrient Agar media, clindamycin 0.1%, DMSO, NaCl 0.9%.

2.2 Procedure

2.2.1 Simplicia Setup

Propolis is taken from Tani Harapan Loa Janan, Kutai Kartanegara, East Kalimantan, which is then wet sorted and targeted to separate propolis from the nest and impurities.

2.2.2 Proceed Extracts

The simplicia obtained was macerated for three days using methanol. Remaceration is carried out on the simplicial dregs and it is filtered to obtain macerate, then evaporated using a rotary evaporator and evaporated over a water bath until a thick extract is obtained (14).

2.2.3 Fractionation

Weighed 5 grams of extract and partitioned using distilled water and n-hexane with a ratio of 1:1 v/v. The sample was shaken repeatedly in a separating funnel until homogeneous and left until a layer of water and a layer of n-hexane were formed. The n-hexane layer was collected in a different container. The n-hexane layer was then heated over a water bath until thick and the n-hexane fraction was obtained.

The water layer was partitioned over using an ethylacetate completed ratio of 1:1 v/v. the following phase is shaken in a separating funnel until homogeneous, left to stand until two layers are formed, namely the water layer and the ethyl acetate layer. Each layer is collected into a different container, heated until thick and the ethyl acetate fraction and residual fraction are obtained (Mujipradana, 2018)

2.2.4 Phytochemical Screening

Alkaloid

0.5 g sample was added to 1 mL of 2 N hydrochloric acid and 9 mL of distilled water heated over a water bath for two minutes and finally cooled and filtered. The filtrate obtained was used for alkaloid testing. Take 3 test tubes, add 0.5 mL of filtrate to each tube. Add 2 drops of reagent of each test tube*mayer*, *bouchardat* and *dragendorf*. Alkaloid is positive if precipitate occurs and at least 2 of the 3 reagents above are positive then sample is defined to contain alkaloids, namely the formation of white precipitate at reagent *mayer*, brown at reagent *Bouchardat* and brick red deposits on the fixer *dragendorf* (Handayani*et al*, 2019; Nafisah *et al*, 2014).

Saponin

0.5 g sample is put into a test tube then 10 mL of hot water is added, cooled briefly after cooling, and shaken vigorously for 15 minutes, if a stable foam form for 10 minutes and the foam is 1-10 cm high and when dripped 1 drop 2 N hydrochloric acid foam is still present, then the sample contains saponin compounds (Supomo, *et al*, 2019).

Flavonoid

1 gram of sample is added to 10 ml of hot water then boiled for 5 minutes, filtered while it is still hot. 5 mL of the filtrate obtained was taken then 0.1 g of magnesium powder, 1 mL of HCl, and 2 mL of amyl alcohol were added, then shaken and allowed to separate. Samples contain flavonoids if there is a red color change at the amyl alcohol layer (Supomo *et al*, 2019).

Tannin

1 mL sample solution is reacted with 10% iron (III) chloride solution, if dark blue, blackish blue or greenish black color occurs, it indicates the presence of tannins (Supomo *et al*, 2019).

Phenol

Several dissolved samples were extracted by 20 mL of 70% ethanol. 1 mL of the resulting solution was taken and then 2 drops of 5% $FeCl_3$ were added. A positive reaction is indicated by the presence of a green or blue-green color (Anisa *et al*, 2022).

Quinones

5 mL sample of the experimental solution obtained from the identification of flavonoids was put into a test tube, and a few drops of 1 N NaOH solution were added. The formation of a red color indicates the presence of quinine group compounds (Lestari and Andriani, 2021).

2.2.5 Antibacterial Testing

Making positive and negative controls

This study used Clindamycin as a positive control of 0.1% and DMSO as a negative control of 1%.

Preparation of extract and fraction concentration series

This study used a series concentration of methanol extract, n-hexane fraction, ethyl acetate fraction and residual fraction of propolis, namely 2.5%, 5%, and 10% completed calculations (w/v), using 1% DMSO as a solvent.

Sterilization of tools
The first phase is using sterilized glassware for antimicrobial activity research at an autoclave at 121 ºC for 15 minutes, the tweezers were burned by burning over a direct flame (Mujipradhana et al, 2018).

Slanted agar media preparation
Weighed 5 g of Nutrient Agar (NA), dissolved in 250 mL of distilled water (20 g/1,000 mL). The homogenized media was then sterilized in an autoclave at 121˚C for 15 minutes. Pour 5 ml of NA media into a test tube, let it sit *Nutrient Agar* at room temperature till preparation solidifies at a 45-degree tilt position for 10 minutes.

Bacterial rejuvenation
Bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis* swabbed into slanted agar media, and incubated in an incubator at 37ºC for 2x24 hours.

Preparation of Mac. Farland solution
Solution H_2SO_4 1% as much as 0.25 ml is mixed completed using $BaCl_2$ solution$_2$ 1% as much as 0.1 gram in an Erlenmeyer. Followed by shaking until a cloudy solution is formed, then the turbidity standard is measured with a spectrophotometer.

Preparation of bacterial suspensions
The test bacterial were taken ± 1 cycle then suspended in a tube containing 10 ml of 0.9% NaCl solution. The turbidity of the solution was then measured using a spectrophotometer *uv-vis* with absorbance that has been determined with standard solutions *Mac. Farland* at a wavelength of 600 nm (Mujipradana,2018).

Antibacterial Activity Test of methanol extract, n-hexane fraction, ethyl acetate fraction and residual fraction of propolis
The paper discs were soaked in methanol extract, n-hexane fraction, ethyl acetate fraction and residual fraction of propolis with concentrations 2,5%, 5%, and 10%, clindamycin 0.1% as positive control, and DMSO 1% as negative control for 3 minutes. Prepare 72 petri dishes, then pour in 20 mL of MHA media, then let it solidify. Dip a sterile cotton swab into each *Propionibacterium acnes* and *Staphylococcus epidermidis* suspension, while it is absorbed, lift the stick and squeeze it by pressing against the wall of the tube. One hundred microliter bacterial suspension was taken using a micropipette and placed in a petri dish containing MHA media, then smeared using a sterile cotton swab until the entire surface of the agar media was tightly covered and left for 5-15 minutes, therefore, bacterial suspension seeped into the agar medium. The soaked paper disc is placed on the surface of the MHA media which has been inoculated with bacteria. The petri dishes were left at room temperature for an hour before being incubated at 37°C for 24 hours and this test was repeated three times (Pratiwi, et al., 2016). Antibacterial activity showed by Inhibition Zone Diameter (mm).

MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration)

While observing the disc paper test, we could see an inhibition zone that forms around the disc paper and after obtaining the inhibition zone, the concentration range of the inhibition zone is used to determine the MIC and MBC using the solid dilution method. Variations in solid dilution concentration were made based on the smallest concentration which still provided an inhibition zone for the antibacterial potential test. Test bacterial suspensions and extracts that have been dissolved according to varying concentrations are inoculated *for flat* in MHA media completed ratio of bacterial suspension, such extract (1:1) incubated at 37°C for 24 hours. Meanwhile, the clearest media is test media completed by the smallest concentration accompanied by no bacterial growth designated as MIC. The results determined as KHM are confirmed by doing the results *streak* on MHA media and incubated at 37°C for 24 hours. On the results *streak* observed based on the turbidity of bacterial growth at media and when the media still looks clear then the results are designated as MBC (Murtiwi, 2014).

3 RESULT AND DISCUSSION

The nest criteria of propolis use at this study which having brittle texture and dark color, taken from Desa Tani Harapan Loa Janan, Kutai Kartanegara, East Kalimantan. Propolis is extracted using methanol. The secondary metabolite groups testing were aimed to determine the presence of secondary metabolites in natural material samples. The results of phytochemical screening tests on total methanol extract of propolis can be seen in Table 1.

Table 1: Phytochemical Screening Test Result.

Compounds	Test	Result
Alkaloid	Mayer	(+)
	Bouchardat	(+)
	Dragendorf	(-)
Saponins	HCl 2N	(+)
Flavonoid	HCl + Mg + Amyl Alcohol	(+)
Tannin	$FeCl_3$	(+)
Fenol	$FeCl_3$ 5%	(+)
Quinone	NaOH 1 N	(+)

Notes:
(-) = Negative result
(+) = Positive result

Groups of compounds which are suspected of being potential antioxidants in the ethanol extract of propolis are including flavonoids. Alkaloid, saponin. Tannin, fenol and quinones. Flavonoid compounds in their structure contain hydroxyl groups that can donate hydrogen atoms to free radicals, so flavonoid compounds have the potential as antioxidants. Flavonoids are reducing compounds that can inhibit many oxidation reactions. Moreover, flavonoids have the ability as antioxidants since they can transfer an electron to free radical compounds as well as quinones (Ridho, 2013).

The method is based on disc diffusion for antibacterial activity. Paper discs containing the antimicrobial agent methanol extract of propolis were placed on MHA media which had previously been planted by bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis*, paper discs that have been soaked in antimicrobial agents from methanol extract of propolis will diffuse on MHA agar media. The antibacterial test results on *Propionibacterium acnes* and *Staphylococcus epidermidis* completed various concentrations, the inhibition zone is obtained shown at table 2.

Identified at antibacterial test, the enterely variations in concentration showed that the propolis extract and fraction had a very active inhibitory response to bacterial growth *Propionibacterium acnes* and *Staphylococcus epidermidis*. Based on measurements of the result bacterial inhibition zone, it shows that the bacteria *Staphylococcus epidermidis* which is a type of gram-positive bacteria has a larger inhibition zone than bacteria *Propionibacterium acnes*.

Based on Table 2, seems that the concentration of propolis methanol extract has the largest inhibitory zone diameter against the bacteria *Staphylococcus epidermidis* namely a concentration of 10% which has an average inhibitory diameter of 0 mm and the smallest concentration at 2.5% is 9.63 mm.

Table 2: Antibacterial Activity Test Results Average Zone of Inhibition (mm)

Bacteria	Treatment	Average Inhibition Zone Diameter (mm)		
		2,5%	5%	10%
P. acnes	Extract	9,34	9,67	10,17
	n-Hexane Fraction	11,83	12	12,67
	Ethylacetate Fraction	11,87	12,26	12,86
	Residual Fraction	7,03	8	9,16
	Clindamycin 0.1%	22,27		
	DMS0 1%	0		
S. epidermidis	Extract	9,63	9,86	10
	n-Hexane Fraction	11	11,67	13,17
	Ethylacetate Fraction	11	11,85	13,27
	Residual Fraction	8,35	9,21	9,78
	Clindamycin 0.1%	22,27		
	DMSO 1%	0		

Table 2 shows the inhibitory power of propolis extracts and fractions on bacterial growth of *Propionibacterium acnes* and *Staphylococcus epidermidis* has a different level of sensitivity. Research finding by Saputera, *et al* (2016), the higher the concentration of the extract used, the larger the inhibition zone produced and it related significantly through research results of each concentration of 2.5% < 5% < 10% completed diameter response of the inhibition zone being successively larger.

That empirical fact shows that the methanol extract and fractions of propolis have antibacterial activity against acne-causing bacteria. It related significantly to previous research by Abdullah et *al*, that propolis from bees (*Heterotrigona itama*) from Brunei Darussalam has antibacterial activity from 20 gram/L propolis extract against bacteria *Pseudomonas aeruginosa* dan *Staphylococcus aureus.* The antibacterial activity is thought to be due to the presence of chemical compounds at the methanol extract of propolis, according to Hotnida *et al* (2011), the chemical content contained in propolis could differ between regions, places where propolis is honeycombed and its biological activity. It described the flora ecosystem surrounding which influences the chemical content of propolis.

Based on the results of phytochemical screening, the methanol extract of propolis contains alkaloids, saponins, tannins, flavonoids, phenols, and quinones.

Alkaloids have an antibacterial mechanism by inhibiting the peptidoglycan components in cells so that the cell walls are not intact and cause cell death (Riyanto *et al*, 2019). The mechanism of flavonoids as antibacterials is by forming complex compounds with extracellular and dissolved proteins, causing phospholipids to be unable to maintain the shape of the bacterial cell membrane, as a result, the bacterial cell membrane will leak and the growth of the bacteria will be hampered until death (Malanggi *et al*, 2012). Based on find research of Hotnida *et al*, (2011), types of flavonoids found in propolis are *pinocembrin*, *respectable*, *quercetin, pinostrobin, kaempferol, pinobaxin*.

The mechanism of tannin as an antibacterial is to disrupt peptidoglycan could be proved that cell wall formation becomes imperfect and causes bacterial cells to lyse, while the mechanism of action of saponin is to reduce the surface tension of the bacterial cell wall, resulting in

increased permeability or cell leakage causing intracellular compounds to come out (Malanggi *et al*, 2012). The mechanism of action of high concentrations of phenol as an antibacterial is by penetrating and disrupting bacterial cell walls and precipitating proteins in bacterial cells. Phenol in lower concentrations inactivates important enzyme systems in bacterial cells (Purwatiningsih *et al* 2014). The mechanism of action of quinones as antibacterials is by forming complex compounds that have properties *irreversible* completed by nucleophilic amino acid residues on plasma transmembrane proteins, cell wall polypeptides, and enzymes found on the surface of cell membranes, thereby disrupting the life of bacterial cells (Sapara *et al*, 2016).

The SPSS statistical tests show the data tested implemented test *Kolmogorov Smirnov* normally distributed at normality test, namely completed significance value ($p>0.05$). meanwhile, the normality test on the data *Pseudomonas aeruginosa* shows a significance value of 0.810 while in *Staphylococcus aureus* The significance value is 0.895. The normality test with normally distributed data is a requirement for carrying out further tests on *One Way ANOVA.*

Meanwhile, homogeneity of variance test shows the data is homogeneously related to each significance value of *Pseudomonas aeruginosa* data ($p>0.005$) $p = 0.054$, and the significance value of *Staphylococcus aureus* data shows $p= 0.081$. Homogeneity testing completed homogeneous data is requirements for carrying out the *One Way Anova* test and while *One Way Anova* at test results shows *Pseudomonas aeruginosa* and *Staphylococcus aerus* of each research data indicated each significance value ($p<0.05$), namely $p= 0.000$ and it concluded there are no similarities in each treatment group or each concentration. This finding related significantly to the hypothesis which described that there are differences in the effectiveness of the antibacterial power of the methanol extract of propolis of each concentration on bacterial growth *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

The following test carried out a*fter this* LSD which shows the antibacterial activity of propolis against bacteria *Pseudomonas aeruginosa,* shows significant differences at the entire concentrations and there was a significant difference between the antibacterial activity of propolis extract and the positive control clindamycin. Meanwhile, bacteria *Staphylococcus aureus* there was no significant difference in the inhibition zone between concentrations of 2.5%, 5%, and 10%. The zone of inhibition of all propolis extract concentrations was significantly different from the positive control clindamycin.

MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration)

Determination of MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) used solid dillution method. This main advantage method is more practical because one concentration of the test antimicrobial agent can be used to test more than one test microbe (Supomo *et al*, 2021). The MIC and MBC testing targeted to determine the number of doses of propolis methanol extract that could inhibit and kill bacteria that cause infection.

Based on finding research Nadhilla (2014), antibacterial substances are divided into two, namely bacteriostatic and bactericidal. Antibacterials that have bacteriostatic activity are substances that could inhibit the growth of bacteria, while bactericides are substances that can kill bacteria. Variations in the test concentrations of propolis extract and methanol fraction were 2.5%, 5%, and 10%.

Table 3: Bacterial Growth After 1x24 hours.

Concentration (%)	**Bacteria**	
	P. acnes	***S. epidermidis***
2,5	-	-
5	-	-
10	-	-

Note : (-)=No bacterial growth
(+)=There is bacterial growth

Results of the first incubation show that there was no bacterial growth at all concentrations and indicated that MIC is the smallest concentration that could inhibit bacterial growth, thus the smallest concentration is 2.5% without bacterial growth *Propionibacterium acnes* and *Staphylococcus epidermidis*. The empirical finding shows the MIC of methanol extract of propolis for bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis* (w/v) is 25 mg/mL. Next, a confirmation test was carried out by streaking again on the media with the bacterial suspension *Propionibacterium acnes* and *Staphylococcus epidermidis* then incubated for 1 x 24 hours at 37°C in a row.

Table 4: Bacterial growth after re-streaking.

Concentration (%)	Bacteria	
	P. acnes	***S.* epidermidis**
2,5	-	-
5	-	-
10	-	-

Note: (-) = No bacterial growth
(+) = There is bacterial growth

Table 4 shows that after re-streaking the media remains clear or there is no bacterial growth at all variations in concentration, then MBC is the minimum concentration that could kill bacteria at a concentration of 2.5%.

4 CONCLUSIONS

Research indicated that bee propolis extracts and fractions of *Homotrigona apicalis* can inhibit bacterial growth. Methanol extract of propolis has inhibitory power against bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis* at concentrations of 2.5%, 5%, and 10%. MIC and MBC of propolis methanol extract on bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis* of 25 mg/mL. Propolis extract has the potential to be an active ingredient in cosmetics as an antibacterial *Propionibacterium acnes* and *Staphylococcus epidermidis* cause of acne.

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