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STRUKTUR SEKRETORI DAN KANDUNGAN HISTOKIMIA PADA TUMBUHAN OBAT DARI FAMILI ANNONACEAE, LAMIACEAE, LYTHRACEAE, DAN MYRTACEAE

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ABSTRAK

Salah satu produk yang dihasilkan oleh lebah adalah propolis. Karakteristik propolis dengan struktur yang kompleks sehingga diperlukan pelarut ekstraksi yang sesuai untuk mendaptkan senyawa kimia yang diinginkan. Tujuan dari penelitian adalah untuk mengetahui kelarutan, golongan senyawa kimia, dan aktivitas antibakteri terhadap bakteri S.aureus dan E.coli dari ekstrak propolis menggunakan pelarut etanol 96%, isopropanol, dan propilen glikol. Propolis diekstraksi menggunakan metode Ultrasound Assisted Extration (UAE). Sembilan rasio pelarut digunakan, yaitu A1(Etanol 96%); A2 (Isopropanol); A3 (Propilen glikol); B1((Etanol 96% : Isopropanol : Propilen glikol (6:3:1)); B2 ((Etanol 96% : Isopropanol : Propilen glikol (1:6:3)); B3 (Etanol 96 % : Isopropanol : Propilen glikol (3:1:6)); C1 ((Isopropanol : Propilen glikol (8:2)); C2 (Etanol 96% : Propilen glikol (2:8)); dan C3 (Etanol 96% : Isopropanol (8:2)). Tinggi endapan ekstrak yang dihasilkan menunjukkan kelarutan propolis. Penapisan golongan senyawa kimia dilakukan pada semua kelompok ekstrak. Pengujian aktivitas antibakteri munggunakan metode sumuran. Data hasil pelarut yang menunjukkan kelarutan paling tinggi hingga terendah terhadap propolis, yaitu isopropanol, etanol 96%, dan propilen glikol. Semua ekstrak propolis positif terhadap alkaloid, saponin, tanin, flavonoid, dan negatif steroid/ triterpenoid. Data uji aktivitas antibakteri terhadap S.aureus menghasilkan ekstrak A1, A2, B1, B2, B3, C1, C2, C3 memiliki aktivitas penghambatan lemah dengan zona hambat berturut-turut yaitu 2,10 ± 0,62; 2,40 ± 0,60; 3,17 ± 0,80; 3,50 ± 0,44; 3,30 ± 0,10; 3,30 ± 1,56; 3,10 ± 0,96; 3,78 ± 0,69. Semua ekstrak tidak menghasilkan diameter zona hambat terhadap bakteri E.coli.

Kata kunci: Antibakteri, Etanol, Isopropanol, Propilen glikol, Propolis

ABSTRACT

Propolis is one of the bee's products that is reported to have many benefits from its compounds. Propolis has a complex compound, therefore it is important to choose a suitable solvent for the extraction process. The aim of this study was to determine propolis' solubility, the secondary metabolite classes, and antibacterial activity against S.aureus and E.coli of propolis extracted using different solvents, namely ethanol 96%, isopropanol, propylene glycol, and the combination of the three solvents. There were nine ratios of used in the extraction process, A1(Ethanol 96%); A2 (Isopropanol); A3 (Propylene glycol); B1((Ethanol 96%:Isopropanol:Propylene glycol (6:3:1)); B2 ((Ethanol 96%:Isopropanol:Propylene glycol (1:6:3)); B3 (Ethanol 96 %:Isopropanol:Propylene glycol (3:1:6)); C1 ((Isopropanol:Propylene glycol (8:2)); C2 (Ethanol 96%:Propylene glycol (2:8)); dan C3 (Ethanol 96%:Isopropanol (8:2)). The best solubility was determined by the height of the precipitate after centrifugation. The secondary metabolite classes were tested on each extract. Antibacterial activity was tested using the diffusion method.
The order of solvents that produced low precipitate was isopropanol, ethanol 96%, and propylene glycol. All propolis extracts were positive alkaloid, saponin, tannin, flavonoid, and negative for steroid/ triterpenoid. The result of antibacterial activity against S.aureus in extracts A1, A2, B1, B2, B3, C1, C2,

C3 showed weak inhibitory with respective inhibition zones 2,10 ± 0,62; 2,40 ± 0,60; 3,17 ± 0,80; 3,50 ± 0,44; 3,30 ± 0,10; 3,30 ± 1,56; 3,10 ± 0,96; 3,78 ± 0,69. All propolis extracts didn't present any inhibition zones against E.coli

Keywords: *Antibacterial, Ethanol, Isopropanol, Propolis, Propylene glycol*

INTRODUCTION

Propolis is a mixture of resin with sticky textures from various plants and trees collected by bees [1]. The component of propolis has 5% pollens, 10% of essential oils, 30% waxes, and 50% plant resins. The biological activity of chemical components from propolis such as polyphenol (phenolic acid and flavonoid) has been reported as antibacterial, antifungal, antivirus, antiparasitic, and anti-inflammatory [2]. Propolis's component content varies according to its geography, type of bee flora, time of harvest, and climate changes [3]. Propolis contains complex mixtures therefore it is important to choose a suitable solvent for the extraction process to obtain a chemical compound of propolis. In general, solvents that have been used for propolis extraction were acetone, ether, dichloromethane, water, chloroform, methanol, and ethanol.

Various concentration of ethanol has been used as a solvent of propolis extraction. The chemical components of 60% ethanol propolis extract were isoquerentine, quercetin, and kaempferol. The chemical components of 70% ethanol propolis extract were pinocembrin and sakuranetin. Kaempferide, acacetin, and isoharnetin were found in 80% ethanol extract propolis [4]. Isoquerentine, quercetin, kaempferol, pinocembrin, sakuranetin, kaempferide, acacetin, and isoharnetin have antibacterial activity [2]. In a previous study, the antibacterial activity of propolis extract against *Staphylococcus aureus* (*S. aureus*) showed inhibition zone 23, 8 ± 0,65 mm at 3,5 ± 0,96 mg/ml concentration and 37 ± 0,66 mm at 5,41 ± 0,96 mg/ml concentration against *Escherichia coli* (*E.coli*) [5].

Some solvents have not been utilized as solvents for propolis extraction such as isopropyl alcohol (isopropanol) and propylene glycol. Propylene glycol is one of the pharmaceutical excipients widely used as a solvent, humectant, parenteral and non-parenteral preservatives. Isopropanol is used as a local antiseptic [6]. Propylene glycol has been studied effectively in inhibiting *Streptococcus mutans*, *S. aureus*, *Enterococcus faecalis*, and *E. coli* growth [7]. Isopropanol at 50% concentration has been reported to show antibacterial activity broad-spectrum against vegetative viruses and fungi with its mechanism of action by degrading cell membranes and protein denaturation [8]. According to the statement above, the author has an interest in exploring isopropanol, ethanol 96%, and propylene glycol as a solvent for *Trigona apicalis* propolis extraction and evaluating their antibacterial activity against *S. aureus* and *E. coli.*

METODE PENELITIAN

The experiment was carried out at Technology and Phytochemistry Laboratorium Pharmacy, Microbiology Laboratorium, Chemistry Laboratorium, Faculty of Science, Institut Teknologi Sumatera during August – February 2024.

The tools used in this study were *Genesys* UV-Vis Spectrophotometer, Ultrasound Assisted Extraction (UAE), centrifuge, Laminar Air Flow (LAF), autoclave, oven, incubator, chiller, petri dish, ose needle, cork borer, hotplate, magnetic stirrer, analytical balance, micropipette, microtips, and laboratory glassware. Materials used in this study were raw propolis *Trigona apicalis* collected from Lembah Suhita, Teluk, Bandar Lampung, *S. aureus* and *E. coli* bacteria culture, isopropanol, ethanol 96%, propylene glycol, aquadest, NaCl, $FeCl_3$, HCl, Mg, hexane, Mayer, Dragendorff, Lieberman Burchad reagent, and nutrient agar.

Extraction

Extraction of propolis was carried out using Ultrasound-Assisted Extraction (UAE) method for 30 minutes at 40℃. One gram of propolis was dissolved in 10 ml of solvent consisting of various ratios (Table 1).

Table 1. Various ratios of solvents

Group	Ethanol 96%	Isopropanol	Propylene Glycol
A1	10	0	0
A2	0	10	0
A3	0	0	10
B1	6	3	1
B2	1	6	3
B3	3	1	6
C1	0	8	2
C2	2	0	8
C3	8	2	0

Measurement of precipitate propolis extract

The measurement was carried out after extraction. Propolis extract was centrifuged for 15 minutes at 4000 rpm. The precipitate after centrifuge was measured in centimeters using a ruler.

Screening secondary metabolite classes

To determine the chemical compound of propolis extract, secondary metabolite classes screening was carried out using supernatant extract after centrifugation.

Antibacterial activity test

Sterilization

Autoclave instrument are used at 121°C for 15 minutes for sterilization to execute any microorganism agents such as bacteria, fungi, and spores from precision glasswares and media cultures and an oven at 160°C for 2 hours for nonprecision glassware [9]. Antibacterial tests were done in LAF.

Media for bacteria

Medium cultures were used to grow *S.aureus* and *E.coli* are Nutrien Agar (NA) and Nutrient Broth (NB). The antibacterial activity was tested using a well diffusion method. 23 gram NA dissolved in 1 L aquadest and 16 gram NB dissolved in 1 L aquadest. The media will be sterilized using an autoclave at 121°C for 15 minutes. Bacterial stock was cultured in NB media. A colony from bacterial stock was taken by ose needle and dipped into NB media and incubated using a sieve shaker at room temperature for 24 hours [10].

Preparation of bacterial inoculation

Bacterial inoculation was performed in NA media using a sterile petri dish. 100μl of bacterial stock was taken and distributed on the surface of the media using the spread plate technique. The inoculum went through incubation for 24 hours at 37°C [11].

Preparation bacterial suspension

The straight tip of an ose was used to take inoculum of *S. aureus* and *E. coli* from stock and was suspended in sterile 0.9% NaCl then homogenized using a vortex. The absorbance of bacterial suspension's turbidity was measured using a UV-Vis spectrophotometer at 600 nm and standardized to 0.5 McFarland or equivalent to 1.5 x 10^8 CFU/ml by adjusting absorbance value in the range of 0.08-0.13 [12] [13].

Antibacterial activity test

Antibacterial activity of extract propolis was carried out using a well diffusion method. The 50 μL of bacterial suspension was poured into NA media in a petri dish and distributed the suspension over the surface using sterile cotton buds. There was four wells made by cork borer with 6 mm diameters in one petri dish. Each of the wells filled with extract samples from 9 various ratios of solvents, solvent control, negative control, and positive control was 1000μg/ml ciprofloxacin. The samples have an incubation time of 24 hours at 37°C. Diameters of inhibition zones showed after incubation and were measured using a vernier capiler. Clear areas around the well which are the diameters of the inhibition zone were observed.

Table 2. Categories of zone inhibition [14]

Inhibition zone (mm)	Antibacterial activity response
>20	Very strong
11-20	Strong
5-10	Medium
<5	Weak

Data analysis

Data of the precipitate propolis extract and inhibition zone obtained were tested for normality using the Saphiro-Wilk test and continued with the homogeneity test. Data is said to be normally distributed if the significance value > 0,05. If the data was normally distributed and homogenous, parametric comparative tests were performed (One Way ANOVA), but if the data was not normally distributed and homogenous, nonparametric tests were performed (Kruskall Wallis) [15].

RESULT AND DISCUSSION

Propolis was extracted using ethanol 96%, isopropanol, propylene glycol, and the combination of three solvents. The similarity of the chosen solvents is included in the type of alcohol. Isopropanol is one of secondary alcohol because its hydroxyl group was bound to two alkyl groups, meanwhile, ethanol is one of primary alcohol because its hydroxyl groups was bound to one alkyl group. Propylene glycol has two hydroxyl group, therefore it is classified as dihydric alcohol [16]. The electric constant exhibits the polarity of the solvents, propylene glycol has the highest electric constant 32, then ethanol 96% 30, and the last one is isopropanol 18,3 [17], [18], [19].

Currently, there are no studies for propolis extraction using propylene glycol and isopropanol as a solvent, but ethanol has been widely used for propolis extraction. In previous studies, propolis has been extracted with ethanol 70% ratio of 1:50, and the yield was 73.45±21.03% [20]. The measurement of propolis precipitate performed the solubility of propolis. The extract with lower precipitate after centrifugation showed good solubility of propolis.

Table 3. Volume of precipitate extract propolis

Group	Propolis' precipitate (cm)
A1	4.43 ± 0.23
A2	3.87 ± 0.15
A3	7.77 ± 0.06
B1	3.93 ± 0.03
B2	4.00 ± 0.10
B3	4.60 ± 0.36
C1	6.43 ± 0.32
C2	8.03 ± 0.06
C3	4.50 ± 0.17

Note: A1(Ethanol 96%); A2 (Isopropanol); A3 (Propylene glycol); B1((Ethanol 96%: Isopropanol: Propylene glycol (6:3:1)); B2 ((Ethanol 96%: Isopropanol: Propylene glycol (1:6:3)); B3 (Ethanol 96 %: Isopropanol:

Propylene glycol (3:1:6)); C1 ((Isopropanol : Propylene glycol (8:2)); C2 (Ethanol 96%: Propylene glycol (2:8)); dan C3 (Ethanol 96%: Isopropanol (8:2)).

Based on the normality test, the significance value obtained shows that precipitate data were not normal and homogenous. Kruskal-Wallis was tested as an alternative for One-Way ANOVA, and the significance value obtained shows that there is a significant difference between the extraction solvent group with the precipitate [13].

All of the supernatant extract groups were tested for secondary metabolite classes. All samples performed positive for flavonoid, alkaloid, tannin, and saponin but negative for the steroid/triterpenoid group. The results were in line with a previous study that propolis in Lampung showed positive for alkaloid and flavonoid, but negative for steroid/triterpenoid [22]. The antibacterial activity test of propolis extract was done to determine the chemical compound of the ethanol 96%, propylene glycol, and isopropanol extract against two bacterials. S.aureus as gram-positive bacteria and E.coli as gram-negative bacteria was examined using the well diffusion method in triple. The method was chosen because of its advantage the ability of the sample directly contact to the bacteria and the osmolarity process was more homogenous than the Kirby-baurer method [20].

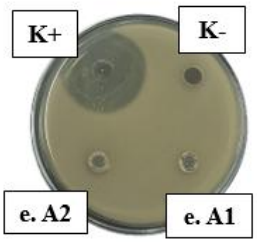

Figure 1. Inhibition zone of positive and negative control

The zone of inhibition growth bacteria showed in the clear zone around the well. Based on results, inhibition zones only showed in sample A1, A2, A3, B1, B2, B3, C1, C2, C3, solvent control A1 and A2, ciprofloxacin 1000 μg/mL (positive control), meanwhile solvent control of B1, B2, B3, C1, C2, C3, and aquadest performed no inhibition zone.

Table 4. Antibacterial activity against *S.aureus*

Sample	Diameter of inhibition zone (mm)*	Inhibition zone categories
Extract A1	2.10 $\pm$ 0.62	Weak
Extract A2	2.40 $\pm$ 0.60	Weak
Extract A3	0.00 $\pm$ 0.00	No inhibition zone
Extract B1	3.17 $\pm$ 0.80	Weak
Extract B2	3.50 $\pm$ 0.44	Weak
Extract B3	3.30 $\pm$ 0.10	Weak
Extract C1	3.30 $\pm$ 1.56	Weak
Extract C2	3.10 $\pm$ 0.96	Weak
Extract C3	3.78 $\pm$ 0.69	Weak
Solvent A1	1.93 $\pm$ 0.67	Weak
Solvent A2	3.33 $\pm$ 1.04	Weak

Solvent A3	0.00 ± 0.00	No inhibition zone
Solvent B1	0.00 ± 0.00	No inhibition zone
Solvent B2	0.00 ± 0.000	No inhibition zone
Solvent B3	0.00 ± 0.00	No inhibition zone
Solvent C1	0.00 ± 0.00	No inhibition zone
Solvent C2	0.00 ± 0.00	No inhibition zone
Solvent C3	0.00 ± 0.00	No inhibition zone
Positive control	32.67 ± 2.08	Very strong
Negative control	0.00 ± 0.000	No inhibition zone

*Average±SD

Note : A1(Ethanol 96%); A2 (Isopropanol); A3 (Propylene glycol); B1((Ethanol 96% : Isopropanol : Propylene glycol (6:3:1)); B2 ((Ethanol 96% : Isopropanol : Propylene glycol (1:6:3)); B3 (Ethanol 96 % : Isopropanol : Propylene glycol (3:1:6)); C1 ((Isopropanol : Propylene glycol (8:2)); C2 (Ethanol 96% : Propylene glycol (2:8)); dan C3 (Ethanol 96% : Isopropanol (8:2)).

Based on the normality test, the significance value obtained shows that the inhibition zone was not normal and homogenous. Kruskal-Wallis was tested as an alternative for One-Way ANOVA, the significance value obtained shows that there is a significant difference between the extraction solvent group with inhibition zones of *S. aureus* [13].

The next step is the antibacterial activity of propolis extract against *E. coli*. Based on the result, all of the propolis extract groups did not show any inhibition zone therefore it can be concluded that the concentration of propolis extract that we used does not have antibacterial activity against *E. coli*. Meanwhile, one of the solvent controls (ethanol 96%) performed a weak inhibition zone, and ciprofloxacin as positive control produced the widest inhibitory zone at 1000μg/mL by 30.66 ± 2.081 mm. The result showed that solvent A2 inhibits more than extract A2 due to several reasons. Propolis are natural products that contain various chemical content, in order to extract the most active antibacterial agent correlated with the extraction method and the solvent. The yield of chemical compound extract, the higher concentration inhibits more bacterial growth. The characteristics of the substance also correlate with the diffusion through the agar medium, propolis extract has sticky and vicious texture, meanwhile the solvent is a light liquid, thus the solvent potentially diffuse more effective thorough the medium than the extract and affects the inhibition zone [24]

There are differences between the result of the antibacterial activity of propolis extract using isopropanol, ethanol 96%, and propylene glycol solvent against *S. aureus* and *E. coli.* All of the group extracts showed weak inhibition zone against *S. aureus*, while *E. coli* did not show any inhibition zone. In the previous study, found that ethanolic extract of propolis performed more effectively against gram-positive bacteria rather than gram-negative bacteria due to different structures. Both bacteria produced hydrolytic enzymes that potentially degrade active components of propolis [21]. Generally, gram-negative bacteria secreted hydrolytic enzymes through the outer membrane [22]. Other studies reported that propolis extract would show an inhibitory effect against gram-negative bacteria at high concentrations [23].

The antibacterial activity of propolis has many mechanisms of action. Polyphenol inhibits bacteria with hydrogen bonds and ionic bonds by interacting with bacterial protein and changing the protein structure which has an impact on changes in protein function. The characteristic of phenolic compounds that have one or more hydroxyl groups will form a hydrogen bond with an atom (N, O) in protein. Ionic bonds were formed in physiological conditions. Phenolic hydroxyl groups will be dissociated into negative phenolic

ions and then bind to positive amino acid groups such as lysine, histidine, and arginine. Generally, the covalent and ionic bonds formed will change the structure of proteins therefore the function of proteins as enzymes, receptors, transport proteins, and transcription proteins will be disrupted [28], [29], [30].

CONCLUSION

The best solubility of propolis was isopropanol, ethanol 96%:isopropanol:propylene glycol (6:3:1), ethanol 96%:isopropanol:propylene glycol (1:6:3), ethanol 96%, ethanol 96%:isopropanol (8:2), ethanol 96 %:isopropanol:propylene glycol (3:1:6), isopropanol:propylene glycol (8:2), propylene glycol, and the last one ethanol 96%:propylene glykol (2:8). The antibacterial extract of isopropanol, ethanol 96%, propylene glycol, ethanol 96%:isopropanol:propylene glycol (6:3:1), ethanol 96%:isopropanol:propylene glycol (1:6:3), ethanol 96 %:isopropanol:propylene glycol (3:1:6), isopropanol:propylene glycol (8:2), ethanol 96%:propylene glycol (2:8), ethanol 96%:isopropanol (8:2) showed weak inhibition zone against *S.aureus* and did not show any inhibition zone against *E.coli.* The most inhibiting *S.aureus* was extract of isopropanol:propylene glycol (8:2).

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