



Antioxidant activity test of propolis trigona sp extract from Cibubur using the dpph (1,1-diphenyl-2 picrylhydrazil) method

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ABSTRACT

Propolis is a resinous substance that comes from the wood and top of the plants gathered by bees, then mixed with wax and bee saliva. Propolis contains several active compounds such as flavonoids that could act as an antioxidant. The content of the active compound in propolis depends on the geographic factor, the bee species, and how to make the extract. The purpose of this research was to determine the antioxidant activity of propolis extracts *Trigona* sp from Cibubur with 96% ethanol using the DPPH (1,1-Diphenyl-2-Picrylhydrazyl) methods. In this study the vitamin C was a positive control. The measurement of antioxidant activity used UV-Vis spectrophotometer with it's wavelength 517 nm. The results showed that the IC₅₀ of propolis extract was 416.486 ppm while vitamin C was 5.96 ppm.

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1. INTRODUCTION

Humans have a very complex body defense system, consisting of various kinds of cells, tissues and organs, which are always related to keeping the body healthy. If the system is not functioning properly, our bodies will quickly get sick. 1 Many factors can affect human health, one of which is the balance between levels of antioxidants and free radicals in the body. Free radicals are highly reactive molecules with one or more unpaired electrons. Free radicals are continuously formed as a result of normal cell metabolism processes, inflammation, malnutrition and responses to influences from outside the body such as pollution, UV, cigarette smoke and others. 2 The human body does not have excess antioxidant reserves. If there are more free radicals than antioxidant levels in the body, this can damage cell-forming macromolecules so that they become a source of pathological conditions in the human body. Therefore the human body requires exogenous antioxidants.

Based on the source of its acquisition, there are 2 types of antioxidants, namely natural antioxidants and synthetic antioxidants. The increasing interest in obtaining natural antioxidants has occurred in recent years. 4 Natural antioxidants can be obtained from vegetable/fruit plants, and we can also obtain them from bee products such as honey, propolis, royal jelly, beeswax, and pollen.

As stated in the Al Quran Surah An-Nahl verses 68-69 which means: "And your Lord revealed to the bees, make nests on the hillshills, in wooden trees, and man-made places. Then eat from every (sort of) fruit and follow the path of your Lord which is made easy (for you), from the belly of the bee comes out a drink of various colors, in which there is medicine, which is healing for humans. Verily in that is really a sign (of God's greatness) for those who think about it "

In these verses it is explained that bees can provide medicine contained in their bodies. There have been many studies related to bees, one of which is propolis. 5 Propolis is a resinous substance (a type of plant sap) derived from bark and shoots of plants, which bees collect and then mix with wax and bee saliva. 8,9 Propolis is used to protect beehive doors and repair their cracked or damaged hives. 10 Propolis is one of the honey bee products whose existence is now being circulated more and more and is consumed by many people in extract form. Many studies have been carried out by various countries in utilizing propolis as a potential drug as an antioxidant, antiviral, antifungal, anti-inflammatory, antiallergic, analgesic,

Propolis contains several chemical components such as polyphenols (flavonoids, phenolic acids and their esters), terpenoids, steroids and amino acids, as well as minerals. 14 Based on research in Egypt it was revealed that propolis contains high levels of flavonoids and can act as antioxidants. 6 In research in India it was also stated that propolis contains active compounds that are antioxidants. 16 The activity test used the DPPH method by comparing different propolis products. The results showed that each propolis has a different antioxidant activity. This is because the chemical components contained in propolis are affected by differences in the place of collection (geographical location, climate, and plants).

which are around the hive), the bees that produce it, and how to make propolis extract.

Indonesia has various kinds of plants and other variations of biodiversity and this can have an impact on the content of active compounds contained in propolis which are antioxidants.,17 One of the methods used to test the antioxidant activity is the DPPH method. 18 There are not many studies in Indonesia that discuss the antioxidant activity contained in propolis in an area in Indonesia with the DPPH method which is an attraction for researchers.

2. RESEARCH METHOD

Propolis extract is prepared using the maceration method (using a solvent with several times of shaking/stirring at room temperature). a. First of all determine in advance the desired final propolis extract concentration, in this study taking a concentration of 10%. 22,30 b. Then weigh the amount of propolis and calculate the volume of ethanol used, namely 100 grams of propolis dissolved in 1000 mL of ethanol c. Propolis that has been cut and weighed then we put it into a beaker that has been filled with 1000 mL of 96% ethanol. Then we shake it every day and soak it for 5 days, but ideally it is soaked for 1-2 weeks and stored in a closed/cold room. 9,17 The use of 96% ethanol as a solvent is because 96% ethanol can attract both polar and non-polar components. . The longer it is soaked in alcohol, the more soluble the propolis will be.9 d. the results of the soaking are then filtered using filter paper and the filtrate is taken for 5 days until the solvent is clear.9,17,22 e. The resulting filtrate is then evaporated using a rotary evaporator at a temperature of ± 40 . f. The concentrated extract is dissolved in ethanol in a ratio of 1:3, then prepares for the antioxidant test

Absorbance Measurement

All blank solutions, test solutions and reference solutions were incubated at 37°C for 30 minutes in the dark, then the absorbance was measured using a spectrophotometer. After getting the absorbance value, calculate the percent resistance of each solution using the formula 23,32:

$$\% \text{ Resistance} = (\text{Abs blank} - \text{Abs sample}) \times 100\%$$

Blank Abs

After getting % of inhibition activity, then look for the probit value by looking at the probit³⁸ table. After obtaining the probit value, the IC₅₀ value is sought through the linear regression equation.

Antioxidant Data Analysis

Data on antioxidant radical scavengers DPPH (% inhibition) of propolis extract were analyzed and its IC₅₀ value was calculated through probit analysis. IC₅₀ is a value that indicates the concentration of

the extract (ppm) which is able to inhibit the oxidation process by 50%. The smaller the IC₅₀ value indicates the higher the antioxidant activity of the compound.

Table 1 Classification of antioxidants

No	IC value	Antioxidant
1	< 50 p.m	Very strong
2	50-100 pm	Strong
3	100-150pm	Currently
4	151-200pmm	Weak

Percentage data was plotted into the probit table to obtain the probit value, then a graph was made between the log concentration (x) and probit (y) to obtain a linear regression equation $y = a+bx$. By entering the value $y = 5$ (probit of 50%) in the equation $y=a+bx$, the IC₅₀ value is determined by the value of x and converted to the anti-log form.

RESULTS AND DISCUSSIONS

Propolis Extraction Results

Samples were taken from raw propolis harvested at the Trigona sp beehive farm in the Cibubur area, West Java. As much as 100 grams of raw propolis was extracted, then 1.9 grams of viscous extract was obtained with a yellowish brown color and was sticky.

Result of mixing the test solution and its absorbance value

The results of mixing the propolis extract test solution with DPPH solution at a concentration of 1ppm are dark purple in color and somewhat look like a blank color, then at concentrations of 10 and 100 the purple color appears to fade, while at a concentration of 1000 ppm the purple color changes to yellow as shown in Appendix 5 The vitamin C solution also changes color to faded purple and yellow. In the absorption measurement, all solutions (blank, propolis extract, and vitamin C) were measured. After the spectrophotometer is set for photometric measurements with a maximum wavelength of 517 nm which is the maximum length of the DPPH, the absorbance measurement begins with the measurement of the blank solution, propolis extract solution 1.10, 100.1000 ppm, then vitamin C 2, 4, 6, and 8 solutions. ppm.

Table 2.Maximum Wavelength and Absorbance of Blank Solution

No	Material	Maximum Wavelength	Absorbance(nm)
1.	Blank	517 nm	0.701

Table 3Calculation of absorbance, inhibition activity, and probit extra value of propolis

No	concentration	absorbance	Activity	Logs	
	(ppm)	(nm)	Obstacle (%)	Concentration	
				Probit Value	
1	1	0.568	18.88	0	4.1147
2	10	0.486	30,67	1	4.4928
3	100	0.402	42,61	2	4.8134
4	1000	0.345	50,69	3	5.1050

No	concentration (ppm)	absorbance (nm)	Activity Obstacle (%)	Logs Concentration	Probit Value
1	2	0.393	27,62	0.3	4.4052
2	4	0.352	35,17	0.6	4.6174
3	6	0.334	38,48	0.7	4.7078
4	8	0.196	63,90	0.9	5.3658

IC₅₀ calculation results and probit analysis

Based on the previous table data, a linear equation and IC₅₀ value will be obtained as shown in table 4.4:

Table 4. Linear Equation and calculation of IC₅₀ values

No	Material	Point a	value b	r value	Linear Equations	IC ₅₀ value (PPM)
1	Propolis Extract	4.13775	0.32915	0.998	$Y=4.13775+0.322915x$	416,48
2	Vitamin C	3.83553	1.50162	0.9	$Y=3.83553+1.50162x$	5.96

To facilitate the process of inputting and calculating data, Microsoft Excel software is used to find linear regression equations with probit analysis. 37 From the calculation results, the IC₅₀ value of propolis extract is 416.486 ppm and vitamin C is 5.96 ppm.

Discussion

To make propolis extract, the maceration method was used with 3 times maceration for 7 days using 96% ethanol and shaking every day. 17 The choice of the maceration method was because it was relatively simple, which did not require complicated tools, was easy, inexpensive, and could avoid damage to the components of the compound. due to heat. The use of ethanol as a solvent is because 96% ethanol can attract both polar and non-polar components. The longer it is soaked in alcohol, the more soluble the propolis will be

This study used the DPPH method to test antioxidant activity. This method was chosen because it has several advantages, including easy, simple, fast, good for certain polarity samples, sensitive and only requires a small number of samples. 18 Test solutions made in various concentrations to be dissolved with DPPH aim to determine antioxidant activity in various concentrations of solutions seen of the color change. DPPH radicals are easily degraded by light, therefore all solutions are wrapped in aluminum foil so that conditions are dark. 35 In order for the solution to become homogeneous, the solution must be shaken with a waterbath shaker. To achieve a steady state condition (the time when the absorbance value is constant), the solution is incubated for 30 minutes at room temperature. 37 During the process of mixing the propolis extract solution and DPPH solution, a color change occurred, namely, the purple color became faded purple and yellow. This change occurs due to a decrease in the molar absorptivity of the DPPH molecule. The color changes based on the number of electrons captured. DPPH free radicals that have unpaired electrons give a purple color. The color change that occurs is due to the bond between the DPPH electrons and the hydrogen atom which indicates an increase in the ability of antioxidants to capture free radicals. 27,37 To find the percentage of free radical inhibition contained in propolis extract, it is necessary to find the absorbance value of the sample and blank first. Therefore a UV-Vis spectrophotometer is used to measure it. Obtained each free radical inhibition contained in propolis extract as in the table below:

Table 5.Obstacle percentage

No	The concentration of the propolis extract test solution	Obstacle percentage
1	1ppm	± 18.88 %
2	10 ppm	± 30, 67 %
3	100 ppm	± 42, 61 %
4	1000 ppm	± 50, 69 %

The results stated that the higher the concentration of propolis, the value of its inhibition on free radicals will increase as seen from the calculation of the percentage of obstacles. This also shows that propolis can inhibit or reduce free radicals. To analyze the antioxidant activity of propolis extract, a linear regression equation with probit analysis was used to find the IC₅₀ value. The probit value is obtained by using the probit table from the value of % inhibition activity.

Based on the classification of Blois antioxidants, propolis extract is a class of antioxidants whose properties cannot be classified, with an IC₅₀ value of 416.486 ppm. Meanwhile, vitamin C as a positive standard/control has been known from previous studies to have an IC₅₀ value of 5.05 ppm. 39 However, from the results of this study the IC₅₀ value of vitamin C was obtained at 5.96 ppm.

Results of research in the country another, India. It was found that the ethanol propolis extract using 80% ethanol solution showed a strong antioxidant. 16 In this study, the IC₅₀ data was obtained at 71 ± 0.44 . Testing of antioxidant activity in this study has similarities in terms of the method and species of bees used, namely the DPPH method and the bee *Trigona* Sp.

The differences in the results of this study can be influenced by the chemical composition contained in propolis in different countries and regions. The chemical composition of propolis depends on the geographical location, climate, and vegetation around where it is taken. 15 Bees collect propolis from plant sources, especially from flowers and leaf buds. Then the bees also make nests through enzymes in their saliva and collected resin materials. This shows that the propolis made by bees is obtained based on the nature that is near them and the content of any active compounds that exist.

As in Gonzales' research, which took propolis samples from 22 regions in Brazil, it showed that differences in the origin of propolis for antibacterials had a relationship with the magnitude levels of flavonoids in propolis. 17 Flavonoids in propolis also function as active compounds that can inhibit free radicals.

The active compounds contained in propolis indicate the characteristics of the region or country where propolis is produced. Geographical differences in European, South American, and Asian countries produce different chemical compositions. 17 Even so, propolis has a percentage value of free radical inhibition at a concentration of 1 ppm of ± 18.88% and there is a color change when the propolis extract is dissolved with DPPH which shows that propolis contains antioxidants although not strong.

Research Limitations

The research conducted this time has limitations and deficiencies that can affect the results of the study, namely: The sample used is a sample purchased from a bee farm and there is no certification or information showing that the propolis is really from *Trigona* Sp bees because it is still a traditional farm. How to harvest and the environment where propolis is taken can also affect the results of this study.

CONCLUSION

From the results of research conducted, propolis extract has antioxidant activity. This is based on the color change and the percentage of free radical inhibition. The propolis extract has an IC₅₀ value of 416.486 ppm and based on the criteria for classifying antioxidants according to Blois, this *Trigona* Spp propolis extract from Cibubur cannot be classified.

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