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Antioxidant Activity of *Apis Mellifera sp*. Propolis Extract from Java (Indonesia)

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Abstract

Propolis was one of natural antioxidant source in the flavonoid and phenolic acid form. The propolis antioxidant potential was influenced by the plant factors that grow in geographic area wherein the bees alive. The propolis biological effect was known and have been used since antiquity. This research was intended to investigate the propolis antioxidant potential originating from Java (Indonesia). The method that was used to test its antioxidant character with the total of flavonoid activity test and the total of phenol. The strength for its antioxidant was measured by DPPH IC_{50} test. The result was obtained that propolis antioxidant potential from Java has a very strong potency with DPPH 35,6 µg/ml. The conclusion was *Apis Mellifera sp* propolis from Java has a high potential to be developed. The high drug activity and propolis therapeutic effectiveness were expected to giving a positive value and contribution to dentistry practice.

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1. Introduction

Propolis is a bee product consisting of a resinous substance that is collected from the flower, bud, and exudate from various plant sources. Then, the bee mixes the resin material with an enzyme that secreted from the bee mandibular gland but the components consisted in the propolis is unchanged [1], [2], [3], [4]. The bees various types can produce propolis. *Apis Mellifera sp* type is the most widely farmed; produce a lot of honey, it also produces propolis although not as much as *Apis Trigona sp* type [1].

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Propolis has been known for health benefits a long time and has done a lot of research about it. The analysis regarding various propolis samples from different geographic regions showed a significant propolis composition distinction [2], [4]; in term of it will also affect its biological activity. Despite, the components diversity in propolis sourced from different parts of the world, propolis has a high antibacterial, antiviral, anti-oxidative, antifungal and anti-atherogenic as well as antiproliferative and proapoptotic activity. Some propolis varieties are able to indicate increased antiproliferative and anti-inflammatory activity, regenerative-reparative, estrogenic, and anesthetic [4].

An antioxidant is defined as inhibitors that act to inhibit oxidation in reacting with the free reactive radicals formed the free radicals that are relatively stable, thus is able to protect the cells from the harmful effects a free radical of the reactive oxygen. An imbalance between oxidants and antioxidants in the body, therefore the over dominant oxidant is called oxidative stress [5]. Nowadays, the natural substances are extensively researched focusing on their ability to counteract the effects of oxidative stress that can lead to various diseases [4].

In the dentistry, the oxidant effects as well as begin to unfold. It has been reported that epigallocatechin-3-gallate antioxidants in the green tea have an effect on caries prevention. The grape seeds and a solution of pine bark extract in the dentistry conservation can increase the strength of declining composite structure. In the orthodontics science, antioxidants of thymoquinone, boron, is able to increase the number of osteoblasts in remodeling the tooth activities. Due to the wide spectrum activity, regarding the research on domestic propolis as an antioxidant is a very important to be conducted in the dentistry development science [2], [7], [8], [9].

2. Research Methods

Materials and Tools

Propolis material is taken from beekeeping in Moyudan area, western Yogyakarta, Central Java, Indonesia. The bee type that farmed is *Apis Mellifera sp.* Propolis is collected by the bee's habitat in the *rambutan* trees, kapok, longan, rubber, mango, and cashew nuts. The instrument that is used in creating on propolis extract is an analytical scales, blender, *Vacuum Rotary Evaporator*, Buchner funnel, *water bath*, Erlenmeyer, porcelain cup, micro pipette.

Research Types

This is a laboratory experimental research. The research was conducted in the Research Laboratory and Integrated Testing (LPPT-1), Gadjah Mada University Yogyakarta.

Research Procedure

Propolis Extraction

Propolis is thinly cut, blended using 96% ethanol. Next, it is silenced 24 hours, then conducted a filtering. The filtrate is evaporated with a *Vacuum Rotary Evaporator* temperature 60˚C water bath heater. The viscous extract result is poured into a porcelain cup. Then, it is heated with a water bath temperature 70 ºC while continuously stirring. The propolis extract result is weighed and packed.

The Total of Flavonoid and the Total of Phenol Test

Propolis extracts that is obtained to be tested for the flavonoid levels using *quercetin*. Whereas, the phenolic acid level using a gallat acid. The solution is measured a flavonoid total and phenol total level by using UV-Vis *spectrofometer* in the wavelength 422.5 nm.

DPPH test (1,1-diphenyl-2-picryl hydrazyl)

An antioxidant activity testing is conducted by capturing the free radical of DPPH within the spectrophotometric method. The principle of antioxidant activity testing is determined by measuring the magnitude of the absorbance decrease for free radical DPPH solution is 517 nm wavelength. IC_{50} is an antioxidant activity parameter that is an extract concentration (fraction) which contributes 50% antioxidant activity compared to control through an equation of linear regression lines. The control solution is DPPH solution is 0.4 nM in the methanol.

The material used is DPPH radical, vitamin E, vitamin C, methanol, and ethanol. The tools used included an analytical balance, glassware, and visible spectrophotometer. The test procedure is as follows:

Preparing the sample test 50 µl with various concentrations; coupled with 1.0 ml DPPH 0.4 mM and 3.950 ml ethanol. (The concentration that contributes IC_{50} value is an extract or fraction concentration which contributes 50% antioxidant activity compared to the control, through an equation of linear regression lines). The mixture itself is then diverted and left for 30 minutes. Furthermore, the solution is

measured its absorbance at 517 nm wavelength towards the blank (the blank consists of 50 µl extracts and 4,950 ml ethanol). Then there is also conducted a control absorbance measurement consisting of 1.0 ml DPPH and 4.0 ml ethanol. Unlike the comparison is used vitamin E and vitamin C.

The procedure and method of making calibration curve are conducted as follow: a) Spectrophotometer tool is conditioned on 517 nm wave, b) An absorbance is measured on the each solution, c) Making a calibration curve for getting the regression line equation. The calculation steps is performed as follows: a) Making a calibration curve equation Y = BX + A (Y = absorbance, X = standard weight), b) IC_{50} activity determination is conducted on calculating the concentration (X) if the value Y = 50%.

3. Results and Analysis

3.1 Propolis Extract

The extract on 564,88 gram, propolis in 96% ethanol producing the thick extracts on weight 89,37 gram and *rendemen* value is 15,8%. An extract consistency is a very thick, sticky, and blackish brown.

3.2 The Total of Flavonoid and Phenol

The measurement result of the total flavonoid on propolis extract is obtained 6,83%, whereas the measurement of the phenol total is obtained 0,62% (Table 1). The test results of propolis extract antioxidant activity can be seen in Table 2, indicating that antioxidant activity towards the free radical DPPH with IC_{50} is 35,56 µg/ml.

Table 1.
The test result of the total of phenol and total flavonoid *Apis Mellifera sp.* propolis extract in Java (Indonesia)

No.	Test Parameter	Result
1.	Equivalent phenol total of gallat acid	0,62 % b/b
2.	Total of quercetin equivalent flavonoid	6,83 % b/b

Table 2.
The test result of capturing free radical DPPH *Apis Mellifera sp.* propolis extract in Java (Indonesia)

Test Parameter	Results & Intensity Value	Unit
Activity of capturing free radical DPPH (IC_{50})	35,6 ± 0,001* (very strong)	µg/ml

* IC_{50} Value < 50 is very strong, 50-100 = strong, 101-150 = moderate, > 150 = weak (in µg/ml)

3.3 Analysis

The results of the present study found that propolis extract from Java is able to inhibit free radical DPPH within its antioxidant activity. In term of this has a relationship due to there is flavonoid compound (6.83%) and phenolic acid (0.62%) in propolis extract. The most important component from Java propolis in this research is polyphenol, including flavonoid and phenolic acid. These compound has a strong antioxidant nature and high biological activity. The polyphenol antioxidant activity also depends on its structure [4].

Due to its high polyphenol compounds content, according to Gorecka propolis also has anti-inflammatory activity [4]. Propolis has a significant effect on arachidonic acid metabolic pathways. In previous experimental studies, it has been reported that the activity of propolis extract inhibition towards cyclooxygenase (COX-1 and COX-2) and its activity on lipoxygenase. The propolis activity effect is a change of prostaglandin concentration E2 and leukotriene [4].

In the dentistry studies, an antioxidant research began to be widely studied. The natural antioxidants sources (herbs), unlike flavonoids, have advantages over artificial antioxidants (chemical). There is a tendency of human habit to nature without sides affect. Some studies on antioxidant in dentistry such as an epigallocatechin-3-gallate role in the green tea in caries preventing [8], the antioxidant availability in the toothpaste, mouthwash, or spray [8], the oxidant role in periodontitis [8], the grape seed can increase restorative binding strength after bleaching process [8]. In orthodontic science, antioxidants included timokuinon [7], boron [10], vitamin C [11], can increase the number of osteoblast in the remodeling of the teeth activities.

4. Conclusion

Propolis has content varieties depending on the geographical situation wherein the bees live. The most significant compounds that make propolis up is in Java (Indonesia). It is flavonoids and phenolic acids. Due to its structure, these compounds exhibit high antioxidant activity. Considering the high antioxidant activity, a new therapeutic possibility related to this bee product is being actively researched.

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