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Indonesian Mount Lawu Propolis as a Potential Antioxidant Ear Drops

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Abstract

Currently available ear drops have shown satisfactory results, but there are various side effects that arise due to the content of anti-inflammatory substances such as the presence of corticosteroids. Propolis is a substance derived from nature with various benefits for the body. Indonesian Mount Lawu Propolis Ear Drop (IMLPED) is a propolis that has currently been developed as an alternative ear drops to treat various ear diseases. This study aims to assess the antioxidant ability activity of IMLPED. This study is an in vitro study to assess the antioxidant ability of the 2% IMLPED with 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) method based on the value of inhibitory concentration at 50% (IC_{50}). The results of this study showed that the IC_{50} value of IMLPED 2% was 30.71 µg/mL. Based on the IC_{50} value, the 2% IMLPED formula showed a very strong antioxidant ability. The implication of this discovery makes the 2% IMLPED a promising candidate as an alternative to ears drops.

Keywords: Antioxidants, ear drops, IC_{50}, Indonesia mount lawu propolis.

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Potensi Penggunaan Propolis Gunung Lawu Indonesia sebagai Tetes Telinga Antioksidan

Abstrak

Obat tetes telinga yang tersedia saat ini telah menunjukkan hasil yang cukup memuaskan, akan tetapi terdapat berbagai efek samping yang timbul akibat kandungan zat antiinflamasinya seperti keberadaan kortikosteroid. Propolis, yang merupakan bahan yang berasal dari alam dengan berbagai manfaat bagi tubuh. *Indonesian Mount Lawu Propolis Ear Drop* (IMLPED) merupakan salah satu jenis propolis yang saat ini telah dikembangkan sebagai alternatif obat tetes telinga untuk mengobati berbagai penyakit telinga. Penelitian ini bertujuan untuk menilai kemampuan antioksidan dari IMLPED. Penelitian ini merupakan penelitian in vitro untuk menilai kemampuan antioksidan IMLPED 2% dengan metode *2,2-Diphenyl-1-Picrylhydrazyl* (DPPH) berdasarkan nilai konsentrasi penghambatan pada 50% (IC_{50}). Hasil dari penelitian ini menunjukkan bahwa nilai IC_{50} dari IMLPED 2% adalah 30,71 µg/mL. Berdasarkan nilai IC_{50}, formula IMLPED 2% menunjukkan efek antioksidan yang sangat kuat. Implikasi dari penemuan ini menjadikan IMLPED sebagai kandidat yang menjanjikan sebagai alternatif obat tetes telinga.

Kata Kunci: Antioksidan, IC_{50}, propolis gunung lawu Indonesia, tetes telinga.

1. Introduction

Propolis is a natural substance produced by bees and has been widely used from various cultures as a medicine such as in ancient Roman and Greek cultures.[1] Propolis is a lipophilic, resinous, natural substance that hosts an extensive assortment of naturally occurring plant-derived organic compounds, with flavonoids being noteworthy among them. The presence of the flavonoid within propolis remains consistent, regardless of the geographical location of the hive. Propolis has been known to have many positive effects, especially its antioxidant abilities.[2–4] Given its antioxidant effects, propolis is currently being developed in various form, including as an ear drop for the treatment of ear diseases. Propolis is a substance with minimal side effects, unlike currently available ear drops that contain steroids or other anti-inflammatory substances.[5]

There are various propolis available today, including Mount Lawu propolis from Indonesia, s derived from the traditional medicinal herbs extract.[6] Indonesian Mount Lawu propolis ear drop (IMLPED) has been reported to contain antioxidant substances such as polyphenols, flavonoids, caffeic acid pethyl ester and quercetin.[7] A study by Primadewi *et al*. reported that the administration of 2% IMLPED in cisplatin model rats can increase the levels of the antioxidant enzyme superoxide dismutase (SOD), while reducing levels of the lipid peroxidation marker, malondialdehyde (MDA).[6] However, the antioxidant ability of the 2% IMLPED has never been studied before. There are various methods to determine the antioxidant ability, but the use of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is the most popular, easy, and affordable method to use. The DPPH method uses free radicals to assess the ability of a compound to act as a hydrogen donor or as a scavenger.[8] The DPPH method is better in determining the antioxidant ability of a compound compared to indirect methods by assessing SOD or MDA level. This is because DPPH is a very stable free radical compound, thus the influence of environmental factors can be minimized.9 Therefore, this study aims to determine the antioxidant ability of the 2% IMLPED. The results of this study are expected to provide the possibility of using ear drops from natural ingredients that have minimal side effects in the treatment of ear diseases.

2. Materials and Methods

2.1. Materials

Materials used in this study are 2% IMLPED (Mount Lawu Beekepers, Indonesia), DPPH powder (HIMEDIA, India), and methanol.

2.2. Methods

2.2.1. Study Design

This research was an *in vitro* study to determine the antioxidant ability of the 2% IMLPED. This study was conducted at the Pharmacy Laboratory of the Faculty of Medicine, Muhammadiyah Surakarta University, Indonesia, during June-July 2023. The antioxidant ability of the 2% IMLPED was assessed using the DPPH method. This method assesses antioxidant activity utilizing the free radical DPPH. The outcome would yield an inhibitory concentration at 50% (IC_{50}) value, representing the concentration of the substances required to reduce 50% of the free radicals DPPH. This study was approved by the Ethics Committee of Dr. Moewardi General Hospital (No. 018/I/HREC/2024).

2.2.2. 2% IMLPED and DPPH Preparation

A total of 10 mg of DPPH powder was weighed and placed into a 10 mL volumetric flask. Methanol then added. The DPPH solution is stored at a controlled temperature and protected from direct sunlight. A total of 542 mg of the 2% IMLPED were put into a 10 mL volumetric flask and methanol was added. If the sample solution is not clear, the sample solution will be filtered.

2.2.3. Free Radical Testing

A total of 100 µL of DPPH was added to the sample solution and then methanol till the volume reached 1000 µL. The solution was incubated for 20 minutes at room temperature under light protection. Subsequently, the solution was read at its maximum wavelength of 516 nm. A blank solution consisting of a mixture of DPPH and methanol was also prepared as a control solution. The absorbance of the control and sample solutions was assessed by observing the color change of the samples after 20 min of incubation using a spectrometer which will then obtain the absorbance of each solution.

The inhibition capacity was obtained by calculating the difference between the absorbance ability of the sample solution and the control solution, and then divided by the absorbance ability of the control solution. The value obtained was then multiplied by 100%. The results of the calculation were then entered into a linear regression calculation with the y value being the % inhibition and x being the concentration of the solution. The IC_{50} value or x value is the result of inserting the value of 50 into the y variable of the linear regression equation that has been obtained.[8] In this study, we both measured the antioxidant activity of the 2% IMLPED and pure propolis as a comparison group.

$$\%\ Inhibition = \{\frac{Control\ soulution\ absorbance - Sample\ solution\ absorbance}{Control\ solution\ absorbance}\} x\ 100\%$$

3. Results

The result of the DPPH test showed that the IC_{50} value of the 2% IMLPED is 30.71 µg/mL, a reflection of its potential in reducing free radicals. This finding underscores the profound ability of the 2% IMLPED formula to effectively mitigate 50% of free radicals, manifesting at a concentration of 30.71 µg/mL. The implications of this revelation establish these ear drops as a promising candidate in combating oxidative stress. The results of the DPPH examination of pure propolis and 2% IMLPED are presented in Figure 1.

4. Discussion

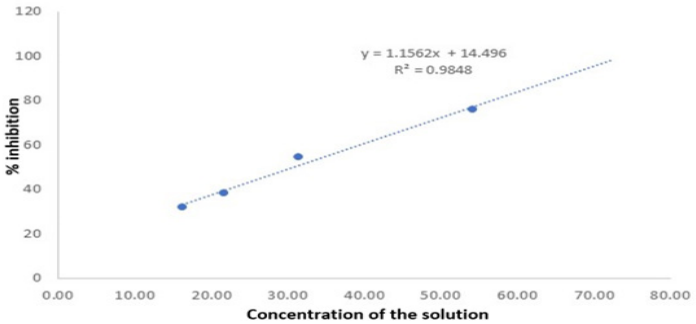
The results of this study indicate that the 2% IMLPED formula has an IC_{50} value of 30.71 µg/mL. This implies that at a concentration of 30.71 µg/mL, the 2% IMLPED can reduce free radicals by up to 50%. The ability of propolis to inhibit free radicals arises from a range of substances such as benzoic acid, phenolics, flavonoids, triterpenoid alcohols, and various enzyme groups. Flavonoids were potent antioxidants that can scavenge free radicals, protect cell membranes against lipid peroxidation, and protect cells from macromolecular damage.[10-12] The antioxidant capacity of propolis depends on the flavonoid composition.[13]

Several factors can influence antioxidant activity, such as geographic location, plant species, harvest time, and selective behavior of bees. These diverse variances contribute to the varying biological activity characteristics of propolis.[14,15] This may lead to differences in the antioxidant ability of different types of propolis. Moh-Yazid *et al*. (2018) reported that propolis from Malaysia had antioxidant capacity with an IC_{50} value of 10 µg/ml.[16] Nadhifah *et al*. (2021) stated the IC_{50} value of propolis from Pandeglang and Kendal, Indonesia, were 68,93 µg/mL and 144,06 µg/mL, respectively.[17] A study by Wiwekowati et al. (2017) reported that propolis from Java has a very strong potency with DPPH with an IC_{50} value of 35,6 µg/ml.[18] An in vitro study by Kumari *et al*. (2016) showed that the antioxidant capacity of propolis extract was found to be comparable to ascorbic acid which help to counteract the damaging effects of oxidative stress.[19] In light of these previous studies, it can be asserted that the antioxidant effectiveness of propolis was undeniably linked to its phytochemical composition, a characteristic subject to variation based not only on the local soil and climate conditions but also on the time of harvest. Nonetheless, propolis has consistently demonstrated robust antioxidant potential.[20]

The antioxidant ability of 2% IMLPED in this study has a very strong antioxidant ability which is on par with propolis from Java. The IC_{50} values were categorized based on their antioxidant ability to very strong, strong, moderate, weak, and very weak. Very strong antioxidants has IC_{50} less than The 50 µg/mL.[17] A small IC_{50} value indicates that only a small amount of a substance is needed to eliminate free radicals, and vice versa. A smaller IC_{50} value implies higher DPPH radical scavenging activity and therefore enhances its role as an antioxidant.[17,20]

The existing results show that propolis of various origins and compositions consistently demonstrate antioxidant properties. Aside from the antioxidant action, bioactive chemicals in propolis have an impact on a wide range of biochemical signaling pathways, and thus physiological and pathological processes. One of the most important aspects of propolis is its antioxidant ability. Although various research studies have confirmed propolis' potential antioxidant effect, there wasn't solid data on the safe amount in humans. As a result, clinical investigations involving propolis are required.

Previous studies on IMLPED had focused on its effect on antioxidant enzymes and oxidative stress markers, while its direct antioxidant ability has never been studied. The limitation of this study was that only one concentration of IMLPED was used, so the concentration with optimal antioxidant ability is not known for sure. This study was also an in vitro study



Note: DPPH = 2,2-Diphenyl-1-Picrylhydrazyl; IMLPED = Indonesian Mount Lawu Propolis Ear Drops

Figure 1. The DPPH test of the 2% IMLPED.

so the safety and side effects that may be caused by the use of the 2% IMLPED cannot be known.

5. Conclusion

The 2% IMLPED employed in this research yielded IC_{50} values of 30.71 µg/mL, thus signifying a pronounced presence of antioxidant activity. The IC_{50} value of 30.71 µg/mL indicates that the 2% IMLPED has a very strong antioxidant ability which opens the possibility of its use as an ear drop in treating ear diseases.

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Conflict of Interest

The author declared no conflict of interest.

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