NASKAH PUBLIKASI

AKTIVITAS ANTIOKSIDAN EKSTRAK ETANOLIK PROPOLIS *Tetragonula testaceitarsis* DIKOMBINASIKAN DENGAN DAUN *Lepisanthes Amoena*

ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT Tetragonula testaceitarsis PROPOLIS COMBINED WITH OF Lepisanthes Amoena LEAVES

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Naskah Publikasi

Aktivitas Antioksidan Ekstrak Etanolik Propolis *Tetragonula testaceitarsis* Dikombinasikan dengan Daun *Lepisanthes Amoena*

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Antioxidant Activity of Ethanolic Extract *Tetragonula testaceitarsis* Propolis Combined with of *Lepisanthes Amoena* Leaves

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ABSTRACT

The illnesses brought on by free radicals are a common source of health issues. Pathogenic processes and tissue damage are both significantly influenced by free radicals. Natural ingredients from Indonesia has been carried out for generations by the community with various kinds of treatments according to existing tribes, for example, such as kelulut bee (*Tetragonula testaceitarsis*) propolis and kokang (*Lepisanthes amoena*) leaves. Stingless bee propolis has several bioactivities to prevent diseases. The research about T. *testaceitarsis* propolis still limited. The purpose of this research to find out whether the combination of T. *testaceitarsis* propolis and L. *amoena* leaves extracts has antioxidant activity. Ethanol extract T. *testaceitarsis*. propolis and ethanol extract L. *amoena* leaves was collected and then tested for antioxidant activity using the DPPH method. The results showed that all three concentrations had very strong antioxidant activity. T. *testaceitarsis* propolis extract (50%) combined with L. *amoena* leaves (50%) showed strong antioxidant activity (92%) in 25 ppm concetration. Then 75% T. *testaceitarsis* propolis extract and 25% L. *amoena* leaves extract had the highest antioxidant activity (95%) at a concentration of 50 ppm concentration. This strong antioxidant activity was prospected to be continued as a pharmaceutical product.

Keywords: Propolis, *Tetragonula testaceitarsis*, *Lepisanthes amoena*, Antioxidant

INTRODUCTION

Health issues was dominated by free radical contribution. Free radicals can develop as a result of bodily metabolic processes as well as external factors including air pollution, chemicals in food and water, alcohol, tobacco, UV radiation, and other environmental factors. Free radicals are molecules with one or more unpaired electrons in their outer shell that are so reactive that in order to maintain stability, it is essential to absorb electrons from other molecules. This causes other molecules to become aberrant and sets off a chain reaction that can harm tissues (Jami'ah, Ifaya, Pusmarani, & Nurhikma, 2018).

The pathogenic processes caused by tissue damage usually was involved radicals mechanism. Free radicals may destroy delicate substances like lipids and proteins and enter the body in abnormal quantities, which can lead to the development of a number of disorders (Pratama & Busman, 2020).

The body's natural antioxidants are powerless to counteract the oxidants that are ingested. Natural antioxidant enzymes including catalase, superoxide dismutase (SOD), glutathione peroxidase, and glutathione transferase are present in the human body. Unfortunately, the body's own antioxidants fall short in their ability to totally shield cells from oxidant damage, necessitating additional antioxidants from outside the body (Suresh, Kunnath, & Reddy, 2022).

The use of natural ingredients has been carried out for generations by the community with various kinds of treatments according to existing tribes in East Kalimantan, such as Kelulut Bee Propolis and Kokang Leaves. Kelulut bees are a type of bee that produces honey but does not sting. In Indonesia, the terms bee kelulut are very diverse, bee klanceng, teweul, galo-galo and ketap. And one of the species of bees is Tetragonula testaceitarsis. Kelulut bees have very significant differences in body shape and production rates compared to other honeyproducing bees. Kelulut bees produce more honey, about 5.8 kg per year, and the taste is usually sour and contains more propolis than other bees (Hamzah, Siregar, Nurwijayanto, Wahyuningrum, & Sari, 2021; Saryanti, 2019; Yusuf, Kawareng, & Indriyanti, 2021).

Propolis is a substance produced by bees to protect their hives from various threats, in the form of plants resin collected by bees. Propolis has been used since ancient times because of its many benefits, one of which is for healing. It was empirically believed that propolis has many properties and is relatively safe to use in various traditional medicines. The composition of propolis itself is influenced by the type, age of the plant, and where the propolis comes from. Compounds contained in propolis include flavonoids, steroids, polyphenols, vitamins, amino acids and terpenoids, indicating that propolis has various antioxidant effects (Khairunnisa, Mardawati, & Putri, 2020).

Kokang leaves (*Lepisanthes amoena*) of the family *Sapindaceae*. L. *amoena* is known as Kukang (Kutai Tribe), Selekop (Dayak Benuaq Tribe), and Rembia (South Kalimantan). The Benuq Dayak tribe uses kokang leaves as a cold powder (pupur) to treat skin and acne scars. Meanwhile, the Kutai Dayak and Tunjung Dayak tribes usually treat skin problems such as dark spots on the face, pockmarks and acne scars (Fajriyati, Arifuddin, & Kuncoro, 2021). Kokang leaves contain several secondary metabolite compounds, such as phenolic compounds as the main group of antioxidants in plants, alkaloids with heterocyclic rings and plant nitrogen compounds that can inhibit the oxidation process, flavanoids as free radical catchers because they have hydroxyl groups, tannins with antioxidant properties as an antidote to disease-causing free radicals, including cancer, steroids classified as lipophilic antioxidants, saponins as superoxide absorbers,

preventing biomolecular damage by free radicals (Arifin & Ibrahim, 2018; Fajriyati et al., 2021; Hardiningtyas, Purwaningsih, & Handharyani, 2014). Unfortunately, there still no scientific report regarding combination of T. *testaceitarsis* propolis and L. *amoena* leaves. In this paper, the antioxidant activity was evaluated to determine their potential as local commodities.

RESEARCH METHOD

A. Materials

The research was used *Tetragonula testaceitarsis* propolis and *Lepisanthes amoena* leaves originated from Lempake, Samarinda and Kutai Kartanegara on June 2022 (Figure 1). Ethanol, Methanol, DPPH, Aquadest was used in extraction and antioxidant determination. The equipment used in this study is analytical balance sheet (Ohauss), Vortex (Thermo), Aluminum foil, UV-Vis Spectrophotometer, Micropipette.

Figure 1. (A) Characteristics of the nest entrance of *Tetragonula testaceitarsis* and (B) *Lepisanthes amoena* leaves

B. The *Tetragonula testaceitarsis* Propolis and *Lepisanthes amoena* Leaves Extraction

The preparation of propolis extract is using the maceration method. The maceration method by soaking it in a solvent at room temperature so that the damage or degradation of metabolites can be minimized. In maceration, a process of equilibrium of concentration occurs between the solution outside and inside the cell so that repeated solvent replacement is needed (Chandra, Asra, & Mevia, 2022). Raw propolis is mashed using a blender then macerated with 96 % ethanol and then shaker for one week. The maceration process is repeated with a filtrate retrieval time of 1x24, the process is carried out until the extract is not concentrated in color. The extract is then filtered with

filter paper which is then evaporated with a rotary evaporator and dried (Sativa & Agustin, 2018).

The 250 g of dry kokang leaves were crushed and macerated in 96% ethanol. The sample's extract solution is evaporated using a rotary evaporator at 45 °C to create a thick extract (Sari, Annisa, & Rusli, 2019).

C. Antioxidant Activity Determination

DPPH powder was weighed as much as 0.0050 mg then dissolved with 10 mL methanol p.a, vortex until dissolved. Furthermore, DPPH solution is taken 1 mL then added methanol p.a to 50 mL and let stand for 30 minutes.

Samples with varying concentrations of *Tetragonula testaceitarsis* propolis extract and kokang leaves were reacted with DPPH in ethanol solution. A sample of 5 mL (in methanol p.a) was mixed with 1 mL of DPPH solution with a concentration of 200 ppm. The blank solution is prepared by mixing 1 mL of DPPH 200 ppm with 10 mL of methanol p.a. The mixture is homogenized using a vortex shaker and incubated for 30 minutes at room temperature under dark conditions. Ascorbic acid was use as positive control. Absorbance was measured at a wavelength of 517 nm using a Uv-Vis Spectrophotometer (Meigaria, Mudianta, & Martiningsih, 2017). Antioxidant activity in the form of a percentage of inhibition, measured using the following formula 1.

$$\%\text{Inhibition} = \frac{\text{Blank absorbance} - \text{Sample Absorbance}}{\text{Blank absorbancel}} \text{ x } 100\%$$

By calculating the % inhibition of DPPH absorption, the amount of DPPH radical absorption resistance of the sample is used to estimate its antioxidant activity. The sample concentration and the percent of inhibition may then be displayed on the x and y axes in the linear regression equation a ± bx after determining the percentage of inhibition of each of these concentrations using the procedure above.

RESULTS AND DISCUSSION

DPPH (2,2-*diphenyl*-1-*pikrihidrazil*) was used as antioxidant determination. Antioxidants was an organic compound that can dampen free radicals in the human body by an oxidation process. Free radicals are relatively unstable molecules with atoms that have one or more unpaired electrons in their outer orbits (Kuncoro H, Naspiah, & Rahmadani, 2022). Both of the extract has potential compound that have antioxidant activity.

This is the first time that *Tetragonula testaceitarsis* propolis extract combined with *Lepisanthes amoena* leaves has been evaluated for antioxidant activity. The T.

testaceitarsis propolis extract combined with L. *amoena* leaves has 3 concentration variations divided into three concentrations (75%; 50%; 25%). The antioxidant activity of the combination was showed in Figure 2.

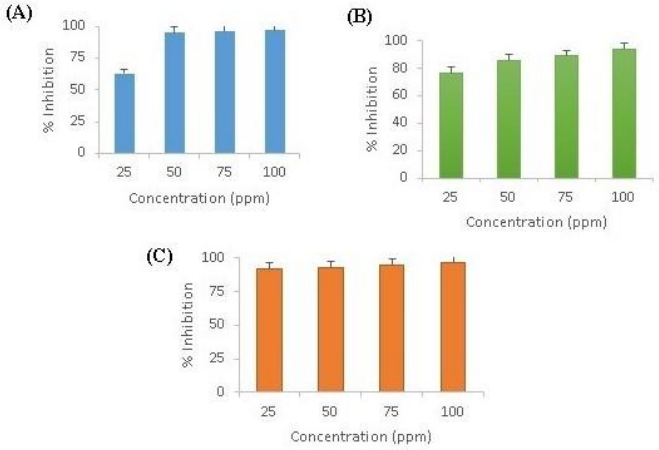

Figure 2. Antioxidant activity of *Tetragonula testaceitarsis* propolis (P) extract combined with *Lepisanthes amoena* leaves (K). Combination comparison was showed on (A) P 75% : K 25 %; (B) P50% : K50%; (C) P25%: K75%.

The *Tetragonula testaceitarsis* propolis extract (P) combined with kokang leaves extract (K) with combination P 75% : K 25 %; P50% : K50%; P25%: K75% all showed scaveging activity, thus P25%: K75% combination showing scaveging activity (92% at 25 ppm) higher than P50% : K50% combination (76%) and P 75% : K 25 % combination (63 %). The P 75% : K 25 % combination showed an increase in free radical inhibitory activity at a concentration of 50 ppm (95%) compared to P25%: K75 % combination (93%) and P50% : K50% combination (87%). This result indicated that propolis has an influence in trapping free radicals in combination with kokang leaves extract. Both have the same potential in antioxidant activity and the combination can increase their effectiveness. The free radical inhibitory activity of the combination of the two extracts can be seen very clearly in the Figure 3. If a substance can donate hydrogen atoms to DPPH, forming a reduced DPPH that changes color from purple to yellow, that substance is said to have antioxidant activity (Pebriarti et al., 2022).

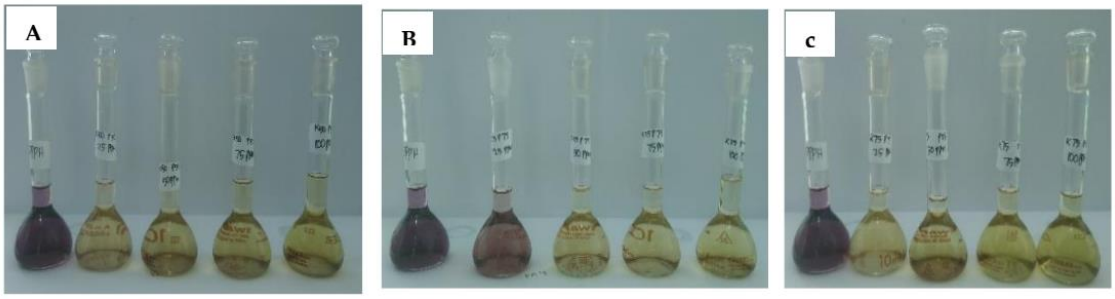

Figure 3. Antioxidant activity of *Tetragonula testaceitarsis* propolis (P) extract combined with *Lepisanthes amoena* leaves (K). Combination comparison was showed on (A) P 75% : K 25 %; (B) P50% : K50%; (C) P25%: K75%.

There are five grades of antioxidant activity such as very strong, strong, moderate, weak, and very weak (Lung & Destiani, 2017). This combination is expected to have antioxidants with an IC50 prospect of less than 50 ppm which is classified as a very strong category (Djaeni, Ariani, Hidayat, & Utari, 2017; Purwanto, Bahri, & Ridhay, 2017; Safrudin & Nurfitasari, 2018). Antioxidant activity is influenced by several factors. The source of *Tetragonula testaceitarsis* propolis is the sap of certain trees, including the damar tree. The active compounds in the collected tree sap also play a role in the synergistic effect of the combination. The *Lepisanthes amoena* also has dominant secondary metabolites such as phenolic compounds, flavonoids, glycosides, terpenes, and tannins (Zulkifli, Ab Ghani, Ismail, Bihud, & Rasol, 2021).

The synergism of the active compounds present in the extract will have a good effect. This is what we can often encounter when an herbal medicine is used in the form of a combination (Archana & Bose, 2022; Malongane, McGAW, & Mudau, 2017; Yang et al., 2014). The prospect of developing this herbal combination product will increase its use as the main function of synergistic and complementary effects.

CONCLUSION

Tetragonula testaceitarsis propolis extract combined with *Lepisanthes amoena* leaves has 3 concentration variations and it can be concluded that from these three concentrations it has a very strong antioxidant activity. The results of this powerful antioxidant activity are expected to be continued as a pharmaceutical product.

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Lampiran 1

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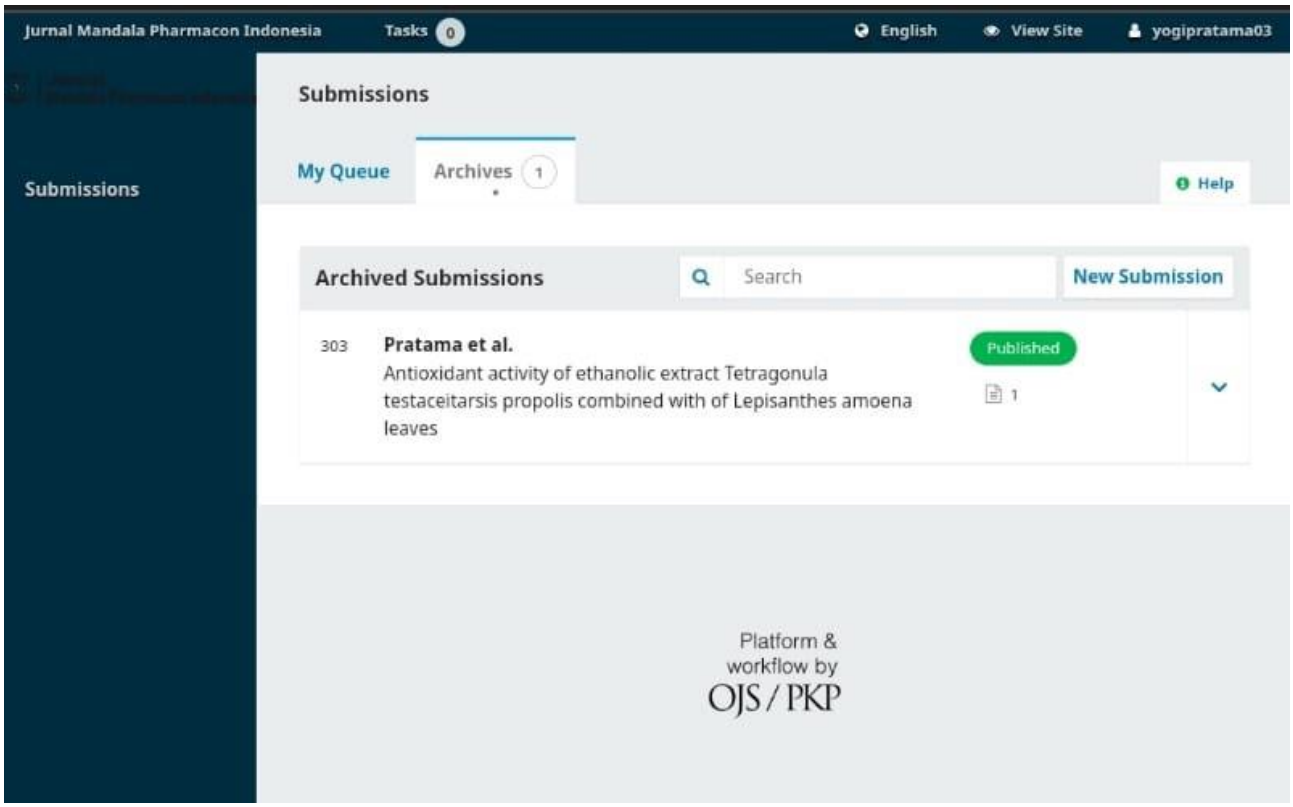
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