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RESEARCH ARTICLE

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QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF ETHANOL EXTRACT KELULUT BEE PROPOLIS (*Trigona laeviceps*)

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Abstract:

Kelulut bees are widely bred in Kalimantan. These bees do not have a stinger and easily adapt to new environments. Another characteristic of the kelulut bee is that it produces more propolis than honey. Kelulut Bee propolis contains tannins, alkaloids, flavonoids, and phenolics bioactive compounds with the potential to heal drugs. Following the concept of 'Back to Nature', namely increasing public interest in using natural ingredients to be used as medicine. Therefore, a test is needed to strengthen the utilization of this potential and quantitative phytochemical analysis is needed to determine the levels of each compound in kelulut bee propolis. The purpose of this research was to analyze the results of the quantitative phytochemical of ethanol extract of kelulut bee propolis (*Trigona laeviceps*). The research used non- experimental research with quantitative laboratory examination to analyze sample content. The results showed he quantitative phytochemical analysis of ethanol extract of kelulut bee propolis (*Trigona laeviceps*) showed a content of tannin compound 1.85 mg/ml, alkaloid 154.31 mg/ml, flavonoid 56.83 mg/ml and phenolic 120.37 mg/ml. ***E***thanol extract of bee propolis (*Trigona laeviceps*) showed a tannin compound content of 1.85 mg/ml, alkaloids 154.31 mg/ml, flavonoids 56.83 mg/ml and phenolic 120.37 mg/ml. Alkaloid had the highest concentration of 154.31 mg/ml, while tannin had the lowest concentration of 1.85 mg/ml.

Keywords: Quantitative analysis; Phytochemical; Propolis; *Trigona laeviceps*,

Introduction

Indonesia is a country with enormous biological natural resource potential. This potential encourages the community to increase their beekeeping. The kelulut bee (*Trigona laeviceps*) is a kind of bee that is frequently cultivated in Kalimantan. These bees are adaptable to new environments and have no stingers. Kelulut bees differ from bees in general in terms of producing honey. The propolis produced is a lot more than honey produced.[1]

Propolis is one of the products of kelulut bees used as self-defence from predators because of its sticky and nest adhesive. Propolis comes from plant resins processed with bee saliva enzymes and mixed with pollen. Research conducted to determine the content of bioactive compounds in kelulut bee propolis, namely qualitative phytochemical screening, has results containing bioactive compounds of tannins, alkaloids, flavonoids and phenolics.[2]

Propolis extract most likely can be used as a wound-healing drug based on the content of bioactive compounds. Related to the concept of 'Back to Nature' is the increasing public interest in using natural materials for medicine. Before being used as a drug, tests are needed to scientifically support these natural ingredients' ability. One of the tests that can be done is a quantitative analysis to determine the total content of bioactive compounds in kelulut bee propolis.[3]

Kelulut Bee propolis is extracted to separate bioactive compounds from natural ingredients. Extraction using the method of maceration with ethanol solvent. Bioactive compounds such as tannins, alkaloids, flavonoids and phenolics are polar compounds that will be easily extracted in polar solvents.[4,5]

Based on this, it is necessary to conduct quantitative phytochemical analysis studies of the ethanolic extract of kelulut bee propolis to determine the content of tannins, alkaloids, flavonoids and phenolic compounds from the ethanolic extract of bee kelulut propolis. The results of this quantitative phytochemical analysis can be used as the basis for further research to strengthen the use of kelulut bee propolis.

Research Method

This is non-experimental research with quantitative laboratory examination to determine the sample content. The Health Research Ethics Commission of Universitas Lambung Mangkurat has approved this study as ethically feasible with No.003/KEPKG- FKGULM/EC/II/2021. The determination test was carried out at the Biology Laboratory, State University of Semarang. Extracts and quantitative phytochemical analysis were carried out at the Biochemistry Laboratory, Faculty of Medicine, Universitas Lambung Mangkurat in Banjarbaru. Testing the content of compounds in the ethanolic extract of bee kelulut propolis was carried out on four compounds: alkaloids, flavonoids, phenolics, and tannins. The quantitative phytochemical analysis was carried out using UV-Vis spectrophotometry for tannins, flavonoids, and phenolics and gravimetric methods for alkaloids. The repetition was carried out three times for each compound. The results obtained are the average value of the repetitions performed.

Materials

The material for the extraction consists of honeybee propolis and 70% ethanol. The quantitative test material consists of aqua bidestilata, Folin-Denis preservative (E. Merck), acetic acid (E. Merck), ammonium hydroxide (E.

Merck), sodium acetate (E. Merck), NaNO2 (E.Merck), AlCl3 (E.Merck), NaOH (E.Merck), aquadest, Folin-Ciocelteau reagent (E.Merck), sodium carbonate (E.Merck).

The research equipment consisted of UV-Vis spectrophotometry (Shimadzu 1800), magnetic stirrer (MSH-3), filter paper (WH40), oven (Memmert), measuring glass (Iwaki), micropipette (Dragon lab), reaction tube (Iwaki), funnel (Iwaki), stirring rod. Tools for extraction consisted of the extractor, filter paper (WH40), analytical balance (Precisa), Erlenmeyer flask (Iwaki), blender (Phillips), oven (Memmert), water bath (Memmert).

Extraction Procedure of Kelulut Bee Propolis

Kelulut bee propolis used taken in small pieces ± 1 cm and then dried using an oven at a temperature of 40℃ for 3x24 hours. Propolis is soaked with 70% ethanol in a closed container for 2x24 hours, stirring occasionally. Solvents are changed daily. The filtration was done using Whatman filter paper no.1. The extract is evaporated to form a thick extract. [6,7]

Quantitative Phytochemical Analysis

Weighing 0.5 grams of the sample and diluting it with aqua bidestilata was used to test for tannin concentration. The model was added to 1 ml in a 10 ml container that already contained 7.5 ml of aqua bidestilata after dissolving. After adding 0.5 ml of Folin-Denis reagent to the solution and letting it sit for 3 minutes, saturated Na_2CO_3 solution was added. It was then left to incubate for 15 minutes. [2,8]

A sample of 10 grams was placed in a 250 ml beaker glass, and 200 ml of 10% acetic acid was added to test the presence of alkaloid compounds. The beaker glass was sealed and left to stand for four hours before filtering. A quarter of the extract was evaporated in a water bath and then precipitated with ammonium hydroxide. Filter and dilute ammonium hydroxide were used to wash the precipitate.

Total flavonoid content was determined by pouring 500 ml of a sample into a test tube with a micropipette and adding 2 ml of distilled water. After adding 150 ml of 5% $NaNO_2$ and letting it stand for 6 minutes, 150 ml of 10% $AlCl_3$ was added and allowed to stand for 6 minutes. The tube was filled with 2 mL of 4% NaOH, which was then diluted with distilled water until the level reached 5 ml.

The test of phenolic compounds used a sample of 0.5 ml, added Folin-Ciocelteau reagent (1:10) and 4 ml of 1 M sodium carbonate was left for 15 minutes. Measurements were carried out using spectrophotometry at a wavelength of 750 nm three times. Total phenol was calculated using the linear regression equation of the gallic acid calibration curve.

Results

Average content of total Tannins, Alkaloids, Flavonoids and Phenolics of Bee Kelulut Propolis Ethanol Extract can be seen in Table 1.

Table 1. Average Content of Total Tannins, Alkaloids, Flavonoids and Phenolics of Bee Kelulut Propolis Ethanol Extract.

No.	Bioactive Compound	Total Content (mg/ml)
1.	Tannins	1.85±0.014
2.	Alkaloids	154.31±5.85
3.	Flavonoids	56.83±0.577
4.	Phenolics	120.37±0.336

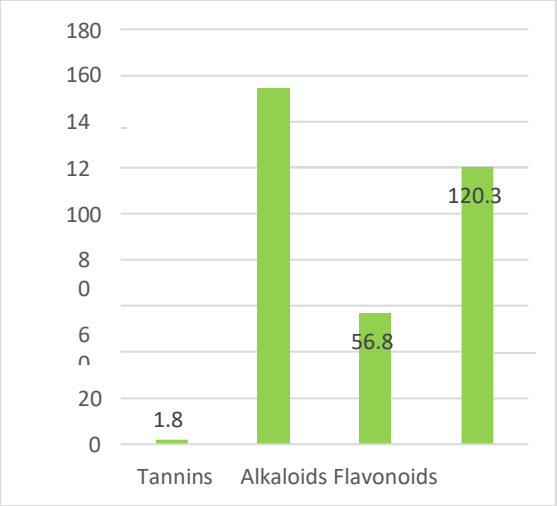

Figure 1. Diagram of the quantitative phytochemical analysis results of ethanol extract of kelulut bee propolis

Based on table 1, the results of the quantitative phytochemical analysis carried out on the ethanolic extract of kelulut bee propolis showed that the total content of bioactive compounds contained were 1.85 mg/ml tannins, 154.31 mg/ml alkaloids, 56.83 mg/ml flavonoids and phenolic of 120.37 mg/ml. The table shows that the highest total levels of bioactive compounds in the ethanolic extract of kelulut bee propolis were found in alkaloid compounds, namely 154.31 mg/ml and the lowest total levels were found in tannins at 1.85 mg/ml.

Discussion

The results of the quantitative phytochemical test of the ethanolic extract of kelulut bee propolis have It is known the highest levels of compounds are alkaloids with 154.31 mg/ml, followed by phenolic content with 120.37 mg/ml, flavonoid content with 56.83 mg/ml, and levels of the lowest was found in the tannin compound 1.85 mg/ml. Research on propolis from different bees, namely *Apis mellifera*, showed that the highest content of alkaloids were alkaloids at 0.06 mg/ml, flavonoids at 0.04 mg/ml and phenolics at 0.03 mg/ml. Research on compound levels secondary metabolites of propolis has also been carried out on the bee type *Trigona sp*. with total phenolic content of 0.03 mg/ml, total flavonoid content of 0.004 mg/ml, and total tannin content of 0.103 mg/ml.

The source of propolis material, whether rubber or resin from plants in the beehive habitat, influences the high quantities of alkaloids in kelulut bee propolis. Each type of bee can fly in search of different food and geographical areas of a habitat that affect the types of plants that grow in the hive environment. This plant affects the content of bioactive compounds in terms of quality and quantity.[9]

Alkaloids are compounds that are not heat resistant. The maceration method is a cold extraction method so that the alkaloid compounds can be extracted maximally. In the extraction process, the solvent was changed every day. This affects the secondary metabolite compounds will be pulled completely. When the immersion is carried out, there is an exchange process of fluid in the cell with a lower concentration solvent. This process continues until the concentration inside and outside the cell reaches balance.[10] In the quantitative phytochemical analysis, acetic acid was used to make an alkaloid salt. Then, a precipitate was created by dumping concentrated ammonium hydroxide onto the extract. Wagner's reagent solution, which reacts in acid, is used to identify alkaloids. This occurs due to the addition of hydrogen ions to the double bond and forms a carbocation. Alkaloids have

potential as antifungals. The antifungal mechanism possessed by alkaloids prevents fungal DNA replication by infiltrating between cell walls or DNA and disrupting fungal growth.11,12

Phenolic chemicals are the second most abundant component in kelulut bee propolis. The flavonoid content, also present in the kelulut bee propolis investigated, contributes to the high phenolic levels. Flavonoids are a group of phenolic compounds. In determining the content, the Folin-Ciocalteu reagent is commonly used for total phenolic analysis in food and beverages. The Folin-Ciocalteu reagent reacts with phenolic compounds and forms a blue colour.[13,14]

The ability of phenolics is a natural antioxidant. The higher the level of phenolic compounds, the higher the antioxidants level. Flavonoid compounds also have the ability as antioxidants because they are phenolic compounds. Flavonoids work to keep the cell cycle regular. Flavonoids can also act as an anti-inflammatory that works by inducing apoptosis and inhibiting proliferation.[15,16]

The content with the lowest total content of kelulut bee propolis is tannins. In measuring total tannin content, a Folin-Denis reagent was added. Colour formation based on oxidation-reduction reaction, tannins as reducer and Folin-Denis as oxidizer.[17,18]

The principle of the Folin-Denis method is the formation of a complex blue compound whose absorption can be measured in the visible ray area. Tannins have antibacterial and antifungal abilities. In addition to being antibacterial and antifungal, tannins also have the potential to an anti-inflammatory. Tannin can increase the activity and number of macrophages. Macrophages will induce the production of growth factors.[19,20]

Conclusions

According to the findings, the ethanol extract of kelulut bee propolis (*Trigona laeviceps*) contained tannins 1.85 mg/ml, alkaloids 154.31 mg/ml, flavonoids 56.83 mg/ml, and phenolic 120.37 mg/ml. The bioactive compound with the highest content, 154.31 mg/ml, was alkaloid, and the bioactive compound with the lowest content, 1.85 mg/ml, was tannin.

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