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Research Paper

Photoprotective and Antioxidant Potential of Indonesia's Klanceng Honey Beehive Waste

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Abstract
A byproduct of the honey processing called Klanceng honey beehive waste is said to still contain secondary metabolites that are beneficial to health. The objective of this research was to assess the photoprotective properties of an ethanol extract, n-hexane fraction, and ethyl acetate fraction obtained from the waste of an Indonesian Klanceng honey beehive that originated in Magelang. The DPPH free radical technique was applied to quantify antioxidant properties, and the Mansur equation's SPF value was used to calculate photoprotective activity. The analysis of the data revealed that the IC50 values for the ethanol extract, n-hexane fraction, and ethyl acetate fraction were, respectively, 470.2935±0.9249, 207.1869± 2.6510, and 216.4892±0.8349. The ethanol extract, n-hexane fraction, and ethyl acetate fraction of the samples had SPF values of 3.872, 3.529, and 9.358, respectively. The ethyl acetate fraction, as opposed to the ethanol extract and the n-hexane fraction, has greater potential as a photoprotective agent as a result.

Keywords
Antioxidant, Extract, Fraction, Honey Beehive, Photoprotective

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1. INTRODUCTION

The human skin serves as a protective border between the internal and exterior environments, guarding against radiation, toxic substances, mechanical trauma, and microbial invasion. It has been abundantly obvious in recent years that the skin plays a significant role in the immune system. Environmental factors like ultraviolet (UV) radiation, oxidative sterss, hazardous and allergic substances, and mechanical damage, as well as intrinsic factors like genetic propensity, immunological and hormonal prestige, and stress, have an impact on the condition and function of the skin. The consequent abnormalities in the skin result in photoaging, inflammation, decreased immune responses, an imbalance of epidermal homeostasis, and other skin problems (Fernandez Garcia, 2014).

Photo-oxidative reactions caused by UV radiation exposure cause biomolecular damage, affecting the authenticity of skin cells and causing skin problems. Damage to these photo-oxidative contributes to the pathological process and is responsible for the oncet of many skin disorders. Continuous sun exposure and other environmental factors can result in the induction of oxidative stress, which has a high reactivity with genetic material, peptides, and fatty acids and frequently causes antioxidant significant damage (Vijayakumar et al., 2020). Photoprotective agents shield the skin from the damage consequences of ultraviolet natural daylight (Latha et al., 2013). Plant-derived phytochemical compounds have the potential to prevent molecular damage by capturing and attempting to destroy ROS raised by biological mechanisms, smog, inhaling, and medicines. Polyphenols are the most important natural product class in dermatology due to their absorption spectrum, which efficiently filters UV radiation, reducing the possibility of radiation penetrating deep into the skin layer (Vijayakumar et al., 2020).

Bee products are regarded as a source of natural antioxidant potential proficient in combating the effects of oxidative stress, which underlies the pathogenesis of several diseases (Kocot et al., 2018). Beehive also contains various phenolic and flavonoid compounds that can be used as antioxidants (Pérez-Pérez et al., 2013). Beehives provide protection for bee colonies from microbial species, mushrooms, infectious agents, and carnivores, as well as a location for honey production, bee pollen, and bee growth and development. The condition of beehives has a significant bearing on the quality of honey (Pérez-Pérez et al., 2013). The content of compounds in

honey beehives serves as a protector and determinant of honey quality, including flavonoids, which are natural phenol compounds, and beeswax. The *Trigona* bee group is a non-rigid bee group that lives socially in a colony that usually tries to live in trunks, wood, bamboo, and soil. One bee colony has only one queen, hundreds of male bees, and thousands of worker bees; one species of *Trigona sp.* is T. Itama, which produces more raw propolis (Hrncir et al., 2016). The content of propolis in the beehive has many benefits, such as drugs and cosmetics (Wagh, 2013). Honey is a sticky substance made by stingless bees. Honey is well-known throughout the world for its excellent nutritional constituents that are advantageous to human health, and propolis is commonly referred to as "bee glue," which is a collective term for the resinous substance gathered by bees from diverse types of plants (Puspawati et al., 2019).

In Muntilan District, Magelang, Indonesia, many Klanceng honey bees, or *Trigona sp*., are cultivated. According to local honey breeders, taking Klanceng honey is extorting honey from the hive, which gives honey and Waste. It is estimated that honey beehive waste still contains phytochemicals that can be used to improve one's health.

The content of compounds in beehives functions as protectors and determinants of honey quality, including phenolic compounds and flavonoids. Honey has lower antioxidant activity than propolis, which is due to the variety of phenolic compounds present in the extract (Mouhoubi Tafinine et al., 2016). Notably, regardless of its composition, propolis extract always contains protective effects. In both animal and cell culture studies, aqueous extracts of propolis have been shown to possess antioxidant capacity (Kocot et al., 2018). Several studies have stated that propolis contains bioflavonoids (Sabir, 2005). The phytochemical content of *Lisotrigona cacciae* propolis extract shows the presence of several compounds of flavonoids, xanthones, alkyl resorcinol, triterpenes, other phenol compounds, fatty acids, esters, and sugar (Georgieva et al., 2019). Phenolic compounds and flavonoids have antioxidant activity Sukweenadhi et al. (2020), antibacterial activity Yuan et al. (2021), and anti-inflammatory activity Candiracci et al. (2012), so it is thought that waste from a honey beehive in Muntilan, Magelang, may have these properties. Based on this description, the purpose of the research is to assess the photoprotective and antioxidant potential of an ethanol extract, n-hexane fraction, and ethyl acetate from honey beehive waste (*Trigona sp*.) that originated in Magelang, Indonesia.

2. EXPERIMENTAL SECTION

2.1 Materials

The substances used in this study were 96% ethanol (Brataco), ethanol p.a. (Merck), Shinoda Reagent (Merck), $FeCl_3$ (Merck), n-hexane (Brataco), ethyl acetate (Merck), HCl 2N, Mayer Reagent (Merck), Bourchard Reagent (Merck), Dragendroff Reagent (Merck), Na_2CO_3 (Merck), Folin-Ciocalteu (Merck), gallic acid (Sigma), Quercetin (Sigma), DPPH (Sigma), Ascorbic Acid (Merck), Anhydride acetate (Merck), Na acetate (Merck). The tools used in this study were a set of glass devices commonly used in laboratories, bransonic brand ultrasonic batch, UV-Vis Shimadzhu 1840 spectrophotometer, a vacuum evaporator, IR-ATR Agilent 630 spectrophotometer.

2.2 Methods

2.2.1 Sample

The sample used was the waste of honey beehive *Trigona sp*. from Muntilan District, Magelang Regency, Central Java Province, Indonesia. Samples were sorted to separate rotten materials and impurities. The sample was reduced in size using a knife.

2.2.2 Extraction and Fractination

A total of 100 g of samples were macerated in the sonicator for 1 hour with 96% ethanol and allowed to stand for 24 hours with a sample and solvent ratio of 1:10. The extract was filtered and separated into filtrates and residues. The residue was remacerated in the same manner. Maceration was performed three times. In a vacuum evaporator set to 50°C, the filtrate was evaporated until a thick extract was obtained. Ten grams of thick extract were fractinated in hexane, ethyl acetate, and ethanol. Using an evaporator, the fractionation results were evaporated to obtain a concentrated n-hexane fraction, an ethyl acetate fraction, and an ethanol extract.

2.2.3 Phytochemical Screening

The chemical content of the sample was confirmed using color reagents. Flavonoid, tannin, saponin, and terpenoid tests were all performed. A shinoda reaction was used for the flavonoid test, which included extract and filtate plus HCl 2 N, magnesium powder, and amyl alcohol. If the amyl alcohol layer is red, the flavonoid sample is positive (Nurhasnawati et al., 2019). The tannin test was performed with a $FeCl_3$ solution that yielded a positive blackish-green color. The alkaloid test was performed using three methods: the dragendroff reagent, the Mayer, and the Bouchard tests (Nurhasnawati et al., 2019). The saponin test was performed by first adding distilled water to the sample and managing it, then adding HCl 2 N and shaking it to determine the presence of stable foam-positive saponin (Nurhasnawati et al., 2019). The terpenoid test was performed by dissolving a number of samples in ether, which was then evaporated and anhydride acetic acid added. The existence of terpenoids was indicated by the presence of a red or green color in this test (Nurhasnawati et al., 2019). An ATR-FTIR spectrophotometer was used to identify the functional groups of ethanol extract, n-hexane fractions, and ethyl acetate fractions. The sample was placed on the prism, and the % transmittance on the wave number 4000-400 cm^{-1} was measured (Liu et al., 2006).

2.2.4 Total Phenolic Content (TPC) Using Visible Spectrophotometry

This method based on Mathur and Vijayvergia (2017) with little modification. The TPC test was performed by pipetting a 1 mL sample (500 – 900 mg/L) solution and 4 mL of Folin-Ciocalteu (F-C) reagent. This mixture was kept for 4 minutes

before adding 0.4 mL of 7% Na_2CO_3 solution and incubating for 120 minutes with distilled water up to 10 ml.. The mixture is measured at its maximum wavelength of 775 nm. The standard solution used was gallic acid with a concentration of 60–100 mg/L in the 10 range.

2.2.5 Total Flavonoid Content (TFC) Using a Visible Spectrophotometry

This test was carried out by the colorimetric method using $AlCl_3$ based on Tristantini and Amalia (2019) with modification. As many as 1 mL of samples plus 0.1 mL of 10% $AlCl_3$ solution and 0.1 mL of 1 M sodium acetate solution was adhered with aquadest to a volume of up to 10 mL and incubated for 30 minutes. The mixture is measured at a maximum wavelength of 436.5 nm.

2.2.6 Antioxidant Activity Test Using DPPH (2,2- Diphenyl-1-Picrylhydrazyl)

With minor modifications, this antioxidant test method is based on Sukweenadhi et al. (2020). Allowing 3 mL of DPPH solution 0.4 mM plus 1 mL of samples to stand for 30 minutes. The wavelength of the solution was 517 nm. Ascorbic acid was used as a positive control, and DPPH in ethanol was used as a blank. Using equation 1, the absorbance obtained from the instrument was used to calculate the percentage inhibition(Syarifah et al., 2021).

$$\%\text{inhibition} = \frac{A_{blank}\text{-}A_{sample}}{A_{blank}} \quad (1)$$

2.2.7 SPF Test Using UV Spectrophotometry

The SPF value is calculated using a modified method based on Sabir (2005). UV spectrophotometry is used to measure absorbance at 290-320 nm in the 5 nm range for samples with concentrations ranging from 100 to 500 ppm (in ethanol solvents). The absorbance is calculated using the Mansur equation (2) to yield the SPF value.

$$\text{SPF} = \text{CF} \times \sum_{290}^{320} EE(\lambda) \times I \times A(\lambda) \quad (2)$$

Where, CF = correction factor (10), EE (λ) = erythmogenic effect of radiation with wavelength λ, Abs (λ) = spectrophotometric absorbance values at wavelength λ. Table 1 shows the values of the EE (λ) x I constants (Khan, 2018).

3. RESULT AND DISCUSSION

Remaceration is used to extract Klanceng honey beehive waste for 1 hour at room temperature, aided by ultrasonic waves. In secondary metabolite extraction, the sonification method is an ecofriendly extraction method that is used to remedy the flaws of the maceration method. Sonication methods can be completed in much less than an hour, and the impact of damage throughout the phase is negligible or can be lessened (Puspawati et al., 2019). Ultrasonic radiation with a frequency greater than 20 kHz makes it easier to extract compounds both natural ingredient and inorganic from porous support utilizing liquid solvents. The sound wave of sonicator creates cavity bubbles close the sample tissue, which disintegrate cell walls and release cell contents, including secondary metabolites (Khoddami et al., 2013). The use of an ultrasonic wave to aid extraction rises compound solubility in the solvent, resulting in a reduction in the volume of solvent required (Alara et al., 2021). The maceration results are evaporated in a vacuum rotary evaporator to produce a concentrated brown color extract with a honey aroma, yielding a 36.09 ± 1.69% yield. The use of a vacuum evaporator can lower the temperature of the solvent because the pressure in the flasks decreases, making the solvent more volatile.

Concentrated extracts are fractionated using n-hexane solvents to obtain nonpolar fractions; the residues are then fractionated with ethyl acetate solvents to obtain semipolar fractions. Water fractions are ethyl acetate fractionation residues. Each fraction is evaporator to obtain a thick fraction of each fraction of the n-hexane fraction; the ethyl acetate fraction and the water fraction are 49.52 ±8.58%, 37.54 ±3.40%, and 2.15 ± 0.26%, respectively.

The secondary metabolite content of the sample was ascertained using phytochemical screening (Gurning et al., 2021). Phytochemical analysis reveals that alkaloid compounds, flavonoids, tannins, and terpenoids are present in ethanol extract, n-hexane fractions, and ethyl acetate fractions (Table 2). The FTIR spectrophotometer results of the ethanol extract, n-hexane fraction, and the ethyl acetate fraction show the same spectrum pattern (Figure 1). The three samples have the same spectrum pattern based on the IR spectrum results. The n-hexane fraction exhibits a lower -OH absorption at wave number 3300 cm^{-1} than the ethanol extract and ethyl acetate fraction. The presence of -OH functional groups in a sample identifies the existence of phenol or alcohol groups (Revathi and Rigley, 2019). The aliphatic CH- alkyl group in the sample was demonstrated by wave number 2800-2900 cm^{-1}. The availability of -C=C- aromatic groups was indicated by the wave number 1700 cm^{-1}, while the presence of carbonyl groups C=O was noted by the wave number 1350-1500 cm^{-1}. The absorption of the C-OH group from alcohol was implied by the wave number at 1025 cm^{-1} (Stuart, 2004). According to this analysis, the samples contained polyphenolic compounds.

Plants produce polyphenols in reaction to external and physiological pressures including pathogenic and insect attacks, UV exposure, and wounds (Khoddami et al., 2013). The basic structure of polyphenols is the benzene ring with hydroxy groups. The phenolic level of the sample is determined by measuring the absorbance at a wavelength of 775 nm using the Folin-Ciocalteu method. The standard used is 60-100 ppm galic acid with a 10 ppm range. The galic acid standard linear regression equation is y = 0.0082x - 0.231, R^2 = 0.9970. The total amount of phenolic is given in milligrams of galic acid

Table 1. The Result of Phytochemical Screening

compound group	Etanol extract	Ethyl Acetate Fraction	n-hexane Fraction
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponin	-	-	-
Tannin	+	+	+
terpenoid	+	+	+

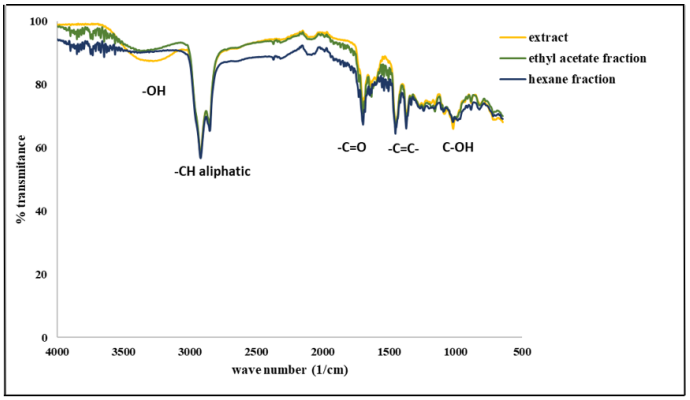

Figure 1. ATR-FTIR Spectrum of Sample

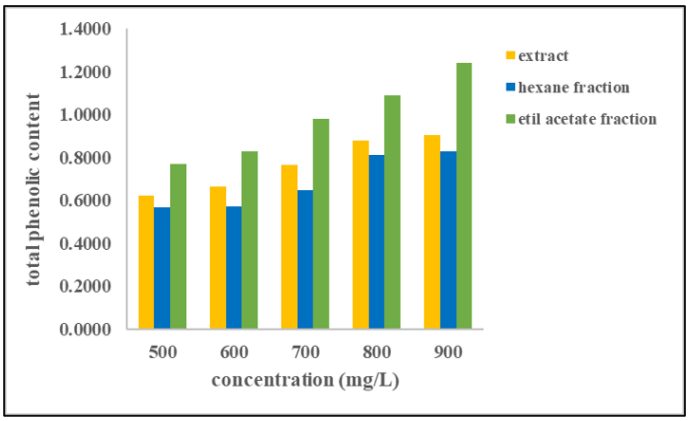

Figure 2. Total Phenolic Content of Sample

per 100 g of sample. Galic acid is used as a standard because its availability and stability (Wabaidur et al., 2020)

According to Figure 2 and Table 3, the total phenolic content of the ethanol extract, as well as the n-hexane and ethyl acetate fractions, increases with increasing sample concentration. This image shows that the ethyl acetate fraction has a higher total phenolic level than the ethanol extract and the n-hexane fraction.

The total flavonoid content (Figure 3) was defined utilising visible spectrophotometry and $AlCl_3$ as a reagent. The construction of a complex among $AlCl_3$ compounds and quercetin compounds is the essence of calculating amount of flavonoid using colorimetry method (Meilawati et al., 2021). The total

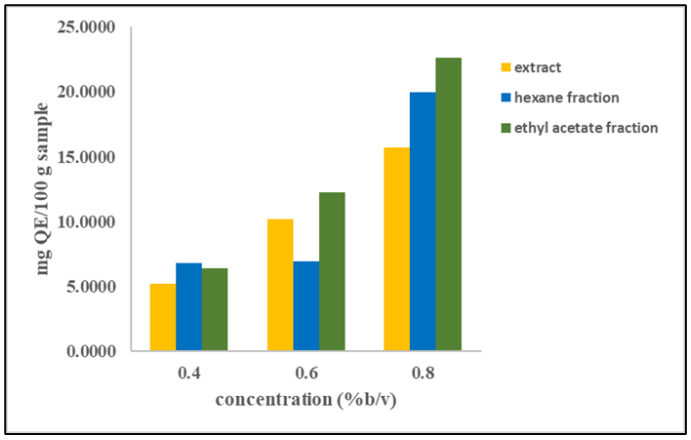

Figure 3. Total Flavonoids Contents of Sample

quantity of flavonoid was described in milligram equivalent of quercetin/100 g sample. Measurement of quercetin resulted in a linear regression equation y=0.0072x+0.0038, r=0.9998.

The antioxidant activity of the ethanol extract, the n-hexane fraction, and the ethyl acetate fraction was tested using the DPPH radical. This method was chosen because DPPH is a stable free radical so it is easy to do. This test was executed by visible spectrophotometry at a wavelength of 517 nm with an operating time of 30 minutes (Gurning et al., 2021; Sembiring et al., 2018). The decrease in DPPH concentration was proportional to the increase in antioxidant concentration. IC50 is the effective concentration that induces a 50% reduction in the previous DPPH concentration (Molole et al., 2022). The IC50 value for the ascorbic acid standard used in this study was 8.875 ppm, indicating very strong antioxidant activity. Ascorbic acid easily and frequently catches superoxide anion species such as superoxide and hydroperoxyl radicals, oxidants, ozone, peroxynitrite, nitrogen dioxide, nitrous oxide radicals, and hypochlorous acid, thereby preventing oxidative stress to other substrates (Birangane et al., 2011).

The sunscreen activity of the sample was carried out by UV spectrophotometry. This measurement is made at a wavelength of 290–320 nm. The SPF value is estimated based on the Mansur equation. Table 3 and Figure 5 illustrate that the greater the sample concentration, the higher the SPF value. The SPF value of the ethyl acetate fraction is greater than that of the extract and greater than that of the n-hexane fraction. The

Table 2. Total Phenolic Content (TFC) of Sample

Concentration (mg/L)	Ethanol Extract	Ethyl Acetate Fraction	n-hexane Fraction
500	0.6227±0.0004	0.7685±0.0014	0.5683±0.0000
600	0.6641±0.0006	0.8307±0.0004	0.5723±0.0022
700	0.7640±0.0006	0.9803±0.0005	0.6466±0.0013
800	0.8788±0.0012	1.0877±0.0007	0.8109±0.0007
900	0.9042±0.0007	1.2389±0.0015	0.8304±0.0006

Table 3. SPF Value of Sample

Concentration of Sample (ppm)	Extract SPF Value	Protection Category	n-hexane Fraction SPF value	Protection Category	Ethyl Acetate Fraction SPF Value	Protection Category
100	1.424	Low	1.991	Low	5.010	Low
200	1.870	Low	2.466	Low	6.535	Low
300	2.700	Low	3.389	Low	6.898	Low
400	3.872	Low	3.529	Low	9.358	Low
500	5.832	Low	4.464	Low	11.898	Low

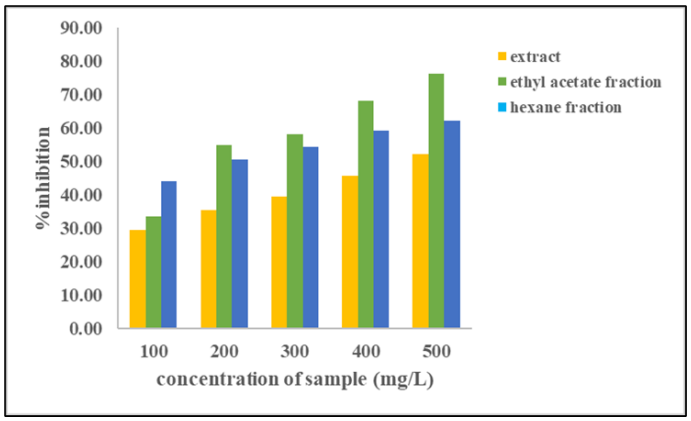

Figure 4. Antioxidants Test of the Sample Using DPPH

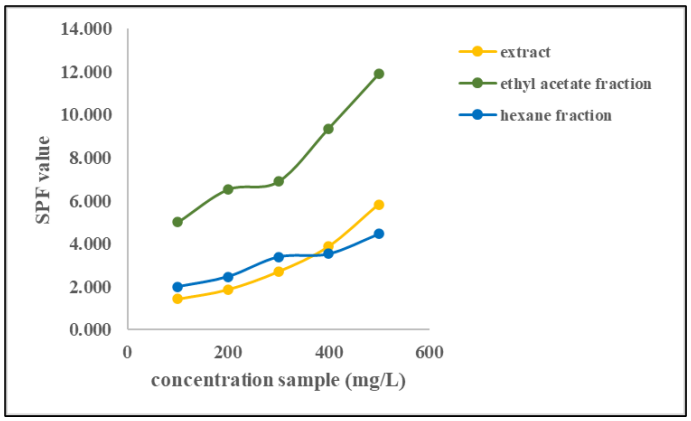

Figure 5. SPF Value of Sample

concentration of 500 ppm of the extract, n-hexane fraction, and ethyl acetate fraction was able to provide low protection against UV B rays, but in this concentration, ethyl acetate had a higher SPF value than the extract and n-hexane fraction. According to Geoffrey et al. (2019), protection from UV rays is graded based on the SPF value: low (SPF 2-15), medium (SPF 15-30), high (SPF 30-50), and highest (SPF>50). Honey and propolis contain active compounds involve polyphenol, flavonoids, carotenoids, and avitamin C (Mouhoubi Tafinine et al., 2016). As a result, it can be assumed that reusing this honey beehive waste reduces the total phenolic and flavonoid content, thereby affecting its SPF activity.

4. CONCLUSION

The honey beehive *Trigona sp.* from Muntilan District has weak or inactive antioxidant activity and a low SPF value, and thus has no development potential.

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