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Klanceng Honey Beehive (*Trigona biroi)* Sunscreen Activity

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ABSTRACT. UV radiation can cause various skin problems, including photoaging and skin cancer. Sunscreen can provide UV radiation protection. Klanceng honey bee nests may contain metabolites that could be employed as sunscreen agents. This research project investigates the sunscreen activity of the extract, n-hexane fraction, ethyl acetate fraction, and aqueous fraction of the Klanceng honey bee hive using SPF, % Te, and Tp values. Honey bee hives are extracted via maceration assisted by ultrasonic waves. As solvents for fractionation, n-hexane, ethyl acetate, and water are used. UV spectrophotometry at a wavelength of 290-375 nm was used to examine the sunscreen activity of the samples in vitro, and the SPF, % *Te*, and % *Tp* values were computed. The extract, n-hexane fraction, ethyl acetate fraction, and aqueous fraction had SPF values of 5.832, 4.464, 11.898, and 2.846, with medium, medium, maximum, and minimal protection categories, respectively. The % Te value indicates that the extract, n-hexane, and aqueous fraction do not protect anti-erythema transmission. However, the ethyl acetate fraction does. The % Tp statistic demonstrates that all samples offer sunblock category protection. Based on this, the availability of ethyl acetate fraction is the most effective defense against UV A and UV B rays, indicating that it has the most significant potential as a sunscreen agent.

Keywords: Erythema, Pigmentation, Honey bee hive, SPF, Sunscreen

INTRODUCTION

The largest organ in the body, the skin makes up 16% of the body's total mass (D'Orazio et al., 2013). Skin condition and function are influenced by internal variables, including background genetics, immunological and hormonal status, stress, and environmental factors like ultraviolet rays, free radicals, toxic compounds and allergens, and mechanical damage. These elements encourage inflammatory conditions, photoaging, immunity decline, a disparity in epidermal stability, and other skin issues (Fernández, 2014).

UV A (315–400 nm), UV B (280–315 nm), and UV C (100–280 nm) are the three different types of UV radiation (D'Orazio et al., 2013; Zou et al., 2022). High levels of UV radiation exposure can cause pigmentation, early aging, and the most serious condition, skin cancer (Vijayakumar et al., 2020). Photoprotective agents shield the skin from the damaging effects of natural light's ultraviolet (UV) rays (Latha et al., 2013). By absorbing and eliminating reactive oxygen species produced by metabolic pathways, contaminants, cigarette consumption, and pharmaceuticals, phytochemical substances from different plant sections can stop molecular damage. Since polyphenols have an absorption spectrum that effectively filters UV rays, they are the most significant class of natural compounds in dermatology because they lessen the likelihood that radiation will penetrate deeply into the skin's layers (Vijayakumar et al., 2020).

It is believed that honey and other honey-based products may provide natural antioxidants that might mitigate the effects of oxidative stress, which is linked to the etiology of several disorders (Kocot et al., 2018). Additionally, Phenolic and flavonoid compounds found in beehives have potential applications as antioxidants (Pérez-Pérez et al., 2013).

The compound content of honey beehive, which includes phenolic compounds and flavonoids, acts as a protector and determines the quality of honey. According to several studies, propolis contains bioflavonoids (Sabir, 2005). Flavonoid components, including xanthones, triterpenes, alkyl resorcinol, other phenolic compounds, fatty acids, esters, and sugars, are found in the phytochemical content of *Lisotrigona cacciae* propolis extract (Georgieva et al., 2019). Antioxidant (Sukweenadhi et al., 2020), antibacterial (Yuan et al., 2021), anti-inflammatory (Candiracci et al., 2012), and sunscreen (Purwaningsih et al., 2023) properties are shared by phenolic compounds and flavonoids.

Based on Stanciauskaite et al. (2022) who researched the SPF value of propolis from honeybees collected in Lithuania, it shows that the propolis extract

has an SPF value of 4.851. This extract contains phenolic acids like p-coumaric acid and cinnamic acid and flavonoids like pinobanksin and pinocembrin. Rohmani and Pangesti (2024) formulated a sunscreen cream containing 16% propolis extract, which showed an SPF value of 5.663, indicating that the cream can act as a sunscreen.

The efficiency of sunscreen is represented by its SPF value, erythema transmission percentage (%Te), and pigmentation transmission percentage (%Tp). It describes the percentage of sunlight that may induce skin erythema, or reddish skin, after applying sunscreen. The percentage of the sun that is transmitted after it strikes the sunscreen and results in skin pigmentation (darkening of the skin) is also represented by the pigmentation transmission (%Tp) (Tjitda et al., 2021).

A 96% ethanol solvent can extract the active ingredients from beehive debris that may be used as sunscreen. Fractionation is required to obtain more specific molecules. Ethyl acetate and n-hexane are the solvents most frequently utilized in fractionation (Tjitda et al., 2021). Because of their varying degrees of polarity, these two solvents are employed to assess each fraction's sunscreen potential and secondary metabolite component concentration.

Researchers are investigating this issue since prior studies have not demonstrated that extracts and fractions from klanceng honey beehive (*Trigona biroi*) waste have been examined for sunscreen activity regarding SPF, %Te, and %Tp values. The focus of this study is to ascertain the sunscreen potential of the klanceng honey beehive (*T. biroi*) ethanol extract, n-hexane fraction, ethyl acetate fraction, and aqueous fraction based on SPF, % Erythema transmission (%Te), and % Pigmentation transmission (%Tp).

EXPERIMENTAL SECTION

Sample Preparation

Trigona biroi beehive waste from Muntilan District, Central Java Province, was used in the study. Rotten materials and contaminants were separated from the samples through sorting. A knife was used to cut the samples down to size.

Material

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), Dragendoff Reagent (Merck), Na_2CO_3 (Merck), Folin-Ciocalteu (Merck), Quercetin (Sigma), Anhydride acetate (Merck), Na acetate (Merck). The set of glass instruments frequently found in lab settings served as the study's instruments, a bransonic brand ultrasonic batch CPX1800H-E type, UV-Vis Shimadzhu 1840 spectrophotometer, a Rotary evaporator, Agilent Cary 630 FTIR spectrophotometer and gas chromatography – Mass Spectra (GC-MS) Shimadzu QP 2010 SE in UII.

Extraction and Fractionation

For sixty minutes, 100 g of the sample was macerated in a sonicator with 96% ethanol, and it was then left to stand for a full day at a ratio of 1:10. The extract was filtered, and the filtrate and residue were separated. In the same manner, the residue was re-macerated. This treatment was repeated three times. Three replications were used for maceration. A vacuum rotary evaporator evaporated the filtrate at 50°C until a thick extract was obtained. Hexane, ethyl acetate, and ethanol were used in that order to fractionate 10 grams of thick extract. A concentrated n-hexane fraction, an ethyl acetate fraction, and an aqueous fraction were obtained by evaporating the fractionation findings using a rotary evaporator.

Screening of Phytochemistry

Color reagents were used to test the chemical content of extracts and fractions of Klanceng honey beehive waste. Tests for flavonoids, tannins, saponins, and terpenoid compounds were conducted.

The flavonoid test using The Shinoda reaction, which involved extracts and filtrates, HCl 2 N, powdered magnesium, and amyl alcohol, was used to conduct the flavonoid test. Flavonoids are present in the sample if the amyl alcohol layer is red. The $FeCl_3$ reagent is used to test for **tannins** in the sample. The solution's dark green hue will show that the sample contains tannins.

Three distinct techniques were used for **the alkaloid** test: the Dragendoff, Mayer, and Bouchardat reagent tests. The sample was heated in 2N HCl before being filtered. The filtrate was separated into three test tubes. If the filtrate in the first test tube is positive for alkaloids, it turns orange when combined with the Dragendoff reagent. The filtrate in the second test tube plus the Mayer reagent shows a white precipitate if alkaloids are present. In contrast, the filtrate in the third test tube plus the Bouchardat reagent is orange-red if alkaloids are present.

The saponin test is performed by shaking the sample with distilled water before adding 2 N HCl and mixing. Saponin responds positively to the presence of stable foam.

The terpenoid test is carried out by dissolving several samples in ether and evaporating them. The result of evaporation combined with acetic acid anhydride A positive sample containing terpenoids is indicated by a red or green color in this assay.

ATR-FTIR spectrophotometry was applied to determine the functional groups of ethanol extracts and fractions.

SPF Value

The modified Khan (2018) research was used to calculate the SPF value. Absorbance at 290–320 nm in the 5 nm range is measured using UV spectrophotometry in samples exhibiting a concentration of 100–500 ppm (in ethanol solvents). The SPF value is determined by applying the Mansur equation (1).

$$SPF = CFx\sum_{290}^{320} EE_{(\lambda)}xIxA_{(\lambda)} \quad (1)$$

where CF = correction factor (10), EE = erythmogenic effect of wavelength radiation, and Abs = spectrophotometric absorbance values at wavelength. **Table 1** (14) shows the values of EE () x I, which are constants (Khan, 2018).

Where, CF = correction factor (Georgieva et al., 2019), EE (λ) = erythmogenic effect of radiation with wavelength λ, Abs (λ) = spectrophotometric absorbance values at wavelength λ. The EE (λ) x I values are constants given in **Table 1**.

Test for % Erythema Transmittance

Each sample was dissolved in ethanol and prepared at 100 to 500 ppm concentrations. The spectrum of the sample solution was then measured using UV-Vis spectrophotometry at a wavelength of 290-320 nm, with ethanol serving as a blank. The transmittance values at different wavelengths were used to calculate the percent transmittance of erythema (% Te), which was calculated using the following formula (2).

$$\%\,Te = \frac{Ee}{\sum Fe} = \frac{\sum(T\,x\,Fe)}{\sum Fe} \quad (2)$$

Description:
T = transmission values at various wavelengths 292.5 - 317.5 nm
Fe = Erythema flux,
Ee = Σ(T.Fe) = the amount of erythema flux transmitted by the sunscreen
ΣFe = total amount of UV light energy that causes erythema.

Pigmentation Transmission Test

Each sample was dissolved in ethanol and prepared at 100 to 500 ppm concentrations. The spectrum of the sample solution was then measured using UV-Vis spectrophotometry at a wavelength of 320-375 nm, with ethanol serving as a blank. The transmittance values at different wavelengths were used to calculate the percent pigmentation transmission (%Tp), which was calculated using the following formula (3).

$$\%\,Tp = \frac{Ep}{\sum Fp} = \frac{\sum(T\,x\,Fp)}{\sum Fp} \quad (3)$$

Description (Tahar et al., 2019):
T = transmission values at various wavelengths 322.5 - 372.5 nm
Fp = Pigmentation flux,
Ee = Σ(T.Fp) = the amount of pigmentation flux transmitted by the sunscreen
ΣFp = total amount of UV light energy that causes pigmentation.

Determination of Flavonoid Levels using a TLC-Densitometer

A GF_{254} silica gel plate containing up to 2 µL of quercetin standard was photographed. Sampel was photographed in as much as 20 µL on a GF_{254} silica gel plate. The standard and sample were eluted in a chamber with a saturated eluent of ethyl acetate, formic acid, glacial acetic acid, and water (100:11:11:26). The area under the curve (AUC) of the quercetin standard and sample was determined using a TLC scanner at a wavelength of 350 nm and UV 254 staining.

RESULTS AND DISCUSSION

Extraction and Fractionation of Klanceng Honey Bee Hive

A thick brown extract with a honey-like scent and a yield of 36.09±1.69% was obtained by utilizing a vacuum rotary evaporator to evaporate the maceration results. A vacuum evaporator can lower the solvent's temperature by lowering the flask's pressure, speeding up the solvent's evaporation. N-hexane solvent was used to extract the nonpolar fraction from the thick extract, and ethyl acetate solvent was used to extract the residue. The residue from the fractionation of ethyl acetate is known as the aqueous fraction. **Table 2** displays the yields of the n-hexane fraction, ethyl acetate fraction, and aqueous fraction, which were 49.52% ±±8.58, 37.54% ±3.40, and 2.15% ±0.26, respectively. The secondary metabolites dissolved in each fraction determine the yield generated by that fraction. **Table 2** shows that, compared to the aqueous and ethyl acetate fractions, the n-hexane fraction has the highest yield. This suggests that nonpolar molecules predominate in honey beehive waste, which aligns with n-hexane's characteristics.

Table 1. Values of EE (λ) x I at a different wavelength

Wavelength (nm)	Value of EE x I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.0180

Profiling Chemical Compound of Klanceng Honey Beehive

Color reagents, IR spectrophotometry, and GC-MS were used to profile the chemical compounds in honey beehives. **Table 3** displays the findings of the samples' phytochemical screening. The phytochemical screening of extracts, n-hexane fractions, and ethyl acetate fractions yielded the same secondary metabolite compounds: alkaloids, flavonoids, tannins, and terpenoids. In contrast, the aqueous fraction yielded alkaloids, flavonoids, and tannins. Due to the terpenoid compound group being nonpolar, it does not exist in the aqueous fraction. This is what distinguishes the four samples' secondary metabolite content.

Based on the infrared spectrum results (**Figure 1**), the spectral pattern is identical for all four samples. The ethanol extract and ethyl acetate fractions have lower OH absorption at wave number 3300 cm^{-1}, but the aqueous fraction has the most significant and widest OH absorption. Because n-hexane is nonpolar, the OH group did not appear in the spectrum of the n-hexane fraction, implying that the compounds contained in this fraction are also nonpolar. A sample's OH functional groups presence indicates phenol or alcohol groups (Revathi et al., 2019). Wave number 2800-2900 cm^{-1} revealed the presence of an aliphatic CH-alkyl group in the sample. In both extracts, the n-hexane and aqueous fractions gave the same absorption in all three samples. Compared to the other samples, the aqueous fraction has a lower absorption at 2900-2800 cm^{-1}. Because the aqueous fraction binds polar compounds, alkyl groups are smaller than other fractions. Wave number 1350-1500 cm^{-1} indicated the availability of -C=C aromatic groups, while wave number 1700 cm^{-1} indicated the presence of carbonyl groups (C=O). The wave number at 1025 cm^{-1} (Stuart, 2005) suggested that the C-OH group was absorbed from alcohol. The aqueous fraction absorbs C-OH more strongly than the others. This is because the compounds in the aqueous fraction are polar and thus bind more hydroxy groups.

Table 2. Percentage (%) yield extract and fractions

Sample	% Yields
Etanol extract	36.09±1.69%.
n-hexane fraction	49.52%±8.58
Ethyl acetate fraction	37.54%±3.40
Aqueous fraction	2.15%±0.26

Table 3. Phytochemical screening

Secondary metabolite	Extract	n-hexane fraction	Ethyl acetate fraction	aqueous fraction
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Saponin	-	-	-	-
Tanin	+	+	+	+
Terpenoid	+	+	+	-

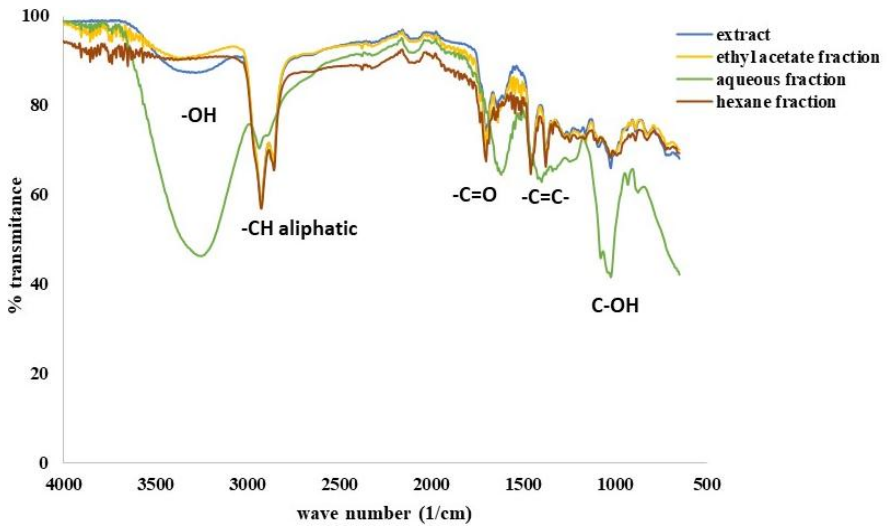

Figure 1. Infrared spectrum of sample

Profiling GC-MS of Klanceng Honey Beehive

The ethanol extract of klanceng honey beehive produced 19 compounds, but only four of them, namely ethyl cetylate, ethyl octadic-9-enoate, Mandel, and tetracosane, had a high similarity index with the WILEY 7 library, according to the GC-MS results. The n-hexane fraction yielded 19 peaks, corresponding to 19 compounds. According to the Wiley 7 Library, the n-hexane fraction contains only two compounds: methyl octadic-10-enoate and heptacosane. The ethyl acetate fraction contains 18 compounds, but only four can be identified with high similarity to the Wiley 7 Library: ethyl octadec-9-enoate, 3-eicosene, lanosterol, and 24-methylene cycloartanol, whereas the aqueous fraction contains two compounds from the 18 compounds: lanosterol and glicerin trilaurate. Ester and steroid groups are the compounds identified by GC-MS in this sample. According to some studies, honey beehive contains various compounds, including flavonoids, biflavonoids, triterpenoids, fatty acids, alcohols, aromatic aldehydes, and sterols. Some of the literature reiterates the outcome of this investigation (Anjum et al., 2019; Lotfy 2006; Pasupuleti et al., 2017).

Based on the infrared spectrum results (**Figure 1**), the spectral pattern is identical for all four samples. The ethanol extract and ethyl acetate fractions have lower OH absorption at wave number 3300 cm^{-1}, but the aqueous fraction has the most significant and widest OH absorption. Because n-hexane is nonpolar, the OH group did not appear in the spectrum of the n-hexane fraction, implying that the compounds contained in this fraction are also nonpolar. A sample's OH functional groups presence indicates phenol or alcohol groups (Revathi et al., 2019). Wave number 2800-2900 cm^{-1} revealed the presence of an aliphatic CH-alkyl group in the sample. In both extracts, the n-hexane and aqueous fractions gave the same absorption in all three samples. Compared to the other samples, the aqueous fraction has a lower absorption at 2900-2800 cm^{-1}. Because the aqueous fraction binds polar compounds, alkyl groups are smaller than other fractions. Wave number 1350-1500 cm^{-1} indicated the availability of -C=C aromatic groups, while wave number 1700 cm^{-1} indicated the presence of carbonyl groups (C=O). The wave number at 1025 cm^{-1} (Stuart, 2005) suggested that the C-OH group was absorbed from alcohol. The aqueous fraction absorbs C-OH more strongly than the others. This is because the compounds in the aqueous fraction are polar and thus bind more hydroxy groups.

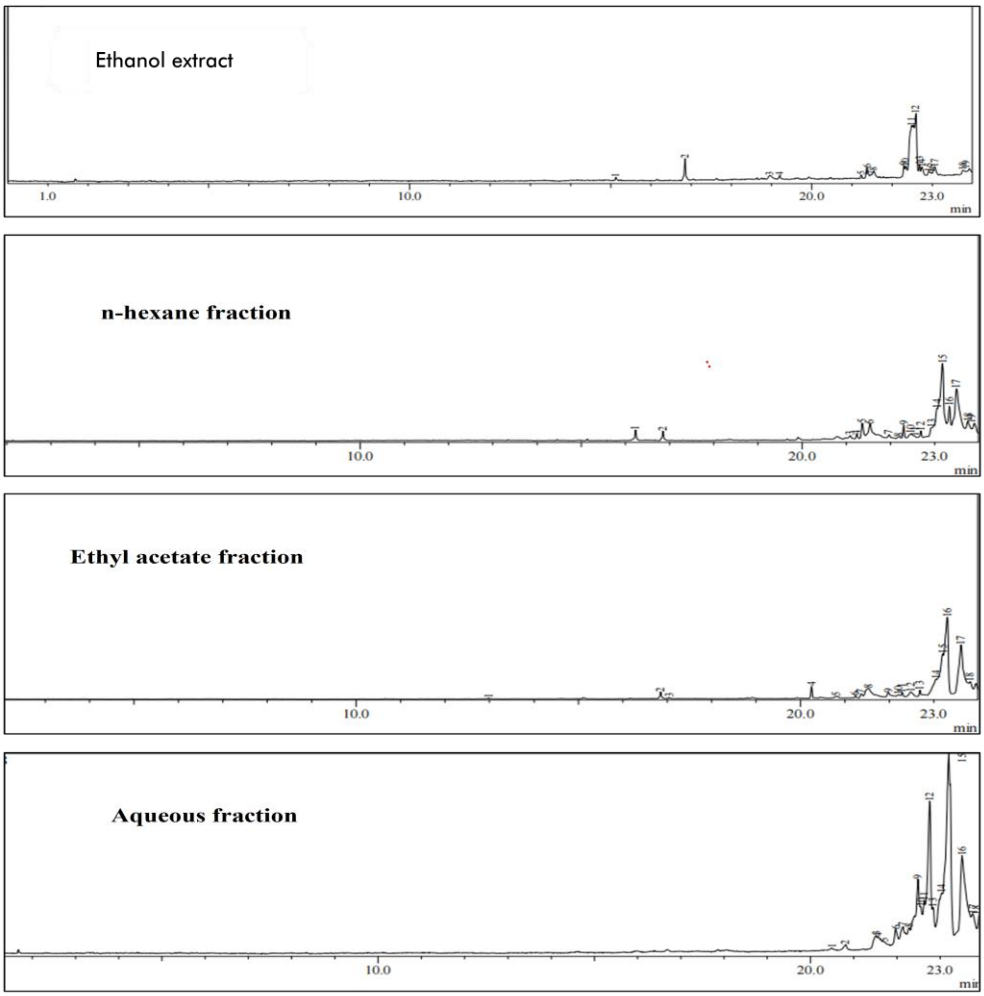

Figure 2. GC chromatogram of sample

Table 4. The GC-MS results of samples

Sample	Peak number	Retardation time (mins)	% area	Similarity	Molecule formula	Molecule weight	Compound
Extract	1	15.125	0.67	95	$C_{18}H_{36}O_2$	284	Ethyl cetylate
	2	16.580	5.29	92	$C_{20}H_{38}O_2$	310	Ethyl octadec-9-enoate
	3	18.959	2.20	87	$C_{20}H_{36}O_2$	308	Mandenol atau ethyl linolate
	5	21.232	0.76	95	$C_{24}H_{50}$	338	tetracosane
n-hexane fraction	1	16.233	1.33	96	$C_{19}H_{36}O_2$	296	Methyl octadic-10-enoate
	4	21.242	0.74	94	$C_{27}H_{56}$	380	heptacosane
Ethyl acetate fraction	2	16.850	1.35	92	$C_{20}H_{38}O_2$	310	Ethyl octadec-9-enoate
	3	17.033	0.25	96	$C_{20}H_{40}$	280	3-eicosene
	4	20.250	1.83	97	$C_{24}H_{38}O_4$	390	Phthalic acid dioctyl ester
	18	23.825	2.56	84	$C_{31}H_{52}O$	440	24-methylene cycloartenol
Aqueous fraction	3	21.517	1.41	84	$C_{30}H_{50}O$	426	lanosterol
	7	22.412	2.65	94	$C_{39}H_{74}O_6$	639	Glicerin trilaurate

Sunscreen Activity of Klanceng Honey Bee Hive

A sunscreen profile is a classification of sunscreen activity that expresses the potential for skin protection against sunlight in UV A and UV B radiation and is used as a sunscreen-related cosmetic ingredient. The UV spectrophotometric method is used in vitro to determine sunscreen activity at wavelengths of UV-B (290-320 nm) and UV-A (290-400 nm). The sunscreen activity of a sample can be observed from the SPF, %Te, and %Tp values. The sample is protected from UV A and B rays if it has a high SPF value and low %Te and %Tp values.

The SPF value profiles of the four samples at quantities ranging from 100 to 500 ppm are displayed in **Figure 3** and **Table 5**. The higher the sample concentration, the higher the SPF value. SPF less than 2 indicates that 100 and 200 ppm extract concentrations do not yet protect UV B rays. At a concentration of 300 ppm with an SPF of 2,700, the extract began to provide minimal UV radiation protection, as did a concentration of 400 ppm with an SPF of 3,872. An additional concentration of 500 ppm provided moderate UV ray protection with an SPF of 5,382. At a concentration of 100 ppm, the ethyl acetate fraction already provides moderate protection. With SPF values of 6.535 and 6.898, the ethyl acetate fraction already provides moderate protection at 100 ppm and extra protection at 200 and 300 ppm. Maximum protection is provided by ethyl acetate fraction concentrations of 400 and 500 ppm. With an SPF value of 1.991, the 100 ppm concentration of the n-hexane fraction does not provide UV protection. In contrast, the 200, 300, and 400 ppm concentrations provide minimal protection with SPF values of 2.466, 3.389, and 3.529, respectively. The n-hexane fraction at 500 ppm provided moderate protection, with an SPF value of 4.464. The aqueous fraction could not provide UV protection at concentrations ranging from 100 to 400 ppm; minimal UV protection began at 500 ppm, with an SPF of 2,846. The ethyl acetate fraction provides the best UV B protection based on each SPF value.

The percentage (%Te) of the sample profile is depicted in **Figure 4** and **Table 6**. The ethanol extract, n-hexane, and water fraction did not protect erythema transmission. In contrast, the ethyl acetate fraction provided erythema transmission protection with a suntan standard category at a concentration of 300 ppm and extra protection at concentrations of 400 and 500 ppm with erythema transmission values of 6.07% and 3.32%, respectively. The lower the %Te value, the better the erythema transmission protection.

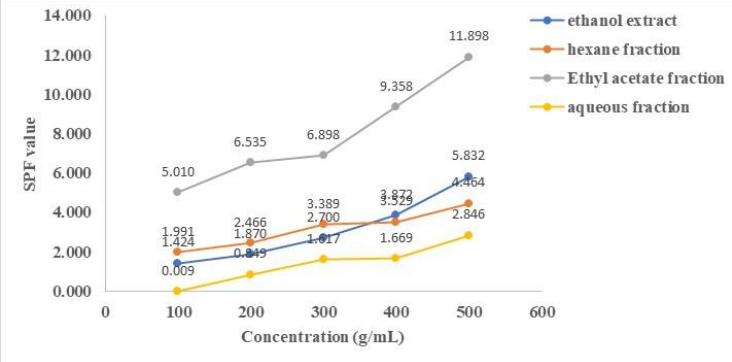

Figure 3. Samples' SPF values

Table 5. SPF Value sample

Concentration sample (ppm)	extract		n-hexane fraction		Ethyl acetate fraction		aqueous fraction	
	SPF	Category*	SPF	Category*	SPF	Category*	SPF	Category*
100	1.424	-	1.991	-	5.010	moderate	0.009	-
200	1.870	-	2.466	minimal	6.535	moderate	0.849	-
300	2.700	minimal	3.389	minimal	6.898	moderate	1.617	-
400	3.872	minimal	3.529	minimal	9.358	maximal	1.669	-
500	5.832	moderate	4.464	moderate	11.898	maximal	2.846	minimal

Description: * minimal= 2-4, moderate = 4-8, maximal= 8-12, Ultra= >12

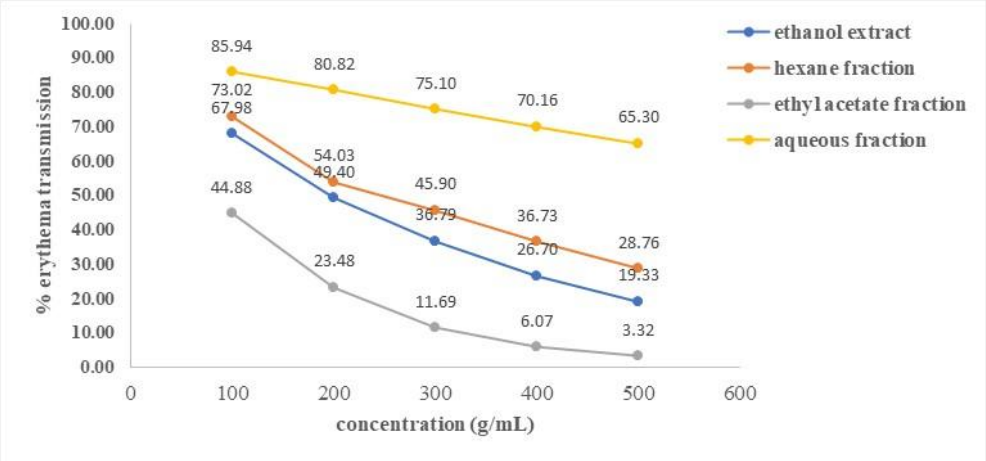

Figure 4. Percentage (%) Te of samples with quantities regarding from 100 to 500 ppm

Table 6. % Erythema transmission of the sample

Concentration sample (ppm)	extract		n-hexane fraction		Ethyl acetate fraction		aqueous fraction	
	%Te	Category*	%Te	Category*	%Te	Category*	%Te	Category*
100	67.98	-	73.02	-	44.88	-	85.94	-
200	49.40	-	54.03	-	23.48	-	80.82	-
300	36.79	-	45.90	-	11.69	Suntan standar	75.10	-
400	26.70	-	36.73	-	6.07	Proteksi extra	70.16	-
500	19.33	-	28.76	-	3.32	Proteksi ekstra	65.30	-

Description : Sunblock : < 1; extra protection : 1 – 6; Suntan Standard : 6 – 12; Fast tanning : 12 – 18

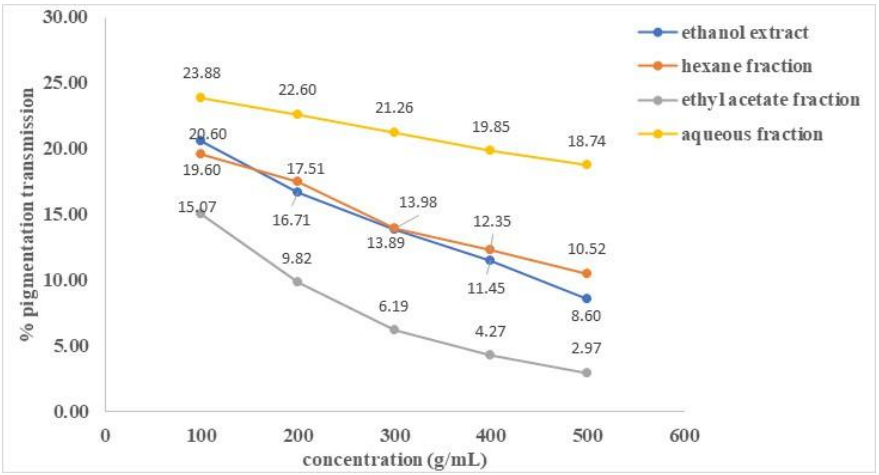

Figure 5. Percentage (%) Tp of samples from 100–500 ppm concentration

Table 7. % Tp sample

Concentration sample (ppm)	extract		n-hexane fraction		Ethyl acetate fraction		aqueous fraction	
	%Tp	Category*	%Tp	Category*	%Tp	Category*	%Tp	Category*
100	20.60	Sunblock	19.60	Sunblock	15.07	Sunblock	23.88	Sunblock
200	16.71	Sunblock	17.51	Sunblock	9.82	Sunblock	22.60	Sunblock
300	13.89	Sunblock	13.98	Sunblock	6.19	Sunblock	21.26	Sunblock
400	11.45	Sunblock	12.35	Sunblock	4.27	Sunblock	19.85	Sunblock
500	8.60	Sunblock	10.52	Sunblock	2.97	Sunblock	18.74	Sunblock

description *: Sunblok: 3-40; proteksi ekstra: 42-86; suntan standard: 45-86; fast tanning: 45-86

Table 8. Sunscreen activity of the sample

sample	SPF	%TE	%Tp	Proteksi UV
Extract	Moderate protection	-	sunblock	UV A and UV B
n-hexane fraction	Moderate protection	-	sunblock	UV A and UV B
Ethyl acetate fraction	Maximal protection	Extra protection	sunblock	UV A and UV B
Aqueous fraction	Minimal protection	-	sunblock	UV A and UV B

Figure 5 and **Table 7** show that all samples protect pigmentation transmission with the sunblock category at different concentration series, with the ethyl acetate fraction having the lowest %Tp value. The lower the %Tp value, the better the resistance to pigmentation transmission.

Table 8 summarizes the sunscreen activity of the extract, *n*-hexane fraction, ethyl acetate fraction, and aqueous fraction based on SPF value, % erythema transmission, and % pigmentation transmission. The extract has a moderate SPF value and does not have anti-erythema, but it does have anti-pigmentation in the sunblock category. The n-hexane fraction has a "medium protection" SPF value, no anti-erythema, and anti-pigmentation in the sunblock category. The ethyl acetate fraction has the best sunscreen activity because it has an SPF value of "maximal protection," anti-erythema, "extra protection," and anti-pigmentation, "sunblock." The aqueous fraction has a low SPF value, no anti-erythema, and is anti-pigmentation in the sunblock category.

High SPF, low erythema transmission, and low pigmentation transmission are numerous signs that a sunscreen is beneficial. The ethyl acetate fraction has the best sunscreen activity, according to **Table 8**, because it has UV A protection with the sunblock category against pigmentation transmission and UV B protection with the maximum category for its SPF value and extra protection against erythema transmission. The ethyl acetate fraction is protected from UV A and B because, according to visible spectrophotometry in the previous section, it has the highest flavonoid and phenolic concentration (Purwaningsih et al., 2023).

The flavonoid content was determined using a TLC-Densitometer and the standard quercetin because the ethyl acetate fraction has the best protection against UV light A and B. The TLC-densitometry data revealed a standard curve for quercetin with the equation $y = 2.10\text{-}5x + 0.0065$; $R^2 = 0.9936$ (**Figure 6**). The linear equation was used to calculate the concentration (ppm) from the area of the ethyl acetate fraction. By dividing the weight of the sample used in the measurement, ppm will be converted to content (%). The flavonoid content in the ethyl acetate fraction was 7.64% ±0.91% (b/b%), as shown in **Table 9**.

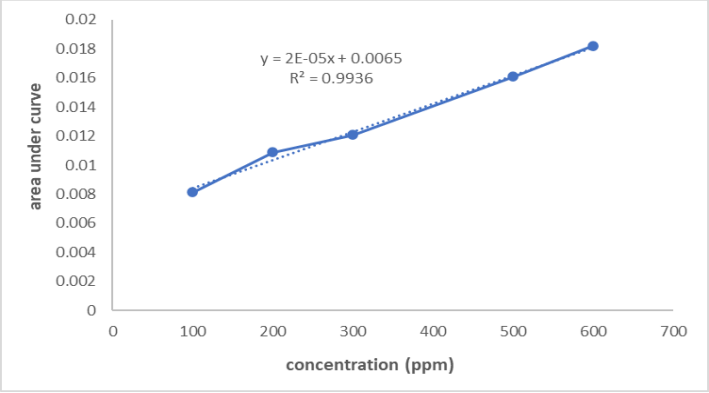

Figure 6. Quercetin standard curve

Table 9. Quercetin levels in ethyl acetate fraction

Replication	AUC	ppm	% (b/b)±SD
1	0.01518	434	8.68
2	0.01346	348	6.96
3	0.01379	364.5	7.29
	average		7.64±0.91

The chemical content of klanceng honey beehive, like ethyl cetylate, ethyl octadec-9-enoate, ethyl linolate, and phthalic acid dioctyl ester, are among the ester chemicals found in the extract and fractions that GC-MS detected. These compounds also aid in UV absorption. According to specific research, ester compounds work well as sunscreens. Based on the results of the phytochemical screening, the sample contains flavonoids and tannins that can act as UV absorbers. These compounds can act as UV absorbers because they contain chromophores and auxochrome groups. Flavonoid compounds' chromophore groups (conjugated double bonds) enable them to capture harmful ultraviolet (UV) rays, including UV A and UV B, minimizing the skin's exposure to UV radiation (Ghazi, 2022). The functional groups in the sample, specifically C=C, C=O, and C-OH, which can function as auxochrome or chromophore groups, further support this.

CONCLUSIONS

The ethyl acetate fraction of klanceng honeycomb has the best sunscreen activity seen from the SPF values of % Te and % Tp, with maximum protection, extra protection, and sunblock categories against UV A and UV B rays. The ethyl acetate fraction has the prospective as a sunscreen agent.

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