Screening Bioactivity of Kersen Fruits (*Muntingia calabura* L.) as a Sunscreens Candidate

*(Skirining Bioaktivitas dari Buah Kersen (Muntingia calabura L.) sebagai Kandidat Bahan Tabir Surya)*

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**ABSTRACT**

Kersen (*Muntingia calabura* L.) fruits have the potential to be used as an active ingredient in sunscreens because of phenolic and flavonoid content that can absorb UV rays. This study aims to determine the percentage of erythema/pigmentation transmission and SPF value as parameters for sunscreen activity. Kersen fruits were extracted by maceration using 96% ethanol. The ethanol extract of Kersen Fruits was also fractionated to separate the components of the active compounds based on the polarity level using n-hexane, ethyl acetate, and ethanol as solvents. The test was carried out using the in vitro method by measuring the ability of the material to absorb ultraviolet light at a wavelength of 292.5-372.5 nm. This research was conducted at concentrations of 100, 200, 400, 600, and 800 µg/mL for ethanol, lyophilisate, n-hexane, and ethanol fractions, while the ethyl acetate fraction concentrations are 50, 100, 150, 200, 250 µg/mL. The results showed the best value at the ethyl acetate fraction concentration of 250 µg/mL with % Te of 5.28 and % Tp of 28.65 and the SPF value of 16.54. Based on the % Te and Tp, the ethyl acetate fraction exhibited protection against erythema and pigmentation with the category of extra protection and based on the SPF value with the category of ultra protection.

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ABSTRAK

Buah Kersen (Muntingia calabura L) memiliki potensi untuk digunakan sebagai bahan aktif tabir surya karena kandungan fenolik dan flavonoid yang dapat menyerap sinar UV. Tujuan penelitian ini untuk mengetahui persentase transmisi eritema/pigmentasi, dan nilai SPF sebagai parameter penentuan aktivitas tabir surya. Buah kersen diekstraksi secara maserasi menggunakan etanol 96 %. Ekstrak etanol Buah Kersen juga di fraksinasi untuk memisahkan komponen senyawa aktif berdasarkan tingkat kepolaran menggunakan pelarut berturut-turut n-heksan, etil asetat, dan etanol. Penelitian ini dilakukan dengan metode in vitro dengan mengukur kemampuan bahan untuk mengabsorpsi sinar ultraviolet pada panjang gelombang 292.5-372.5 nm. Penelitian ini dilakukan pada konsentrasi 100, 200, 400, 600, dan 800 µg/mL untuk ekstrak etanol, liofilisat, fraksi n-heksan, dan fraksi etanol sedangkan fraksi etil asetat konsentrasi 50, 100, 150, 200, 250 µg/mL. Fraksi etil asetat konsentrasi 250 ppm menunjukkan nilai paling baik yaitu %Te 5,28 dan % Tp 28,65 serta nilai SPF 16,54. Berdasarkan % Te dan Tp yang diperoleh, fraksi etil asetat dapat memberikan perlindungan terhadap eritema dan pigmentasi dengan kategori proteksi ekstra dan berdasarkan nilai SPF kategori proteksi ultra.

Kata kunci: Kersen, Muntingia calabura L, Tabir Surya, Transmisi Eritema, Transmisi Pigmentasi.

PENDAHULUAN

One of the sun's rays is UV (ultraviolet) radiation which has many bad effects, especially on the skin. UV radiation is known by various types including UV-A (320-400 nm), UV-B (290-320 nm), and UV-C (259 nm). UV-A and UV-B rays are sunlight that reaches the earth's surface and has an impact on the skin (Adhi & Aida, 2018). Exposure to sunlight when the insufficient time will be beneficial for the health as the content of vitamin D. Adequate levels of vitamin D in the body also play a role in the prevention of various diseases, ranging from degenerative diseases to malignancy (Fiannisa, 2019). However, excessive sun exposure will provide risk factors for skin cancer such as malignant melanoma, basal cell carcinoma, and squamous cell carcinoma. In addition, it is a major contributor to skin aging (Halliwell & Gutteridge, 2015).

The use of sunscreen is necessary to protect the skin thereby reducing the damaging effects of ultraviolet radiation. Sunscreen is an active ingredient that can absorb, reflect and/or dissipate solar energy that hits human skin (D’Orazio et al., 2013; Goswami et al., 2013). Sunscreens derived from synthetic chemicals are generally allergenic and can cause skin problems including photo irritation, photosensitization, and contact dermatitis (Cefali et al., 2016; Saewan & Jimtaisong, 2013). Sunscreens whose active ingredients come from natural resources can avoid the adverse effects caused by the use of synthetic sunscreen cosmetics. Phenolic compounds derived from natural ingredients such as flavonoids can potentially be used as active ingredients in sunscreens. Phenolic compounds can experience resonance after interaction with ultraviolet light because they have a conjugated double bond so that they have acted as a photoprotector (Prasiddha et al., 2016).

Kersen (Muntingia calabura L) is one of the natural ingredients that have the potential to be used as a sunscreen, especially in the fruit, which is enriched with phenolic compounds related to their antioxidant capacity. It is reported to contain phenolic compounds include gallocatechin, epigallocatechin, catechin, flavanol, naringenin, quercetin, gallic acid, vanilla acid, chlorogenic acid, caffeic acid, β-coumaric acid, ferulic acid, ε-hydroxycinnamic acid, and myricetin (Mahmood et al., 2014; Mastuki et al., 2019).

Activity as a sunscreen can be observed based on several parameters such as the percentage of erythema/pigmentation transmission (% Te/p) as well as the sun protection factor (SPF) value which can be spectrophotometrically determined (Moore & Wilkinson, 1982). Based on the value of % erythema/pigmentation transmission can be classified into several categories of sunscreens, namely total block which is a category of sunscreen preparations with a percentage of erythema transmission of 1% and a percentage of transmission of pigmentation 3–40% which can absorb almost all UV-B rays and UVA-rays and is followed by extra protection, regular suntan, and fast tanning category. Wasitaatmadja (1997) reported that the amount of retention of the skin from exposure to UV rays from...
sunscreen was determined by the value of SPF (Sun Protection Factor), which is the ratio between the smallest doses that can cause erythema on the skin with the use of sunscreen compared to those without using sunscreen. Therefore, a study was conducted on the bioactivity screening of kersen fruit as a sunscreen candidate.

MATERIAL AND METHODS

Materials

Kersen (Muntingia calabura L.) fruits, 96% ethanol (Brataco), ethanol pro-analisis (JT-Baker), hydrochloric acid (Merck), iron (III) chloride (Merck), n-Hexane (Merck), ethyl acetate (Merck), distilled water.

Plant Collection

The sample used was Kersen fruits from Biring Kanaya, Makassar City, South Sulawesi. The specimen was identified by Dr. A. Mu’nisa from Botanical Laboratory, Department of Biology, Mathematics and Science Faculty, State Makassar University, Indonesia. Kersen fruits with almost ripe characteristics were collected then wet sorted. The next step was dried using an oven with a temperature of 40 °C then weighed as the dried weight.

Extraction and Lyophilization Process

Dried simplicia of Kersen fruits was pollinated and extracted by maceration method using 3.2 L of 96 % (w/w) ethanol solvent for 3 x 24 hours in a closed vessel protected from light and stirring occasionally with a stirring rod. Then filtering was carried out, and the filtrate was evaporated by a rotary vacuum evaporator until a thick extract was obtained (ethanol extract). For the lyophilization process, fresh kersen fruits were washed thoroughly and then put into a juicer. Then the kersen fruit juice was frozen in the freezer (-20° C) and lyophilized using a freeze dryer until the Kersen fruits lyophilisate was obtained.

Liquid-liquid Extraction

The ethanol extract was fractionated by sequential liquid-liquid extraction using n-hexane, ethyl acetate, and 70% ethanol as solvents. Amount 5 g of crude extract was dissolved with 50 mL of 70% ethanol and stirred. The mixture was put into a separatory funnel, then fractionated with 50 mL of n-hexane solvent, then shaken and left to stand until completely separated. The addition of the solvent was carried out two times until the solution obtained was clearing (colorless). The same thing was done with ethyl acetate solvent follows by the level of polarity ranging from non-polar to polar. The liquid fractions of n-hexane, ethyl acetate, and ethanol (residue) were evaporated to obtain the dry fraction.

Phytochemical Screening

For phenolic analysis, each extract as much as 0.5 grams were weighed and then put it into a test tube and 5% (w/v) FeCl₃ was added. If a dark blue or black color is formed, it indicates a phenol compound (Harborne, 1998). Meanwhile, for flavonoid analysis, each extract as much as 2 grams were weighed and then put it into a test tube and added with 2-3 drops of AlCl₃. If a yellow color is formed, it shows the flavonoid compound (Harborne, 1998; Sri Mulyani dan Toga Laksana, 2011).
**Evaluation of Percent Erythema and Pigmentation Transmission**

Determination of the percentage value of erythema/pigmentation transmission was carried out in vitro with a UV spectrophotometer (Cumpelik, 1972; Pakki et al., 2018; Sami et al., 2015). The test was carried out by weighing 25 mg for each sample, then dissolved with distilled water for lyophilisate, while the extract and fractions of Kersen fruit were dissolved by ethanol p.a. The mixture was homogenized, and the final volume was added with each solvent up to 25 ml (1000 µg/mL) in a volumetric flask. A series of samples concentrations of 100; 200; 400; 600; and 800 µg/mL for ethanol extract, n-hexane fraction and ethanol fraction, while the ethyl acetate fraction with a concentration series of 50; 100; 150; 200; and 250 µg/mL were made. Ethanol p.a was used as a blank. The absorbance of each concentration was measured using UV spectrophotometry in the wavelength range 292.5 nm - 372.5 nm that could cause erythema and pigmentation. From the measurement results, the absorbance value (A) was obtained, which can then be calculated for the transmission (T) using the equation:

\[ A = -\log T \]

Equations for obtaining erythema transmission (Te):

\[ TE = T \times Fe \]

where Te = Erythema transmission; Fe = erythema flux (Balsam & Sagarin, 1972)

The amount of erythema flux transmitted by the sunscreen (Ee) obtained by the equation:

\[ Ee = \Sigma (T \times Fe) \]

While the (%) value of erythema transmission obtained using the equation:

\[ \% Erythema Transmission = \frac{Ee}{\Sigma Fe} = \frac{\Sigma (T \times Fe)}{\Sigma Fe} \]

Equations for obtaining transmission pigmentation (Tp):

\[ Tp = T \times Fp \]

Where

Tp = Transmission of pigmentation; Fp = Flux pigmentation

Pigmentation flux has a value at a specific wavelength. The amount of flux pigmentation transmitted by the sunscreens (Ep) was calculated by the equation:

\[ Ep = \Sigma (T \times Fp) \]

Where

Ep = the amount of pigmentation flux that sunscreen transmits. The pigmentation % transmission value was calculated using the equation:

\[ \% Pigmentation Transmission = \frac{Ep}{\Sigma Fp} = \frac{\Sigma (T \times Fp)}{\Sigma Fp} \]

**Evaluation of Sun Protection Factor (SPF) Value**

Determination of the effectiveness of sunscreens in protecting the skin is known by calculating the SPF value in vitro using the spectrophotometric method (Donglikar & Deore, 2016). Each concentration of the sample solutions was measured of the absorbance value (A) at a wavelength of 290-320 nm with 5 nm intervals. The SPF value is obtained by the equation:

\[ SPF = CF \times \Sigma^{320}_{290} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \]

Where EE is the erythema effect spectrum, I is the spectrum of light intensity, Abs is the absorption of the sunscreen material and CF is the correction factor (value is 10).
Data Analysis

Analysis and processing of data were conducted by using Microsoft Excel and calculated using mathematical equations based on the absorbance of the sample at a certain wavelength to obtain the percent of Erythema and Pigmentation Transmission and the SPF value.

RESULTS AND DISCUSSION

Extraction and Fractionation

The yield percentage of Kersen (Muntingia calabura L.) fruits was presented in Table 1.

Table 1. The yield percentage of ethanolic extract and lyophilisate of kersen

| Sample            | Initial weight (g) | Extract weight (g) | Rendement (%) |
|-------------------|--------------------|--------------------|---------------|
| Ethanol Extract   | 431.3              | 125.1              | 29            |
| lyophilisate      | 668                | 18.18              | 2.72          |

Lyophilisates were obtained by using the freeze-drying/lyophilization method (freeze dryer). This method was done to reduce the water content from the sample at very low pressure so that the moisture content of the sample is reduced. According to Januari and Martin, (2014) lyophilization is a process of drying material by sublimating water from frozen samples. The advantage of lyophilization compared to other methods is maintaining product stability as well as avoiding changes in color, odor, and other organoleptic elements. Besides, the stable material structure can prevent deformation and shrinkage after drying.

For the ethanolic extract, the simplicia of the Kersen fruit was then extracted by maceration using 96% (w/w) ethanol. Ethanol is non-toxic solvent and insufficiently contaminated by microbes (Ahmad Najib, 2018). The results of calculating the percentage yield fractionation of Kersen fruits were presented in Table 2.

Table 2. The yield percentage of kersen fruit fractions

| Sample            | Extract weight (g) | Fraction weight (g) | Rendement (%) |
|-------------------|--------------------|---------------------|---------------|
| n-hexane fraction | 6                  | 0.34                | 5.70          |
| Ethyl Acetate     | 6                  | 1.15                | 19.11         |
| Ethanol fraction  | 6                  | 4.32                | 72.04         |

The crude extract was extracted by the liquid-liquid partition method to separate compounds based on the phenomenon of distribution or partition of an analyte between two immiscible solvents. In the liquid-liquid extraction, the highest yield was found in the ethanol fraction followed by ethyl acetate and n-hexane fractions, respectively. These results indicate that the components of the kersen fruit compound are more interested in polar to semi-polar solvents. Phytochemical screening was carried out to identify the chemical compounds contained in the sample. The extracted secondary metabolite profile was influenced by differences in the degree of polarity of the solvent. The results of the phytochemical screening of each sample are presented in Table 3.

Table 3 Phytochemical test results from extract, lyophilisate and fraction of kersen fruit

| Sample            | Phytochemical Screening |
|-------------------|-------------------------|
|                   | Flavonoid | Phenolic |
| Ethanol Extract   | +         | +        |
| lyophilisate      | +         | +        |
| n-hexane fraction | +         | -        |
| Ethyl Acetate     | +         | +        |
| Ethanol fraction  | +         | +        |
Evaluation of Erythema and Pigmentation Transmission

Sunscreen activity is based on the sample's ability to absorb light in the ultraviolet spectrum. After obtaining the absorbance value with an interval of 5 nm observation scale in the wavelength range that can cause erythema or pigmentation, the transmittance value was obtained. The transmittance value was used to calculate of % Te and % Tp. The percentage value of transmission pigmentation (% Tp) is a value that indicates the ability of a sample to protect the skin from UV rays on the spectrum that can cause pigmentation. While the percentage value of erythema transmission (% Te) is a value that shows the ability of a material to protect the skin from UV rays on the spectrum that can cause erythema (Cumpelik, 1972; Sami et al., 2015). The results of the calculation of the percentage value of erythema/pigmentation transmission of ethanol extract, lyophilisate, and fractions are presented in Table 4.

Table 4 The results of the percentage (%) erythema and pigmentation transmission of the extract, lyophilisate, and fraction of Kersen fruit with the category of assessment.

| Sample            | Concentration (µg/mL) | %Te    | %Tp     | Category          |
|-------------------|-----------------------|--------|---------|-------------------|
| Ethanolic Extract | 100                   | 65.62  | 94.62   | -                 |
|                   | 200                   | 45.01  | 77.51   | -                 |
|                   | 400                   | 19.43  | 64.69   | Fast tanning      |
|                   | 600                   | 9.06   | 52.37   | Suntan regular    |
|                   | 800                   | 5.05   | 41.20   | Extra protection  |
| Lyophilisate      | 100                   | 93.96  | 107.38  | -                 |
|                   | 200                   | 83.61  | 102.78  | -                 |
|                   | 400                   | 78.55  | 98.36   | -                 |
|                   | 600                   | 76.41  | 96.71   | -                 |
|                   | 800                   | 57.79  | 93.12   | -                 |
| n-hexane fraction | 100                   | 80.46  | 92.72   | -                 |
|                   | 200                   | 75.14  | 96.16   | -                 |
|                   | 400                   | 59.83  | 82.57   | -                 |
|                   | 600                   | 44.36  | 70.63   | -                 |
|                   | 800                   | 38.20  | 61.85   | -                 |
| Ethyl acetate     | 50                    | 43.38  | 83.86   | -                 |
| fraction          | 100                   | 20.75  | 55.56   | Fast tanning      |
|                   | 150                   | 13.57  | 57.64   | Fast tanning      |
|                   | 200                   | 5.36   | 37.05   | Extra protection  |
|                   | 250                   | 5.28   | 28.65   | Extra protection  |
| Ethanol fraction  | 100                   | 47.11  | 79.80   | -                 |
|                   | 200                   | 33.60  | 71.39   | -                 |
|                   | 400                   | 9.70   | 39.01   | Suntan regular    |
|                   | 600                   | 3.40   | 23.13   | Extra protection  |
|                   | 800                   | 1.26   | 16.32   | Total block       |

It can be seen that the ethanol extract, ethyl acetate fraction, and ethanol fraction have sunscreen activity at various concentrations and can be categorized as sunscreens while lyophilisate and n-hexane fraction are not provided sunscreen activity (Table 4). It can be seen that the higher the concentration, the percentage of Te and Tp are decreases and the effect are various from weak to strong. The smaller the transmission value (T), the better the sunscreen's potential to protect the skin because the lower UV rays transmitted into the skin (Cumpelik, 1972; Hasanah et al., 2015; Sami et al., 2015).
Evaluation of Sun Protection Factor (SPF) Value

The results of the Sun Protecting Factor value of ethanolic extract, lyophilisate, and fractions of Kersen fruit are presented in Table 5. The classification of suntan products based on the percentage value of erythema/pigmentation transmission and the effectiveness of sunscreen preparations based on the Sun Protecting Factor value were according to Moore & Wilkinson, (1982) and Balsam & Sagarin. (1972) and presented in Table 6.

Table 5 The SPF value from ethanol extract, lyophilisate, and fractions of Kersen fruit

| Material test      | Concentrations (ppm) | SPF Value | Category           |
|--------------------|-----------------------|-----------|--------------------|
| Ethanolic Extract  | 100                   | 1.71      | -                  |
|                    | 200                   | 3.09      | Minimal protection |
|                    | 400                   | 6.51      | Extra protection   |
|                    | 600                   | 9.83      | Maximal protection |
|                    | 800                   | 12.63     | Maximal protection |
| Lyophilisate       | 100                   | 0.42      | -                  |
|                    | 200                   | 0.90      | -                  |
|                    | 400                   | 1.17      | -                  |
|                    | 600                   | 1.18      | Minimal protection |
|                    | 800                   | 2.94      | Minimal protection |
| n-hexane fraction  | 100                   | 1.03      | -                  |
|                    | 200                   | 1.25      | -                  |
|                    | 400                   | 2.14      | Minimal protection |
|                    | 600                   | 3.31      | Minimal protection |
|                    | 800                   | 3.89      | Minimal protection |
| Ethyl acetate      | 50                    | 3.19      | Minimal protection |
| fraction           | 100                   | 6.12      | Extra protection   |
|                    | 150                   | 7.84      | Extra protection   |
|                    | 200                   | 12.09     | Maximal protection |
|                    | 250                   | 16.54     | Ultra protection   |
| Ethanol fraction   | 100                   | 3.13      | Minimal protection |
|                    | 200                   | 4.40      | Moderate protection |
|                    | 400                   | 9.49      | Maximal protection |
|                    | 600                   | 14.11     | Maximal protection |
|                    | 800                   | 18.76     | Ultra protection   |

Table 6. Suntan product classification

| SPF value & Sunscreen protection category | Erythema / pigmentation transmission range & Classification |
|-----------------------------------------|------------------------------------------------------------|
| Value                     | Classification                     | % TE | % TP | Category             |
| 2-4                       | Minimal protection               | 1.0  | 3-40 | Total block          |
| 4-6                       | Moderate protection              | 1-6  | 42-86| Extra protection     |
| 6-8                       | Extra protection                 | 6-12 | 45-86| Suntan regular      |
| 8-15                      | Maximal protection               | 10-18| 45-86| Fast tanning        |
| >15                       | Ultra protection                 | -    | -    | -                   |
It can be seen that the SPF value of each sample has sunscreen activity. It can also be seen that the higher the SPF value of a product or active sunscreen substance, the better the sunscreen's ability to protect the skin from the harmful effects of ultraviolet rays (Donglikar & Deore. 2016). The bioactivity screening of kersen fruit with the highest SPF value (18.76) was obtained in the ethanol fraction with a concentration of 800µg/mL with the ultra protection category. However, in the ethyl acetate fraction with a low concentration (250 µg/mL), the SPF value was 16.54 with the ultra protection category (Table 6). The ethyl acetate fraction has good activity compared to the ethanol fraction and other samples. Also, based on the percentage value of erythema/pigmentation transmission, it shows that the ethyl acetate fraction at low concentration (100 µg/mL) compared to other samples has obtained results with the fast tanning category. It can be assumed that the ethyl acetate fraction has the potential to be used as a cosmetic active ingredient related to sunscreens. The ethyl acetate fraction that has the best activity in absorbing UV light is influenced by the ability of the ethyl acetate solvent to attract chemical compounds in kersen fruit. Wungkana et al. (2013) stated that the ethyl acetate fraction had the best sunscreen activity due to the polarity of ethyl acetate, which is semi-polar so that it dissolves more phenolic and flavonoid compounds. Phenolic compounds are aromatic compounds that can absorb photon energy in the ultraviolet spectrum because of the presence of conjugated double bonds giving a similar sunscreen activity in this study. Also, Prasiddha et al. (2016) also reported that flavonoids have the ability as active ingredients in sunscreens because of the presence of chromophore groups. Conjugated aromatic systems that can absorb strong light in the wavelength range of UV rays both at UVA and UVB. Several studies have reported that the ethyl acetate fraction has the highest total phenolic and flavonoid levels compared to other fractions that are 74.90±1.32 mg/g GAE and 10.91±0.50 mg/g QE, respectively (Lasboi et al., 2017). Preethi & Sasikuma. (2011) also reported that the ethyl acetate fraction had a phenolic content of 1140±0.03 mg/100 g GAE. The amount of total phenolic and flavonoid content of the ethyl acetate fraction of kersen fruit correlates with its activity as a sunscreen. This allows that the ethyl acetate fraction can be used as an additive to increase the effectiveness of sunscreen preparations.

CONCLUSION

The ethanol extract, ethyl acetate fraction, and ethanol fraction have sunscreen activity. The ethanol fraction at a concentration of 800 µg/mL showed a total block category, while the ethyl acetate fraction at a concentration of 250 µg/mL showed the ultra protection category. Ethyl acetate fraction is the best fraction because at a small concentration of 250 µg/mL it displays ultra protective activity so it has the potential to be used as a sunscreen material.

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