Sunscreen Activity Test and Fragmentation Analysis of the Active Compound Ethyl Acetate Fraction of Pidada Merah (Sonneratia caseolaris L.)

Eka Siswanto Syamsul1,2*, Salman2, Meri Susanti2, Dachriyanus2

1 Sekolah Tinggi Ilmu Kesehatan Samarinda, East Borneo, Indonesia
2 Faculty of Pharmacy, Universitas Andalas, Padang, West Sumatra, Indonesia, 25163
*Corresponding author. Email: eka8382@gmail.com

ABSTRACT

The leaves of the pidada merah (Sonneratia caseolaris L.) are traditionally used by the people of Borneo as a composition of chilly powder. They are used by applying it to the face when doing outdoor activities exposure for a long period of time. This study aims to determine the sunscreen activity, and the chemical profile of the ethyl acetate extract of Pidada Merah leaves. The SPF value was determined by UV-Vis Spectrophotometer while chemical profile by LC-HRMS (Liquid Chromatography - High Resolution Mass Spectrometry). The results showed that the lowest concentrations of ethyl acetate extract that gave moderate protection was 50 ppm. The concentration of 100 ppm and 150 ppm gave maximum protection. The concentration of 200 ppm and 250 ppm gave ultra protection. This activity could be related to Luteolin 7-O-ß-D-Glucoside and luteolin in this extract. It was concluded that the ethyl acetate fraction of pidada merah leaves had potential as a sunscreen

Keywords: Pidada Merah, Sonneratia caseolaris L., SPF, luteolin, sunscreen.

1. INTRODUCTION

Sunscreen is a cosmetic preparation used to effectively diffuse or absorb sunlight, especially in the emission areas of ultraviolet and infrared waves, to prevent skin disorders caused by sunlight. [1]. Sunscreen can protect the skin by spreading sunlight or absorbing solar radiation energy that hits the skin, so that the radiation energy does not directly hit the skin. Sunscreen is a substance that contains ingredients that protect the skin against sunlight so that UV rays cannot enter the skin [2]. The effectiveness of sunscreen can be expressed by the sun protection factor (SPF), the percentage of erythema and the percentage of pigmentation transition[2]. Pidada merah leaf or perepat merah (Sonneratia caseolaris L.) is one of the natural ingredients that is efficacious as a sunscreen[3,4]. Pidada merah leaf is a mangrove plant known to have efficacy as a traditional medicine to treat various diseases [5]. Pidada Merah leaves in South Kalimantan are used as a mixture of cold powder [6]. Pidada merah leaves contain alkaloids, flavonoids, glycosides, saponins, and phenols [7].

This plant can be used for food, its young leaves can be processed into food and a mixture of dishes, and the old fruit can be used as a drink, and the wood can be used as firewood. Pidada merah plant is traditionally used as a cold powder ingredient [8]. The Dayak people also traditionally use the leaves for healing wounds, swelling, and bruises. The ethanolic extract of perepat merah leaves 2.5% had the effectiveness of sunscreen in the Preparation and was included in the category of Extra Protection for the percentage of erythema transmission [9]. Pidada of fresh leaves showed that phenols, flavonoids, and tannins were highly accumulated in the epidermis and some parts of the mesophyll [6].

2. RESEARCH AND METHODS

2.1. Materials and Equipment

The tools are analytical balance, dropper pipette, test tube, beaker glass, tube clamp, stirring rod, spiritus, tripod, 60 mesh sieve, 10 ml measuring cup, 100 ml measuring cup, funnel, filter paper, macerator, spatial, micropipette, cuvette,
spectrophotometer, porcelain dish, water bath, aluminum, and jars. The materials are distilled water, pidada merah leaves, ethanol 70%, n-hexane, ethyl acetate, 2N HCl, Mg powder, amyl alcohol, Meyer reagent, Bouchardat reagent, and Dragendrof reagent.

2.2. Research Procedure

2.2.1. Preparation of ethyl acetate fraction

The ethanol extract was made by the maceration method using ethanol 70%. The ethanol extract was fractionated in stages by the liquid-liquid fractionation method using n-hexane and ethyl acetate solvent. After that, it was allowed to stand for a while until two layers were formed, namely the n-hexane layer (top layer) and the water layer (bottom layer). The n-hexane fraction was separated and accommodated to evaporate the solvent. In contrast, the water fraction was put back into a separating funnel to continue the following fractionation process to obtain the ethyl acetate fraction.

2.2.2. Preparation of test solution

In this study, the researchers made concentration of ethyl acetate fraction. One thousand ppm extract was diluted, with five concentration variants, namely 50, 100, 150, 200, and 250 ppm.

2.2.3. Determination of SPF value of the ethyl acetate fraction in vitro.

Determination of effectiveness is done by determining SPF value in vitro using UV-Vis spectrophotometry. UV-Vis spectrophotometry was used, calibrated first using 1 mL of ethyl acetate into a cuvette, and put into UV-Vis spectrophotometry for the calibration process.

A test absorption curve was made with a wavelength between 290-320 nm, using ethyl acetate as a blank. Then determine the average absorption at 5 nm intervals. Weighing 0.01 g of ethyl acetate fraction into a 10 mL volumetric flask, dissolved with ethyl acetate, and then added ethyl acetate to the mark. SPF Value calculated the absorbance result with the concentration of ethyl acetate fraction. The formula for calculating SPF:

$$\text{SPF} = \text{CF} + \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times \text{absorbance}$$

Information:
CF = Correlation Factor (10)
EE = Efficiency Erythema
I = Solar Simulation Spectrum
Category:
<2 = No Protection
2-3 = Minimum Protection
4-5 = Medium Protection
6-7 = Extra Protection
8-14 = Maximum Protection
>15 = Ultra [10]

3. RESULTS AND DISCUSSION

Table 1. Phytochemical Screening

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Steroids</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2. SPF Value Ethyl Acetate Fraction of Sonneratia caseolaris L.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Mean±SD</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>3.86±0.12</td>
<td>Medium Protection</td>
</tr>
<tr>
<td>100</td>
<td>9.02±0.11</td>
<td>Maximum Protection</td>
</tr>
<tr>
<td>150</td>
<td>14.83±0.05</td>
<td>Maximum Protection</td>
</tr>
<tr>
<td>200</td>
<td>18.8±0.23</td>
<td>Ultra</td>
</tr>
<tr>
<td>250</td>
<td>25.08±0.35</td>
<td>Ultra</td>
</tr>
</tbody>
</table>

The data obtained that the lowest concentration of 50 ppm is included in the category of moderate protection, concentrations of 100 ppm and 150 ppm are included in the type of maximum protection, concentrations of 200 ppm and 250 ppm are included in ultra protection.

According to [11] in the category of moderate protection and maximum protection, the extract absorbs less UV B rays. It has a short time to absorb sunlight, so it can still cause erythema and absorb UVA rays which can cause the skin to turn brown. And in the ultra protection category, the extract can reflect UV A and UV B rays and has a very long time to block UV rays from entering the skin.

a. LC-HRMS Testing

LC-HRMS (Liquid Chromatography–High-Resolution Mass Spectrometry) can determine the chemical profile of the compound of ethyl acetate fraction of Pidada Merah leaves at the Central Laboratory of Biological Sciences, Universitas Brawijaya Malang.
Isolated leaves of Sonneratia caseolaris L. contain fatty acid compounds, sterol hydrocarbons, and two flavonoids, namely luteolin and luteolin 7-O-β-D-glucoside, which have high antioxidant power [12]. Some studies of Pidada merah leaves show as an antioxidant with an IC50 value in methanol extract of 21.62 ppm, n-hexane fraction 82.36 ppm, ethyl acetate fraction 13.41 ppm, and the n-butanol fraction of 13.04 ppm this plant has very strong antioxidant activity [13,14].

4. CONCLUSION
This activity can be attributed to the Luteolin 7-O-β-D-Glucoside and luteolin in this extract. The conclusion is that the ethyl acetate fraction of red pidada leaves has the potential as a sunscreen.

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REFERENCES