



Potential Bengle (*Zingiber cassumunar* Roxb.) Rhizomes for Sunscreen and Antioxidant Compounds

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Abstract : Bengle(*Zingiber cassumunar* Roxb.)rhizome is one of rhizomes commonly used to maintain healthy for skin, this activities are possibly due to its abilities to inhibit the harmful effect of the sun light and the occurrence of oxidative reaction. Therefore, this study was aimed to determine the ability to protect skin from the sun (sun screen) and antioxidant activity of *Z. cassumunar* ethanol extract (Et-B) compared to that of Temu giring (*Curcuma heyneana*) extract (Et-Tg) that has been clinically used. Sunscreen activities were evaluated by spectrophotometric method as the Sun Protection Factor (SPF) values. Antioxidant potential was determined by 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging method, and vitamin C used as reference. Et-B showed activity as a sunscreen having SPF value 8.81 ± 0.25 and antioxidant activity at IC_{50} value 0.793 ± 0.105 mg/ml that were better than that of Et-Tg (SPF value, 2.33 ± 0.31 and IC_{50} , 1.119 ± 0.195 mg/ml). Et-B was then fractionated in a mixture of n-hexane: ethanol: water (28:8:2v/v), to give 2 layers, the upper (non-polar) and lower (polar) layers. The upper layer shows the SPF value of 36.30 ± 0.35 , that is better than that of the lower layer (12.27 ± 1.68), and the lower layer is more antioxidant active ($IC_{50} = 0.996 \pm 0.121$ mg/ml) than that of the upper layer ($IC_{50} = 2.842 \pm 0.228$ mg/ml). Research results indicates that *Z. cassumunar* potential to be developed in preparation for sunscreen represented by the non-polar substances of the upper layer and antioxidants represented by the more polar substances in the lower layer.

Key Words : Antioxidant, 1,1-diphenyl-2-picrylhydrazil, Sunscreen, Sun Protection Factor, *Zingiber cassumunar* Roxb.

Introduction

Indonesia is one of tropical countries on the world that has sun exposure longer than those countries with four seasons. Sunlight with ultraviolet (UV) radiation can cause adverse effects on the skin. The skin is the outermost part of the body that exposed to UV light, so that UV radiation can penetrate the skin layers. Depletion of the ozone layer will lead to an increase in UV radiation at the Earth's surface. The reduction of ozone layer will increase UV radiation exposure to the earth, causing the incidence of skin cancer¹. Acute exposure of UV radiation to the skin can cause sunburn, inflammation, and immune system suppression, whereas chronic exposure to UV radiation will change the structure of the skin, causing skin cancer². Sunscreen agent is necessary to filter or withstand sunlight, thereby reducing the negative effects of UV radiation on the skin.

The UV radiation forms Reactive Oxygen Species(ROS), which causes oxidative damages to the cells. Oxidative damage occurs due to a lack of antioxidants in the body, so it can not compensate for reactivity oxidants³. Antioxidants inhibit oxidative reactions in the body through the mechanism of reduction of free radicals⁴. Although the body has had antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, but exogenous antioxidants are still required in balancing the reactivity oxidants that arise due to external factors that can form the ROS⁵. The use of natural sources is needed to obtain the antioxidant agent as alternative materials for UV protection to the skin, by neutralizing free radicals⁶.

Bengle (*Zingiber cassumunar* Roxb.) is one of the plants of *Zingiberaceae* family that has been used by Java community to treat skin redness. Some traditional herbal preparations also use *Z. Cassumunar* rhizomes in addition to Temu Giring rhizome (*Curcuma heyneana* Val & Zijp) as a mixture. Sunscreen and antioxidants may also be compounds that have conjugated double bonds or an aromatic group, such as phenolic compounds⁷, and *Z. cassumunar* containing some of these compounds. *Z. cassumunar* has been analyzed to contain terpenes in the volatile oil^{8,9} and phenylbutanoids¹⁰, which are responsible for some of the biological activities. *Z. cassumunar* benefits in the form of biological activity such as anti-inflammatory¹¹, anti-allergic¹², and anticancer^{13,14}.

The aims of this study are to determine the potential of *Z. cassumunar* rhizome as sun screens and antioxidants compared to that with *C. Heyneana* rhizome that has been used extensively to treat skin abnormalities. Fractionation is able to separate the *Z. Cassumunar* extract to the non-polar and polar fractions, in that case of potential activities can be located. The antioxidant potentials were determined using of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, while the potential of sunscreens were evaluated by the spectrophotometric method.

Materials and Method

Plant Material.

Z. cassumunar rhizomes obtained from Karanganyar, Central Java, and *C. heyneana* rhizomes obtained from Purworejo, Central Java, in the month of May, 2015.

Preparation of simplisia.

Z. cassumunar and *C. heyneana* determined in Pharmaceutical Biology Laboratory of Faculty of Pharmacy, Gadjah Mada University. Rhizomes are washed with water, cut into pieces, and then dried in an oven set at 50°C. The dried rhizome was grounded and sieved to 30/40 mesh.

Extraction.

Extracts were made by maceration at room temperature. A total of 900 g each rhizome powder was soaked with 70% ethanol (6.3 L), and macerated for 4 days. Upon filtration, the aliquot obtained was evaporated to dry with a rotary vacuum evaporator to give ethanol extract of *Z. cassumunar* (Et-B). Similarly, as Et-Tg for dried ethanol residue *C. heyneana*.

DPPH free radical scavenging assay.

The antioxidant potentials of Et-B and Et-Tg were measured using DPPH method, a free radical scavenging assay¹⁵. The DPPH solution (0.004%) and extract with a concentration of stock solution 2mg/ml in methanol p.a were prepared. Each extract is diluted and made a series of concentration. A total of 0.3ml of the extract solution is added to a solution of 3.0 ml of DPPH. Absorbance of the solution is observed at a wavelength of 516nm, after 30 minutes with methanol without the addition of DPPH (Shimadzu spectrophotometer UV1800). A total of 0.3ml of methanol coupled with 3.0 ml of DPPH solution, used as a control solution. Percent antioxidant activity (%AA) was calculated with the following equation

$$\%AA = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The data obtained were analyzed with linear regression equation with concentration as x-axis and %A, A as the y-ordinate. Antioxidant activity was measured in the form of concentration that can reduce 50% of the free radical DPPH (IC₅₀). Tests were carried out in triplicate, and averaged. Vitamin C is used as a reference compound.

Determination of absorbance profile and SPF in vitro.

Solution of Et-B and Et-Tg with a concentration of 1g/L in ethanol were prepared, and diluted to make solutions with concentration of 20, 40, 60, 80 and 100mg/L. Spectrophotometric absorption profile of the extract solution was observed in the wavelength range 290-400nm at intervals of 5nm. SPF value of each extract at concentrations of 20, 40, 60, 80 and 100mg/L is determined by spectrophotometry according to the method that has been used previously¹⁶. The test was performed using 1cm quartz cuvette with ethanol p.a used as blank. SPF value is calculated using the equation:

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

Which CF-correction factor (= 10), EE(λ) - erythemal effect spectrum, I (λ) -solar intensity spectrum, and Abs(λ) –absorbance of sample. E E value(λ) x I (λ) is a constant n accordance with the calculation. The determinations of the SPF were done in triplicate, and then were averaged.

Z. cassumunar extract partition.

Partition method was selected to obtain the initial fractions of the Et-B. Partitioning was done by partitioning Et-B with a mixture n-hexane-ethanol-water (28:8:2v/v) in a separating funnel to give upper layer representing non-polar substances and lower layer representing polar substances. Each of both layers obtained, were separately evaporated using a vacuum rotary evaporator to dryness. The DPPH scavenging assay, spectrophotometric absorption profiles, and SPF value determination of the upper layer and lower layer were done by using similar procedures applied for the EtOH extracts.

Result and Discussion

Ultraviolet spectrum provides variety effects depending on the UV wavelength region, which is UV-A (320-400 nm), UV-B (290-320 nm) and UV-C (200-290 nm)¹⁷. The UV-C radiation can be filtered out by the atmosphere before reaching the earth. The UV-B radiation not be completely filtered out by the ozone layer, and UV-A radiation can reach the earth's surface and penetrate the dermi layer of the skin. In order to get an overview of a sunscreen potential, absorbance profile of Et-B observed at a wavelength of UV-A and UV-B, which is at a wavelength of 290-400 nm. Spectrophotometric absorption profile of Et-B indicates that absorbance greater than Et-Tg at the identical concentrations (Figure 1). Although there is no maximum absorbance peaks in the wavelength range 290-400 nm, but Et-B provides absorbance in the UV-B. The content of phenylbutanoids in *Z. cassumunar* giving absorbance peak around 260-290nm, may affect the absorbance profile of *Z. cassumunar* extract at the UV-B region.

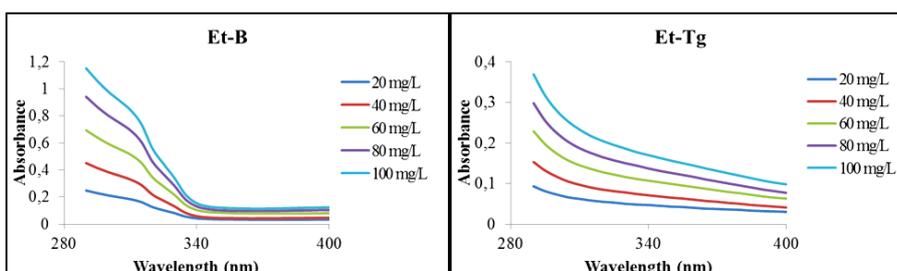


Figure 1: Spectrophotometric absorption profile of *Z. cassumunar* extract (Et-B) and *C. heyneana* extract (Et-Tg)

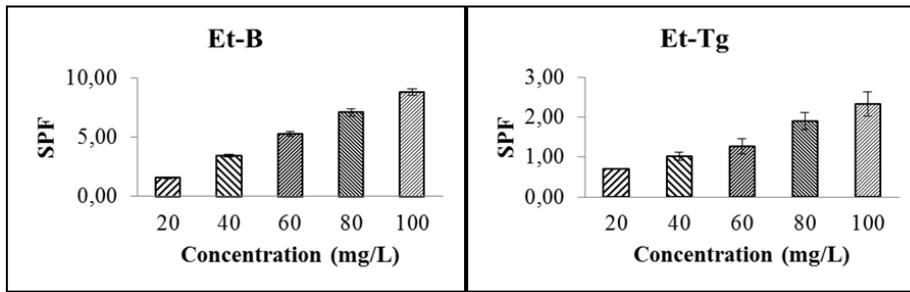


Figure 2:The SPF values of *Z. cassumunar* extract (Et-B)and *C. heyneana* extract (Et-Tg).

The UV-B radiation is called as a sunburn radiation causes immediate damage to the skin such as erythema. The long exposure of UV-B radiation can cause harmful effects to the skin, therefore in the determination of the SPF value by spectrophotometry using wavelength region of UV-B according to the method used previously. Although the tests performed *in vitro*, but this method is very much indicative to that of *in vivo* tests. Figure 2 shows the SPF value of *Z. cassumunar* extract (Et-B) at a concentration of 100 mg/L is 8.81 ± 0.25 , while the *C. heyneana* extract (Et-Tg) provides SPF value of 2.33 ± 0.31 , it means that Et-B is considered more potential than that of Et-Tg as sunscreen. However, which parts of Et-B is more potential for sunscreen or antioxidant should be able to be determined when the test is applied to both upper and lower layers.

The antioxidant activity of the *Z. cassumunar*(Et-B) and *C. heyneana* extracts (Et-Tg) was determined by *in vitro* method using DPPH. The DPPH free radical scavenging method is a quick method and has been widely used to measure the antioxidant activity of natural materials¹⁸, and it is an organic nitrogen radical that showing maximum peak at 515-518 nm. When the DPPH solution is mixed with a substance that able to provide one hydrogen atom, it will be reduced to a form that is characterized by discoloration, interpreted as the parameter IC_{50} ¹⁹. The IC_{50} value of Et-B is 0.793 ± 0.105 mg/ml, it means that Et-B is having ability to reduce 50% of DPPH radicals; greater than that of Et-Tg ($IC_{50} 1.119 \pm 0.195$ mg/ml). Vitamin C is used as a reference compound or positive control with IC_{50} at 0.046 ± 0.001 mg/ml. Vitamin C is an antioxidant vitamin that has free hydroxyl groups which capable of capturing free radicals.

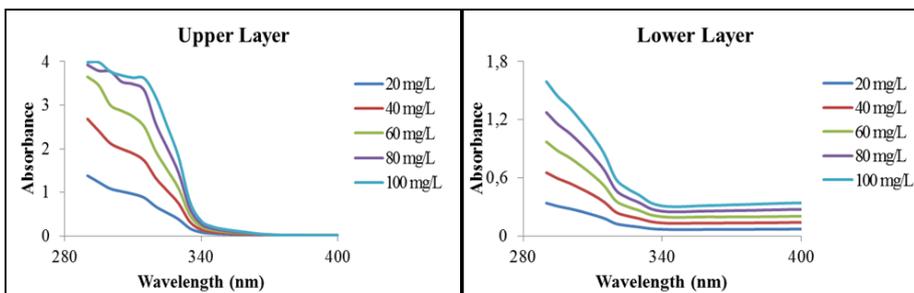


Figure 3:Spectrophotometric absorption profile of upper layer and lower layer

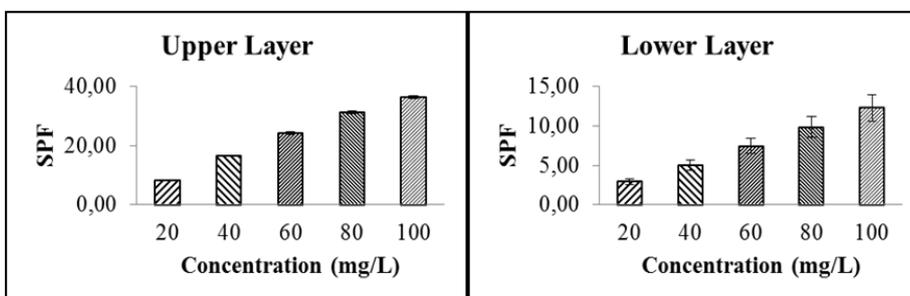


Figure 4:SPF value of upper layer and lower layer

Based on the value of SPF and IC_{50} , then *Z. cassumunar* extract fractionation, obtained the upper (non-polar) and lower (polar) layer. The upper layer shows the absorbance profile at a wavelength of 290-400nm,

especially in the UV-B region, as well as the lower layer(Figure 3). The resulting absorbance in the equal concentration of upper layer is greater than that of the lower layer, so it will provide an SPF greater value. The upper layer with a concentration of 100mg/L showed the largest SPF value of 36.3 ± 0.35 compared with the lower layer of 12.27 ± 1.68 (Figure 4). Chemical compound that gives the UV absorbance at less of 320nm is integrated to the upper layer, because the simple phenolics are generally dissolved in a non polar solvent such as n-hexane.

The antioxidant activity of the lower layer is greater than the upper layer, with $IC_{50} 0.996\pm 0.121$ mg/ml. The IC_{50} of all samples according to this research is still higher than that of vitamin C (Table 1), but it can be considered that there is still a potential natural antioxidant compounds in the lower layer.

Table 1 The IC_{50} value of *Z. cassumunar* extract (Et-B), *C. heyneana* (Et-Tg), and Et-B fractions

Sample	IC_{50} (mg/ml)
Et-B	0.793 ± 0.105
Et-Tg	1.119 ± 0.195
Upper Layer	2.842 ± 0.228
Lower Layer	0.996 ± 0.121
Vitamin C	0.046 ± 0.001

Conclusion

In summary, this research illustrate that the rhizome of *Z. cassumunar* extract has potential source of the active ingredient for sunscreen and natural antioxidants. Compounds having activity to block the sunlight is located in the non-polar portion while compounds having antioxidant activity located on the polar portion. Further study is needed to isolate and identified active compounds individually from upper layer for skin protection and also from lower layer for antioxidant compounds.

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