Effect of ethanol polarity on extraction yield, antioxidant, and sunscreen activities of phytochemicals from *Gyrinops versteegii* leaves

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Abstract. *Gyrinops versteegii* is one of the agarwood-producing species that is popularly cultivated in Indonesia. This research aimed to determine the yield, in vitro antioxidant and sunscreen activities of *G. versteegii* leaf phytochemicals (extracts) from the maceration with ethanol solvents at various polarities (E100%, E50%, E0%) and to analyze the total phenol content (TPC) and total flavonoid content (TFC) of the extract. The result shows that the polarity of ethanol affects the yield, antioxidant and sunscreen activities, and TPC of the extract. The E50% dissolved extract is the highest yield (19.14%), antioxidant activity (DPPH and CUPRAC test value are 35.82 and 389.52 µmol trolox/g sample), sunscreen activity (sun protection value is 14.9) and TPC (89.35 mg/g AGE) with TFC 0.485%.

1. Introduction
Agarwood is one of the potential Non Timber Forest Products (NTFPs) in Indonesia. Agarwood is very expensive compared to other NTFPs commodities. The export value of agarwood in September 2017 to USD 11.92 million with a volume of 847,392 kg [1]. However, the increase in demand for agarwood cannot be fulfilled due to the decreased availability of agarwood in the natural forests. Therefore, CITES appendix II limits the export of agarwood [2]. For this reason, Indonesia is developing the cultivation of agarwood-producing trees. *Gyrinops versteegii* is one of the agarwood-producing species that is popularly cultivated in Indonesia. The *G. versteegii* trees that had been planted [3].

One of the prospective parts of *G. versteegii* is the utilization of leaf part as a natural antioxidant source. The antioxidant activity of the methanol extract of *G. versteegii* leaf is very strong (IC₅₀ 14.46 µg/mL). The extract also has a very high sunscreen effectiveness with sun protection factor (SPF) value of 16.28 [4] and according to Wilkinson and Moore [5] belong to the ultra-protection category. However, for commercial utilization, the extraction with ethanol is more recommended than methanol because methanol is flammable, toxic, and smelly compared to ethanol solvents [6]. Therefore, the research on the antioxidant activity and SPF of *G. versteegii* leaf extracts from the extraction using ethanol at various polarity needs to be done.
This research aimed to analyze the effect of solvent polarity on the yield, antioxidant and sunscreen activities, the Total Phenol Content (TPC), and Total Flavonoid Content (TFC) of *G. versteegii* leaf phytochemicals (extracts) from the maceration with ethanol solvents at various polarities. In order to achieve these objectives, the study was started with the determination of the extraction yield, antioxidant activity, SPF value TCF, and TFC.

2. Materials and methods

2.1. Raw material preparation
The research sample is an old leaf from Depok, West Java. The dried leaves were then mashed by grinding and then sieved to be 40-60 mesh in size. The water content of samples was measured by taking a sample of ± 2 g and dried in an oven at a temperature of 103±2ºC to achieve a constant kiln-dry weight.

2.2. Extraction
*G. versteegii* leaf powder that has known water content was weighed 200 g x 3 replications. The sample extraction used the maceration method with ethanol at various polarities, such as the ethanol concentration of 100% (E100%), 50% (E50%), and 0% (E0%/only aquadest). Dilution of ethanol into an E50% solvent is done by mixing 50% ethanol with 50% aquadest. The extraction filtrate was evaporated with a rotary evaporator and dried at 40ºC.

2.3. In vitro antioxidant and sun protection assay
The antioxidant activity assay using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method refers to Salazar et al. [7] and the Cupric ion reducing antioxidant (CUPRAC) method refer to Ozturk et al. [8]. The antioxidant capacity is expressed in μmol trolox/g dry powder. The higher value of μmol trolox/g values indicates the higher antioxidant activity. The determination of the SPF value refers to Kawira [9].

2.4. Phytochemical analysis
The phytochemical analysis is carried out quantitatively. Analysis of total phenol content and total flavonoid content refers to Indrayani et al. [10].

2.5. Data analysis
Data analysis using complete random design with treatment is ethanol solvent at different polarities (ethanol concentration), such as E100%, E50%, and E0%. Data analysis uses an analysis of variance (ANOVA). The responses analyzed were the yield, antioxidant activity, sunscreen activity, TPC, and TFC extracts which are being produced from the extraction process with ethanol solvents at various polarities. If the treatment has a significant effects then Duncan performs further tests using the SPSS application.

3. Results and Discussion

3.1. Extract yield
The results of statistical analysis showed that the difference in polarity of ethanol had a significant effect on the extract yield. Duncan test results (α = 0.05) showed that the highest yield is the E50% dissolved extract (E50E) and significantly different from the E100% dissolved extract (E100E) and E50% dissolved extract (E0E) (Fig 1). This shows that the difference in polarity affects the phytochemicals of the extracted leaves. This is confirmed by the results of research showing the difference in polarity of the solvent affects the yield of *Pluchea indicia* leaf extract [11]. Some chemical compounds can dissolve using a mixture of ethanol and water including amino acids, sugar, some phytochemical compounds such as alkaloids, flavonoids, flavonoid glycosides, and chlorophyll [12].
3.2. Antioxidant activity

The antioxidant activity of G. versteegii leaf extracts using two antioxidant assays, namely the DPPH and CUPRAC methods. The antioxidant activity of testing with the CUPRAC method on all extracts has a higher value than the DPPH method (Table 1). This can be caused by the CUPRAC method which has more selective properties and more stable reagents than chromogenic reagents in measuring the antioxidant activity of DPPH. Also besides, the CUPRAC method can simultaneously measure antioxidant and lipophilic antioxidant compounds [13]. Besides that, according to Irawati [14], the DPPH method has a narrow range of absorbance and concentration linearity compared to the CUPRAC method which is able to react with large molecules so that it has a wide range of absorbance and concentration linearity.

Table 1. Antioxidant activity of G. versteegii leaf extracts from the maceration with the ethanol solvents at various polarities (concentration of ethanol)

| Kind of antioxidant assay | Kind of extract (µmol trolox/g sample)¹,² |
|---------------------------|-----------------------------------------|
|                           | E100E  | E50E   | E0E   |
| DPPH                      | 27.42±0.00⁹ | 35.82±0.03⁹ | 25.92±0.20⁹ |
| CUPRAC                    | 342.95±0.00⁹ | 389.52±0.05⁹ | 286.06±0.06⁹ |

Note: *) = 1): average of 3 replications, 2) the different letters in the lane show significantly different antioxidant activity values (α = 0.05)

The results of statistical analysis showed that the type of solvent had a significant effect (α = 0.05) on antioxidant activity based on tests using the DPPH and CUPRAC method. Duncan's further test results showed that the E50E had the highest antioxidant activity and was significantly different from other extracts and followed by the E100E. But, the antioxidant activity of the E100E was different from the E0E which had the lowest antioxidant activity (Table 1). If compared with the methanol extract of Mint leaves (Mentha piperita) which is known as an antioxidants source [15], the antioxidant activity of Mint leaves is almost the same (antioxidant value of 386 µmol trolox/g powder) with the E50% dissolved extract (Table 1). This shows the potential of G. versteegii leaves is developed as a source of antioxidants compounds.

3.3. Sun protection factor value

The results of the analysis of the variance showed that the differences in ethanol polarity influenced the sunscreen activity of the extract. Duncan's further test results showed that the sunscreen activity (SPF value) of the E50E is not significantly different from the SPF value of E100E. However, the SPF value of the E50E and E100E are higher and significantly different from the E0E (Figure 2). The difference
in the SPF value of these extracts is influenced by the content of secondary metabolites from phenolic groups such as flavonoids, isoflavonoids, and tannins which can absorb UV rays and potentially as sunscreens. Wolf et al. [16] stated that flavonoid compounds have the potential to be a high sunscreen due to the presence of chromophore groups (conjugated double bonds) that are able to absorb UV so that it can reduce its intensity on the skin. Based on the sunscreen classification according to Wilkinson and Moore [5], all of the extracts are classified as maximum because the SPF value is in the range of 10-15.

Figure 2. The SPF value of G. versteegii leaf extracts from the maceration with the type of solvent (E100, E50, and E0). The different letters in the lane show significantly different antioxidant activity values (α = 0.05).

3.4. Total Phenol Content (TPC) and Total Flavonoid Content (TFC) of extract

The results of the analysis of variance showed that the solvent type factor had a significant effect (α = 0.05) on the total phenol content of the extract. The TPC of the E50 extract is significantly higher than E100 extract and E0 extract (Table 2). But, the results of the analysis of variance showed that the solvent type factor had not a significant effect (α = 0.05) on the total flavonoid content of the extract. The difference in total phenol content can be due to the total concentration of phenol depending on the material and its solubility in the various polarities of the extraction solvent.

Table 2. TPC and TFC of G. versteegii leaf extracts from the maceration with the ethanol solvents at various polarities

| Phytochemical test | Kind of extract |
|--------------------|-----------------|
|                    | E100            | E50            | E0              |
| TPC (mg/g AGE)*    | 79.840±0.004a   | 89.350±0.001a  | 34.070±0.006c   |
| TFC (%b/b)         | 0.496±0.001     | 0.485±0.004    | 0.490±0.003     |

Note: *) the different letters in the lane show significantly different antioxidant activity values (α = 0.05)

The E50 extract had a higher antioxidant activity than E100 (Table 1). This has the positive correlation with the total phenol content compared to E100% extract and E50% (Table 2). This can be caused by antioxidant activity was influenced by total phenol content. This is in line with Wolf et al. [16] research which reported that the strongest positive correlation between total phenolics and antioxidant activity of microalga.

4. Conclusion

The yield, antioxidant activity, SPF value, and TPC of the G. versteegii leaf extract were affected by the polarity of the solvent (ethanol concentration). The highest yield is the E50E (19.1%) and significantly different from the E100E (15.7%) and E0E (12.6%). The highest antioxidant activity is the E50E with DPPH and CUPRAC value of 35.8 and 389.5 µmol trolox/g sample and followed by E100E and E0E with DPPH values of 27.4 and 25.9 µmol trolox/g sample and CUPRAC value is 342.9 and 286.1 µmol
trolox/g sample, respectively. The highest sunscreen activity is the E50E with SPF value of 14.9, and followed by E100E and E0E with SPF value of 14.7 and 11.6. The highest TPC is the E50E (89.4 mg/g AGE) and followed by E100E and E0E with TPC of 79.8 and 34.1 mg/g AGE. However, the TFC values of the three extracts are relatively the same (0.5%).

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Acknowledgment

The author would like to thank the Directorate of Higher Education of the Ministry of Education and Culture of the Republic of Indonesia with the ‘IPB Higher Education Research Grant’ scheme 2019 (contract No. 3/EI/KP.PT.PTNBH/2019) for funding support for some parts of this research.