Research Article

Antioxidant activity from ethanol extract and fractions of red flame ivy (*Hemigraphis colorata* Hall. F.) leaf using 1,1-diphenyl-2-picrylhydrazyl

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ABSTRACT

Context: Antioxidants can prevent free radical formation. Natural antioxidants found in many plants, such as red flame ivy (*Hemigraphis colorata* Hall.F.) **Aims:** The aim of the study was to determine the antioxidant activity extract and fractions of red flame ivy (*Hemigraphis colorata* Hall. F.) leaf. **Methods:** The sample was extracted with ethanol 70% in reflux. The antioxidant activity red flame ivy was determined by 1,1-diphenyl-2-picrylhydrazyl method. **Results:** The IC_{50} value was 98.52 ppm for ethyl acetate fraction, 140.41 ppm for n-hexane fraction, 218.4 ppm for water fraction, and 374.3 ppm for extract. **Conclusions:** Ethyl acetate fraction of *H. colorata* leaf is a strong category for antioxidant activity.

KEY WORDS: 1,1-diphenyl-2-picrylhydrazyl, Antioxidant, *Hemigraphis colorata* Hall. F. leaf

INTRODUCTION

Free radicals defined as chemical species possessing unpaired electrons.[1] Free radicals are responsible for damage of lipids, proteins, and nucleic acid in cell which leads to cardiovascular diseases, cancers, and other age-related degenerative diseases.[2]

Red flame ivy (*Hemigraphis colorata* Hall. F.) is a versatile tropical low creeping perennial herb that reaches a height of 15–30 cm. The leaf has metallic purple luster on upper surface and a solid dark purple on ventral side.[3] The phytoconstituents present in *H. colorata* are saponins, flavonoids, terpenoids, coumarins, carbohydrates, carboxylic acid, xanthoproteins, phenols, tannins, proteins, alkaloids, steroids, and sterol.[4] According to the literature, the antioxidant activity *H. colorata* mainly due to the presence of phenolic compounds.[4] Phenolic compounds are effective hydrogen donor which makes them a good antioxidant. The phenolic acids such as chlorogenate, cinnamate, coumarate, gallate, and ferulate present in the plant act as prooxidants and exhibit free radical scavenging activity.[3]

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The study of antioxidant activity from *H. colorata* that has been done was using Infus extract and the sample was collected from Balai Penelitian Obat dan Aromatik, Bogor, West Java, Indonesia.[6] The aim of this study was to determine the antioxidant activity extract and fractions of *H. colorata* leaf by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method.

MATERIALS AND METHODS

Materials

Red flame ivy leaves were collected from Manoko Garden, Lembang Subdistrict, West Java, Indonesia. The plants were identified at Laboratory of Plant Taxonomy, Department of Biology, Universitas Padjadjaran, with No.487/HB/01/2017. All chemicals are analytical grade (Merck, Germany).

Extraction

Simplicia is extracted with 70% ethanol by reflux method for 6 h. Each 2 h, the solvent changed with the fresh one. Liquid extract collected and evaporated with rotary Rotavapor. Dissolve 10 g of ethanolic extract with aquadest to obtain 100 ml solution, then done liquid–liquid extraction with n-hexane and ethyl acetate, 3 times for each solvent. All fractions collected and evaporated.

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Antioxidant Activity Determination

The modified method of Molyneux (2004) was conducted.[1,210,11] A total of 1.5 ml of 40 µg/ml DPPH were added to various concentrations of 1.5 ml of Vitamin C or extract and fractions. The mixture was incubated in a dark chamber for 30 min, and absorbance was measured at 517 nm using spectrophotometer. The blank was 96% ethanol. Percentage of antioxidant activity was calculated using the formula:

$$\%\ \text{of DPPH inhibition} = \frac{(\text{Ab-Aa})}{(\text{Ab})} \times 100$$

Aa and Ab are absorbance values of the sample and the blank, respectively. A percent inhibition versus concentration was plotted, and the concentration of sample required for 50% inhibition was determined and expressed as inhibitory concentration 50% value.

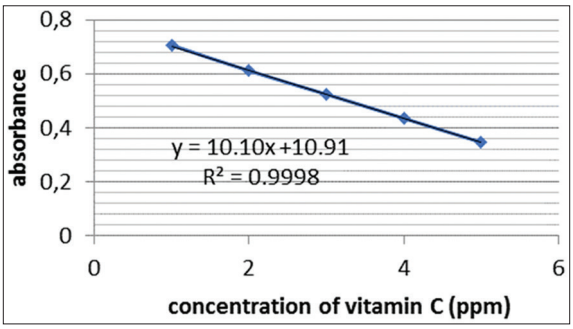

Figure 1: Calibration of Vitamin C (n = 3)

Table 1: Fraction yield

n-hexane (%)	Ethyl acetate (%)	Water (%)
22.3	32.6	45.1

The IC_{50} value of extract and fractions were counted from the linear regression equation of the curve of concentrations versus absorbance [Figure 1].

RESULTS AND DISCUSSION

Reflux is a hot extraction method. This method was conducted to maximal extraction of entire secondary metabolites in the sample. Liquid–liquid extraction was conducted to separate the secondary metabolites based on its polarity.[5] Ethyl acetate fraction has bigger yield than n-hexane fraction [Table 1]. We concluded that the majority of secondary metabolites were semipolar and polar secondary metabolites.

The antioxidant activity of the extract and fractions was lower than ascorbic acid as a positive control [Table 2] since extract and fractions were not pure compounds. Ethyl acetate fraction has the best antioxidant activity compared to the other fractions and extract. This fraction contains flavonoids, tannins, and alkaloids. All these structures having the hydroxyl group which can donate hydrogen to interact with DPPH radical to produce the DPPH-H (2,2-diphenyl-1-picrylhydrazyl).

Natural antioxidant from medicinal plants is a good choice to control oxidative stress. Due to natural origin, these compounds are usually non-toxic. Antioxidants on interaction with DPPH radicals transfer a proton to

Table 2: The antioxidant activity

Sample	Concentration (µg/ml)	Absorbance	% inhibition	Linier regression equation	IC_{50} (µg/ml)
Vitamin C standard	2	0.705±0.00057735	42.13	y=10.10x+10.91	3.87
	4	0.61±0.001154701	54.25	R^2=0.9998	
	6	0.52±0.001732051	64.82		
	8	0.435±0.00057735	75.28		
	10	0.345±0	86.4		
Red flame ivy extract	100	0.504±0.001154701	42	y=0.067x+35.265	218.4
	200	0.462±0.001154701	46.89	R^2=0.9931	
	400	0.309±0.001527525	64.48		
	600	0.209±0.00057735	75.9		
	800	0.106±0.001154701	87.81		
n-hexane fraction	100	0.601±0	30.9	y=0.0612x+27.09	140.41
	200	0.511±0.001	41.3	R^2=0.9912	
	400	0.412±0.001527525	52.64		
	600	0.314±0.002081666	63.9		
	800	0.215±0.00057735	75.28		
Ethyl acetate fraction	100	0.445±0.00057735	48.8	y=0.059x+44.187	98.52
	200	0.38±0.00057735	56.3	R^2=0.9942	
	400	0.269±0.00057735	69		
	600	0.167±0	80.8		
	800	0.087±0	90		
Water fraction	100	0.474±0	45.5	y=0.633x+41.112	374.3
	200	0.398±0	54.4	R^2=0.9912	
	400	0.275±0.00057735	68.39		
	600	0.173±0.002081666	80.11		
	800	0.086±0.001527525	90.11		

IC_{50}: Inhibitory concentration 50%

DPPH radicals by direct abstraction of phenol H-atoms and electron transfer process, thus neutralizing its free radical character, which produces DPPH-H i.e., DPPH with less reactivity.

The antioxidant activity with the DPPH method was characterized by the color alteration, from purple to yellow after incubation for 20 min. It is due to the free radical DPPH was reduced to DPPH. The category of antioxidant activity which is based on criteria[7] was strong for ethyl acetate (50–100 ppm), weak for extract, n-hexane, and water fractions (150–200 ppm).

CONCLUSION

Ethyl acetate fraction of *H. colorata* leaf is a strong category for antioxidant activity.

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REFERENCES

1. Saptarini MN, Herawati EI. Comparative antioxidant activity on the *Ficus benjamina* and *Anona reticulate* leaves. Int J Public Health Sci 2015;4:21-6.
2. Saptarini MN, Herawati EI. Antioxidant activity of water apple (*Syzigium aqueum*) fruit and fragrant mango (*Mangifera odorata*) fruit. Asian J Pharm Clin Res 2017;Special issue (May):54-6.
3. Devi MP. Review on pharmacological activity of Hemigraphis colorata (Blume) H. G. Hallier. Int J Herb Med 2013;1:120-1.
4. Joyson A, Krishnakumar K, Hareeshbabu E. Hemigraphis colorata: A review. J Biol Innov 2107;6:557-61.
5. Okawa M, Kinjo, Nohara, Ono. DPPH (1,1-Diphenyl-2-picrylhydrazil) radical Scavenging Activity of Flavonoid Obtained from Some Medicinal Plants, Biol Pharm Bull, 24(10), 1202-5.
6. Available from: http://www.dosen.univpancasila.ac.id/dosenfile/20102110581405993788 22July2014.pdf. Sandhiutami NM, Rahayu L, Oktaviani T, Sari LY. Uji Aktivitas Antioksidan Rebusan Daun Sambang Getih (Hemigraphis bicolor Boerl.) Dan Sambang Solok (Aerva Sanguinolenta (L.) Blume) Secara In vitro. Fakultas Farmasi Universitas Pancasila; 2014. [Last cited on 2018 August 20]
7. Houghton P, Raman A. Laboratory Handbook of the Fractination of Natural Extracts. London: Chapman & Hall; 1998.
8. Reichardt C. Solvents and Solvent Effects in Organic Chemistry. 3rd ed. New York: Wiley-VCH Publishers; 2003.
9. Fransworth N. Biological and phtyocemical screening of plants. J Pharm Sci 1966;1:247-68.
10. Saptarini MN, Herawati EI. The Effect of acetic acid on total anthocyanins content and antioxidant activity of tamarillo (*Solanum betaceum* Cav). J Pharm Res 2018;12:398-401.
11. Saptarini NM, Suryasaputra D, Nurumamah N. Antioxidant activity of extract and fractions of dragon scales (*Drymoglossum piloselloides* C. Presl). Res J Pharm Biol Chem Sci 2018;9:757-63.
12. Molyneux P. The use of the stable free radical diphenylpicrylhydrazil (DPPH) for estimating antioxidant activity. Songklankarin J Sci Tech 2004;26:211-9.

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