

ANTIOXIDANT ACTIVITY TEST OF ANDROGRAPHOLIDE IN BITTER HERBS USING DPPH SCAVENGING

¹ Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Udayana University, Bukit Jimbaran, Badung Telp/Fax : 0361 224133, dekpitsusanti@unud.ac.id

Ni Made Pitri Susanti¹, Ni Kadek Warditiani¹, I Made Agus Gelgel Wirasuta¹

Abstract Bitter herbs (Sambiloto) have many pharmacological activities including antioxidant, antidiabetic, anticancer, antihyperlipidemic and anti-inflammation. Andrographolide is a diterpene compounds contained in bitter herbs. It is known that andrographolide compound responsible for the pharmacological activity of the bitter herbs. This study investigated DPPH free radical scavenging activity from andrographolide diterpene lactone. This study was initiated with the isolation of andrographolide compound from bitter herbs and then testing their DPPH free radical scavenging. The results suggested that andrographolide had IC₅₀ value of 5.45 mg. This means andrographolide has 50% DPPH inhibition effect, i.e 5.45 mg.

Keywords: *andrographolide, antioxidant, bitter herbs, DPPH, free radical*

I. INTRODUCTION

In our environment, there are many sources of free radicals which cannot be avoided. Among them are motor vehicle fumes, cigarette smoke, food fried in cooking oil, baked foods, certain drugs, and air pollution. Free radical is a molecule having one or more unpaired electrons. These free or unpaired electron is highly reactive to the body's cells. This suggests that the cells in our bodies are oxidized [1], [2].

The presence of free radicals in the body that lead to oxidation reaction will cause damage to the nucleic acids, fat and DNA cell. This event will initiate the occurrence of degenerative diseases such as memory loss, cataracts, cancer and cardiovascular disorders [1], [3]. Our body has ability to protect cells against oxidation reactions that may occur in the presence of peroxidase, catalase, glutathione, histidine-peptidine. More over, the intake of foods containing antioxidants can also protect our body against free radical attack. Consumption of foods such as fruits and vegetables, contain antioxidants compounds like vitamins A (beta-carotene), C and E [4].

Secondary metabolites contained in the plant have potential effect as free radical scavenger such as carotene, lycopene, polyphenols and flavonoids [5], [6]. The main mechanism of antioxidant compounds from secondary metabolites contained in the plant is the capability to bind, stabilize and prevent free radicals and not damage the body's cells [5].

Dragon fruit (*Hylocereus undatus* (Haw.) Britt. & Rose; Cactaceae family) contains vitamin C and carotene that have potential antioxidants. Based on the research of DPPH free radical in methanol and water extracted from dragon fruit, 4.49% of water extract (equivalent to 3.08 g of fresh dragon fruit) has the ability to scavenge free radicals by

87.11% in the 5th minute and 90, 83% in the 30th minute; while 6.47% of methanol extract (equivalent to 4.44 g fresh dragon fruit) have the ability to scavenge free radicals by 79.18% in the 5th minute and 90.46 in the 30th minute. EC₅₀ value of the water extract is 1.08%. Bitter herbs (sambiloto) play a role as an antioxidant against free radicals oxidative damage. Reference [7] suggested that the methanol extract of bitter herb (*Andrographis paniculata*) inhibit DPPH free radicals so they have an antioxidant potential. Inhibition of DPPH free radicals measurement by this bitter methanol extract is compared with gallic acid. But the study did not calculate the IC₅₀ value. Thus, it is important to measure inhibition of DPPH free radicals from andrographolide compound of bitter herbs and calculate the IC₅₀ value. The IC₅₀ value is the concentration of a compound that shows an ability to inhibit free radicals by 50%.

II. METHODS AND PROCEDURE

A. Bitter Herbs Preparation

Bitter herbs obtained is dried then downsized to simplify and accelerate the extraction. Dried process is to reduce the water content in bitter herbs to prevent damage to the herbs. Damage herbs can be triggered by the growth of microbes or fungi and the enzymatic reaction due to high water content.

B. Extraction and Isolation

Bitter herbs powder was extracted with 96% ethanol by maceration method for 24 hours. After 2 times re-

maceration by using the same solvent, evaporation of the solvent in order to obtain a thick extract was done. Condensed extract of bitter herbs re-extracted with a *n*-hexane solvent to remove chlorophyll which were contained in the ethanol extract of bitter herbs. We separated the non soluble part in *n*-hexane. The process was continued with extract purification process in order to obtain purified extract. Re-crystalization was done by dissolving the purified extract in hot methanol, and then cooling until it formed crystals andrographolide.

C. DPPH Level Measurement

Samples were prepared by weighing 10 mg of extract and dissolved in 1 mL of pro analysis methanol. An amount of 200 μ L then was added with 1800 μ L methanol. From this solution then pipette 100, 300, 500, 700 and 900 μ L, respectively added by methanol to a series volume of 1 mL. Each sample was pipetted 500 μ L and was added with 500 μ L DPPH, incubated for 1 hour. DPPH crystals were dissolved in ethanol to get a concentration of 0.025% in methanol for immediate use and kept in light-protected low temperature.

Samples were then spotted on a TLC plate of silica gel GF 254. Plate was scanned at a wavelength of 530 nm.

D. IC₅₀ Measurement

Inhibition of free radicals was calculated based on the result of reduction in area under the curve (AUC) forms by the sample AUC toward AUC at a wavelength of 530 nm using a calculation as refer to (1).

$$\frac{(AUC_{blanko} - AUC_{sample})}{AUC_{blanko}} \times 100\% \quad (1)$$

III. RESULTS AND DISCUSSION

Based on andrographolide isolation results, obtained andrographolide crystals was amorphous as shown in Fig. 1. Visible light spectra profile of andrographolide in various levels and DPPH solution can be seen in Fig. 2.



Fig.1. Andrographolide crystal from bitter herbs

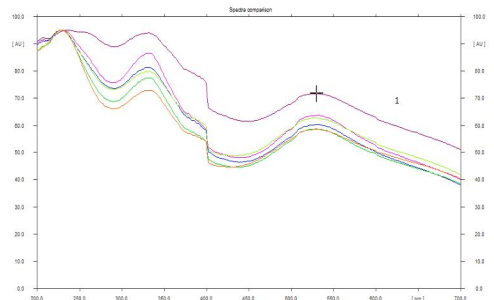


Fig. 2. DPPH and andrographolide spectrum at 530 nm wavelength
1 = DPPH spectrum

Andrographolide levels in extract were quite a lot. Extraction using *n*-hexane was able to eliminate non-polar compounds compared to andrographolide chlorophyll. Andrographolide effect on inhibit DPPH was tested by adding andrographolide and reagents DPPH and performed incubation for 1 hour. Appropriate incubation time is very important note to get the value of AUC and a good spectrum at a 530 nm wavelength, maximum wavelength for DPPH. Test for free radical scavenging begins with making spectrum in the range of 400-600 nm visible light from DPPH solution and andrographolide. This measurement is done to look at the pattern of the blank DPPH and DPPH added andrographolide spectrum. Measurements were made by looking at the value of AUC (Area Under the Curve) contained at a 530 nm wavelength. The AUC values result from the blank DPPH and DPPH added andrographolide shown in Table 1.

Table 1.
AUC Value from Blank DPPH and Andrographolide

No	Blank	50 μ g	150 μ g	250 μ g	350 μ g	450 μ g
1	7430,5	6304,5	6799,7	6483,7	6266,3	6293,0
2	7522,5	6209,6	6473,3	6451,5	6198,0	6149,0
3	7074,0	6634,7	6288,6	6371,1	6402,8	6277,4
4	7463,9	6680,4	6185,7	6416,5	6486,8	6230,4

The smaller the value of AUC in addition of andrographolide showed greater andrographolide concentrations was added. Conversely, increase andrographolide concentration was added, will increase the % inhibition of DPPH free radicals. The result of percentage inhibition calculation can be seen in Table 2.

According to Table 2, an equation to calculate the IC₅₀ value of andrographolide was created. The calculation of IC₅₀ was done by interpolating percent reduction (50%) in the test solution concentration curve with the percent reduction. Andrographolide concentration and the percent reduction data obtained regression curve as in Fig. 3.

Table 2.
DPPH Inhibition from Andrographolide Compound

No	% Free Radical Inhibition				
	50 μ g	150 μ g	250 μ g	350 μ g	450 μ g
1	14,489	7,772	12,058	15,007	14,645
2	15,776	12,199	12,495	15,933	16,598
3	10,010	14,705	13,586	13,156	14,856
4	9,390	16,100	12,970	12,016	15,494
Mean	12,416	12,694	12,777	14,028	15,398
SD	3,190	3,657	0,655	1,770	0,877

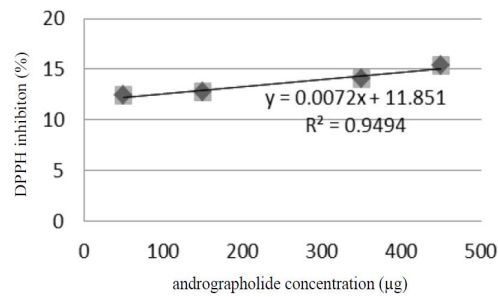


Fig. 3. Percentage of DPPH inhibition and andrographolide concentration (µg)

Based on % DPPH inhibition and andrographolide concentration (µg) correlation curve, a straight-line equation, namely $y = 0,007x + 11.85$ was constructed. The value of y is % DPPH inhibition, while the value of x is andrographolide concentration. Based on calculations, andrographolide 50% inhibition or IC_{50} is 5.45 mg. A value of 0% means that the compound does not have a free radical or antioxidant activity, while a value of 100% means a total reduction of a compound.

IV. CONCLUSION

Andrographolide compounds can be separated from bitter herbs by re-crystallization. IC_{50} value of DPPH radical inhibition by andrographolide is 5.45 mg.

ACKNOWLEDGMENT

The authors thank the Ministry of Research Technology and Higher Education and Udayana University for the opportunity to get competitive grant in 2016.

REFERENCES

- [1] Pietta P-G., 1999. Flavonoids as Antioxidants, Reviews, *J. Nat. Prod.*, 63, 1035-1042
- [2] Wijaya A., 1996. Radikal Bebas dan Parameter Status Antioksidan, Forum Diagnosticum, Prodia Diagnostic Educational Services, No. 1 : 1-12.
- [3] Leong L.P., Shui, G., 2001. An Investigation of Antioxidant Capacity of Fruits in Singapore Markets, *Food Chemistry* 76 : 69-75.
- [4] Clarkson, P. M., Thompson, H. S. 2000, Antioxidants: what role do they play in physical activity and health, *J. Clin Nutr. Biochem*, 72.: 637S-46S.
- [5] Prakash A., 2001. Antioxidant Activity, *Medallion Laboratories Analytical Progress*, Vol. 19 (2).
- [6] Okawa M., Kinjo, J., Nohara, T., Ono, M., 2001. DPPH (1,1-Diphenyl-2-Picrylhydrazyl) Radical Scavenging Activity of Flavonoids Obtained from Some Medicinal Plants, *Biol. Pharm. Bull* 24 (10), 1202-1205.
- [7] Huidrom, Sangeeta and Deka, Manab, 2012, Determination of antioxidant property of *Andrographis paniculata*, *IJDAD*, 1(1): 12-17