Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (18):67-71
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Antioxidant activity and total flavonoid content of fractions of piladang (*Solenostemon scutellarioides* (L) Codd) leaf extract

Verawati, Mimi Aria, Afdhil Arel and Efi Ryanto

STIFI Perintis Padang, Jl. Adinegoro/Simp. Kalumpang Km7 Lubuk Buaya Padang - 25173

ABSTRACT

Flavonoids may function as an antioxidant by neutralizing free radicals. This study was aimed to determine total flavonoid content and antioxidant activity from fraction of Piladang (Solenostemon scutellarioides (L) Codd) leaf extract. Total flavonoid was performed with colorimetric method while antioxidant activity of fractions were determined by in-vitro method using DPPH assay. The total flavonoid content was expressed in quercetin equivalent for each fraction was 1.75 mg/g in hexane, 9.63 mg/g in ethyl acetate and 16.78 mg/g in butanol. Antioxidant activity was expressed as 50% inhibitory concentration (IC_{50}) in fractions were 364.73 µg/ml in hexane; 29.15 µg/ml in ethyl acetate and 66.21 µg/ml in butanol. The results indicated a direct correlation between the antioxidant activity and the flavonoid content of the fractions. This study confirmed that the ethyl acetate fraction had a highest activity.

Keywords: *Solenostemon scutellarioides*, flavonoids, antioxidant, DPPH

INTRODUCTION

Advances in technology, changes in lifestyle and environment can accumulate to produce the radical oxygen species (ROS) that are harmful to human health. These ROS trigger the onset of degenerative diseases such as cardiovascular disease, premature aging, cancer, diabetes, gout , etc [1]. ROS can be neutralized by antioxidant compounds that can be derived from natural and synthetic. Natural antioxidant produced by plants for example flavonoids that are the largest group of polyphenols with diphenyl propane as a general structure. Many flavonoid compounds have been isolated from plants such as quercetin, catechin, hesperidin, naringenin and others. Aside from being antioxidant this class of compounds also have a variety of pharmacological activities such as antibacterial, anti-inflammatory, antidiabetic, hepatoprotective, anticancer and treatment various cardiovascular diseases [2,3].

Solenostemon scutellarioides is a plant that has been used as traditional medicine in Indonesia. Leaves of this plant were used to treat fever, diabetes, hemorrhoids, ulcer, diarhea and many inflammatory condition [4]. *Solenostemon scutellarioides* leaves have been investigated contain flavonoids, polyphenos, volatile oils, alkaloids and saponin [5,6]. This study was aimed to determine the total flavonoid content and antioxidant activity of fractions obtained from leaf extract of *S. scutellarioides*.

MATERIALS AND METHODS

Plant materials

Leaves of *Solenostemon scutellarioides* (L) Codd. were collected from Bukittinggi West Sumatra Province, Indonesia. Botanical identification was performed by Herbarium Andalas Of Biology Faculty of Andalas University.

Plant Extraction and fractionation

S. scutellarioides leaves were air dried for a week then make powder leaves by grinding. Leaf powder was extracted with ethanol using maceration method. The extract obtained was concentrated with a rotary evaporator (RV 10 IKA®). Thick extract was diluted with distilled water and then gradually fractionated with a solvent that has a polarity at a different level by using hexane, ethyl acetate and butanol. Each fraction was concentrated by rotary evaporator to obtain viscous fractions.

Chemicals

Quercetin, 1,1-diphenyl-2-pikril hidrazil (DPPH), ascorbic acid, aluminum chloride, were obtained from Sigma chemical Co. (USA), methanol, ethanol, ethyl acetate, butanol, hexane, sodium acetate were obtained from Brataco Chem (Indonesia).

Determination of total flavonoid content

Determination of total flavonoid performed by colorimetric methods using $AlCl_3$ as complexing color [7]. Standard solution of quercetin (25, 50, 75, 100 and 125 µg / ml) and the fraction of each solution (1000 µg / ml) 0.5 ml pipette into the vial and then mixed with 1.5 ml of methanol; 0.1 ml of 10% aluminum chloride and add 0.1 mL of 1 M sodium acetate and 2.8 ml of distilled water. Let stand for 30 minutes at room temperature.All samples and standard solution were measured at a wavelength of 428 nm using a UV-Vis spectrophotometer (6850 double beam Jenway®). Based on data of concentration and absorption value of standar solution that was made standard calibration curve in order to obtain a regression equation. Quercetin standard regression equation used to determine the total flavonoid content in leaves of *S. scutellarioides* fraction.

Determination of antioxidant activity

Antioxidant activity was determined by the method of DPPH radical scavenging according to modified method Sahu 2013 [8]. Ascorbic acid was used as a standard antioxidant to compare with antioxidant ability of each fraction [8]. Ascorbic acid and each fraction of *S.scutellarioides* were prepared with several concentration. 2 ml of each ascorbic acid solution (2, 4, 6, 8 and 10 µg / ml), hexane fraction (100, 200, 300, 400, 500 µg/ml), ethyl acetate fraction (10, 20, 30, 40, 50 µg / ml) and butanol fraction (20, 40, 60, 80, 100 µg / ml) was pipetted into a vial and added 4 ml of DPPH solution 35 mg/ml. 2 ml of methanol and 4 ml DPPH 35 ug / ml mixed and used as a control. All mixture were allowed to stand in a dark place for 30 minutes. Absorbance of each solution was measured at a wavelength of 517 nm using UV-Vis spectrophotometer. The percentage of inhibition of antioxidant toward to DPPH radicals was calculated by the following equation;

$$\%\ inhibition = \frac{Abs\ control - Abs\ sample}{Abs\ control}\ x\ 100\%$$

IC_{50} value is concentration of sample (fraction) that required to scavenge or inhibite 50% of radical DPPH [9]. That value can be calculate from the regression equation of the calibration curve of the absorbance of samples against concentration of fractions.

RESULTS AND DISCUSSION

2100 g of *S. scutellarioides* leaves were dried for a week and then grinded to obtain 240 g leaf powder. Leaf powder was macerated with ethanol and obtained 54.95 g (22.89%) thick ethanol extract. Ethanol extract was diluted with distilled water and gradually fractionated with hexane, ethyl acetate and butanol. Each fraction was obtained by hexane fraction 1.3072 g (3.268%), ethyl acetate fraction of 6.8367 g (17.09%) and butanol fraction 15.459 g (38.64%). Each fraction was identified with the content of flavonoids by cyanidine test and showed positive results on a fraction of ethyl acetate and butanol.

Quercetin was used as the standard compound to calculate the total flavonoid content of each fraction. From examination of the standard solution of quercetin derived calibration curve with the equation y = - 0.03575 + 0,00685x and the correlation coefficient (R^2) is 0.9970; standard deviation = 0.02425; the limit of detection (LOD) = 10, 62 µg/ml, and the limits of quantitation (LOQ) = 35, 41 µg / ml. R value close to 1 proved that the regression equation was linear and little standard deviation indicates higher accuracy. The calibration curve of quercetin to determine flavonoid content was shown in Figure 1.

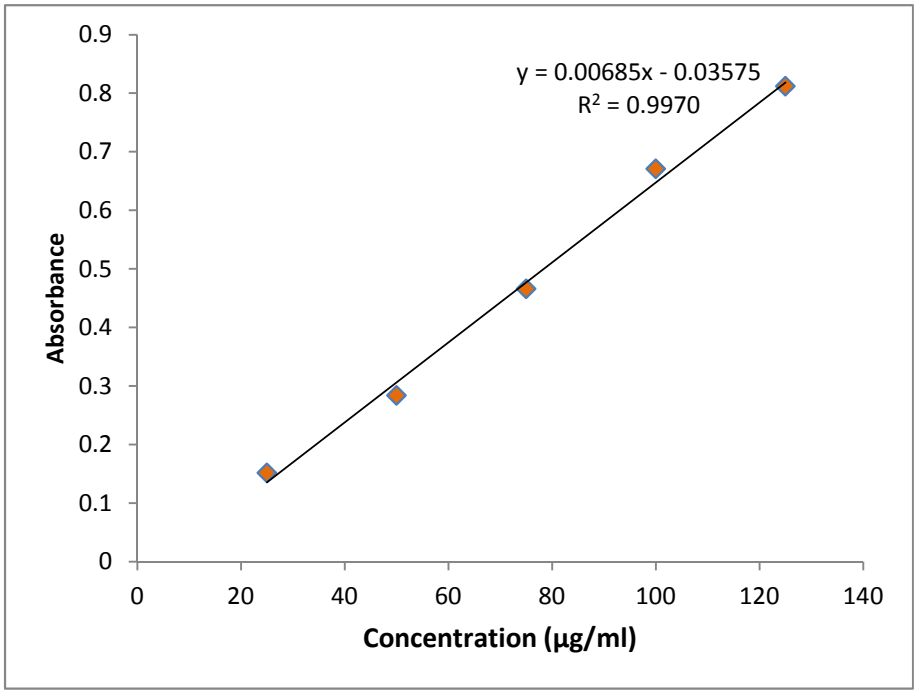

Figure 1. Calibration curve of quercetin to determine total flavonoid content

Total flavonoids contained in each fraction was expressed as quercetin equivalents in mg per g fractions. Among all the fractions, butanol fraction containing the highest flavonoid that may be in the form of flavonoid glycosides which is polar. Flavonoid total value of all fractions can be seen in Table 1.

Antioxidant activity was determined by radical DPPH scavenging method. Compounds that have the ability as an antioxidant will donate electrons or a hydrogen atom to the DPPH radical so that single free electron of DPPH be duplicate and stable. Visually, reduction process of DPPH radical will be shown on the color change of DPPH solution which was originally blue-violet to yellow after reacting with the electron donor compounds (flavonoids) [10]. The antioxidant activity of the fractions were expressed as IC_{50} The lower the IC_{50} value means higher power inhibition of antioxidant compounds against DPPH radical. Antioxidant power of each fraction are shown in Table 1. Ascorbic acid was used as a reference antioxidant which the IC_{50} was at 7.85 µg/ml.

Table 1. Total flavonoid content and antioxidant activity of fractions *S. scutellarioides*

Fractions	Flavonoid content (mg/g)	IC50 of antioxidant Activity (µg/ml)	Antioxidant equivalence toward ascorbic acid (mg)
Fr. Hexane	1.75±0.77	364.73 ± 7.35	46.44
Fr. Ethyl acetate	9.63±1.10	29.15 ±5.21	3.71
Fr. Butanol	16.78±1.63	66.21 ± 1.87	8.84

IC_{50} value of antioxidant activity obtained from the calibration curve of each fraction. The calibration curve was plotted from inhibition percentage value against concentration of each fraction. Combined calibration curve of each fraction can be seen in Figure 2.

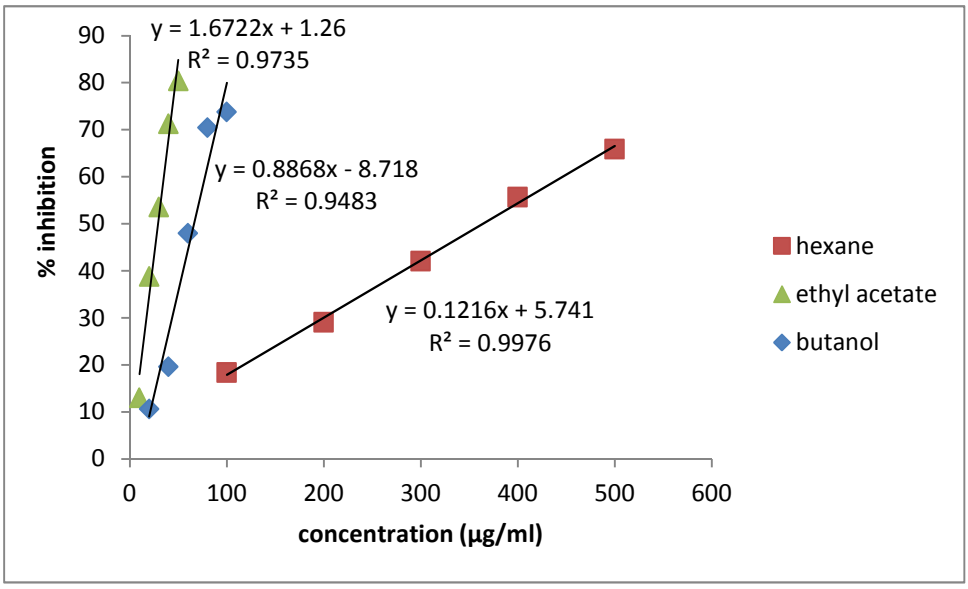

Figure 2. Calibration curve of antioxidant activity for each fraction

Each fraction of *S. scutellarioides* contains different phytochemical compound and that was responsible for the antioxidant activity of fractions. High flavonoid content in butanol fraction proved to have lower antioxidant activity than the fraction of ethyl acetate. Correlation levels of flavonoids with antioxidant activity of *S. scutellarioides* fractions shown in Figure 3.

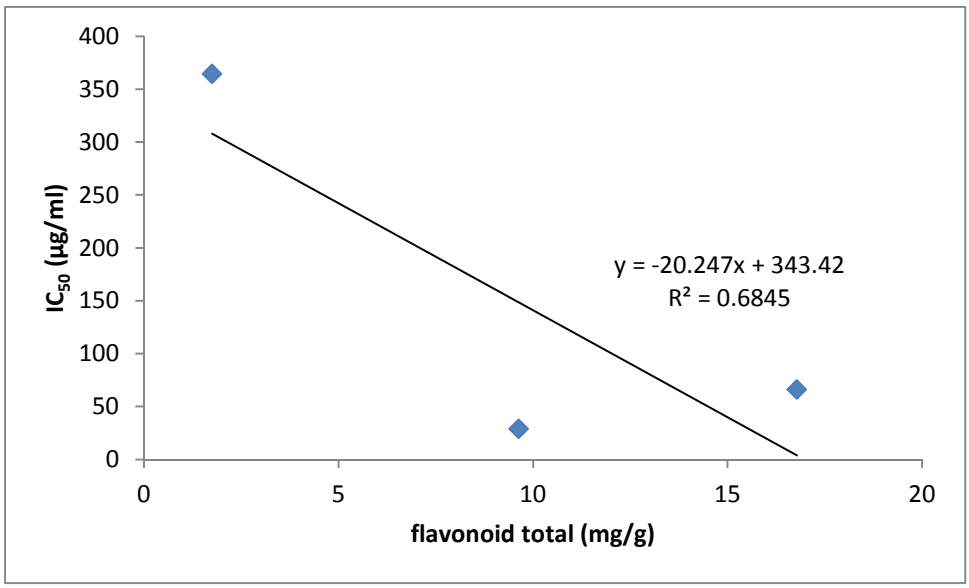

Figure 3. Correlation between antioxidant activity and total flavonoid content

Graph correlation between the levels of flavonoids with antioxidant activity of fraction had a coefficient of determination (R^2) 0.6845 . This result suggested that flavonoid compounds accounted for 68.45% of the radical scavenging ability of fractions. In other words can be interpreted that the antioxidant activity of the leaves of *S. scutellarioides* not only caused by the flavonoids alone but can also came from other compounds contained in them such as essential oils, vitamins and terpenoids. Polarity of solvent could influence and had significant impact on chemical profile of secondary metabolite of fraction.

CONCLUSION

Butanol fraction of *S. scutellarioides* contains highest flavonoids among fractions and it could be suggested that *S. scutellarioides* contains more polar flavonoids due to glicisidic flavonoid form. The highest antioxidant activity was

showed by ethyl acetate fraction. This fact expressed not only flavonoids had responsibility on DPPH radical scavenging ability of *S. scutellarioides* leaves.

Acknowledgements
Researchers would like to thank to the Directorate for Higher Education Kemristekdikti for its financial support through Hibah Bersaing scheme DIPA Dirjen Dikti 2015 to perform this research.

REFERENCES

[1] R. P. Singh, S. Sharad, S. Kapur, *Journal Indian Academy of Clinical Medicine*, **2004**, 5, 3, 218-25.
[2] S. Chakkaravarthi, D. Philoma, P. Bharathi, *Indian J. Pharm. Sci*., **2005**, 67, 4, 399-403.
[3] A.R Tapas, D. M. Sakarkar, R. B. Kakde, *Tropical Journal of Pharmaceutical Research*, **2008**, 7, 3, 1089-1099.
[4] Zulfahmi, B. Solfan, *Jurnal agroteknologi*, **2010**, 1, 1, 31-38.
[5] H. Soni, A.K. Singhai, *Asian Journal of Pharmaceutical and Clinical Research*, **2012**, 5, 1, 12-17
[6] A. Ahmad, M.N. Massi, *Int. J. Pharm Bio Sci*, **2014**, 5, 1, 758 – 764.
[7] F. Pourmorad, S. J. Hosseinmehr, N. Shahabimajd, *African Journal of Biotechnology*, **2006**, 5, 11, 1142-1145.
[8] R. K. Sahu, M. Kar, R. Routray, *Journal of Medicinal Plants Studies*, **2013**, 1, 4, 21-27.
[9] P.K. Ramamoorthy, A. Bono, *Journal of Engineering Science*, **2007**, 2, 1, 70-80.
[10] P. Molyneux, *J. Sci Technol*., **2004**, 26, 2, 211-219.