https://doi.org/10.46344/JBINO.2021.v10i06.23

ANTIOXIDANT OF *COLEUS SCUTELLARIOIDES* L. WATER EXTRACTS BY DPPH METHOD

Resmi Mustarichie[1*] & Danni Ramdhani

[1]Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia

Email : resmi.mustarichie@unpad.ac.id

ABSTRACT

Background: is commonly used as an ornamental plant or medicinal plant. The pattern, shape, and color of the leaf are varied, but the nutritious as a medicine is a brownish-red leaf. According to various studies, this leaf contains many chemical compounds but the main ingredient is a potent antioxidant flavonoid compound. **Objectives:** This study aims to determine the antioxidant activity of water extract of *C. scutellarioides* leaf using DPPH method with comparative standard of vitamin C. **Material and Methods:** Leaves of *C.scutellarioides* obtained from Lembang, West Java and determined in Biology Department FMIPA Universitas Padjadjaran. The leaves was macerated with ethanol and water and and concentrated with rotary evaporator and freeze dryer, respectively. The concentrated extract was analyzed by UV-spectrophotomer to determine its antioxidant properties. **Results:** From phytochemical screening, it was found that *C. scutellarioides* contained flavonoid, saponin and polyphenols. This study obtained antioxidant activity of IC_{50} 244,42 µg/mL). **Conclusion:** Water extract of *C. scutellarioides* belong to the weak antioxidant group because it has an IC*50* value of over 150 µg / mL and is also weaker than vitamin C (IC_{50} 7,27 µg/mL).

Keywords: flavonoid, antioxidant, DPPH, UV-Spectrophotometer, Coleus scutellarioides.

INTRODUCTION

Coleus scutellarioides L. is generally grown in the yard as an ornamental plant or medicinal plant. Herbs from Southeast Asia are found to grow wild in places as moist and open as the edge of a ditch, paddy fields, or on the edge of rural roads at a height of 1-1300 above sea level. Commonly known as coleus, is a species of flowering plant in the family Lamiaceae, native to southeast Asia through to Australia. Typically growing to 60–75 cm tall and wide, it is a bushy, woody-based evergreen perennial, widely grown for the highly decorative variegated leaves found in cultivated varieties. Another common name is painted nettle, reflecting its relationship to deadnettles, which are in the same family. The synonyms Coleus blumei, Coleus scutellarioidesand Solenostemon scutellarioides are also widely used for this species The pattern, shape, and color of this leaf are varied, but the medicinal merit is a brownish red leaf[1-2]. Coleus has the composition of chemical compounds useful include: flavonoids, tannins, saponins, rosmarinic acid, caffeic acid, gallic acid, quercetin, and p-coumaric acid, alkaloids, ethyl acetate, methyl eugenol, eugenol, thymol, phenol, carvacrol, phytosterols and minerals[3-5]. The use of *C. scutellarioides*leaf empirically in the community generally in the form of fresh and boiled. Leaves of C. scutellarioides have many uses such as for the medicine of ulcers, obses, ulcers, ulcers, ear and eye inflammation, while the roots are used for diarrhea and heartburn[7,8].

According to Middleton and Kandaswami[9], flavonoids play an important role in plant biochemistry and physiology, such as antioxidants, enzyme inhibitors, and precursors for toxic components. According to Johnson[10], flavonoids are very effectively used as antioxidants. Groups of flavonoids that have antioxidant activity include flavones, flavonols, flavonones, isoflavones, catechins and kalkon [11]. In recent years there has been an increased interest in getting natural antioxidants. Studies show phenolic compounds such as flavonoids have radical catcher antioxidant activity[12-13]. Polyphenol compounds such as flavonoids are able to inhibit oxidation reactions through radical scavenging by donating an electron to unpaired electrons in free radicals so that the amount of free radicals becomes reduced[14].

There are several ways of determining the antioxidant activity that is often used in vitro, the method of 1,1-diphenyl-2-picrilhidrazil (DPPH), thiocyanate, xanthine oxidase, and deoxyribose[14-19]. The DPPH method provides the reactivity information of a compound with a stable radical. DPPH provides strong absorption at 517 nm wavelength with dark violet color. The free radical catcher causes the electrons to be paired which then causes the color removal proportional to the number of electrons taken[20]. This article reports on the antioxidant activity of water extract of *C. scutellarioides* leaf with DPPH method (1,1-diphenyl-2-picrilhidrazil).

MATERIALS AND METHODS

Plant material: Plant material used in this study obtained from Flower Plantation, Lembang, West Java and was determined in Taxonomy laboratory, Biology Department, Faculty Mathematics and Natural Sciences, Universitas Padjadjaran. The collection and treatment of simplicia followed the method of Mustarichie et.al [21] in which the fresh sample was collected in sufficient quantities (~ 10 kg) at a time. The sample was washed thoroughly with running tap

water, followed by rinsing with distilled water and then each part was cut into small pieces and powdered. They were dried (~ 30 °C) without sun, at open area with active ventilation until they attained constant weight (around one month).

Extraction

Extraction method refers to Herbal Pharmacopoeia Indonesia [22].

Ethanol extract: The chopped mixture was weighed and extracted by maceration for 3 x 24 hours with solvent replacement every 1 x 24 hours using 70% ethanol solvent. The macerate was then evaporated using a rotary evaporator and then followed by using a water bath (50^0C).

Water extract: The chopped simplicia was extracted by boiling with water in a container for 30 minutes at 90 °C. then filtered hot. The juice of the decoctation was concentrated by freeze drying as it impossible to use water bath.

Phytochemical screening

Phytochemical screening based on a modification of the Mustarichie et.al method[16,21] based on the Farnsworth method[23] on water extract to determine the presence of secondary metabolite compounds such as alkaloid compounds, polyphenols, flavonoids, saponins, tannins, quinones , steroids, triterpenoids, monoterpenoids and sesquiterpenoids.

Thin Layer Chromatography (TLC)

TLC plates were cut with a size of 10x1 cm. The bottom was marked 1 cm to bottle the test sample and the top 1 cm as a boundary mark. The extract was bottled on the lower bound of the plate. The chamber was prepared, then a solution of n-hexane:ethyl acetate (7:3) was added to the ethanol extract and n-butanol:glacial acetic acid:water (4:1:5) for the water extract. The developer solution was allowed to saturate. Plates put in a chamber that has been saturated, silenced until there was development to the upper limit. The observed spots were marked, and the plates were sprayed with DPPH patches. The color and spots that appear were observed.

Antioxidant Activity Test

The antioxidant activity of leaf of *C. scutellarioides* and vitamin C was done by spectrophotometric method using DPPH reagent which was a modification of Mustarichie *et.al* method[16].

The value of absorbance obtained calculated percent inhibition by using the equation:

$$\%\ Inhibition = \left[1 - \frac{A_{sample}}{A_{DPPH}}\right] x\ 100$$

Notes: A_{DPPH} = absorbance DPPH control

A_{sampel} = absorbance sample

% inhibition = percentage capcity inhibition of free radicals

From the value of % inhibition, a linear regression curve between concentration and percent of inhibition was obtained, so that a linear regression equation was obtained. The concentration value of 50% (IC_{50}) of the extract was obtained by substituting the value of y with a value of 50.

RESULTS AND DISCUSSION

Collection, Processing, and Determination of simplicia: The results of determination showed that the plants used in the study were *C. scutellarioides*) included into the Lamiaceae tribe.

Preparation of Water extract: The extraction method used was decoctation by boiling in a container with water for 30 minutes at 90 °C and then filtered hot. The juice of the decoctation was concentrated by freeze drying. Retrieved yield of 6.6% W/W.

Phytochemical Screening: Phytochemical screening was performed on ethanol and

water extracts of all test plants to determine the presence of secondary group compounds of metabolites in the plant. The results of phytochemical screening are shown in Table 1.

Tabel 1. Phytochemical screening of C. scutellarioides

Metabolite group	Ethanol extract	Water extract
Alkaloids	-	-
Polyphenol	+	+
Tannin	-	-
Flavonoids	+	+
Monoterpenoid and Sesquiterpenoid	-	-
Steroids	-	-
Triterpenoids	-	-
Saponins	+	+
Quinone	-	-

Notes: (−) = not detected, (+) = detcted

The results of phytochemical screening showed that the antioxidant compounds contained in the water extract of *C. scutellarioides* leaf were secondary metabolites of flavonoids and polyphenols. Ethanol extract and water extract gave positive result for flavonoids and polyphenols which activity as antioxidant while positive result on saponins because of C. scutellarioidesleaf besides containing antioxidant compound in the form of flavonoids and polyphenols, Jawer kotok leaf also contains compound saponins[6]. These results differ from those reported by Bole and Jayashree[24] who reported finding alkaloids by using the Wgners test and tannins using Gelatin test. Laurente *et.al*[25]. The Philippine species as Lagerstroemia speciosa (Lythraceae), *Syzygium cumini* (Myrtaceae), Plectranthus amboinicus (Fam Lamiaceae), Jasminum sambac (Fam Oleaceae) Punica granatum *(Punicaceae), Apium graveolens* Linn. (Apiaceae), Carmona retusa (Boraginaceae), Coleus scutellarioides(Lamiaceae), *Senna alata* (Fabaceae), *Orthosiphone aristatus* (Lamiaceae), *Leucaena leucocephala* (Fabaceae), *Morinda citrifolia* (Rubiaceae), *Andrographis paniculata* (Acanthaceae), and *Peperomia pellucida* Piperaceae), contained alkaloids, saponins and tannins. Alkaloids, terpenoids, cardiac glycosides, saponins, tannins and flavonoid was found in the species of *Plectranthus amboinicus*[26]. Lisdawati *et.al*[27] did not find alkaloids, saponins and steroids in their *C.scutellarioides* growth in Indonesian North Sulawesi. The difference in phytochemical screening finding most likely due to different species, different location and different parts of plant used.

Thin Layer Chromatography (TLC)

The thin layer chromatography profile of water extracts of *C. scutellarioides* was seen using silica gel GF 254 plate, n-hexane:ethyl acetate (7:3) developer for ethanol extract, while for water extract using n-butanol:acetic acid glacial (BAA):water (4:1), then observed in visible light, UV light 254 nm, 366 nm, and DPPH solution spray. Visible patches can be seen in Figure 1.

I II III IV

Notes : I. Visible light, II. UV 254 nm, III. UV 366 nm
IV. DPPH solution spray

From the TLC results found that ethanol extract had more spots that was able to reduce free radicals compared with water extracts. TLC in many was only used to find out how many compounds contained in the tested extract, unless there was a reference substance[29].

Antioxidant Activity Test

1. Determination of the maximum wavelength of DPPH: From the results of the study it was found that the maximum wavelength of DPPH was 517 nm.
2. Determination of DPPH Operating Time: Determination of DPPH operating time in ethanol aims to determine the best or stable working time of DPPH solution.

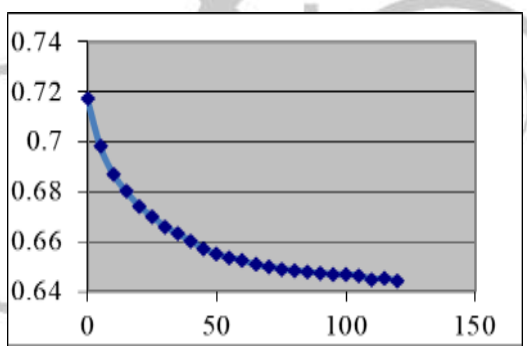

Figure 2. Operating time curve of DPPH solution in ethanol

From the results of the study obtained DPPH absorbance was stable in the range 65-80 minutes and the analysis should be done on the operating time range.

3. Sample Concentration Orientation: The results of concentration orientation can be seen in Table 2.

Table 2. Orientation concentration results

No	Extract	Variation of concentration (µg/mL)
1	Water	50,100,150,200,250
2	Vitamin C	2, 4, 6, 8, 10

Antioxidant Activity Test

Measurements were made by mixing 3 ml of 40 µg/mL DPPH solution and 2 mL of test solution. The controls used were 3 mLs of DPPH ppm and 2 mL ethanol. As the blanks of testing used 2 mL solution test and 3 mL of ethanol. Vitamin C was used as a comparison solution. Parameter

result of interpretation of antioxidant activity testing method with DPPH was IC_{50} or Inhibition concentration 50 that was concentration where sample able to damp DPPH activity equal to 50% from initial concentration. The value of IC_{50} was obtained by using the linear regression equation for the water extract.

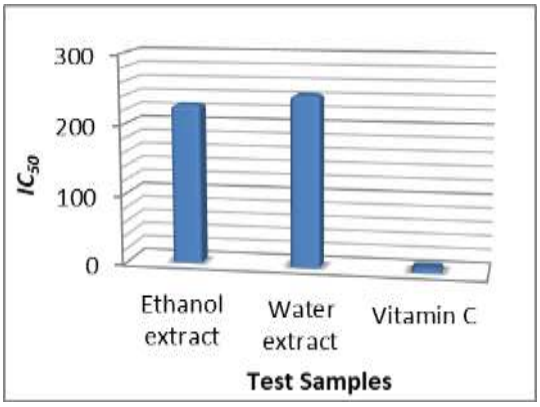

Figure 3. Diagram of IC_{50} samples

The IC_{50} value chart of the test sample and vitamin C can be seen in Figure 3. The test results showed IC_{50} values for each ethanol extract, water extract, and vitamin C were 227.84 µg/mL and 244.42 µg/mL. Water extract provided a higher free radical damping activity with an IC_{50} value of 244.42 µg/mL that was 33.62 times lower than vitamin C. From the diagram obtained IC_{50} ethanol extract ethanol and water extract were almost the same, the antioxidant activity, IC_{50} of *C. scutelllarioides* leaf about 200 µg/mL. Based on IC_{50} the antioxidant properties of a compound were grouped into four parts: very strong (below 50 µg/mL), strong (50-100 µg/mL), moderate (100-150 µg/mL), and weak (above 150 µg/mL)[16, 28,30].

The small amount of antioxidant activity provided by the water extract could also be caused at the time of extraction, the ethanol solvent was better to attract secondary metabolites, the water was too polar to attract secondary metabolite compounds or because of the possibility of the active compound being damaged or lost during heating.

CONCLUSION

Based on the research results it could be concluded that water extract had antioxidant activity with ability to capture free radical from DPPH. The water extract belong to a weak antioxidant class because the IC_{50} value was more than 150 µg/mL. However, the antioxidant activity of water extract was of 244,42 µg/mL.

REFERENCES

1. Dalimartha S. 2000. Atlas of Indonesia Plant (*Atlas Tumbuhan Indonesia)*.Vol.2. Jakarta:Trubus Agriwidya.
2. Departemen Kesehatan. 2000. Coleus blumei Benth. Available at: http//:bebas.vlsm.orgv12artikelttg_tanaman_obat/depkesbuku22-072.pdf.
3. Thomas ANS. T Traditional Medicinal Plants 2 (*Tanaman Obat Tradisional 2)*, Yogyakarta: Kanisius , 1992
4. Bhatt P, Joseph GS, Negi PS, and Varadaraj MC, Chemical Composition and Nutraceutical Potential of Indian Borage (Plectranthus amboinicus) Stem Extract, *Journal of Chemistry* 2013; Volume 2013 (2013), Article ID 320329, 7 pages
5. Winarto WP. Indonesian Medicinal Plant for Herbal Medicine (*Tanaman Obat Indonesia untuk Pengobatan Herba*) Volume I. Jakarta: Karyasari Herba Media. 2007

6. Heryana S. Preliminary examination of the chemical content of iler leaf Coleus antropurpureus Benth. (*Pemeriksaan pendahuluan kandungan kimia daun iler (Coleus antropurpureus Benth)*. Thesis. Fakultas Matematika dan Ilmu Pengetahuan Alam. Universitas Padjadjaran. 1987
7. Wijakusuma HMH, Wirian AS, Yaputra T, Dalimartha S, Wibow B. Medicinal Plants in Indonesia (*Tanaman Berkhasiat Obat di Indonesia*) Volume 4. Jakarta: Pustaka Kartini. 1996
8. Rice LJ, Brits GJ, Potgieterc CJ, Van Staden J. Plectranthus: A plant for the future?, *South African Journal of Botany* 2011; 77(4): 947-959
9. Middleton E and Kandaswani C. The Impact of Plant flavonoids on Mammalian Biology: Implication for Immunity, Inflammation, and Cancer. Di dalam Harborne, J. B. (ed.). The Flavonoids. Chapman and Hall. London. 1994
10. Johnson IT. Antioxidative and Antitumors Properties. In : Pokorny, J., M. Yanishileva, M. Gordon. CRC Press, Cambridge. 2001
11. Markham KR. How to Identify Flavonoids (*Cara Mengidentifikasi Flavonoid*). Translated by K. Padmawinata. Bandung: Penerbit ITB. 1998.
12. Cos P, Calomme M, Sindambiwe JB, Bruyne TD, Cimanga K, et.al. Cytotoxicity and Lipid Peroxidation-Inhibiting Activity of Flavonoids, *Planta Med*. 2001; 67: 515-519.
13. Gulcin I, Uguz MT, Oktay M, Beydemir S, and Kufrevioglu O.I,. Evaluation of the Antioxidant and Antimicrobial Activities of Clary Sage (Salvia sclarea L.), *Turk I. Agric.For.,* 2004; 28: 25-33.
14. Pokorni J, Yanishlieva N, and Gordon M. Antioxidant in Food; Practical Applications, CRC Press, New York. 2001.
15. Sharma S. In vitro evaluation of antioxidant activity of methanolic and petroleum ether extracts from seeds of Benincasa hispida. *J Nat Plant Resour.* 2014;4(4):31-4.
16. Mustarichie R, Runadi D, Ramdhani D. The antioxidant activity and phytochemical screening of ethanol extract, fractions of water, ethyl acetate, and n-hexane from mistletoe tea (Surrula atropurpurea BL. Dans), *Asian J Pharm Clin Res*, 2017; 10(2): 343-347
17. Hanani E, Mun'im A, Sekarini R, dan Wiryowidagdo S. Antioxidant Activity Test Some Sea Sponges from the Thousand Islands (*Uji Aktivitas Antioksidan Beberapa Spons Laut dari Kepulauan Seribu*), *Jurnal Bahan Alam Indonesia* 2006; 6:1-3.
18. Atun S. Activity Test of Some Oligoresveratrol Compounds Result of Isolation From Planta Bark Skin Hopea odorata As a Prevention of 2-Deoxyribose Degradation (*Uji Aktivitas Beberapa Senyawa Oligoresveratrol Hasil Isolasi Dari Kulit Batang Tumbuhan Hopea odorata Sebagai Pencegah Degradasi 2-Deoksiribosa*). *Jurnal Penelitian Saintek.* 2010; 13(1)
19. Atun S. Relationship of Structure and Antioxidant Activity of Some Compounds Resveratrol and Derivatives (*Hubungan Struktur dan Aktivitas Antioksidan Beberapa Senyawa Resveratrol dan Turunannya)*. Yogyakarta: UNY; 2010.
20. Sunardi IK. Antioxidant Activity Test of Wuluh Belimbing Extract (Averrhoa bilimbi, L) to DPPH (*Uji Aktivitas Antioksidan Ekstrak Belimbing Wuluh (Averrhoa bilimbi, L) terhadap DPPH)*.Available at: http://p3m.amikom.ac.id/p3m .
21. Mustarichie R, Warya S, Saptarini NM, Musfiroh I. Acute and Subchronic Toxicities of Indonesian Mistletoes Dendrophthoe pentandra L.(Miq.) Ethanol Extract, *Journal of Applied Pharmaceutical Science* 2016; 6(09): 109-11
22. Departement Kesehatan RI. Indonesian Herbal Pharmacopea, Edisi I. Jakarta: Departement Kesehtaan RI; 2015. p. 17-8.
23. Farnsworth NR. Biological and phytochemical screening of plant. *J Pharm Sci* 1966;55(3):243-69.

24. Bole S, Jayashree K. Phytochemical screening and biological activities of medicinal plant Coleus aromaticus, *World Journal of Pharmacy and Pharmaceutical Sciences* 2014; 3(6): 974-986
25. Laurente MR, Adegoke JA, Ademakinwa EAA, Afable JCC, Aguam DH, *et.al.* Qualitative Phytochemical Screening of Selected Medicinal Plant Species of the Philippines, *JAASP* 2017; 6(1): 10-14
26. Sathasivam A, Elangovan K. Evaluation of phytochemical and antibacterial activity of Plectranthus amboinicus, *IJRAP* 2011; 2(1): 292-294
27. Lisdawati V, Mutiatikum D, Alegantina S, Astuti Y. Characterization of Miana leaves (Coleus scutellarioides(L.) Bth.) And betel fruit (Piper betle L.) are physically chemical from local antimalarial herbs of northern Sulawesi *(Karakterisasi daun Miana (Coleus scutellarioides (L.) Bth.) dan buah sirih (Piper betle L.) secara fisiko kimia dari ramuan lokal antimalarial daerah Sulawesi utara, , Media Litbang Kesehatan* 2008; 18(4): 213-225
28. Hihlati, I, Abdul Rohman dan Triana Hertiani. Antioxidant Power of Ethanol Extract Rimpang Temu Kunci [Boesenbergia pandurata (Roxb.) Schlecth] With DPPH Radical Capture Method (1,1-diphenyl-2-picrylhydrazyl) *(Daya Antioksidan Ekstrak Etanol Rimpang Temu Kunci [Boesenbergia pandurata (Roxb.) Schlecth] Dengan Metode Penangkapan Radikal DPPH(1,1-difenil-2-pikrilhidrazil)*. *Jurnal Fakultas Farmasi UGM*. 2011.
29. Mutiatikum D, Alegantina S and Astuti Y. Standardization of simplicia from the fruit of Miana (Plectranthus Seutellaroides (L) R.Btlz) originating from 3 growing places of Menado, Kupang and Papua *(Standarisasi simplisia dari buah Miana (Plectranthus Seutellaroides (L) R.Btlz) yang berasal dari 3 tempat tumbuh Menado, Kupang dan Papua)*, *Bul. Penelit. Kesehat*, 2010; 38(1): 1-16
30. Kuete V and Efferth T.Cameeroonian medicinal plants: Pharmacology and derived natural products. *Frontiers Pharm* 2010; 1: 123.