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Antioxidant activity and phytochemical screening of *Plectranthus scutellarioides* L. leaves ethanol and water extracts by DPPH method.

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ABSTRACT

Jawer Kotok leaf (Plectranthus scutellarioides) is commonly used as an ornamental plant or medicinal plant. The pattern, shape, and color of this 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. This study aims to determine the antioxidant activity of ethanol extract and water extract of P. scutellarioides leaf using DPPH method with a comparative standard of vitamin C. Leaves of Jawer Kotok obtained from Balitro, West Java and determined in Biology Department FMIPA Universitas Padjadjaran. Leaves of P. scutellarioides was macerated with ethanol and water and concentrated with the rotary evaporator and freeze dryer, respectively. The concentrated extract was analyzed by UV-spectrophotometer to determine its antioxidant properties. From phytochemical screening, it was found that P. scutellarioides contained flavonoid, saponin, and polyphenols. This study obtained extracts that had higher antioxidant activity was ethanol extract because it had a smaller IC50 value (IC_{50} 227,84 µg/mL) than water extract (IC_{50} 244,42 µg/mL). Both the ethanol extract and the water extract from P.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: Jawer kotok, flavonoid, antioxidant, DPPH, Plectranthus scutellarioides

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INTRODUCTION

Jawer kotok (Indonesian) (*Plectranthus scutellarioides* [L.] R.Br) 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. The pattern, shape, and color of this leaf are varied, but the medicinal merit is a brownish red leaf[1-2]. Jawer kotok 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 P. scutellarioides leaf empirically in the community generally in the form of fresh and boiled. Leaves of P. scutellarioides have many uses such as for the medicine of ulcers, obsess, 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, flavanones, 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-picrylhydrazyl (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 ethanol extract and water extract of P. scutellarioides leaf with DPPH method (1,1-diphenyl-2-picrylhydrazyl).

MATERIALS AND METHODS

Plant material

Plant material used in this study, namely Jawer Kotok leaf obtained from Experimental Garden of Research Institute of Medicinal and Aromatic Plants (BALITRO) located in Manoko, District 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 an open area with active ventilation until they attained constant weight (around three weeks).

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 decoction was concentrated by freeze-drying as it impossible to use a 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 ethanol extract and 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 is bottled on the lower bound of the plate. The chamber was prepared, then a solution of n-hexane: ethyl acetate (7:3) is 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 Jawer Kotok and vitamin C was done by spectrophotometric method using DPPH reagent which is a modification of Mustarichie *et.al* method[16]

1. Determination of the maximum wavelength of DPPH: 3 mL of 40 µg / mL DPPH solution was dissolved in 2 ml of ethanol incorporated into the chocolate vial, then the absorbance was seen at λmax 450-600 nm.
2. Determination of DPPH Operating Time: 3 mL of 40 µg / mL DPPH solution was dissolved in 2 mL of ethanol inserted into the chocolate vial, then the absorbance was observed at λmax 450-600 nm for 120 min at intervals of 5 min. DPPH operating time was obtained when the DPPH solution absorbance was stable.
3. The orientation of Sample Concentration: Each test sample was prepared in certain concentrations and DPPH was then prepared with a concentration of 40 µg/mL. Into the vials were inserted a sample solution and DPPH with a ratio of 2:3 and idle during the operating time. Then measured the absorbance.
4. Antioxidant Activity Test: 2 mL test solution of various concentrations plus 3 mL DPPH solution was inserted into the chocolate vial, homogenized, then incubated during the operating time. Absorbance measured on λmax did triple. Vitamin C is used as a comparison solution.

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

The collection, Processing, and Determination of simplicia: The results of determination showed that the plants used in the study were Jawer Kotok (Plectranthus scutellarioides) included into the Lamiaceae tribe.

Preparation of ethanol extract: The method of extraction was maceration with 70% ethanol solvent for 3x24 hours with solvent replacement every 1x24 hours. The yield was 9.37% W/W

Water extract: The extraction method used was decoction by boiling in a container with water for 30 minutes at 90 °C and then filtered hot. The juice of the decoction was concentrated by freeze drying. The yield was 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 results of P. scutellarioides

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

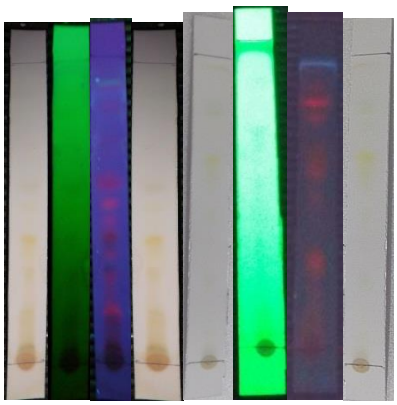
Notes: (−) = not detected, (+) = detected

The results of phytochemical screening showed that the antioxidant compounds contained in the ethanol extract and water extract of Jawer kotok leaf were secondary metabolites of flavonoids and polyphenols. Ethanol extract and water extract gave the positive result for flavonoids and polyphenols which act as an antioxidant while positive result on saponins because of P. scutellarioides leaf beside containing an 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 Wagners 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), Plectranthus scutellarioides (Lamiaceae), Senna alata (Fabaceae), Orthosiphon 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 Plectranthus scutellarioides growth in North Sulawesi. The difference in phytochemical screening finding most likely due to different species, different location and different parts of the plant used.

Thin Layer Chromatography (TLC)

The thin layer chromatography profile of ethanol and water extracts of P. 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.

Fig 1.



I II III IV I II III IV

Ethanol extract **Water extract**

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 were 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.

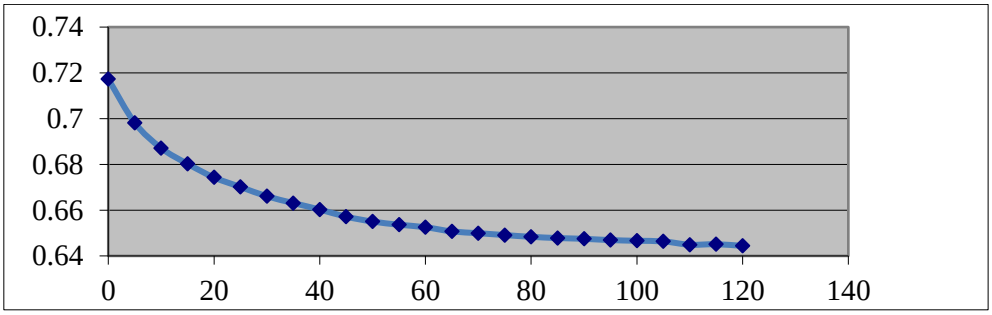


Figure 2. Operating time curve of DPPH solution in ethanol

From the results of the study obtained DPPH absorbance was stable in the range 65-80 minutes and the analysis should be done in 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	Ethanol	50,100,150,200,250
2	Water	50,100,150,200,250
3	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 mL 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 each extract.

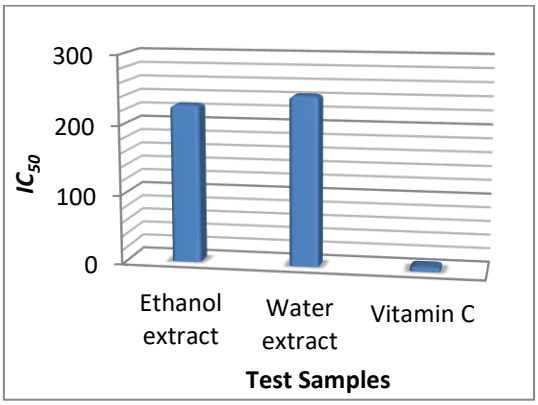


Figure 3. Diagram of IC_{50} samples

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. The lowest IC_{50} value was owned by ethanol extract which showed that ethanol extract had DPPH free radical damping activity which was stronger than water extract, but 31.33 times weaker than vitamin C with the IC_{50} value of 7.27 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 was almost the same, the antioxidant activity, IC_{50} of P. 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 ethanol extract and water extract had antioxidant activity with the ability to capture free radical from DPPH. The ethanol extract and water extract both belong to a weak antioxidant class because the IC_{50} value was more than 150 µg/mL. However, the antioxidant activity of ethanol extract was higher than water extract because it had a smaller IC_{50} value of 227,84 µg/mL compared with water extract of 244,42 µg/mL.

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