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Original Article

DETERMINATION OF QUALITY PARAMETERS AND TEST ANTIOXIDANT ACTIVITIES OF 70% ETHANOL EXTRACT OF SEROJA LEAVES (*NELUMBO NUCIFERA* GAERTN.)

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ABSTRACT

Objective: The purpose of this study was to evaluated the quality parameters and analyzed the antioxidant activity of seroja leaves *Nelumbo nucifera* Gaertn.

Methods: The quantification of the chemical compound was determined by its total phenol and flavonoid levels. The evaluate the antioxidant activity was determined by the comparability of the four common radical scavenging assays using 2,2̀ -azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS); 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical; cupric ion reducing antioxidant capacity (CUPRAC); ferric reducing antioxidant power (FRAP); and 2-thiobarbituric acid (TBA) methods.

Results: The results of phytochemical screening for simplicia powder and 70% ethanol extract of seroja leaves contain secondary metabolites of alkaloids, flavonoids, saponins, tannins, coumarin, quinones, and triterpenoid steroids. The results of the determination of the quality parameters meet the requirements of quality and safety standard of the medicinal herb. The result of the determination of total phenol content from 70% ethanol extract of Seroja leaves was 181.62±0.82 mg GAE/g extract. The results of the determination of total flavonoid levels from 70% ethanol extract of seroja leaves amounted to 289.83±1.04 mg QE/g extract. The results of antioxidant activity tests using the ABTS, DPPH, and TBA methods showed IC_{50} respectively 287.7 mg/l, 22.3 mg/l, and 352.6 mg/l and CUPRAC and FRAP methods had an antioxidant capacity of 160.76±0.35 and 253.36±0.48 mg AAE/g extract.

Conclusion: Seroja leaves (*Nelumbo nucifera* Gaertn.) have the potential to be used as an antioxidant medicinal herb and its extract meet the standard of quality control and safety.

Keywords: Seroja (*Nelumbo nucifera* Gaertn.), Antioxidants, ABTS, CUPRAC, DPPH, FRAP, TBA

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INTRODUCTION

Currently, the use of antioxidants from natural ingredients to ward off free radicals is becoming a popular research material in the world of health. Free radicals are molecular atoms that have unpaired electrons in their outer orbitals so their reactivity is very high. An increase in the amount of free radicals in the body can cause oxidative stress, which according to some studies if it occurs continuously, can cause degenerative diseases. Antioxidants are compounds that can inhibit the oxidation process of free radicals by the mechanism of action, namely donating one of the hydrogen atoms (H) or protons to free radical compounds so that free radical compounds can be more stable. According to several studies that have been done, the use of synthetic antioxidants in the long term can potentially cause carcinogenic [1]. The use of natural ingredients for treatment tends to increase with the people's thinking back to nature and the belief that the use of natural materials has minimum side effects. Lotus plants (*Nelumbo nucifera* Gaertn.) better known as swamp plants which are found in Indonesia have many benefits, one of which is as an antioxidant [2, 3].

MATERIALS AND METHODS

Plant material

The material used for the study was the leaves of the lotus (*Nelumbo nucifera* Gaertn.) obtained from the Bogor Institute of Spice and Medicinal Research (BALITRO), Bogor, West Java, Indonesia. The plant determination of the leaves of Seroja (*Nelumbo nucifera* Gaertn.) is carried out with the aim of macroscopically identifying the truth of the plant to be used. Determination was carried out at the Botanical Gardens Conservation Center, Bogor, West Java, Indonesia (No: B-542/IPH.3/KS/III/2019).

Chemical and reagent

neocuproin (2,9-dimethyl-1,10-phenanthroline), Copper(II) chloride dihydrate ($CuCl_2$), ammonium acetate, DPPH (1,1-diphenyl-2-picrylhydrazyl), TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), sodium acetate trihydrate, Fe (III) chlorida hexahydrate ($FeCl_3$), gallic acid, sodium carbonate (Na_2CO_3), Folin-Ciocalteu, methanol pro analysis, ethanol pro analysis, ascorbic acid, ammonium hydroxide 30%, chloroform, HCl, amyl alcohol, H_2SO_4, CH_3COOH anhydrate, petroleum eter, sodium hydroxide 1 N, ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), quersetin, potassium asetate, potassium persulfate, Thiobarbituric acid (TBA), phosphate buffer pH 7, linoleic acid, trichloroacetate (TCA), acetate buffer pH 3.6 were purchased from Sigma-Aldrich (Merck KGaA, Missouri, United States). Dragendorff reagent, Mayer reagent, Libermann-Buchard reagent, magnesium, and Gelatin solution 1% were purchased from Q-Lab laboratory (Pancasila University, Jakarta, Indonesia).

The extraction of *Nelumbo nucifera* gaertn

Simplicia of seroja leaf powder was extracted by kinetic maceration using 70% ethanol solvent. The filtrate is filtered with filter paper coated with cotton to separate the pulp and filtrate. Remaceration was then concentrated using a vacuum rotary evaporator to obtain 70% ethanol viscous extract [4, 5].

Phytochemical screening

Preliminary examinations of secondary metabolite compounds on 70% ethanol extracts of the leaves of the lotus leaf included identification of the alkaloid, flavonoid, saponin, tannin, quinone, triterpenoid steroid, coumarin, and essential oil groups [6].

Determination of extract quality parameters

Determination of the quality parameters of 70% ethanol extract of seroja leaves includes specific parameters: organoleptic test (appearance, color, smell and taste) and determination of the percentage of dissolved compounds in certain solvents. The non-specific parameters: determination of ash content, drying losses, water content, heavy metal contamination, microbial contamination, and residual solvents [4].

Determination of total phenol levels

0.4 ml of 70% ethanol extract of Seroja leaves added 0.4 ml of the Folin Ciocalteu reagent (allowed to stand for 3 min in a dark place), 4 ml of 7.5% Na_2CO_3, and aquadest added to 10.0 ml. The solution is allowed to stand for 70 min at room temperature. The absorption was measured with a UV-Vis spectrophotometer at a wavelength of 759.5 nm. The total phenol content of the 70% ethanol extract of the seroja leaf is expressed in mg Gallic Acid Equivalent/gram extract (mg GAE/g) [7].

Determination of total flavonoid levels

1.0 ml of 70% ethanol extract of seroja leaves were added with 3 ml of methanol, 200 µl of potassium acetate, 200 µL of $AlCl_3$ (aluminum chloride), and aquadest was added to 10.0 ml. The solution is allowed to stand for 30 min at room temperature. The absorption was measured with a UV-Vis spectrophotometer at a wavelength of 371.0 nm. The total flavonoid content of 70% ethanol extract of seroja leaves is expressed in mg Quersetin Equivalent/gram extract (mg QE/g) [7].

Vitamin C standard solution

Testing the antioxidant activity of the extract with the ABTS, DPPH, and TBA methods using vitamin C as a positive control. Antioxidant activity is determined from the IC_{50} value. Measurement of the antioxidant capacity of the extract by the CUPRAC and FRAP methods uses vitamin C as a standard curve whose results are expressed in mg Ascorbic Acid Equivalent/gram extract (mg AAE/g).

Antioxidant activity test ABTS method

The test was carried out by piping a number of 70% ethanol extract sample stock solution of 1000 mg/l lotus leaves, then adding 1.0 ml of ABTS then 5.0 ml of volume was sufficient with ethanol. The solution is allowed to stand for 30 min at room temperature. The absorption was measured with a UV-Vis spectrophotometer at a wavelength of 412.0 nm. Antioxidant activity is determined from the IC_{50} value [3, 7, 8].

Antioxidant activity test CUPRAC method

As much as 0.5 ml of 70% ethanol extract of seroja leaves were added 1 ml of $CuCl_2.2H_2O$ solution, 1 ml of neokuproin solution, 1 ml of NH_4 Ac buffer solution pH 7, and 4.1 ml volume was sufficient with aquadest. The solution is allowed to stand for 30 min at room temperature. The absorption was measured with a UV-Vis spectrophotometer at a wavelength of 453.5 nm. The antioxidant capacity of 70% ethanol extract of seroja leaves is expressed in mg Ascorbic Acid Equivalent/gram extract (mg AAE/g) [9, 10].

Antioxidant activity test DPPH method

The test is carried out by piping a number of 70% ethanol extract sample stock solution of 1000 mg/l leaves, then adding 1.0 ml DPPH 0.4 mmol and then 5.0 ml of methanol are sufficient. The solution is allowed to stand for 30 min at room temperature. The absorption was measured with a UV-Vis spectrophotometer at a wavelength of 516.5 nm. Antioxidant activity is determined from the IC_{50} value [7, 9, 11].

Antioxidant activity test FRAP method

1.0 ml of 70% ethanol extract of seroja leaves was added with 3.0 ml of aquadest solution, 3.0 ml of FRAP solution. FRAP solution was prepared by mixing 25 ml of acetate buffer pH 3.6, 2.5 ml of TPTZ solution, and 2.5 iron (III) chloride hexahydrate solution. The solution is allowed to stand for 30 min at room temperature. The absorption was measured with a UV-Vis spectrophotometer at a wavelength of 595.0 nm. The antioxidant capacity of 70% ethanol extract of seroja leaves is expressed in mg Ascorbic Acid Equivalent/gram extract (mg AAE/g) [7, 9, 12].

Antioxidant activity test TBA method

The test was carried out by piping a number of 70% ethanol extract sample stock solution of 1000 mg/l leaves, then adding 2.0 ml of 0.1 M phosphate buffer pH 7 and 2 ml of 50 mmol linoleic acid. The solution is allowed to stand for 30 min at room temperature. The absorption was measured with a UV-Vis spectrophotometer at a wavelength of 532.0 nm. Antioxidant activity is determined from the IC_{50} value [3, 8].

RESULTS AND DISCUSSION

Phytochemical screening

Simplisia powder and 70% ethanol extract of leaves of the seroja leaves were analyzed phytochemically to determine the content of the samples used.

Table 1: Phytochemical screening results of lotus leaves

No.	Group identification	Results
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannins	+
4.	Saponins	+
5.	Quinones	+
6.	Steroids/triterpenoids	+/+
7.	Coumarins	+
8.	Essential oil	-

(+) contains secondary metabolites; (-) does not contain secondary metabolites

Organoleptic determination

Organoleptic determination aims to determine the identity of the initial introduction to the extract that can be observed visually.

Determination of compounds dissolved in certain solvents

Determination of dissolved compounds in certain solvents aims to determine the amount of secondary methanolite dissolved in water and ethanol solvents.

Table 2: The result of organoleptic extract determination

No.	Extract identity	Result
1.	Shape	Thick extract
2.	Color	Blackish green
3.	Smell	Odorless
4.	Taste	Weak

Table 3: The result of determining the level of dissolved compounds in certain solvents

No.	Determination	Results
1.	Water soluble compounds	7.59%
2.	Ethanol soluble compounds	7.85%

Based on the results above, there are more secondary metabolites found in ethanol solvents than those found in water solvents. This result was obtained due to the use of 70% ethanol as a solvent which is a universal solvent that can attract compounds of non-polar, semi-polar, and polar properties.

Determination of non-specific quality parameters of the extract

Determination of non-specific quality parameters aims to determine the quality of extracts that have standards and safe limits of extracts as a safe and quality natural material product.

Table 4: The results of determining non-specific quality parameters

Parameters	Results
Loss on drying	4.97%
Water content	5.28%
Total ash content	6.32%
Water soluble ash content	5.13%
Ash content not acid solubility	1.17%
Residual solvent	0.54%
Lead metal contamination	0.3684 mg/kg
Cadmium metal contamination	0.1748 mg/kg
Total Plate Count	Not detected
Yeast And Mold Plate Count	Not detected

Table 5: The results of the determination of total phenol and flavonoid levels

Content determination	Results
Phenol	181.62±0.82 mg GAE/g extract
Flavonoid	289.83±1.04 mg QE/g extract

The data was given in mean+SD, n=3

Determination of total phenol and flavonoid levels

Determination of total phenol and flavonoid levels was carried out to determine the amount of compounds contained in the extract using the colorimetric principle. Determination of total phenol and flavonoid levels in the ethanol extract of 70% of Seroja leaves by the UV-VIS spectrophotometer method [13].

Antioxidant activity test

Testing the antioxidant activity with the ABTS method based on the ability of the neutralization of the sample to the amount of free radicals in the ABTS solution, this process is characterized by the fading color of the ABTS solution from yellow to purple whose absorbance can be measured using a UV-Vis spectrophotometer [3, 7].

Testing of antioxidant activity by DPPH method based on the reaction of hydrogen capture by DPPH radicals from antioxidants, this process is characterized by the fading color of DPPH solution from purple to yellow whose absorbance can be measured using a UV-Vis spectrophotometer [11, 14, 15].

Testing of antioxidant activity by the TBA method is based on the reaction that occurs between 1 MDA molecule and 2 TBA molecules to produce a pink MDA-TBA complex compound. At low pH and high temperatures, the malondialdehyde-TBA bond will change to red MDA-TBA, whose absorption can be measured using a UV-Vis spectrophotometer [3, 7].

Determination of the antioxidant activity of 70% ethanol extract of Seroja leaves by the ABTS, DPPH and TBA methods can be determined from the IC_{50} value, namely the concentration of antioxidants that can inhibit 50% of free radicals. IC_{50} values were obtained from linear regression equations by entering extract concentration data as x values and% inhibition data as Y values [7]. The result of IC_{50} can be seen in fig. 1.

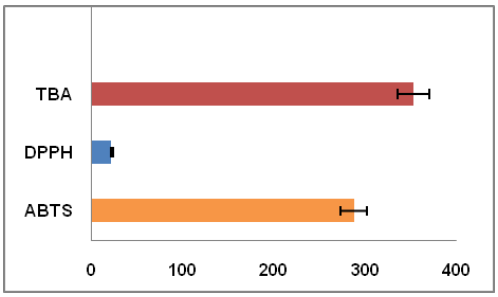

Fig. 1: Antioxidant activity test results (IC_{50})

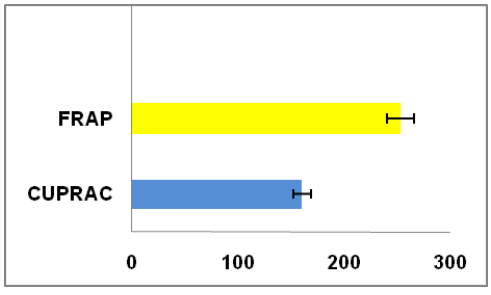

Fig. 2: Antioxidant activity test results (mg AAE/g)

Testing the antioxidant activity by the CUPRAC method based on the principle of the electron transfer mechanism by forming the bisneokuproin-copper(II) ($Cu(Nc)2^{2+}$) complex will oxidize the antioxidant compounds in plant extracts then undergo reduction to form the complex bisneokuproin-copper(I) ($Cu(Nc)_2^{+}$). Blue turquoise

$(Cu(Nc)_2^{2+})$ reagents will be reduced to yellow $(Cu(Nc)_2^{+})$ whose absorbance can be measured using a UV-Vis spectrophotometer [9, 10].

Testing of antioxidant activity by the FRAP method is based on the principle of the electron transfer mechanism by reducing the Fe^{3+}complex from tripiridiltriazine $Fe(TPTZ)^{3+}$to the Fe^{2+}complex. $Fe(TPTZ)^{2+}$will be intensely blue by antioxidants in an acidic atmosphere where absorbance can be measured using a UV-Vis spectrophotometer [9, 12].

Determination of the antioxidant capacity of 70% ethanol extract of Seroja leaves by the CUPRAC and FRAP methods is expressed in mg Ascorbic Acid Equivalent/gram extract (mg AAE/g). The antioxidant capacity of CUPRAC and FRAP methods can be seen in fig. 2.

Based on the research that has been done, the results of testing the activity and antioxidant capacity of 70% ethanol extract of Seroja leaves by the ABTS, CUPRAC, DPPH, FRAP and TBA methods can be seen in table 6.

Table 6: Antioxidant activity test results

No.	Test	Results
1.	ABTS (IC_{50})	287.7 mg/l
2.	CUPRAC	160.76±0.35 mg AAE/g extract
3.	DPPH (IC_{50})	22.26 mg/l
4.	FRAP	253.36±0.48 mg AAE/g extract
5.	TBA (IC_{50})	352.6 mg/l
6.	DPPH Vitamin C (IC_{50})	3.76 mg/l

The data was given in mean+SD, n=3

CONCLUSION

The results of phytochemical screening for simplicia powder and 70% ethanol extract of Seroja leaves contain secondary metabolites of alkaloids, flavonoids, saponins, tannins, coumarin, quinones, and triterpenoid steroids. The results of determining the quality parameters of the extract obtained percentage of dissolved compounds in water by 7.59%, dissolved compounds in ethanol 7.85%, drying losses 4.97%, moisture content 5.28%, total ash content 6.32%, content acid insoluble ash 1.17%, water soluble ash content 5.13%, lead metal contamination 0.3684 mg/kg, cadmium metal contamination 0.1748 mg/kg, residual solvent 0.54%, and microbial contaminants that meet the requirements. The result of the determination of total phenol content from 70% ethanol extract of Seroja leaves was 181.62±0.82 mg GAE/g extract. The results of the determination of total flavonoid levels from 70% ethanol extract of Seroja leaves amounted to 289.83±1.04 mg QE/g extract. The results of antioxidant activity tests using the ABTS, DPPH, and TBA methods showed IC_{50} respectively 287.7 mg/l, 22.3 mg/l, and 352.6 mg/l and CUPRAC and FRAP methods had antioxidant capacity of 160.76±0.35 and 253.36±0.48 mg AAE/g extract. Seroja leaves (*Nelumbo nucifera* Gaertn.) have the potential to be used as an antioxidant medicinal herb and its extract meet the standard of quality control and safety.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

1. Wojcik M, Burzynska Pedziwiatr I, Wozniak LA. A review of natural and synthetic antioxidants important for health and longevity. Curr Med Chem 2010;17:3262–88.
2. Jarmkom K, Techaoei S, Wisidsri N, Khobjai W. Evaluation of acetylcholinesterase activity and cytotoxicity of different parts of *Nelumbo nucifera* Gaertn on human neuroblastoma cell line (SH-SY5Y). Int J Appl Pharm 2019;11:103-5.
3. Salmiyah S, Ainunnisa A, Nurmilasari N, Handayani E, Firdaus F. Identification of organic compounds from extract lotus seeds (*Nelumbo nucifera*). Indonesia Chim Act 2019;10:19-24.
4. Zheng LJ, Wu YB, Wu JG, Tan CJ, Yi J, Chen TQ, *et al.* Antioxidant activity of lotus (*Nelumbo nucifera* Gaertn.) receptacles of eleven cultivars grown in China. J Med Plants Res 2012;6:1902–11.
5. Djamil R, Rahmat D, Zaidan S, Latifah MN. Anticholesterol activity of okra fruit extract (*Abelmoschus esculentus* (L) Moench) and its nanoemulsion *in vivo*. Pharmacogn J 2020;12:316–20.
6. Farnsworth NR. Biological and phytochemical screening of plants. J Pharm Sci 1966;55:225–76.
7. Apak R, Gorinstein S, Böhm V, Schaich KM, Ozyurek M, Guclu K. Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). Pure Appl Chem 2013;85:957-98.
8. Moharram HA, MM Youssef. Methods for determining the antioxidant activity. Alex J Fd Sci Technol 2014;11:31-9.
9. Choirunnisa AR, Fidrianny I, Ruslan K. Comparison of five antioxidant assays for estimating antioxidant capacity from three *Solanum sp.* extracts. Asian J Pharm Clin Res 2016;9:123-8.
10. Apak R, Guclu K, Demirata B, Ozyurek M, Celik SE, Bektaşoglu B, *et al.* Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules 2007;12:1496–547.
11. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 2004;26:211–9.
12. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996;239:70–6.
13. Limwachiranon J, Huang H, Shi Z, Li L, Luo Z. Lotus flavonoids and phenolic acids: health promotion and safe consumption dosages. Compr Rev Food Sci Food Saf 2018;17:458–71.
14. Djamil R, Anelia T. Penapisan fitokimia, uji BSLT, dan uji antioksidan ekstrak metanol beberapa spesies papilionaceae. J Ilmu Kefarmasian Indones 2009;7:65-71.
15. Djamil R, Wahyudi PS, Wahono S, Hanafi M. Antioxidant activity of flavonoid from *Anredera cordifolia* (Ten) steen is leaves. Int Res J Pharm 2012;3:241-3.