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 evaluate 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 2,2'-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 289.38±1.04 mg QE/g extract. The results of antioxidant activity tests using the ABTS, DPPH, and TBA methods showed IC₅₀ 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

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 become 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 (CuCl₂), ammonium acetate, DPPH (1,1-diphenyl-2-picrylhydrazyl), TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), sodium acetate trihydrate, Fe (III) chloride hexahydrate (FeCl₃), gallic acid, sodium carbonate (Na₂CO₃), Folin-Ciocalteu, methanol pro analysis, ethanol pro analysis, ascorbic acid, ammonium hydroxide 30%, chloroform, HCl, amyl alcohol, H₂SO₄, CH₃COOH anhydrate, petroleum ether, 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, microbical contamination, and residual solvents [4].

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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% Na2CO3, and aquadest added to 1.0 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 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].

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 AlCl3 (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 Quercetin 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 IC50 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 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 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) [7, 9, 11].

Antioxidant activity test TBA method

As much as 0.5 ml of 70% ethanol extract of Seroja leaves were added 1 ml of CuCl2·2H2O solution, 1 ml of neocuproin solution, 1 ml of NH4 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 532.0 nm. The antioxidant capacity of 70% ethanol extract of Seroja leaves is expressed in mg Ascorbic Acid Equivalent/gram extract (mg AAE/g) [7, 10].

Determinant of compounds dissolved in certain solvents

Organoleptic determination

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

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

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 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].

Testing the antioxidant activity by the CUPRAC method based on the principle of the electron transfer mechanism by forming the bisneokuproin-copper(I) (Cu(Nc)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⁺) reagents will be reduced to yellow (Cu(Nc)⁺) 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 Fe³⁺-complex from tris(2,2′-bipyridyl)triazine Fe(TPTZ)²⁺ to the Fe²⁺-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 (IC₅₀)| 287.7 mg/l                       |
| 2.  | CUPRAC    | 160.76±0.35 mg AAE/g extract     |
| 3.  | DPPH (IC₅₀)| 22.26 mg/l                       |
| 4.  | FRAP      | 253.36±0.48 mg AAE/g extract     |
| 5.  | TBA (IC₅₀)| 352.6 mg/l                       |
| 6.  | DPPH Vitamin C (IC₅₀)| 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 0.1740 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 IC₅₀ 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.

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