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Research Article

Determination of Total Flavonoid Levels and Antioxidant Activity from Ethanol Extracts of Stone Parasite (*Begonia* sp.) with DPPH Method (2,2-diphenyl-1-picrylhydrazyl)

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Abstract

Stone Parasite (*Begonia* sp.) has long been known by the people of Kabaena, Bombana Regency, Southeast Sulawesi, a plant known as a natural herb because of its ability to treat various diseases. People use this plant for the treatment of tumors and cancer. Stone Parasite contains secondary metabolites including saponins, tannins, flavonoids and polyphenols. Flavonoids are one of the largest natural phenolic group antioxidants found in all plants. This study aims to determine the levels of flavonoid compounds and test the antioxidant activity of ethanol extract of Stone Parasite using UV-Vis spectrophotometry. Extraction was carried out by maceration method using ethanol 96% with a yield of 14.8%. The results of the determination of the Stone Parasite water content was 2.6%. The determination of the total Flavonoid content of the ethanol extract of Stone Parasite was determined based on the absorbance value measured at a wavelength of 437 nm using a quercetin comparison. The results of the determination of total flavonoid levels were 16.68 mg QE/gr. The results of the antioxidant activity test on Stone parasite extract at a wavelength of 517 nm showed an IC_{50} value of 6.7357µg/ml. Stone Parasit ethanol extract has a very strong antioxidant activity with the DPPH method

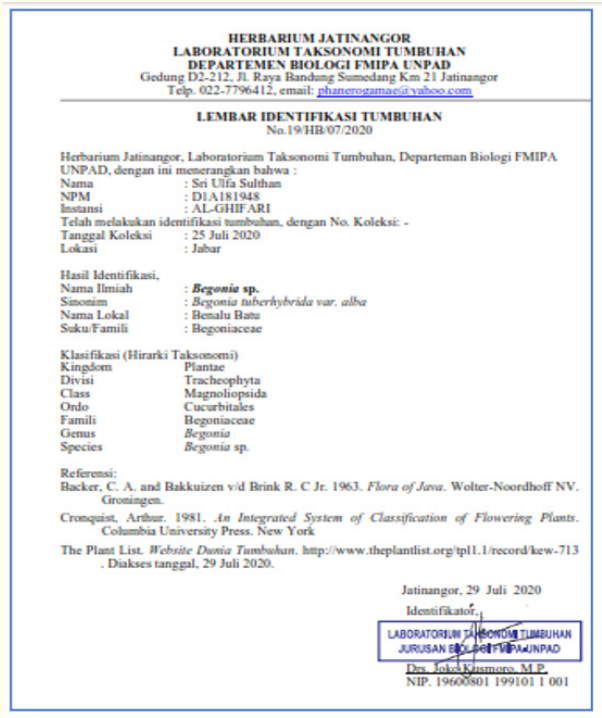
Keywords: Stone Parasite; Total Flavonoids; Antioxidants; DPPH

Introduction

Stone parasite plant (*Begonia* sp.) (Figure 1) has long been known by the people of Kabaena, Bombana Regency, Southeast Sulawesi as pokkokajang-kajang known as a natural herbal plant because of its ability to treat various diseases. Stone parasite (*Begonia* sp.) is an important component as an ingredient of this traditional medicine in Indonesia. People use this plant for the treatment of tumors, cancer [1].

The results of the phytochemical screening test for the methanol extract of stone parasite were found to be positive for flavonoid compounds [2]. Flavonoids are one of the largest natural phenolic antioxidant compounds and are found in all plants, so it is certain that there are flavonoids in every study of plant extracts [3]. A number of medicinal plants containing flavonoids have antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, and anticancer activities [4].

Free radicals are one form of reactive oxygen compounds, which are known as compounds that have unpaired electrons. Free radicals in the body are very reactive and will interact destructively through the oxidation process with the inside of the body and certain cells that are composed of fat, protein, carbohydrates, DNA, and RNA so that they can trigger various diseases such as coronary heart disease, premature aging and cancer. Therefore, antioxidants are needed to overcome free radicals [5]. Antioxidants work by donating one electron to compounds that are oxidants so that the activity of these oxidant compounds can



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Nama : Sri Ulfa Sulthan
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Telah melakukan identifikasi tumbuhan, dengan No. Koleksi: -
Tanggal Koleksi : 25 Juli 2020
Lokasi : Jabar

Hasil Identifikasi,
Nama Ilmiah : ***Begonia* sp.**
Sinonim : *Begonia tuberhybrida var. alba*
Nama Lokal : Benalu Batu
Suku/Famili : Begoniaceae

Klasifikasi (Hirarki Taksonomi)
Kingdom Plantae
Divisi Tracheophyta
Class Magnoliopsida
Ordo Cucurbitales
Famili Begoniaceae
Genus *Begonia*
Species *Begonia* sp.

Referensi:
Backer, C. A. and Bakkuizen v/d Brink R. C Jr. 1963. *Flora of Java*. Wolter-Noordhoff NV. Groningen.
Cronquist, Arthur. 1981. *An Integrated System of Classification of Flowering Plants*. Columbia University Press. New York
The Plant List. *Website Dunia Tumbuhan*. http://www.theplantlist.org/tpl1.1/record/kew-713 . Diakses tanggal, 29 Juli 2020.

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Figure 1: Result of determination.

be inhibited. Chemically, antioxidants are compounds that donate electrons (electron donors), and biologically, the definition of antioxidants are compounds that can counteract or reduce the negative effects of oxidants [6].

One of the methods used to test the antioxidant activity is the DPPH method (2,2-diphenyl-1-picrylhydrazyl). The DPPH method is easy to use, fast, fairly thorough and good for use in organic solvents.

UV-Vis spectrophotometry is an analytical technique using a visible light radiation source (380-800 nm) with a spectrophotometer instrument. This method is a simple, easy method and has a fairly high level of accuracy [7].

Experiment

Materials

Erlenmeyer, analytical balance, brown bottle, knife, spatel, watch glass, porcelain cup,volumetric flask, funnel, beaker, measuring cup, drop pipette, volume pipette, oven, evaporator, bath water, test tube, moisture balance, UV –Vis spectrophotometer.

Ethanol 96%, $AlCl_3$, quercetin, HCL, acetic acid, DPPH, Ascorbic acid, aluminum foil, filter paper, distilled water, H_2SO_4, Dragendroff's reagent, Mayer's reagent, Mg, NaCl 10%, $FeCl_3$.

Preparation of sample

Stone parasite obtained from Kabaena Island, Bombana Regency, Southeast Sulawesi. A total of 1 kg of parasite stone samples were cleaned of dirt, washed with running water, drained and then cut into small pieces. Stone parasite is dried and then put into a container.

Extraction

150 grams of stone parasite coarse powder was extracted using 96% ethanol solvent by maceration method for 3 x 24 hours protected from light while stirring occasionally, then filtered using filter paper to obtain the filtrate. Then evaporated using a Rotary Vacuum Evaporator at a temperature of 40°C, obtained a thick extract and then evaporated using a water bath at a temperature of < 50°C to obtain a concentrated extract of stone parasite as much as 22,2 g with a yield of 14.8%.

Phytochemical screening

Phytochemical screening is a preliminary stage that can provide an overview of the content of certain compounds in natural materials to be studied [8]. Screening for alkaloids, flavonoids, tannins and saponins was carried out.

Total flavonoid content (TFC)

Total flavonoid content with quercetin as a comparison, 15 mg of extract, dissolved in 10 mL of ethanol, in order to obtain a concentration of 1500 ppm. From this solution, 1 mL of pipette was added, 1 mL of 2% $AlCl_3$ solution and 1 mL of 120 mM potassium acetate were added. Samples were incubated for one hour at room temperature. The absorbance was determined using UV-Vis spectrophotometry at a maximum wavelength of 437 nm [9]. The total flavonoid content was figured as g quercetin equivalent per 100 g extract.

DPPH scavenging activity

Preparation of DPPH solution by mixing 4 mg of DPPH with 96% ethanol in a 100 mL volumetric flask to obtain a concentration of 100 ppm. Then the absorption was measured at a wavelength of 400-600 nm using UV-Vis spectrophotometry.

20 mg of ethanol extract of stone parasites was dissolved with ethanol in a 10 mL volumetric flask and then centrifuged. Various concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm were made, homogenized and incubated for 30 minutes in a dark place at 37°C. The absorbance was measured at a wavelength of 517 nm using UV-Vis spectrophotometry. The IC_{50} value is calculated using the linear regression equation formula.

Results and Discussion

- Plant determination was done in Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA) Universitas Padjadjaran Jatinangor and stated that the plants used were stone parasite (*Begonia* sp.) (Figure 1).
- Characterization of simplicia exhibited that water content 2,6% and loss drying 16,68%.
- Results pumpkin seed extraction 22,2 g with a yield of 14,8%.
- The chemical screening was performed in extracts to find out the presence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins in extracts. The results of the phytochemical screening can be seen in table 1.
- The total flavonoid test results were calculated in mg QE/100g and the total flavonoid content of the ethanol extract of stone parasite was 16,92 mg QE/100g.
- Measurement of antioxidants using the DPPH method was characterized by a purple to yellow color change after being incubated for 30 minutes. The DPPH method was chosen because it is simple, easy, fast and sensitive and requires a small sample to determine the antioxidant activity of natural compounds [10]. Ascorbic acid was used as a comparison because it has very strong antioxidant properties. The IC_{50} calculation results for Ascorbic acid is 1,211 µg/mL exhibit very strong antioxidant activity and ethanol extract of stone parasite is 6,731 µg/mL, exhibit very strong antioxidant activity.
- The standard linear regression curve of inhibition of antioxidant activity of Ascorbic Acid and ethanol extract of stone parasite using DPPH can be seen in figures 3 and 4.

Figure 2: Stone Parasite (Begonia sp.).

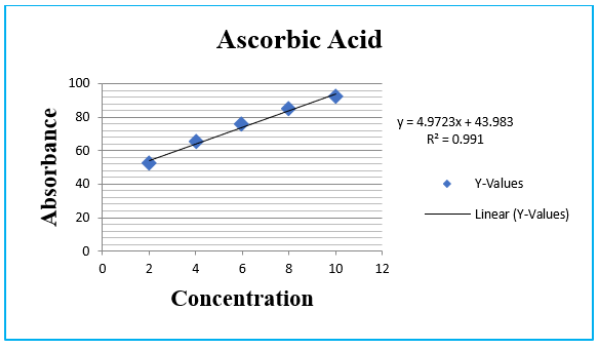

Figure 3: Standard Curve of Linear Regression % Inhibition of Antioxidant Activity of Vitamin C with DPPH.

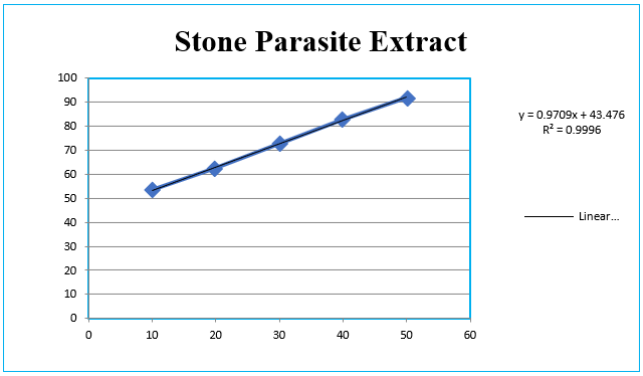

Figure 4: Standard Curve of Linear Regression % Antioxidant Activity Stone Parasite Extract with DPPH.

Secondary metabolites	Solvent	Extract
Alkaloid	Dragendroff	+
	Mayer	+
Flavonoid	Magnesium	+
Tannin	$FeCl_3$	+
Saponin	HCl	+

Table 1: Chemical screening of extract.
(+) = detected.

Conclusion

The total flavonoid content of the ethanol extract of stone parasite (*Begonia* sp.) was 16,92 mg QE/100g and IC_{50} antioxidant activity 6,731 µg/mL, indicating a very strong antioxidant activity using the DPPH method.

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