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Research Article

# Pharmacognostic Characteristics and Antioxidant Activity of Gendola Stem (*Basella Rubra* L.) Ethanol Extract from South Kalimantan

**Arnida Arnida*, Dian Kurnia, Sutomo Sutomo**

## ABSTRACT

**Background**: Gendola (*Basella rubra L.*) is a medicinal plant native to South Kalimantan. Therefore, this research aims to determine the antioxidant activity of *B. rubra* and the specific as well as nonspecific parameters of its ethanolic stem extract through pharmacognostic tests. **Objective**: The specific parameters comprise of the extract composition, phytochemical screening, TLC profile, organoleptic, and microscopic variables. Meanwhile, drying shrinkage and ash content were the non-specific parameters. **Materials and Methods**: The Indonesian Herbal Pharmacopoeia was used as a reference for the pharmacognostic test method. Also, the antioxidant activity was determined through the DPPH method, which was based on the $IC_{50}$ value. **Results**: A tasteless, brownish-purple powder with a characteristic smell, was obtained from the simple organoleptic assay while the epidermis, cortex, endodermis, pith, xylem, phloem, cambium, cell walls, stoma, epidermal, guard, and neighboring cells were observed through microscopic examinations. The *B. rubra* stem contains phenolic compounds, flavonoids, steroids, tannins, and saponins. A good TLC profile was shown by the eluents of n-hexane: ethyl acetate (3:7) and chloroform: methanol (9:1). The *B. rubra* simplicia stem had a water- and ethanol-soluble extract, drying shrinkage, total ash, and acid insoluble ash contents of 16.433% ± 0.252, 10.5% ± 0.173, 8.467% ± 0.153, 6.5% ± 0.1, and 0.517% ± 0.115, respectively. **Conclusion:** Moreover, the pharmacognostic test results were acceptable. The *B. rubra* stem ethanol extract had an antioxidant activity of 344,096 ppm based on the $IC_{50}$ value.

**Key words**: *Basella rubra L.,* Gendola, Stem, Pharmacognostic, Antioxidant.

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## INTRODUCTION

Indonesia is a country rich in biodiversity, with the majority of plants used as traditional medicines by the community to prevent and treat diseases. *Basella rubra L.* (Gendola) is an example of these medicinal plants and is indigenous to South Kalimantan. The leaves and fruits of this vegetable are the most used parts[1] while the stem has been investigated to have antimicrobial activity. Furthermore, the ethanol extract of this stem is known to contain carbohydrates, tannins, terpenes, steroids, and saponins. These contents are responsible for inhibiting the activity of microorganisms, such as *Staphylococcus aureus*, *Escherichia col*i, *Candida albican*s, and *Trichophyton rubrum*.[2] The ethanolic and methanolic extracts of the leaves and fruit were found to have a significant antioxidant activity with Inhibitory Concentration 50 ($IC_{50}$) values of 84.70 ppm for the leaf and 35.20 ppm for the fruit.[3,4]

Pharmacognostic tests were conducted to determine the identity and characteristics of the medicinal plant ingredients through a series of parameters that ensure quality, safety, and prevent counterfeiting.[5] Meanwhile, the antioxidant activity assay on natural ingredients was performed to discover new sources of natural antioxidants. The DPPH (1,1-diphenyl-2-picrylhydrazyl) method is a common type of this test, which was chosen because it is easy, fast, simple, sensitive, and requires a small sample.[6] Also, this parameter was measured based on the $IC_{50}$ value.

According to the previous description, this is noteworthy research on the pharmacognostic tests and antioxidant activity of the ethanol extract of South Kalimantan *B. rubra* stems. The pharmacognostic tests were conducted to determine the stem's characteristics, while the antioxidant activity assays were performed to discover natural sources of antioxidants. This research is expected to add to the information regarding the *B. rubra* plant and serve as data for further investigation.

## MATERIAL AND METHODS

The materials used included ethanol extract B rubra rod, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ammonia ($NH_3$), aqua dest ($H_2O$), dilute hydrochloric acid (HCl), iron (III) chloride ($FeC_{l3}$), and 96% technical ethanol ($C_2H_5OH$). Ethyl acetate pa, 1% gelatin, filter paper, chloroform ($CH_3Cl$), quercetin, silica gel plate $GF_{254}$, methanol pa ($CH_3OH$), n-hexane pa, Dragendorff reagent, Liebermann-Burchard reagent, and Mayer reagent were also utilized. Tools, such as furnace (Ney-Vulcan D-550) for farmakognostic assay, UV-Vis spectrophotometer (Spectronic Genesys 10 uv) for antioxidant activity Assay,

### Simplicia processing and preparation of *B. rubra* ethanol stem extract

The wet *B. rubra* stems were obtained from the yard of a Guntung Manggis Village house, South Kalimantan Province, and then sorted, washed, and chopped. Subsequently, these stems were dried using

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a drying cabinet at a temperature of 55°C and then sorted, as well as mashed with the aid of a blender. A total of 167.90 grams of simplicia powder was extracted through the maceration method using 96% ethanol. This process was conducted for 7x24 hours (re-maceration) by changing the solvent hourly and stirring every 8 hours. The liquid extract of the stems was thickened with a water bath at a temperature of 50°C until a constant weight was obtained.[7]

## Pharmacognostic test of *B. rubra* stem

Furthermore, the Pharmacognostic tests consisted of specific and non-specific parameters. The specific parameter tests conducted in this research comprised of the organoleptic testing of Simplicia, microscopic cross-sectional, longitudinal, and paradermal stems analysis, phytochemical screening of the plant and its extracts, extract TLC profile, water-soluble, and ethanol-soluble extract contents. Meanwhile, the non-specific parameters included the determination of drying shrinkage, total ash composition, and acid insoluble ash content of Simplicia.

### *Qualitative antioxidant activity test*

The thin-layer chromatography (TLC) plates were sprayed with the DPPH specific reagent after being washed with the appropriate eluent. Subsequently, the formation of yellow TLC spots on a purple background indicates a positive antioxidant result.[8]

## Quantitative antioxidant activity test

Determination of the maximum DPPH wavelength: Determination is carried out 0.5 mL of 0.4 mM DPPH solution was reacted with 2 mL of methanol p.a. and then allowed to stand for 30 minutes in a dark environment. Subsequently, the solution's absorbance was read at a wavelength of 450-550 nm.[9]

Operational timing: Determination is carried out 0.5 mL of 0.4 mM DPPH solution was reacted with 2 mL of 6 ppm quercetin. The absorbance of the mixture was then read at the maximum DPPH wavelength at an interval of 2 minutes for an hour.[8]

Determination of the $IC_{50}$ value of quercetin comparison solution: Quercetin mother liquor was formulated with a concentration of 100 ppm and then diluted into a series solution of 2, 3, 4, 5, and 6 ppm. Subsequently, 2 mL per concentration series solution was collected and reacted with 0.5 mL of 0.4 mM DPPH solution. The solution was then placed in a dark environment for an operational time before the absorbance was measured at the maximum DPPH wavelength.[10]

Determination of $IC_{50}$ value of B. rubra stem ethanol extract: The extract solution was formulated with a concentration of 500 ppm and then diluted to 40, 80, 120, and 160 ppm. Consequently, 2 mL of each extract solution was reacted with 0.5 mL of 0.4 mM DPPH. The solution was then left for an operational time in a dark environment and the absorbance read at the maximum DPPH wavelength.[10]

# RESULTS

## Plant determination

Furthermore, the Center for Plant Conservation of the Botanical Gardens – LIPI conducted the plant determination analysis, which revealed gendola as part of the Basellaceae family with the species name *Basella rubra* L (Figure 1), specimen number B-3550/IPH.3/KS/X/2018.

## Simplicia processing and ethanol extract B. *rubra* stem making

The *B. rubra* stems were collected from the house yard, which is a home cultivation media of the *B. rubra* plant with medicinal properties. Also, 254.98 grams of the Simplicia stem powder was obtained while a total of 167.90g was extracted by the maceration method using 96% ethanol. This process was chosen because it is a safe method to test samples that have not been exposed to heat. Moreover, 96% ethanol was used to attract secondary metabolites that are polar, semi, or non-polar. A dark green-black ethanolic extract of the *B. rubra* stems was obtained with a weight and percent yield of 28.1 grams and16.736%, respectively.

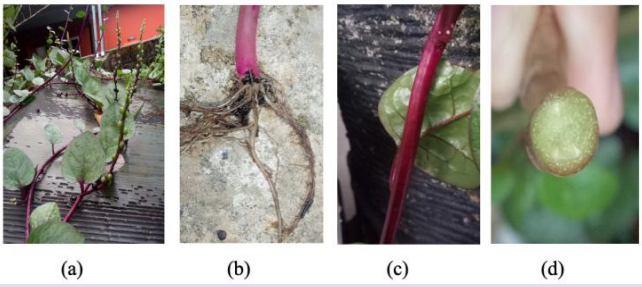


**Figure 1:** *Basella rubra* plant; (a) whole parts; (b) root; (c) stems; (d) stem slices (Personal documentation, 2019).

## Pharmacognostic test of B. *rubra* stems

According to the organoleptic examination, the *B. rubra* stem Simplicia powder is tasteless, brownish-purple, with a characteristic odor. This sample was originally reddish-purple, however, the color changed due to the drying process. Also, the distinctive odor produced may be influenced by the presence of volatile oil compounds.[11]

The *B. rubra* stems were observed in cross, longitudinal, and paradermal sections (Figure 2). Consequently, the epidermis, cortex, endodermis, pith, xylem, phloem, and cambium were visible in cross-section, while the longitudinal section only showed the epidermis, cortex, endodermis, and pith. The paradermal cross-section illustrated the presence of cell walls, stoma, guard, epidermal, and neighboring cells. Moreover, the epidermal cells are polyglonal in shape with parasitic stomata.

Phytochemical screening was conducted on simplicia powder samples and B. *rubra* stems ethanol extract. These samples were tested for color using the appropriate reagents to determine the class of compounds. Table 1 shows that both variables were positive for phenolic compounds, flavonoids, steroids, saponins, and tannins.

The eluents of *n*-hexane: ethyl acetate (3:7) and chloroform: methanol (9:1) display the thin-layer chromatography (TLC) profile of the *B. rubra* stems ethanolic extract, which produced good separation. However, Table 2 illustrates the separation for the chloroform eluent: methanol (5:5) was inadequate. The ability of the compound components in the sample to separate was influenced by the polarity of the eluent. Furthermore, fluorescent spots were only visible on a 254 nm and not on a 366 nm UV lamp.

Extract content testing was performed to provide an initial description of the number of compounds that can be extracted with certain solvents.[12] According to the testing levels of *B. rubra* simplicia stem extract, the compounds in this plant are more concentrated in water solvents, which are more polar than in less polar ethanol.

The drying shrinkage was measured to obtain an overview of the compounds lost due to the 105 °C drying process. This value was influenced by the simplicia drying process, which aimed to remove water from fresh samples. Consequently, a value greater than 10% indicates that the water content in simplicia is higher (Tabel 3). Therefore, this condition can result in a microbe-dense plant and trigger enzymatic processes that convert chemical compounds into pharmacologically inactive products.[13,14]

Furthermore, the purpose of testing the simplicia ash content was to obtain an overview of its internal and external mineral composition.[15] This

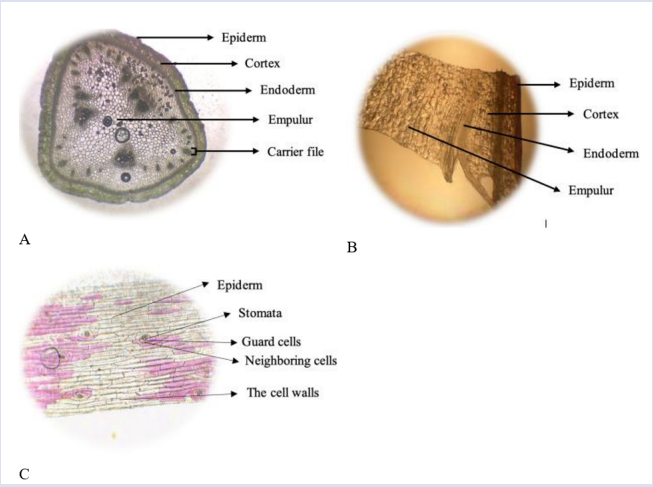


**Figure 2:** Microscopic results of *B. rubra* L. stem; (A) Transverse cross -section; (B) Longitudinal cross -section; (C) Paradermal cross section (4x10 magnification).

**Table 1: Results of phytochemical screening of *B. rubra* stems.**

| Number | Phytochemical Compounds | Test Results | |
|---|---|---|---|
| | | Simplicia Powder | Ethanolic Extract |
| 1 | Phenolic | + | + |
| 2 | Flavonoids | + | + |
| 3 | Steroids | + | + |
| 4 | Saponins | + | + |
| 5 | Alkaloids<br>a. Mayer's reagent<br>b. Dragendorff's reagent | <br>-<br>- | <br>-<br>- |
| 6 | Tannins | + | + |

Note: + is available, and – is not available

**Table 2: Rf value of *B. rubra* stems ethanol extract.**

| Number | $R_f$ Value on eluent | | | Information |
|---|---|---|---|---|
| | n-hexane: ethyl acetate (3:7) | chloroform: methanol (5:5) | chloroform: methanol (9:1) | |
| 1 | 0,98 | 0,8 | 0,96 | stationary phase: Silica gel $GF_{254}$ spot viewer: UV 254 nm |
| 2 | 0,9 | | 0,88 | |
| 3 | 0,6 | | 0,68 | |
| 4 | 0,54 | | 0,52 | |
| 5 | 0,3 | | 0,46 | |
| 6 | | | 0,34 | |

value is influenced by plant cultivation and simplicia drying factors.[16,17] The acid-insoluble ash content shows the cleanliness level of simplicia processing, which is indicated by the presence of insoluble mineral or metal contamination, such as soil, sand, dirt, metal elements Ag, Pb, and Hb.[18,19]

### *Qualitative antioxidant activity test*

The antioxidant activity was assessed qualitatively as a basis for conducting the quantitative tests. Also, yellow TLC spots were produced on the stem ethanol extract of *B. rubra* after spraying with DPPH on a purple background. This indicates the presence of antioxidant activity in the sample.[20]

### Quantitative antioxidant activity test

Determination of the DPPH maximum wavelength was conducted to establish the wavelength with the highest sensitivity. Consequently, a value of 516 nm was obtained from the analysis. However, the operational period was ascertained to acquire the optimal stability time of the test compound during a reaction. This was indicated by a stable absorbance with an operational time between the 24th and 52nd minute.

Quercetin is a flavonoid compound with strong antioxidant activity, which was used as a positive control or comparison. The results of the linear regression equation between the quercetin concentration and the inhibition percentage were y = 14.4903x + 2.99 with a correlation coefficient (r) of 0.999, hence, the $IC_{50}$ value of linear regression is 3.244 ppm. However, Table 4 shows the $IC_{50}$ probit SPSS quercetin value is 4.189 ppm. According to the results, this compound has very strong antioxidant activity because its value was below 50 ppm.[21]

## DISCUSSION / CONCLUSION

The antioxidant activity of the ethanolic stem extract of *B. rubra* was determined quantitatively using the $IC_{50}$ value. The results of the linear regression equation between the extract concentration and the inhibition percentage were y = 0.14042x -1.682 with a correlation coefficient (r) of 0.989, hence, the linear regression IC50 value is 344.096 ppm. However, Table 5 shows that the $IC_{50}$ value of the probit SPSS stem ethanol extract of *B. rubra* is 406.909 ppm. The difference in the resulting $IC_{50}$ values was influenced by the lack of sample data available for analysis. Based on the value obtained, the ethanolic extract of *B. rubra* stems has antioxidant activity with a value above 200 ppm.[21] The absorbance response data and percent inhibition used to determine the $IC_{50}$ value of the ethanolic stem extract of *B. rubra* are yet to be in the positive control response range.

The pharmacognostic tests results of *B. rubra* stem specific parameters, which include organoleptic and microscopic tests, determination of extract levels, phytochemical screening, and TLC profiles met the requirements of the Indonesian Herbal Pharmacopoeia and Materia

**Table 3: Results of pharmacognostic tests of *B. rubra* stem simplicia powder.**

| Number | Assaying | Results | Requirements (MMI & FHI) |
|---|---|---|---|
| 1 | Water soluble essence | 16,433% ± 0,252 | ≥16% |
| 2 | Ethanol soluble extract content | 10,5% ± 0,173 | ≥8% |
| 3 | Drying shrink | 8,467% ± 0,153 | <10% |
| 4 | Total ash content | 6,5% ± 0,1 | <16,6% |
| 5 | Acid insoluble ash content | 0,517% ± 0,115 | <2% |

**Table 4: IC50 value of quercetin comparison solution.**

| Concentration (ppm) | % Inhibition | $IC_{50}$ Linear Regression | $IC_{50}$ Probit |
|---|---|---|---|
| 2 | 32,219% | 3,244 ppm | 4,189 ppm |
| 3 | 45,287% | | |
| 4 | 61,697% | | |
| 5 | 76,478% | | |
| 6 | 89,075% | | |

**Table 5: IC50 Value of ethanol extract of B. rubra stem.**

| Concentration (ppm) | % Inhibition | $IC_{50}$ Linear Regression | $IC_{50}$ Probit |
|---|---|---|---|
| 40 | 4,393% | 344,096 ppm | 406,909 ppm |
| 80 | 9,626% | | |
| 120 | 13,645% | | |
| 160 | 21,776% | | |

Medika Indonesia. Also, the non-specific parameters, including determination of drying shrinkage and ash content were acceptable. The ethanolic extract of *B. rubra* stem was also recognized to exhibit a relatively poor antioxidant activity with a linear regression IC50 value of 344.096 ppm.

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## AUTHOR CONTRIBUTIONS

Concept – A.A., D.K., S.S.; Design – A.A., D.K.; Supervision – S.S.; Resources – S.S.; Materials: A.A., D.K., S.S.; Data Collection and/or Processing – A.A., D.K., S.S.; Analysis and/or Interpretation – A.A., D.K., S.S.; Literature Search – A.A., D.K., S.S.; Writing – A.A., D.K., S.S.; Critical Reviewers – A.A., S.S.

## CONFLICTS OF INTEREST STATEMENT

The authors declared no conflicts of interest in the manuscript.

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