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***Research paper***

# Total Phenolic Test and Antioxidant Activity of Bajakah Stem Extract (*Spatholobus littoralis Hassk.*)

# Uji Total Fenolik dan Aktivitas Antioksidan Ekstrak Batang Bajakah (*Spatholobus littoralis* Hassk.)

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## ABSTRACT

Diseases in the human body can be caused by free radicals. Environmental conditions and unhealthy lifestyles cause the human body's defense system against free radicals to become weaker, so it is necessary to have external antioxidants to help ward off free radicals. Therefore, it is necessary to have natural antioxidants from plants in overcoming free radicals. This study aims to determine the levels of phenolic contained in the ethanol extract of bajakah stems and their potential bioactivity as antioxidant. Determination of phenolic content was carried out using the Folin-Ciocalteau method and antioxidant testing was carried out using the DPPH method. The total phenolic test results obtained were 21.535 mg EAG/g. The antioxidant test of the ethanol extract of the bajakah stem showed weak activity with an average $IC_{50}$ value of 281.717 g/mL.

**Keywords:** *Bajakah stem, phenolic, antioxidant, Spatholobus littoralis Hassk.*

## INTRODUCTION

Free radicals are molecules that have unpaired electrons so they are reactive. Diseases caused by free radicals are degenerative diseases such as cancer, cardiovascular disease, stroke, and premature aging. Currently free radicals are increasing due to several things such as UV rays, consumption of fast food, cigarette smoke and environmental pollution. Environmental conditions and an unhealthy lifestyle cause the human body's defense system against free radicals to weaken, so it is necessary to have antioxidants from outside to help ward off free radicals (Wahdaningsih *et. al*., 2011).

Antioxidants are compounds that can counteract free radicals. Antioxidants counteract free radicals by giving electrons to free radicals so that the radicals become stable and cell damage can be avoided (Winarsi, 2007). However, the use of synthetic antioxidants is dangerous because they are suspected of causing cancer (Permatasari, 2011). So it is necessary to have natural antioxidants from plants in overcoming free radicals.

This plant belongs to the *Spatholobus* genus. The pirate plant was originally well known in the Central Kalimantan area and is also found in East Kalimantan and the Bangka Belitung Islands. Inland communities in Central Kalimantan have been using the stem parts of this plant for generations as a sore medicine, dysentery medicine, wound healing medicine and to minimize breast cancer (Saputera dan Ayuchecaria., 2018). Currently, people in Bangka Belitung are also starting to use parts of the Bajakah stem (*Spatholobus littoralis* Hassk)

as herbal medicine for the body. However, not much has been done about the content of the stem from Bangka Belitung, so its bioactivity is not known.

Based on several studies, Bajakah stems have potential as antioxidants. Research on pirate stems from Central Kalimantan, Bajakah stems contain phenolics, tannins and saponins (Ayuchecaria *et. al.,* 2020). Fitriani *et. al.,* (2020) Bajakah stems from East Kalimantan contain phenolics, flavonoids and tannins. Research by Abdulrahman *et. al*., (2021) Regarding the stem of the Bajakah from Bangka Belitung, it was stated that the extract of the stem of the Bajakah contains alkaloids, flavonoids and phenolics.

According to Sen *et. al*., (2010) Alkaloids, flavonoids and tannins from plants can potentially act as natural antioxidants. Therefore, testing the total phenolic and antioxidant activity of the extract of the Bajakah stem from Bangka Belitung was carried out. Testing the potential as an antioxidant from plant extracts needs to be done to minimize the use of synthetic antioxidants which have negative effects when used continuously. This study aims to calculate the total phenolic content and antioxidant strength of the ethanol extract of *S. littoralis* stem from Bangka Belitung.

## METHODOLOGY

### Material

Materials used: powdered stems of Bajakah, aquades , technical ethanol MKR Chemicals, gallic acid (Merck), DPPH (*2,2-difenil-1-pikrilhidrazil*) (Sigma), methanol p.a (Merck), reagen Folin–Ciocalteau (Merck) dan Natrium carbonate (Merck).

### Equipment

Equipment used: blender, glassware, aluminum foil, analytical balance, rotary evaporator IKA RV 10 DIGITAL V-GERMANY, incubator, spektrofotometer UV-Vis SHIMADZU UV-1800, vortex, wrapping plastic, and filter paper.

### Procedure

#### Bajakah Stem Sample Preparation (*Spatholobus littoralis Hassk*.)

Bajakah stem samples were obtained from Berbura Village, Riau Silip District, Bangka Regency. Bajakah stems are dried at room temperature. Then, it is cut into smaller sizes, blended until smooth and sieved using a 100 mesh sieve to produce a fine powder of pirated stems.

#### Extraction

250 grams of fine powder of pirated stems was taken and macerated using ethanol solvent with a ratio of sample: solvent (1:10) for 3x24 hours. The filtrate obtained was concentrated using a rotary evaporator vacuum to obtain a concentrated ethanol extract of the Bajakah stem (Dahmoune *et al*., 2015). This extraction was carried out at the FPPB UBB MIPA Laboratory.

#### Total Phenolic Testing

A standard solution of 1000 ppm gallic acid was prepared by dissolving 10 mg of gallic acid in 10 ml of methanol p.a. The solution is then taken and diluted. The concentration variations used were 20, 40, 60, 80 and 100 ppm. Then each concentration was put into a 10 ml volumetric flask. Added 0.5 ml of Folin–Ciocalteau reagent and 2.5 ml of 7% Na2CO3 solution in each concentration, then shaken homogeneously using a vortexer. Then let it stand for 30 minutes at room temperature. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 760 (Khadijah *et. al.,* 2017).

Total phenolic determination was carried out in duplo. A total of 30 mg of the extract was added to 10 ml of methanol. Then pipette 0.5 ml and add 0.5 ml of Folin–Ciocalteau reagent and then vortex for 30 seconds. Then add 2.5 ml of 7% Na2CO3 solution into the mixture and vortex again. Leave the solution for 30 minutes at room temperature. Measure the absorbance using a UV-Vis spectrophotometer at a wavelength of 760 nm (Khadijah *et. al.,* 2017).

#### Antioxidant Testing

The test was carried out in duplicate. As much as 5 mg of the extract of the pirated stem was dissolved in 50 ml of methanol to obtain a concentration of 100 ppm. The solution was then pipetted 0.5, 1, 2 and 4 ml, diluted in a 10 ml volumetric flask. Then put 1 ml of each solution into a test tube, add 2 ml of methanol and 1 ml of DPPH with a concentration of 100 ppm. After that, prepare a blank solution by mixing 3 ml of methanol and 1 ml of DPPH. Each solution and blank was stirred using a vortexer for 30 seconds. Then incubate for 30 minutes at 37$^{o}$C. The absorbance was measured at a wavelength of 515 nm with a UV-Vis spectrophotometer (Mahardika dan Roanisca,

2018). Antioxidant strength is determined from the $IC_{50}$ value. If the value of $IC_{50} \leq 50$ µg/ml (very strong), 50-100 µg/ml (strong), 101-250 µg/ml (medium), ≥ 250 µg/ml (weak) (Molyneux, 2004).

## RESULT AND DISCUSSION

### Extraction

Extraction of pirated stem samples was carried out by maceration process. The maceration method is preferred in extracting a material because maceration does not use heating so that the active substance to be extracted is not damaged (Pratiwi, 2010). The result of maceration is in the form of a brownish brown pirate stem extract solution. The extract obtained is then concentrated with a rotary evaporator which is useful for obtaining a concentrated ethanol extract of the Bajakah stem. The concentrated extract obtained was then calculated for its yield value. The yield of the extract of the pirated stems obtained is 12,028 %.

### Total Phenolic Testing

Determination of total phenolic was carried out using the Folin-Ciocalteau method. The working principle of this method is the oxidation reduction of phenolic-hydroxyl groups. The phenolic compounds undergo oxidation to become phenolic ions, while the Folin-Ciocalteau reagent is reduced to form a phosphotungstate-phosphomolybdate complex to form a molybdenum blue complex. (Azlim, *et. al*., 2010).

The standard solution used in the determination of total phenolic is gallic acid. Gallic acid reacts with folin's reagent to form a yellow color and turns blue when sodium carbonate ($Na_2CO_3$). The following is the reaction of gallic acid with Na2CO3 and Folin–Ciocalteau reagent.

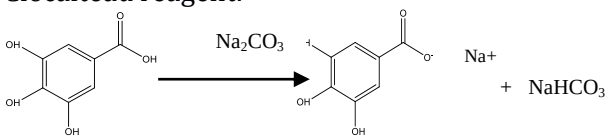


**Figure 1.** Reaction of Gallic Acid with $Na_2CO_3$ (Nunes *et. al*., 2012)

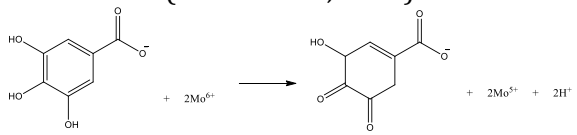


**Figure 2.** Reaction of Gallic Acid with Folin–Ciocalteau reagent (Nunes *et. al*., 2012)

Absorbance measurements were carried out at a wavelength of 760 nm. The gallic acid concentrations used were 20, 40, 60, 80 and 100 ppm. The results of the total phenolic test of the ethanol extract of the Bajakah stem can be seen in Table 1 below.

**Table 1.** Total Phenolic Test Results

| Equations Linier | Sample Absorbance | Total Phenolic Content (mg EAG/g) | Average Total Phenolic Content (mg EAG/g) |
|---|---|---|---|
| y = 0,0059x + 0,8781<br>$R^2$ = 0.9829 | (1) 1,257 | (1) 21.4 | 21.535 |
| | (2) 1,262 | (2) 21.62 | |

Based on the test results, the ethanol extract of Bajakah stems which was tested in duplicate yielded total phenolic content of 21.4 mg EAG/g and 21.62 mg EAG/g, respectively. The average total phenolic content obtained was 21.535 mg EAG/g. Based on a review of *S. littoralis* species, the ethanol extract of the Bajakah stem from Bangka Belitung has a higher total phenolic content than the ethanol extract of the Bajakah stem from Central Kalimantan, which is 12.33 mg EAG/g. However, the total phenolic content obtained was lower than the ethanol extract of the Bajakah stem from East Kalimantan, which was 131.40 mg EAG/g. The different total phenolic yields were due to environmental factors in the area including temperature, soil pH and soil moisture. This is in accordance with research Utomo *et. al.,* (2020) namely environmental conditions affect the levels of phenolic, flavonoid and antioxidant activity of an extract.

### Antioxidant Testing

Determination of antioxidant strength in this study using the DPPH (1,1-diphenyl-2-picrylhidrazyl) method. The principle of measuring antioxidants using DPPH is that the change in concentration of DPPH solution is

proportional to the intensity of the purple color of DPPH. The reaction of DPPH with antioxidant compounds is as follows.

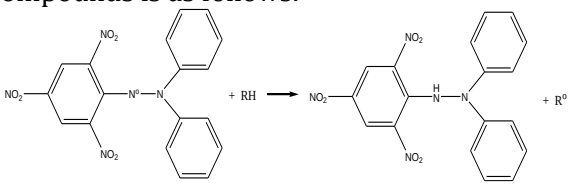

**Figure 3.** DPPH reaction with antioxidants

Testing of antioxidant activity was carried out in duplicate. The concentrations used were 5, 10, 20 and 40 ppm. In this test a blank solution was also used. Blank is a solution without a sample that serves as a reference solution. The absorbance was measured using a UV–Vis spectrophotometer at a wavelength of 515 nm to obtain an $IC_{50}$ value of the test solution and blank. $IC_{50}$ is the ability to reduce 50% of DPPH free radicals. The results of the antioxidant activity test can be seen in Table 2 below.

**Tabel 2.** Hasil Pengujian Aktivitas Antioksidan Ekstrak Etanol Batang Bajakah

| Sample | $IC_{50}$ (µg/mL) | Information |
|---|---|---|
| Ethanol extract of the Bajakah stem | 281.717 | Weak |

Based on the test results, the ethanol extract of the Bajakah stem from Bangka Belitung has an antioxidant activity value of 281.717 µg/mL which is weak. The following is a comparison of the antioxidant power of *S. littoralis* stem extract. .

**Table 3.** Comparison of the Antioxidant Strength of *S. littoralis*

| Area name | Solvent | $IC_{50}$ (µg/mL) | Reference |
|---|---|---|---|
| Borneo East | Ethanol | 26.29 | Fitriani *et. al.,* (2020) |
| Borneo Central | Etyl Acetate | 24.163 | Nopian, (2020) |

The result of weak antioxidant activity in the ethanol extract of the Bajakah stem from Bangka Belitung could be due to the small total phenolic content of 21.535 mg EAG/g. According to Nurwaini *et. al.,* (2006) The strength of the antioxidant activity of an extract is influenced by the content of phenolic compounds in it. The greater the phenolic content of an extract, the greater its antioxidant power, marked by the smaller the $IC_{50}$ value.

## CONCLUSION

The total phenolic test for the ethanol extract of the Bajakah stem (*S. littoralis*) from Bangka Belitung was found to be 21.535 mg EAG/g. The antioxidant power of the ethanol extract of the Bajakah stem showed weak activity with an average $IC_{50}$ value of 281.717 µg/mL.

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