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Antioxidant and Antibacterial Activity of The Stem Bark Extract of *Sterculia Foetida* L.

Aktivitas Antioksidan dan Antibakteri Ekstrak Kulit Batang Tumbuhan *Sterculia Foetida* L.

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ABSTRACT

Free radicals are compounds that contain one or more unpaired electrons which are very reactive, causing damage to cells or tissues and implication in the emergence of various degenerative diseases. Several methods of exploring medicinal plants have been carried out to find new sources of natural antioxidants and antibacterials that can reduce the use of synthetic drugs. This study aimed to examine the antioxidant and antibacterial activity of the stem bark extract of the plant Sterculia foetida L. The antioxidant activity of S. foetida was tested using the ABTS radical method (2,2-Azinobis 3-ethyl benzothiazoline 6-sulfonic acid). The principle of this method is that the color intensity or absorbance of the ABTS solution is inversely proportional to the concentration of antioxidant compounds. ABTS absorbance measurements were carried out at a wavelength of 734 nm. The results showed that the bark extract of the plant S. foetida could inhibit ABTS radicals with an IC50 value of 5.54 g/mL. The antibacterial activity of S. foetida was tested by measuring the optical density (OD) at a wavelength of 630 nm and expressed by the Inhibition Concentration 50 (IC50) value of 10442.29 g/mL.

Keywords: *Sterculia foetida L.* plant, antioxidant, antibacterial, ABTS, phytochemical

ABSTRAK

Radikal bebas merupakan suatu senyawa yang mengandung satu atau lebih elektron yang tidak berpasangan yang sangat reaktif sehingga menyebabkan kerusakan sel atau jaringan, dan berimplikasi pada timbulnya berbagai penyakit degeneratif. Beberapa metode eksplorasi tumbuhan obat dilakukan untuk menemukan sumber baru antioksidan dan antibakteri alami yang mampu mengurangi penggunaan obat sintetik. Penelitian ini bertujuan untuk menguji aktivitas antioksidan dan antibakteri dari ekstrak kulit batang tumbuhan Sterculia foetida L. Pengujian aktivitas antioksidan S. foetida dilakukan menggunakan metoda radikal ABTS (2,2-Azinobis 3-ethyl benzothiazoline 6-sulfonic acid). Prinsip dari metode ini adalahintensitas warna atau absorbansi larutan ABTS berbanding terbalik dengan konsentrasi senyawa antioksidan. Pengukuran absorbansi ABTS dilakukan pada panjang gelombang 734 nm. Hasil penelitian menunjukkan bahwa ekstrak kulit batang tumbuhan S. foetida memiliki kemampuan menghambat radikal ABTS dengan nilai IC_{50} sebesar 5,54 µg/mL. Pengujian aktivitas antibakteri S. foetida dilakukan dengan mengukur densitas optik (OD) pada panjang gelombang 630 nm dan dinyatakan dengan nilai Inhibition Concentration 50 (IC_{50}) sebesar 10442,29 µg/mL.

Kata Kunci: Tumbuhan *Sterculia foetida L.*, antioksidan, antibakteri, ABTS, fitokimia

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1. INTRODUCTION

In recent years, the use of natural ingredients as traditional medicine in Indonesia has increased, and some natural ingredients have even been mass-produced. Traditional medicine is believed to have fewer side effects compared to chemically-derived drugs. Additionally, raw materials for traditional medicine are readily available and relatively inexpensive (Alam et al., 2012; Muchtadi et al., 2013).

Many studies have been conducted to test the active ingredients found in plants or natural ingredients. Purgiyanti (2019) tested the antioxidant mechanism of the active ingredients of *Centella asiatica* extract against free radicals. Torokano et al. (2018) tested the inhibitory capacity of red jatropha leaf extract to inhibit the growth of *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli* bacteria, while Monalisa et al. (2011) tested *Elephantopus scaber* leaves to inhibit *Staphylococcus aureus* and *Salmonella typhi* bacteria.

Antioxidants stabilize free radicals by interrupting chain reactions so that free radical reactions can be halted (Winarsi, 2007; Babalola, 2012; El-sherei et al., 2016). Free radical chain reactions due to unpaired electrons cause free radicals to become highly reactive compounds against body cells, causing various chronic, degenerative diseases to cell or tissue damage (Bosenberg & Zyl, 2008; Tuba & Gülçin, 2008). Antibacterial is a substance that kills or suppresses bacteria's growth and reproduction (Vital et al., 2010; Hebbar et al., 2014). Based on their activity, antibacterial substances can be bactericidal (kill bacteria), bacteriostatic (inhibit bacterial growth) or inhibit the germination of bacterial spores (Murray et al., 2003). Some bacteria, such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhosa*, and *Vibrio cholera*, can cause various diseases for living things (Griffin, 1981; Silalahi, 2006).

Medicinal plants have the potential to contain secondary metabolites such as alkaloids, flavonoids, terpenoids, and steroids that can act as antioxidants, antibacterial, antifungal, and antidiabetic compounds (Gressler et al., 2008; Li et al., 2009; Lin et al., 2010; Widodo & Rahayu, 2010; Tarak et al., 2011; Wibowo et al., 2014; Hairani et al., 2016;). One of the medicinal plants that have the potential as natural antioxidants and antibacterial agents is *Sterculia foetida* L. (Heyne et al., 1987; Forest Watch Indonesia, 2001; Ministry of Forestry, 2002). This plant is intriguing to examine because it is proven to be efficacious in overcoming various health problems based on the empirical experience of the local NTT community. *S. foetida* is known by the people of NTT as the Nitas tree (Hall for the Establishment of Forest Areas Region XIV, 2008). The leaves, bark, and roots of *S. foetida* are used to treat wounds in livestock. People also take advantage of the ripe fruit skin of *S. foetida* to treat stomach and kidney diseases (Njurumana, 2011; Siswadi et al., 2013).

Other studies have shown that *S. foetida* has antibacterial and antioxidant activity. An antioxidant test by Gunawan and Karda (2015) proved that the bark essential oil from the stem of *S. foetida* has the potential as an antioxidant compound. The antifeedant activity test by Rani and Rajaskharreddy (2009) proved that the leaves of *S. foetida* contain triterpene and alkaloid compounds that have potential as antifeedant compounds. An anti-inflammatory test by Naik et al. (2003) proved that *S. foetida* contains the taraxer-14-en-3β-ol, which has the potential to be an anti-inflammatory compound. Based on these studies, it is known that the *S. foetida* plant contains phenolic compounds that have the potential as antioxidant agents (Asih et al., 2010). It is known that plants with a high concentration of phenolic compounds possess antioxidant properties (Mujumdar et al., 2011; Huang et al., 2013; Cahyani et al., 2019). Based on the data from the literature study that has been carried out, this research will determine the antioxidant and antibacterial activity test of the stem bark extract of the plant *Sterculia foetida* L. originating from East Nusa Tenggara.

2. MATERIALS AND METHODS

2.1. Materials

The materials used were stem bark samples of *S. foetida*, ABTS and quercetin (Sigma-Aldrich), $K_2S_2O_8$, sodium chloride, potassium chloride, sodium hydrogen phosphate, potassium dihydrogen phosphate, $AlCl_3$, Mayer's reagent, Wagner's reagent, Dragendorff's reagent, magnesium, chloroform, DMSO, anhydrous acetic acid, $HgCl_2$, KI, and Iodine from Merck (Darmstadt, Germany). In addition, 10% $FeCl_3$, 25% NH_3, methanol, ethanol, 2M HCl, concentrated sulfuric acid, distilled water, and Whatman filter paper were also used on an analytical scale.

The tools used were knife, oven, blender, sieve, analytical balance, extraction equipment, test tube, test tube rack, dropper, beaker, measuring cup, rotary evaporator, incubator, and UV-Vis Thermo Genesys 10S spectrophotometer.

2.2. Extraction

A total of 600 grams of *S. foetida* bark powder was extracted by maceration using 1.5 liters of methanol for 3 x 24 hours. The results of the macerate were filtered with filter paper to obtain the filtrate. The filtrate was then evaporated using a rotary evaporator at a temperature of 60°C.

2.3. Phytochemical Test

A. Alkaloids

A total of 500 mg of extract was dissolved with methanol in a test tube, and then 3 - 5 drops of Wagner reagent were added. The sample was then observed until it was cloudy or a precipitate was formed. Positive alkaloids are characterized by brown deposits (Tiwari et al., 2011; Atun, 2014).

B. Flavonoids

A total of 500 mg of extract was dissolved with methanol in a test tube, then two drops of concentrated HCl and Mg metal were added and shaken vigorously. Positive flavonoids are indicated by the presence of foam and a red/yellow/orange color solution (Atun, 2014).

C. Saponins

A total of 500 mg of extract was dissolved with methanol in a test tube, then two drops of 1 M HCl were added and shaken. Positive saponins are indicated by the presence of foam with much intensity and are consistent for 10 minutes (Atun, 2014).

D. Tannins

A total of 500 mg of extract was dissolved in methanol in a test tube, then ten drops of 10% $FeCl_3$ were added. Positive tannins are indicated by a dark blue and blue-black/greenish-black color solution (Atun, 2014).

E. Triterpenoids/steroids

A total of 500 mg of extract was dissolved in methanol in a test tube. Ten drops of glacial acetic acid and two drops of concentrated H_2SO_4 were added, then shaken slowly. Positive triterpenoids are indicated by a red/brown color solution, while steroid positives are indicated by a blue, purple/green color solution (Atun, 2014).

2.4. Determining Antioxidant Activity Preparation of Quercetin Stock Solution

A stock solution of 1000 g/mL quercetin was prepared by dissolving 10 mg of quercetin with ethanol p.a in a 10 mL volumetric flask.

2.5. Preparation of ABTS Solution and Reagents

a. ABTS solution: 18 mg ABTS (7 mM) was dissolved with distilled water in a 5 mL volumetric flask.
b. $K_2S_2O_8$ solution: 14 mg potassium persulfate (2.45 mM) was dissolved with distilled water in a bottle to 20 mL.
c. ABTS stock solution: 5 mL of ABTS solution was added with 5 mL of potassium persulfate solution and incubated in a dark room at 22-24°C for 12-16 hours before use to produce ABTS with a dark blue color.
d. Phosphate Buffer Saline (PBS) solution pH 7.4: 8 g sodium chloride, 0.2 g potassium chloride, 1.42 g sodium hydrogen phosphate, 0.24 potassium dihydrogen phosphate were dissolved in distilled water to 1 L.

2.6. ABTS Maximum Absorption

Wavelength Measurement

A total of 0.1 mL of PBS solution pH 7.4 was mixed with 2 mL of ABTS stock solution and incubated for six minutes. Then the absorbance of the solution was measured by UV-Vis spectrophotometry at the maximum wavelength.

2.7. ABTS Test with Quercetin (Comparison)

Quercetin stock solution 100 g/mL was pipetted into a 5 mL volumetric flask as much as 3.09 g/mL, 6.19 g/mL, 12.38 g/mL, 24.75 g/mL, and 49.5µg/mL, or pipette 0.15 mL, 0.31 mL, 0.62 mL, 1.24 mL, and 2,475 mL, respectively. Then the volume was added up to the mark with ethanol p.a. From each concentration, 0.1 mL of solution was taken and added with 2 mL of ABTS stock solution. The solution was incubated for six minutes, and the absorbance was measured using UV-Vis spectrophotometry at the maximum wavelength.

2.8. Measurement of Antioxidant Activity

The stock solution of 1000 g/mL methanol extract sample was pipetted into a 5 mL volumetric flask as much as 25 g/mL, 50 g/mL, 100 g/mL, 200 g/mL, and 400 g/mL or as much as 0.125 mL, 0.25 mL, 0.5 mL, 1 mL and 2 mL. Then the volume was added up to the limit with ethanol p.a. From each concentration, 0.1 mL of the extract solution was added with 2 mL of ABTS stock solution, incubated for six minutes, and the absorbance was measured using UV-Vis spectrophotometry at the maximum wavelength. Sample concentration and percent inhibition were plotted on the x and y axes of the linear regression equation (Y = ax + b, respectively). This equation is used to determine the IC_{50} value. The amount of antioxidant activity was calculated by the formula:

$$Percent\ Inhibition\ (\%) = \left(\frac{control\ absorbance\ \ sample\ absorbance}{control\ absrbance}\right) \times 100\%$$

2.9. Evaluating Antibacterial Activity

A. Media

In the antibacterial bioactivity test, tools and media were sterilized using an autoclave for 15 minutes at 120°C and 1 atm. Solid media was prepared by inserting 20 g of Mueller Hinton Agar into 500 mL of distilled water, then put into an Erlenmeyer, covered with cotton, and heated until it boiled and thickened. The agar medium was then sterilized using an autoclave at 105°C for 15 minutes.

Liquid media (Nutrient Agar) was prepared by dissolving 20 g of Nutrient Agar (NA) in 250 mL of distilled water, then put into an Erlenmeyer and covered with cotton. The suspension was then heated to boiling while stirring and cooled to room temperature. The media was sterilized in an autoclave at 105°C for 15 minutes.

2.10. Preparation of Isolated Bacterial Colony

The tube containing the bacteria was removed from the –80°C freezer and thawed. A total of 5 mL of Nutrient Broth (NB) was poured into a sterile test tube and added with 50 µl of bacteria. The test tubes were then covered with parafilm and incubated at 37°C for 18 hours. A vortex was used to even out the bacterial suspension. A total of 200 µl of bacterial suspension was spread evenly on the NA plate in a machine, covered with parafilm, and incubated at 37°C for 18 hours to obtain isolated colonies.

2.11. Preparation of Bacterial Suspension For Antibacterial Evaluation

A total of 5 mL of NB was poured into a sterile test tube. One colony of bacteria with a sterile yellow tip was taken and put into a 5 mL NB tube. The bottom of the test tube was pressed to dissolve the bacteria in the NB. The tube was then covered with parafilm and incubated on a shaker at 37°C for 18 hours.

2.12. Sample Preparation

The extract was dissolved in a solvent (e.g., 1 mL) to the highest concentration (record the amount of extract to reach the maximum concentration). Dissolve the ethanol extract in DMSO (e.g., 1 mL) to the highest concentration (record the amount of extract to reach the maximum concentration). Sonification was carried out so that all extracts were wholly dissolved.

2.13. Measurement of Optical Density (OD) of Cell Suspensions

A total of 500 µL of bacterial suspension

was mixed with 500 µL of NB, and the OD630 was measured at 630 nm. The bacterial suspension was then prepared at OD630 = 0.4. Then the calculations were carried out.

2.14. Colony Inspection

Serial dilutions of the bacterial suspension were carried out: 10^9, 10^8 10^7 10^6 10^5 10^4 10^3 for each bacteria used. A total of 100 µL of 10^3 bacteria were spread on NA, covered with parafilm, and incubated at 37°C for 18 hours. After that, the colonies of each bacterium were counted.

2.15. Experiment

A 500 L mixture was made from 445 µL NB + 50 µL bacterial suspension (using 104 cells and the remaining 105 cells) + 5 µL samples, then repeated for the rest of the samples. Vortex was used for leveling. A premix of 150 µL was used for each sample, three replication for each sample, and twice for the blank. It was then tightly closed with sealing paper and fitted with a well cover. It was incubated at 37°C for 18 hours, and the cell density was measured at OD630.

3. RESULTS AND DISCUSSION

Based on Table 1, phytochemical tests on the methanol extract of Nitas stem bark showed the presence of secondary metabolites, namely flavonoids and tannins. The results of the antioxidant activity test of the methanol extract of the Nitas stem bark against ABTS are presented in Table 2, with a percent radical inhibition of 88.3%.

From Table 2 and Fig. 1, it can be explained that the greater the sample concentration, the greater the inhibition of the sample against free radicals. Fig. 1 shows the logarithmic regression equation that can be used to calculate the IC_{50} value (5.54 g/mL). These results indicate that the methanol extract of the stem bark of *S. foetida* has a powerful antioxidant activity. Antibacterial activity of methanol extract of Nitas bark against *Staphylococcus aureus* bacteria is presented in Fig. 2.

The results of the measurement of bacterial growth inhibition are presented in Fig. 2. From Fig. 2, it can be seen that the effectiveness of the methanol extract of Nitas stem bark as an antibacterial against *S. aureus* bacteria was 47.11%, indicated by an IC_{50} value of 10442.29 g/mL.

Based on the research that has been done, the methanol extract of the bark of Nitas can inhibit the growth of *S. aureus* bacteria. It is supported by the presence of tannins and flavonoids in the extract. As an antibacterial, flavonoids work by inhibiting the synthesis of nucleic acids located in ring B, which plays a vital role in the intercalation process or hydrogen bonding by accumulating nucleic acid bases, which can inhibit the formation of DNA and RNA (Cushnie et al., 2005). As an antibacterial, tannins work by inhibiting the reverse transcriptase and DNA topoisomerase enzymes, causing bacterial cells to fail to form (Nuria et al., 2009). In *S. aureus* bacteria, administration of antibacterial compounds from the methanol extract of Nitas stem bark can inhibit the assembly of the cell wall and fail to join the glycan chain with the peptidoglycan of the cell wall causing bacterial death. Without a cell wall, bacteria will not be able to survive outside influences, so bacteria tend to die shortly.

Table 1. Phytochemical test results of methanol extract of Nitas bark

Identification	Result	Description

Alkaloids	Reagen wagner: brick-red	-
Flavonoids	Orange color solution	+
Saponins	Golden-brown	-
Tannins	Greenish black	+
Triterpenoids/Steroids	Turbid yellow	-

*Note: (+) = positive contains compounds
(-) = negative contains compounds

Table 2. Data on the percentage of inhibition of samples from various concentrations

Sample concentration (µg/mL)	(%) inhibition
3.09	33.88
6.19	51.28
12.38	73.91
24.75	85.73
49.5	88.3

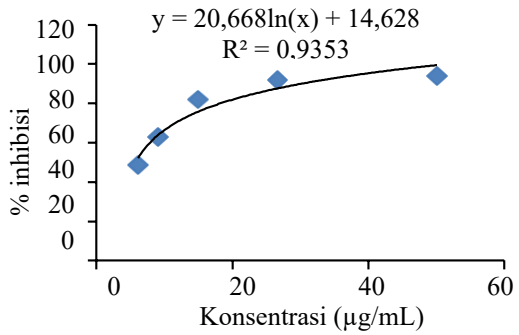

Figure 1. Antioxidant activity of plant extract of *S. Foetida*

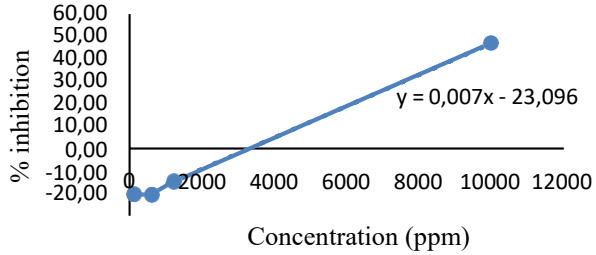

Figure 2. Antibacterial activity of the plant extract of *S. foetida*

4. CONCLUSIONS

Based on the results of the research conducted, it can be concluded that:

1. The methanolic extract of the bark of Nitas (*Sterculia foetida* L.) contains secondary metabolites in the form of flavonoids and tannins that have the potential as antioxidants and

antibacterials.

2. Antioxidant and antibacterial activity of the methanolic extract of the bark of Nitas (*Sterculia foetida* L.) with inhibition of 88.3% and 47.11%, respectively, with IC50 values of 5.54 g/mL and 10.442.29 g/mL.

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