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Secondary Metabolites and Antioxidant Activity of Ethanolic Extract of Faloak (*Sterculia quadrifida*)

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Abstract

Faloak (*Sterculia quadrifida*) is a medicinal plant used by the people of East Nusa Tenggara to treat lumbago, liver dysfunction and to restore the stamina. The research aims were to determine the qualitative and quantitative content of flavonoids, phenols, and tannins, as well as to examine the antioxidant activity of roots, stem barks, leaves, fruits and seeds extracts of faloak plant. Each organ was extracted with ethanol 70% using the maceration method. The qualitative content of bioactive compounds was determined using the phytochemical screening method. The determination of bioactive compounds concentration was using spectrophotometric methods and antioxidant activity was using the DPPH method. The result of phytochemical screening showed that all of the extracts were exhibit phenols compounds, but the flavonoids and tannins were only found in roots, barks, leaves, and fruits extracts. The quantitative content of total flavonoids of roots, barks, leaves, fruits, and seeds was 48.09; 62.76; 12.56; 11.91 and 1.55 mg/g, while the phenols total content were 82.90; 45.37; 3.43; 29.50 and 2.89 mg/g. Tannins total content were 71.26; 59.64; 10.52; 13.18 and 14.12 mg/g samples respectively. The stem barks and roots extracts showed a very strong antioxidant activity, while leaves, fruits, and seeds extracts belong to the strong category. The potential of faloak as an antioxidant has been widely studied, especially in the stem bark. Studies on the antioxidant activity of roots, leaves, fruits, and seeds can provide new information about the benefits of phaloac plants as a source of natural antioxidants.

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INTRODUCTION

Sterculia quadrifida is an important medicinal plant, which has been used to cure various ailments by people on the island of Timor, East Nusa Tenggara (NTT) for generations in traditional system of medicine. The plant belongs to the family of Sterculiaceae and is commonly known as faloak. Based on survey of the ethnobotany study, it was reported that the faloak was used by people in Timor Island (NTT) for treatment various ailments such as liver dysfunction (55%), to restore the stamina (13%), to treat lumbago (7%), ulcers (7%), illness waist (6%), malaria (6%), and cleaning/blood-booster (6%) (Siswadi et al., 2016). Ranta et al., (2012) reported that based on the experience of NTT people who consuming bark routinely, it can increase the stamina (reduce fatigue for heavy workers), liver disfunction, ulcer, typhus, vaginal discharge, shedding debris after giving birth, and menstrual decay. In addition, it was also reported that faloak leaves are used by Aboriginal people in Australia to treat wounds, skin complaints, eye aches, and stings (Akter et al., 2016). Although the faloak has been widely used in traditional medicine, however, research and scientific evidence about the characteristics and activities of faloak as a source of natural ingredients for medicines are still very limited.

The faloak can be used in traditional medicine because it contains various secondary metabolites such as terpenes, alkaloids, flavonoids, phenols, and tannins. The secondary metabolite has been found to have a broad range of therapeutic properties including antioxidant activities (Alamgir et al., 2014). Flavonoids and tannins are the most abundant phenolic compounds in plants and exhibit various bioactivities mostly due to their antioxidant potential. The plant antioxidants have the ability to inhibit or delay oxidative damages that caused many degenerative diseases (Ojha et al., 2018). Antioxidants can be used to protect the body from free radicals which cause pathological conditions such as neurodegenerative diseases and cardiovascular conditions. The antioxidant also can destroy and neutralize free radicals that damage biomolecules such as proteins, lipoproteins and DNA in the body as triggers for degenerative diseases such as arthritis, cataracts, liver, and diabetes. Degenerative diseases occur because of an increase in free radicals in the body while antioxidants in the body are unable to centralize it (Filberth et al., 2014; Sembiring et al., 2017). In order to prevent this condition, exogenous antioxidants or additional antioxidants are needed to supply the lack of antioxidants in the body in their function to neutralize or destroy free radicals by inhibiting the initiation and propagation of oxidation chains. Faloak has the potential as antioxidant agents to prevent degenerative diseases such as diabetes, cancer, hepatitis, immunogenic, typhus, vaginal discharge, etc.

The antioxidant activities of several *Sterculia* species that have been studied are *S. foetida* (Mannivanan et al., 2011), *S. villosa* (Haque et al., 2014), *S. quadrifida* (Rollando & Monica, 2017; Soeharto &Tenda, 2018; Saragih & Siswadi, 2019), *S. setigera* (Taha & Mudawi, 2018), *S. tragacantha* (Sayuti et al., 2018), *S. apetala* (Mosca et al., 2018), *S. nobilis* (Zhang et al., 2018), *S. populifolia* (Khairi et al., 2018), and *S. oblongata* (Murningsih et al., 2019). Part of the plant is one of the factors that affect the yield of extracts and the content of secondary metabolites. Faloak bark is the most widely used in traditional medicine. Research on faloak that has been widely reported is also related to the potential of stem bark as an antibacterial (Tenda et al., 2017), antifungal (Ranta et al., 2012), antitumor (Rollando & Prillianti 2017), antiviral (Dean et al., 2019), and antioxidant agent (Saefudin et al., 2013; Rollando & Monica, 2017).

Faloak stem bark is reported to have antioxidant activity with IC_{50} of 91.72% (Saefudin et al., 2013) and 45.63 µg/ml (Rollando & Monica, 2017), and both are classified in very strong antioxidant activity. The other part of faloak plant that have been investigated for their antioxidant activity is leaf, with IC_{50} of 69.19% (Saefudin et al., 2013) and 4.96 µg/ml (Saragih & Siswadi, 2019), classified as strong antioxidant activity. In addition to the stem bark and leaf of faloak, the other parts can be investigated for their secondary metabolite content and biological activity, especially as a source of antioxidants. The study on secondary metabolites and the antioxidant activity of roots, leaves, fruits, and seeds are expected to provide new information about the benefits of faloak plant. In addition, it also can support the use of faloak as natural antioxidant source. The research aims were to determine the qualitative and quantitative content of flavonoids, phenols, and tannins, as well as to examine the antioxidant activity of roots, barks, leaves, fruits and seeds extracts of faloak plant.

METHODS

The research was conducted for 3-months starting from June-September at the Biology Chemistry and Molecular Biology Laboratory, Satya

Wacana Christian University. The steps in this research included sample preparation and extraction with ethanolic solvents. Then, next step was phytochemical screening and the determination of secondary metabolites from flavonoids, phenols and tannins quantitatively followed by antioxidant activity measurement.

Sample collection and Preparation

S. quadrifida was collected from Kolobolon Village, Lobalain District, Rote Ndao Regency, Nusa Tenggara Timur, Indonesia. The organs used were root, stem bark, leave, fruit and seed. The samples were washed in tap water, air dried in shade, and dried using the oven (Memmert V 30) at 30^{O}C for 4-5 days. The samples were grinded using a blender (Philip HR1538) and macerated using ethanol 70% two times. All supernatants were concentrated using a rotary evaporator (Rotavapor RE 100 Pro) with a vacuum pump (China 51089).

Qualitative analysis of bioactive compounds

Analysis of flavonoid compounds

0.05 g extract was dissolved in 4-5 drops of concentrated HCl. The positive test results for flavon were indicated by the formation of red or red purple color, while the positive test results for flavonon were indicated by the formation of orange color.

Analysis of phenolic compounds

0.05 g of extract was shaken strongly with 10 ml of chloroform. Then, 10 ml of distilled water was added to the solution and, allowed to form two layers, chloroform and water. After the layers formed, iron (III) chloride was added to the test tube. The positive test results for phenol were indicated by the formation of green and purple color.

Analysis of condensed tannin compounds

0.05 g of extract was dissolved in 10 ml of methanol until the extract was completely submerged. Then 2-3 drops of iron (III) chloride solution were added. The positive test results for condensed tannins were indicated by the formation of bluish black or green color.

Quantitative analysis of bioactive compounds

Total Flavonoids Content

Total flavonoids analysis was conducted using aluminum chloride method (John et al., 2014). The concentration series of quercetin used were 20.0; 40.0; 60.0; 80.0; and 100.0 µg/ml. Using 10 ml volumetric flask, 1 ml sample or quercetin and 0.30 ml of 5% $NaNO_2$ were mixed and incubated for 5 minutes. The mixture was added with 0.3 ml of 10% aluminum chloride hydrate and incubated for 5 minutes. After that, the mixture was added with 2.0 ml of NaOH 1 M and distilled water to exactly 10 ml. The absorbance of mixture was measured at 510 nm wavelength using UV-Vis spectrophotometer (Hitachi UV mini 1240). Quercetin were used as flavonoid standard. The conversion of absorbance to concentration was using the equation QE = c (V / m) where QE is flavonoid total concentration of the quercetin standard curve (mg/ l), v is volume of extract (l), and m is extract weight (g). The determination of flavonoid total concentration in sample was based on linear regression equation quercetin.

Total Phenols Content

Total phenols analysis was conducted using folin-ciocalteu method (John et al., 2014). The concentration series of gallic acid were 100.0; 200.0; 300.0; 400.0; and 500.0. 10 ml of Na_2CO_3 7% and distilled water exactly 25 ml and incubated for 90 minutes. The absorbance of mixture was measured at 550 nm wavelength using UV-Vis spectrophotometer (Hitachi UV mini 1240) and Gallic acid were used as phenolic standard. The conversion of absorbance to concentration was using the equation GAE = c (V/m) where c is phenolic total concentration of the gallic acid standard curve (mg/l), v is volume of extract (l) and m = extract weight (g). The determination of total concentration in sample was based on gallic acid linear regression equation.

Total Tannins Content

Total Tannins analysis was conducted using folin-ciocalteu method (Tambe & Bhambar, 2014). The concentration series of gallic acid were 20.0; 40.0; 60.0; 0; 80.0; and 100.0 µg/ml. Using 10 ml volumetric flask, 0.1 ml sample or gallic acid, 7.5 ml distilled water and 0,5 ml of folin-ciocalteu reagent were mixed. Then the mixture was added with 1 ml of Na_2C0_3 35% and 10 ml distilled water and incubated for 30 minutes. The absorbance of mixture was measured at 725 nm wavelength using UV-Vis spectrophotometer (Hitachi UV mini 1240) and Gallic acid were used as tannin standard. The conversion of absorbance to concentration was using the equation GAE = c (V/m) where c is tannin total concentration of the standard curve of gallic acid (mg/l), v is volume of extract (l) and m = extract weight (g). The determination of total tannin concentration in sample was based on gallic acid linear

regression equation.

Antioxidant Activity

The antioxidant activity was conducted using DPPH (1,1-difenyl-2-picrylhydrazyl) method (Gomes de Melo et al., 2010). Each extract was re-dissolved using ethanol 96%. The concentration series of sample were 10.0; 15.0; 20.0; 25,0; 50.0; and 100.0 µg/ml. The concentration series of ascorbic acid used were 5.0; 10.0; 15.0; 20.0; 25.0; and 50 µg/ml. 3.5 ml sample was added with 0.5 ml DPPH 4 mM and incubated for 30 minutes in the dark room. The calculation of antioxidant activity was using the equation antioxidant activity (%) = (($A_{-blank} - A_{-sample}$)/A_{-blank}) x 100%, where a blank is absorbance of 0.4 mM of DPPH while a sample is absorbance 4 mM of DPPH after the treatment. The IC_{50} value was determined based on the linear regression equation of each sample. The IC_{50} value was used to categorize the antioxidant activity (Armala, 2009).

Data Analysis

Data of total flavonoids, phenols, tannins and antioxidant activity were analysed using Microsoft Excel and reported as mean ± standard deviation of triplicate determination.

RESULTS AND DISCUSSION

Qualitative analysis of bioactive compounds

Determination of secondary metabolite compounds in plants qualitatively can be done through phytochemical screening on the five extracts. The results of phytochemical screening can be seen in Table 1.

The test results showed that the faloak sample from Rote Island was positively containing various secondary metabolites, such as flavonoids, phenols and tannins which can act as antioxidant agents. Determination of secondary metabolite compounds in plants qualitatively can be done through phytochemical screening of each extract. Phytochemical screening was carried out to identify the types of compounds contained in the plant sample studied (Maryono et al., 2015).

Table 1. Results of Phytochemical Screening

Organs	Test Compound		
	Flavonoids	Phenolics	Tannins
Root	++	+++	+++
Stem bark	++	+++	+++
Leave	++	++	+
Fruit	++	++	+
Seed	+	+	-

Ethanol was used as a solvent to extract secondary metabolites contained in faloak because it is a universal solvent which could extract both polar and non-polar compounds. Flavonoids and phenols are compounds that tend to be polar because their -OH group, so that both compounds will be extracted using ethanol in the extraction process. Tannin is a water-soluble phenolic compound and tends to be polar so it can also be extracted by ethanol. Munte et al., (2015) suggest that ethanol which has a polarity index of 5.2 can dissolve all organic components in extract both polar or non-polar compounds such as flavonoids, tannins, alkaloids, saponins, and steroids.

The determination of total Flavonoid, Phenols and Tannins Content

The five ethanol extracts of faloak plants showed that all extracts had varying levels of flavonoids, phenols, and tannins (Table 2).

Based on the the results in Table 2, it shows that the highest total flavonoid content was found in stem barks extract (62.76 mg/g), while the lowest was in seeds extract (1.55 mg/g). In leave and fruit, total flavonoid was in the range of 11-12 mg/g. The high level of flavonoids on the stem barks appeared from the dominance of red pigment in the extract. The extract of leaves, fruits, and seeds did not produce striking color indicated that the content of flavonoid compounds

Table 2. The total content of flavonoids, phenols, and tannins in ethanolic extract

Sample	Compounds in plant organs *S. quadrifida* (mg /g Sample)		
	Flavonoids	Phenols	Tannins
Roots	48.09 ± 12.47	82.90 ± 2.50	71.26 ± 10.21
Stem barks	62.76 ± 4.84	45.37 ± 3.82	59.64 ± 9.64
Leaves	12.56 ± 1.28	3.43 ± 1.44	10.52 ± 3.61
Fruits	11.91 ± 1.11	29.50 ± 9.01	13.18 ± 1.53
Seeds	1.55 ± 1.44	2.89 ± 1.40	14.12 ± 5.29

was small and there were other compounds that were more dominant. Raharjo (2013) explained that flavonoid compound has a role to produce yellow, blue, purple and red color in plants. Several studies have shown that the highest level of flavonoids in faloak is in the stem bark (Siswadi, 2015; Munawaroh et al., 2018).

Tannins are members of secondary metabolites of a group of phenolic compounds. The results of this study indicate the roots have highest phenol and tannin. The highest phenols (82.90 mg/g) and tannins (71.26 mg/g) were found in the roots while the smallest phenols were in the seeds (2.89 mg/g) and tannins were in the leaves (10.52 mg/g). The presence of tannin is functioned as a protective agent against infection by herbivorous attacks (Furlan et al., 2011). Faloak habitat in dry land is in accordance with the conditions of the NTT region, this affects the pressure high enough on the faloak roots so that the presence of tannin on the roots can cope with environmental stresses due to drought. Andriani et al., (2015) explained that the presence of tannin in the extract is thought to be related to the benefits of tannin as a poison for herbivores.

The antioxidant activity

The ability of the extract to inhibit the oxidation process was shown with the IC_{50} value (Inhibitory Concentration 50%). IC_{50} value is the extract concentration needed to reduce 50% of free radical activity. Result of antioxidant activity measurement of each test extract using DPPH method is presented in table 3.

Table 3. The antioxidant activity and characteristics based on IC_{50} values.

Extracts	IC_{50} Value (µg/ml)	Antioxidant Characteristic*)
Roots	20.55 ± 0.42	Very Strong
Stem barks	14.17 ± 0.55	Very Strong
Leaves	52.59 ± 0.75	Strong
Fruits	61.36 ± 0.37	Strong
Seeds	76.62 ± 0.32	Strong

Note: *) Very strong if IC_{50} value is < 50 µg/ml, Strong if IC_{50} value is 51-100 µg/ml, Moderate if IC_{50} value is 101-150 µg/ml, Weak if IC_{50} value is > 150 µg/ml (Armala, 2009)

The result in Table 3 shows that all of the organs of faloak have the potential to be used as antioxidant agents. The extracts of roots and barks have high antioxidant content because IC_{50} values were 20.55 ± 0.42 and 14.17 ± 0.55 µg/ml, respectively. Leaf, fruit and seed extracts had IC_{50} values of 52.59 ± 0.75, 61.36 ± 0.37, and 76.62 ± 0.32 µg / ml, respectively. In the antioxidant process, the antioxidant agent give a hydrogen atom to DDPH radicals and causes the change in color of DPPH from purple to yellow. The smaller the value of IC_{50}, the greater the antioxidant ability. The results indicate that the antioxidant content of the leaves, fruits and seeds extracts is lower than in the roots and bark. Based on IC_{50} values, the antioxidant activity of root and stem bark extract is classified as very strong while leaves, fruit and seed extract were strong. The antioxidant activity of faloak barks extract with an IC_{50} value of 45.63±1.47 µg/ml which was classified as very high had also been reported previously by Rollando & Monica (2017).

The IC_{50} value of the five tested faloak extracts (roots, bark, leaves, fruit, and seeds) was lower than the IC_{50} value of ascorbic acid of 6.60 µg / ml, which was also determined in this study. These results indicate that pure ascorbic acid has higher antioxidant activity than the five extracts of faloak. The higher antioxidant activity of ascorbic acid compared to faloak extract was due to the higher purity of ascorbic acid compound compared to the five crude extracts of faloak tested. To increase the antioxidant activity of the five faloak extracts, it is necessary to purify active compounds that play an important role as antioxidants such as flavonoids, phenolics, and tannins.

The result in Tables 2 and 3 showed that there is a relationship between flavonoid, phenolic and tannin levels with antioxidant ability. It appears that the three compounds play a role in the antioxidant activity of a substance. It is supported by Costa- A et al., (2014), Baba & Malik (2015), and Ahmed et al., (2017) statement that flavonoids, phenols, and tannins were compounds that are thought to act as an antioxidant. Flavonoid is the largest group of phenolic compounds possess many biological activities such as antioxidant, antiulcer, anti-arthritic, antiangiogenic, anticancer, anti-mutagenic, etc. Flavonoids are particularly beneficial, acting as antioxidants and giving protection against cardiovascular disease, cancer and degenerative diseases (John et al., 2014). Tannins are active compounds of secondary metabolites that are known to have several properties, namely as astringent, antibacterial, antioxidant and antidiarrhea (Kumari & Jain 2012; Sangi et al., 2012; Maisetta et al., 2019).

The antioxidant activity of flavonoids, phenols and tannins contained in the sample is caused by the ability of bioactive compounds

from these three compounds in contribute hydrogen atoms to DPPH so that the free radicals of DPPH are reduced and their form becomes more stable (Behera et al., 2009; Santoso et al., 2017; Priska et al., 2019). In this study, the total of the three compounds found in the roots was about 200 µg/ml while in the stem was about 160 µg/ml but the antioxidant ability of the stem was greater than the root. This is due to the flavonoid compounds generally have more -OH groups than phenolic compounds. The activity of antioxidant agents in reducing free radicals of oxidant compounds is influenced by the amount and position of hydrogen in molecules (Prasonto et al., 2017; Sulistyaningtyas & Wilson, 2018). The results of this study as scientific evidence about the habits of the people of NTT to consume faloak stem bark to cure hepatitis. It also gives scientific evidence that bioactive compounds from the extract of roots, stem bark, leaves, and seed faloak can be used as antioxidant agents.

CONCLUSION

The extracts of roots, barks, leaves, fruits, and seeds of faloak were exhibit the phenols compounds, but the flavonoids and tannins were only found in roots, barks, leaves, and fruits extracts on phytochemical screening. The highest total flavonoid content was found in stem barks extract (62.76± 4.84 mg/g), while the lowest was in seeds extract (1.55± 1.44 mg/g). The highest phenols (82.90±2.50 mg/g) and tannins (71.26±10.21 mg/g) compound content were found in the roots while the smallest phenols were in the seeds (2.89 mg/g) and tannins were found in leaves (10.52±3.61 mg/g). Based on IC50 values, the antioxidant activity of root and stem bark of faloak extract was classified as very strong (IC50 value < 50 µg/ml), while the leaves, fruit and seed extract were classified as strong (IC50 value 51-100 µg/ml). Faloak plant has the opportunity to be used as an antioxidant agent.

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