Phytochemical Contents and Antioxidant Activity of *Dysoxylum densiflorum* Extract and Fractions

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Abstract. Many studies have been carried out on the use of natural product compounds as an alternative medicine. *Dysoxylum densiflorum* is a plant that is commonly found in tropical countries and is used as an alternative treatment in local communities for several diseases. Our current study aims to determine the content of phytochemicals, total phenol, and total flavonoids from *D. densiflorum* leaves and stem bark methanol extract. The extraction was performed by the maceration method using methanol solvent and then fractionated into hexane, ethyl acetate, and methanol. A phytochemical screening was performed to determine the content of alkaloids, flavonoids, terpenoids, tannins, and saponins. The total phenol contents of the extract were determined by Folin-Ciocalteu using gallic acid as a standard. Total flavonoid were determined by alumunium chloride colorimetric methods, with quercetin as the standard. The antioxidant activity was determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and 2,2'-azino-bis-(3 ethylbenzothiazoline)-6-sulfonic Acid (ABTS) assay. The results of phytochemical screening showed that the extract and fractions of *D. densiflorum* leaves contained flavonoids, terpenoids, tannins, and saponins. The methanol extract and fractions of *D. densiflorum* stem bark contained flavonoids, tannins, and saponins. The total phenol analysis showed that leaves and stem bark methanol fractions had the highest content, at 4.631 mg GAE/g and 3.011 mg GAE/g, respectively. While the total flavonoids analysis showed that leaves methanol extract and bark ethyl acetate fraction had the highest content compared to other samples from the same origin, which are 49.585 mg QE/g and 63.432 mg QE/g, respectively. Antioxidant test results using DPPH radical scavenging and the ABTS assay obtained the highest IC_{50} values, respectively, in the stem bark methanol fraction of 104.092 µg/mL and the leaves ethyl acetate fraction of 8.49 µg/mL. The ethyl acetate fraction of *D. densiflorum* leaves contains the highest antioxidant activity. The results indicate that *D. densiflorum*

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extracts and fractions have antioxidant potential. Further research is required to identify compounds that act as antioxidants.

1 Introduction

Many studies have been carried out regarding the use of natural compounds as alternative medicine. Research on traditional plants and active compounds that have the potential for prevention and treatment is carried out with the aim of reducing doses and side effects due to consumption of standard medicines. *Dysoxylum densiflorum* is a commonly found plant in tropical countries that local communities use as an alternative treatment for multiple ailments. The majegau plant, known as gaharu in Indonesian and cempaga in Java, this plant belongs to the Meliaceae family (mahogany-mahonian tribe). Dysoxylum genus is widespread across India, Sri Lanka, Australia, New Zealand, Malaysia, and Indonesia.[1,2]
Meliaceae is a vast plant family, widely spread across tropical and subtropical regions, encompassing approximately 50 genera and over 1400 species. Among these, *D. densiflorum* (Meliaceae) is an evergreen tree traditionally utilized in the treatment of various ailments in children, including fever, limb stiffness, convulsions, bleeding, and facial deformation. [3]

Several previous studies have reported various biological activities exhibited by compounds isolated from the genus Dysoxylum. Several of these genera have pharmacological activity due to the compounds they contain. These compounds have been found to exhibit various properties including being anti-breast cancer MCF-7, antiplasmodium, anti-inflammatory, antibacterial, antifeedant, and others.[4] The pharmacological activity of each Dysoxylum plant is derived from its constituent compounds. In each species, the Dysoxylum genus contains various active compounds. While these compounds differ among species, the majority can be categorized as steroids, triterpenoids, and alkaloids.[5]
This study aims to determine the phytochemical content, total phenolics, total flavonoids and antioxidant activity of extracts and fractions of leaves and stem bark of *D. densiflorum*.

2 Materials and Methods

2.1 Materials

The leaves and stem barks of *D. densiflorum* were collected from Bogor Botanical Garden, Bogor, West Java, Indonesia. Chemicals such as chloric acid (HCl), bouchardat reagent, mayer's reagent, dragendorf, sulfuric acid (H_2SO_4), chloroform, $FeCl_3$, anhydrous acetate, Folin-ciocalteau, gallic acid, quercetin, ascorbic acid, $NaNO_2$, $AlCl_3$, NaOH, DPPH (2, 2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis-(3 ethylbenzothiazoline)-6-sulfonic Acid) were analitical grade. The solvents used for extraction and fractionation of *D. densiflorum* leaves and stem barks were methanol, n-hexane and ethyl acetate.

2.2 Extraction

Leaves of *D. densiflorum* were extracted with methanol by maceration method. Maceration is done for 24 hours to 3 repetitions. The extract was dried by using a forced fan oven at 50° C to constant weight. The methanol extract was partitioned with solvent of hexane, ethyl acetate and methanol respectively.

2.3 Phytochemical screening

2.3.1 Alkaloid Identification [6,7]

Test solution was prepared by adding 1 mL of 2N HCl and 9 mL water to 500 mg of extract then heated in a water bath for 2 minutes, cooled and filtered to obtain the filtrate.

- Bouchardat reagent

Boucchardat reagent consist of 2 g of iodine and 4 g of potassium iodide which dissolved in 100.0 mL of aquadest. The 1 mL of sample filtrate was added with 2 drops of Bouchardat reagent. Positive alkaloid content was observed if the brown to black precipitate was formed.

- Mayer's reagent

Mayer's reagent was consist of a mixture of mercury (II) chloride (1,358 g $HgCl_2$ in 60 mL distilled water) with potassium iodide solution (5 g potassium iodide in 10 mL distilled water) and made up to 100.0 mL with distilled water. The 1 mL of sample filtrate was added with 2 drops Mayer reagent. Positive alkaloid content was observed if a white or yellow deposited precipitate was formed and could dissolves in methanol.

- Dragendorf Reagents

Dragendorf reagents was consist of a mixture of bismuth nitrate (8 g bismuth nitrate in 20 mL of nitric acid) and potassium iodide (27.2 g potassium iodide in 50.0 mL distilled water) solution and made up to 100 ml with distilled water. The 1 mL of sample filtrate was added with 2 drops Dragendorf reagent. Positive alkaloid content was observed if a brown orange precipitate was formed then it contains a positive alkaloids.

The sample was consider contains alkaloids if at least formed a precipitate with two classes of experimental solution used.

2.3.2 Identification of flavonoid

Several mg of extract were added 4 mL of ethanol to the soluble extract. The results of identification compared with the standard of the cat's whiskers. 2 mL of solution added 0.5 grams of zinc powder and 2 mL of 2N HCl, allowed to stand for 1 minute. Then added 10 drops of concentrated HCl. If intensive red color formation within 2-5 minutes indicates the presence of flavonoids (glycosides-3-flavonols).[6]

2.3.3 Identification of tannin

Several mg of viscous extract was boiled in 15 mL of water. Afew drops of 1% $FeCl_3$ was added in filtrate and observed for colour change to green violet [7]. Several mg extract was put into the test tube and was added 10 mL of hot water, cooled and shaken firmly for 10 seconds. Form of steady foam as high as 1 to 10 cm for not less than 10 minutes. Addition of 1 drop of 2N HCl froth is not lost shows the presence of saponins.[6]

2.3.4 Identification of saponin

Several mg extract was put into the test tube and was added 10 mL of hot water, cooled and shaken firmly for 10 seconds. Form of steady foam as high as 1 to 10 cm for not less than 10 minutes. Addition of 1 drop of 2N HCl froth is not lost shows the presence of saponins.[6]

2.3.5 Identification of terpenoid

The crude extract was separately shaken with 2 mL of chloroform followed by the addition of concentrated H_2SO_4 along the side of the test tube, a reddish brown coloration of the

interface indicates the presence of terpenoid [8].

2.4 Total Phenolic Procedure

The content of total phenolic was determined using the Folin-ciocalteau method [9] with some modification. 2 mg of sample was dissolved in 2 mL methanol (1000 ug / mL). 500 µL of extract solution and 1000 ug / mL standard solution gallic acid of 50, 100, 150, 200 and 250 µL respectively was added into the test tube, then added 3.5 mL of aquadest and 250 µL of Folin -ciocalteau and shaken. After 8 minutes added 750 µL Na_2CO_3 20% and shaken until homogeneous. The solution allowed to stand for 2 hours at room temperature and then the absorbance was measured by spectrophotometer at 765 nm. The resulting phenol content was obtained as equivalent of mg the gallic acid (GAE) / gram sample.

2.5 Total Flavonoid Procedure

The content of total flavonoids was determined using aluminium chloride colorimetric assay method [10] with modification. The extract solution 1000 ppm (1000 µL) and the standard solution quercetin (50, 125, 250 and 500 µL) were added into the test tube, and then added 2 mL of aquadest. Into the tube is added 150 uL $NaNO_2$ 5%. After 5 minutes added 150 uL $AlCl_3$ 10%. After 6 min was added 2 mL of 1 M NaOH and added aquadest to 5 mL and was shaken until homogeneous and measured uptake at a wavelength of 510 nm. Levels of flavonoids obtained equivalent with mg quercetin (QE) / gram sample.

2.6 Antioxidant Activity

2.6.1 DPPH Method

The antioxidant activity test was performed using DPPH free radical scavenging activity method [11]. An extract of 4 mg was dissolved in 4 mL methanol to obtain 1000 µg/mL as mother solution of test sample. This test samples were diluted with methanol to concentrations of 10, 50, 100 and 200 µg/mL respectively. The test samples were mixed with the methanol solution of 500 µL DPPH and incubated at 37 °C for 30 minutes. The absorbance was measured at 515 nm.
Inhibition percentage of the test samples was compared to that of control (methanol). The positive control test was use solutions of ascorbic acid and quercetin. Percentage of inhibition (free radical scavenging activity) was calculated by the equation :

$$\%\ inhibition = \frac{\mathrm{Ac-As}}{\mathrm{As}}\ x\ 100 \quad (1)$$

Where Ac is absorbance of blank solution and As is absorbance of sample or standard

2.6.2 ABTS Method

ABTS reagent was prepared by combining 5 ml of 7 mM ABTS with 88 µL of 140 mM potassium persulfate. The resulting mixture was stored in the dark at room temperature for 18-20 hours to allow the formation of free radicals, and then diluted with water (1:44, v/v). To determine the inhibition activity, 1000 µL of ABTS was mixed with 1000 µL of the

sample in test tube and incubated for 6 minutes. Following incubation, the absorbance was measured at 734 nm using spectrophotometer. The negative control involved mixing 100% methanol with ABTS [12].nPercentage of inhibition (free radical scavenging activity) was calculated by the equation (2) :

$$\% \, inhibition = \frac{\text{Ac}-\text{As}}{\text{As}} \, x \, 100 \quad (2)$$

where Ac is absorbance of blank solution and As is absorbance of sample or standard

3 Result and Discussion

The yield of extracts and fractions of *D. densiflorum* leaves and stem bark can be seen in Table 1. The partition with ethyl acetate fraction of leaves resulted in the highest amount of total extractable compounds compared with other solvents with a total yield of 58 %.

Table 1. The yield of extracts and fractions of *D. densiflorum* leaves and stem bark

Sample	Yield (%)	
	Leaves	Stem bark
Methanol Extract (ME)	17.8	12.4
Hexane fraction (HF)	16.67	16.67
Ethyl acetat fraction (EAF)	58	28
Methanol fraction (MF)	25.33	55.33

3.1 Phytochemical screening

Phytochemical screening was carried out to determine the presence of secondary metabolites in each extract and fraction of *D. densiflorum* leaves and stem bark , such as alkaloids, flavonoids, terpenoids, steroids, saponins and tannins. In this study, the results of *D. densiflorum* phytochemical screening can be seen in Table 2, with the highest content is flavonoid.

Table 2. Phytochemical screening extract and fraction of *D. densiflorum* leaves and stem bark

Parameter	*D. densiflorum* leaves				*D. densiflorum* stem bark			
	ME	HF	EAF	MF	ME	HF	EAF	MF
Alkaloid								
-Bouchardat	-	-	-	-	-	-	-	-
-Meyer	-	-	-	-	-	-	-	-
-Dragendorf	-	-	-	-	-	-	-	-
Flavonoid	++	+	+++	+++	+++	++	++	+
Tannin	+	-	+	+	-	-	-	-
Saponin	++	-	+	+	++	-	-	-
Terpenoid	-	++	-	+	++	+++	+	++

From the results obtained all extracts and fractions did not contain alkaloid. The phytochemical analysis showed the presence of flavonoid in all extracts and fractions. Flavonoid and phenolic were found in all parts of the plant samples, and these components are produced by plants to protect or promote growth under unfavourable conditions. Moreover, flavonoid and phenolic compounds are generally known for their antioxidant properties [13]. Phytochemical screening is the examination of the chemical content qualitatively to know the class of compounds contained in a plant. The examination is directed to secondary metabolite compounds that have health benefits such as, alkaloids, flavonoid compounds, terpenes, tannins, saponins, glycosides, quinones and anthraquinones. Plants contain numerous phytochemical constituents, many of which are known to be biologically active compounds and are responsible for exhibiting diverse pharmacological activities [14]. Different bioactive compounds, such as alkaloid, flavonoid, phenolic, saponin and terpenoid show that these plants may have the potential as medicinal plants.

3.2 Total phenolic and flavonoid

The total phenolic compound content in the extract was determined using the Follin-Ciocalteu method used gallic acid as a standard. The total phenolic compound content in the extract is expressed as gallic acid equivalent (mg/g extract). The total flavonoid content was calculated based on the aluminum chloride method. The total flavonoid compound content is expressed as quercetin equivalent (mg/g extract). The results of total phenolic and flavonoid contents are shown in Table 3**.**

Table 3. The results of total phenolic and flavonoid of extract and fraction of *D. densiflorum* leaves and stem bark

Sample	*D. densiflorum* leaves				*D. densiflorum* stem bark			
	ME	HF	EAF	MF	ME	HF	EAF	MF
Total phenolic (mg GAE/g)	1.841	0.674	0.669	4.631	1.453	1.078	0.237	3.011
Total Flavonoid (mg QE/g)	49.585	-	30.814	20.034	46.076	-	63.432	9.593

From the results obtained the highest total phenol value was found in the leaves methanol fraction at 4.631 mg GAE/g and in the stem bark methanol fraction at 3.011 mg GAE/g. Meanwhile, the highest total flavonoid value was found in the methanol extract of both leaves and stem bark, 49.585 mg QE/g and 46.076 mg QE/g respectively. Phenolic compounds act as reducing agents, hydrogen donors, and are the dominant antioxidants that exhibit scavenging efficiency on free radicals and reactive oxygen species are numerous and widely distributed in the plant kingdom. Presence of considerably good amount of phenolics in the fruits, seeds, and bark extracts of *Z. armatum* may contribute significantly to the antioxidant properties. Because of these properties, this plant might have been used in several traditional herbal medications [15, 16]. Phenolic substances are secondary metabolites present in plants having a variety of biological functions, including protection from oxidative damage. Plants have been shown to produce phenolic chemicals in response to oxidative stress. On the other

hand, flavonoids, a class of phenolic compounds are well known for antioxidant property. Besides acting as antioxidants, flavonoids function as stabilizers of scavenging chemicals that are otherwise washed away by ROS flooding. This dual role is acclaimed to large quantity of free OH groups, notably 3-OH and resultant higher reactivity of flavonoids hydroxyl groups with oxidants [17].

3.3 Antioxidant Activity

The radical scavenging activity of extract and fractions of *D. densiflorum* was determined by using DPPH and ABTS assay. Antioxidants activity measurement methods can determine the characteristics which is different from the antioxidants in sample. There are various methods that can be done used to measure totals antioxidant characteristics, but not yet no one method is considered as the most ideal. Various methods different activity measurements can be produce antioxidant mechanisms of different action.
The results of antioxidant activities are shown in Table 4

Table 4. The results of antioxidant activities of extract and fraction of *D. densiflorum* leaves and stem bark

Sample	*D. densiflorum* leaves				*D. densiflorum* stem bark			
	ME	HF	EAF	MF	ME	HF	EAF	MF
DPPH IC_{50} (µg/mL)	289.211	*NA	*NA	264.625	289.576	*NA	*NA	104.092
ABTS IC_{50} (µg/mL)	118.85	*NA	8.49	14.15	93.52	*NA	135.54	144.59

*NA = not active

A compound is have very strong antioxidant activity if the IC_{50} value less than 50 ppm, the strong IC_{50} group is between 50-100 ppm, the medium group if the IC_{50} value is 101-150 ppm, and the weak group if the IC_{50} value is between 150-200 ppm [18]. According to the results, in the DPPH method only the methanol fraction of the stem bark has antioxidant activity. Meanwhile, in the ABTS method, almost all samples had antioxidant activity except the leaves hexane fraction and the stem bark hexane fraction. The antioxidant inhibition pattern was differed might be due the difference content of antioxidant compounds and possible antagonistic or synergistic interaction among compound present in the fractions and extract.

The ABTS and DPPH methods have the advantages of each. ABTS method can be used on based systems water or organic, and has reaction time faster and can work over a wide pH range. However, this method is very sensitive to light and takes time. The incubation is quite long 12-16 hours in dark conditions. The DPPH method can be used for both solid and liquid samples, however doesn't work specifically for certain antioxidant components. This method measuring the antioxidant capacity of samples overall with the hydrogen scavenging reaction by DPPH from antioxidant substances [19].

Based on the ability of antioxidant compounds between DPPH and ABTS have different mechanisms the reaction. In DPPH antioxidant ability a compound is seen based on its ability antioxidant compounds for donation hydrogen. Meanwhile in the ABTS test the ability of antioxidant compounds based on the ability of antioxidant compounds to stabilize free radical compounds with donating proton radical [20].

4 Conclusions

The results of phytochemical screening showed *D. densiflorum* leaves containing of secondary metabolite class compounds were flavonoid, tannin, saponin and terpenoid. *D. densiflorum* stem bark containing of secondary metabolite class compounds were flavonoid, saponin and terpenoid The highest total phenol were found in the methanol fraction of *D. densiflorum* leaves and the highest total flavonoids were found in the ethyl asetat fraction of *D. densiflorum* stem bark. Ethyl acetate fraction of *D. densiflorum* leaves have highest antioxidant activity. Based on the results, the extracts and fractions of *D. densiflorum* has potential as an antioxidant. Further research is needed to obtain compounds that are active as antioxidant

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