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Antioxidant Capacity Fraction of the Pelawan Stems (Tristaniopsis merguensis Griff)

Robby Gus Mahardika [1*], Khairul Fajri[1], Henri[2]

[1]Departement of Chemistry, Faculty of Engineering, Universitas Bangka Belitung Jl. Kampus Peradaban, Merawang, Bangka, 33172

[2]Departement of Biology, Faculty of Engineering, Universitas Bangka Belitung Jl. Kampus Peradaban, Merawang, Bangka, 33172

[*]*Corresponding Author: robby@ubb.ac.id*

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Abstract

Free radicals destroy other molecules such as proteins, fats, carbohydrates, or DNA. The impact of reactions from free radical compounds can cause various health problems such as inflammation, aging, and cancer. Therefore, to avoid damage caused by free radicals, the body needs an important substance, antioxidants. Natural antioxidants are more recommended than synthetic antioxidants because synthetic antioxidants must be in accordance with the prescribed dose because they can cause side effects that are harmful to the body. This study aimed to determine the antioxidant activity of the extract and fraction of the Pelawan (Tristaniopsis merguensis Grifft.) stem extract. Antioxidant testing was carried out using the DPPH method on extracts and fractions of Pelawan stem extract (T. merguensis Grifft.). In addition, qualitative phytochemical testing was also carried out on extracts of the stems of Pelawan (T. merguensis). The results of the three solvent fractions, n-hexane, ethyl acetate, and methanol, were positive for flavonoid and phenolic secondary metabolites. Meanwhile, the antioxidant activity in reducing DPPH radicals in the methanol extract fraction of pelawan stems had a very strong activity with an IC50 value of 36.33 g/mL, while the ethyl acetate and n-hexane extract fractions had a very weak antioxidant activity with an IC50 value of 387.43 µg/mL dan 759.88 µg/mL.

Keywords: Pelawan Stems, Antioxidants, Phytochemicals, DPPH, IC_{50}

INTRODUCTION

Currently, many people use private vehicles to make their activities easier. Increased use of vehicles can result in much pollution generated by vehicle exhaust. Free radicals are one of the things caused by pollution (Septiana, Muchtadi, & Zakaria, 2002). The pollution from vehicle fumes can increase free radicals, where excess free radicals can cause various diseases.

Free radicals are unstable compounds in which the outer orbit of the atom has one or more unpaired electrons (Khaira, 2016). Free radicals damage cell-forming macromolecules such as proteins, fats, carbohydrates, and DNA. The behavior of these free radicals is destructive to other molecules whose electrons are taken. Taking electrons by free radicals causes a reaction, forming more free radicals. The effects of reactions from free radical compounds can cause various health problems such as inflammation, aging, and cancer. Therefore, to avoid damage caused by free radicals, the body needs important substances, namely antioxidants, which can protect the body from free radical attacks (Khaira, 2016).

Antioxidants act as inhibitors that inhibit the oxidation process by reacting with reactive free radicals to form non-reactive free radicals, which are relatively stable so that they can protect cells from the harmful effects of reactive oxygen free radicals (Sofia, Prabowo, & Rijai, 2016). Natural antioxidants are more recommended than synthetic antioxidants because synthetic antioxidants must be in accordance with the prescribed dose because they can cause side effects that are harmful to the body. Sources of natural antioxidants come from plants which generally contain phenolic secondary metabolites scattered in plant parts such as fruit, seeds, flowers, leaves, pollen, wood, and roots (Sarastani, Soekarto, Muchtadi, & Fardiaz, 2002). Phenolic compounds and polyphenols can change or reduce free radicals and act as anti-free radicals (Pietta, 2000; Agatonovic-Kustrin, W. Morton, & Ristivojevic, 2017).

Based on previous studies, the major content of secondary metabolites was found in the *Tristaniopsis* genus is tannins and phenolics. The content of phenolic compounds in the genus *Tristaniopsis* has the uniqueness of

glycosylated phenolics (Verotta, 2001). Pelawan leaves contain secondary metabolites such as alkaloids, phenol, hydroquinones/tannins, and flavonoids. Another study found that the total phenolic content in pelawan leaves was 215.22 mg GAE/g dry extract and had a strong antioxidant activity with an IC_{50} value of 22.1454 µg/mL (Roanisca, Mahardika, & Sari, 2019; Souhoka, Hattu, & Huliselan, 2019)). The research above shows that pelawan plants can be a source of antioxidants.

Various studies have been conducted to determine plant stems and leaves' antioxidant activity and toxicity. Based on the results of research on extracts of karamunting (*Rhodomyrtus tomentosa*), it is known that the stems have a lower toxicity value of >500 ppm compared to the leaves of 43.38 ppm. However, for antioxidant activity in stems and leaves (*Rhodomyrtus tomentosa*), both have strong antioxidants with IC_{50} values of 6-50 ppm (Kusuma, Ainiyati, & Suwinarti, 2016)\. This study showed that stems have high antioxidant activity with lower toxicity than leaves. In addition, the choice of solvent is important in extracting the active components in plants. Therefore this study aims to determine the antioxidant activity of extracts and fractions of Pelawan stem extract (*T. merguensis* Grifft.)

METHODOLOGY

Materials

The tools used in this study were measuring cups, beaker glasses, Erlenmeyer, dropper pipettes, volume pipettes, test tubes, filter paper, stirring rods, vials, measuring flasks, aluminum foil, vortex, analytical balance, rotary evaporator IKA RV 10 and a Shimadzu UV-1800 UV-Vis spectrophotometer, and an FTIR spectrophotometer. At the same time, the materials used in this study were Pelawan stem Simplicia, technical methanol, technical n-hexane, technical ethyl acetate, distilled water, acetone, 2 N hydrochloric acid, Wagner reagent, Meyer reagent, magnesium metal powder, amyl alcohol (a mixture of hydrochloric acid 37% and 95% ethanol), ethanol, chloroform, glacial acetic acid, sulfuric acid, 1% ferric chloride, DPPH (Sigma).

Extraction

The Pelawan stems fine powder was taken 250 grams and then macerated using three solvents, including n-hexane, ethyl acetate, and methanol. 2500 mL of each solvent was used, and Pelawan stem extract from each solvent was macerated for 3 x 24 hours. Then the filtrate obtained from each solvent will then be concentrated using a rotary evaporator to obtain a thick extract. The extract and the three fractions were subjected to FT-IR analysis to determine the functional groups in the extract.

Antioxidant Testing

This antioxidant test was carried out based on the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. First, a test solution was prepared by weighing each pelawan stem extract from each solvent as much as 0.005 g dissolved in 50 mL methanol to obtain a concentration of 100 ppm. The solution is then pipetted as much as 0.1; 0.5; 1, 2, and 5 mL and diluted in a 10 mL volumetric flask. Then each of these solutions was put into a test tube of 1 mL and added 2 mL of methanol, and 1 mL of DPPH (0.005 gram in 50 mL of methanol). Then prepare the test tube again for the blank solution, 3 mL of methanol added 1 mL of DPPH. Each solution and blank was stirred using a vortex for 30 seconds and incubated for 30 minutes at 37^{o}C. After that, the solution and blank were tested using a UV-Vis spectrophotometer at 515 nm (Mahardika & Roanisca, 2018; Mahardika, Roanisca, & Sari, 2020; Fadiyah, Lestari, & Mahardika, 2020)).

RESULTS AND DISCUSSION

The Pelawan stems refine using a wood-crushing machine and a blender (Heriani, Sari, & Oktasari, 2021). In general, extraction is divided into two: single and multilevel. Single extraction can be carried out using only one solvent, whereas, in multilevel extraction, two or more solvents are used. Stratified extraction will produce certain compounds extracted specifically for each solvent used.

In contrast, single extraction produces the extracted compounds, total extracts that can be extracted with solvents. The solvent used in multilevel extraction uses solvents with different polarity levels. Non-polar solvents can extract terpenoid groups, including triterpenoid compounds, steroids, and saponins, while polar solvents will extract compounds such as alkaloids and phenolic groups, including flavonoids and tannins (Gong et al., 2016). In comparison, semi-polar solvents will attract compounds such as flavonoids, terpenoids, aglycones, and glycosides (Harborne, 1987).

The Pelawan stem maceration using multilevel extraction method. Multilevel extraction was done by sequentially soaking the sample in different solvents, starting from non-polar, semi-polar, and polar solvents. The solvents used in the extraction of Pelawan stems are solvents with increasing polarity, namely n-hexane (non-polar), ethyl acetate (semi-

polar), and methanol (polar). The first maceration was done by soaking Pelawan stem simplicial powder in n-hexane solvent for 3x24 hours. The filtrate is separated by filtering, while the dregs are macerated again with ethyl acetate solvent, and then separation is carried out again. The same treatment is for methanol solvent. All the extracts were then concentrated using a rotary evaporator to evaporate the solvent to produce a thick extract. The yield of the Pelawan stem extract with n-hexane, ethyl acetate, and methanol solvents were 4.232; 3.0917; and 9.7145 grams with a yield percentage of 1.69; 1.56; and 3.88%. These data indicate that the highest extract yield is methanol. It is because the active compounds contained in pelawan stems are polar.

Phytochemical identification was carried out qualitatively on Pelawan stem extract. The results of identifying secondary metabolites of Pelawan stem extract are shown in Table 1. The results of the phytochemical tests showed that Pelawan stem extract in each solvent was positive, containing phenolic compounds and flavonoids. At the same time, the negative results on the test are alkaloids, steroids, and saponins. Tests on pelawan leaves have been carried out by Enggiwanto, Istiqomah, Daniati, Roanisca, & Mahardika, (2018), where the Pelawan leaves were positive for alkaloid, flavonoid and phenolic secondary metabolite compounds. Based on the biosynthesis of secondary metabolites in plants, they are divided into three main groups: the terpenoid group, including triterpenoid compounds, steroids, and saponins. The phenolic group, including flavonoids and tannins, and the alkaloid group is an alkaline secondary metabolite compound containing one or more nitrogen atoms and usually in a heterocyclic ring.

FT-IR analysis was carried out to determine the functional groups in the Pelawan stem extract as indicated by the presence of absorption at certain wave numbers by giving different absorptions to each sample. The FTIR results can be seen in Figure 1. Based on the FTIR results of the Pelawan stem extract, the results were analyzed for wave numbers with functional groups, which are presented in Table 2.The results of functional group analysis of Pelawan stem extract using FTIR spectrophotometry found a broad absorption at wave numbers 3000-3600 cm^{-1}, which indicated the presence of O–H groups. The stretching C-H group is in the range of 2850-2960 cm^{-1}. The C=O stretch ranges from 1700-1780 cm^{-1}. the C=C group stretches 1500-1680 cm^{-1}, the C-H groups bending 1350-1470 cm^{-1}. The C-O group ranges from 1060-1300 cm^{-1}. At the same time, C-H is aromatic was found at wave numbers 675-995 cm^{-1}. Based on the results of functional groups that have been analyzed, pelawan stem extract from n-hexane, ethyl acetate, and solvents have the C=O functional group, which indicates that pelawan stem extract contains flavonoid compounds and is also supported by the presence of O-H groups found in wave numbers 3000-3600 cm^{-1},

Table 1. Results of identification of secondary metabolites of Pelawan stem extract

Test	Test Method	Pelawan Stem Extract			Ethanol Extract of Pelawan Leaves (Enggiwanto et al., 2018) and the fraction of ethyl acetate extract of methanol leaves (Samsiar, Mahardika, & Roanisca, 2021)
		n-hexane	Ethyl Acetate	Methanol	
Alkaloid	Mayer	-	-	-	+
	Wagner	-	-	-	+
Flavonoid	$FeCl_3$	+	+	+	+
Phenolic	Wilstater Sianidin	+	+	+	+
Steroid	Forth	-	-	-	-
Saponin	Liebermann-Burchard	-	-	-	-

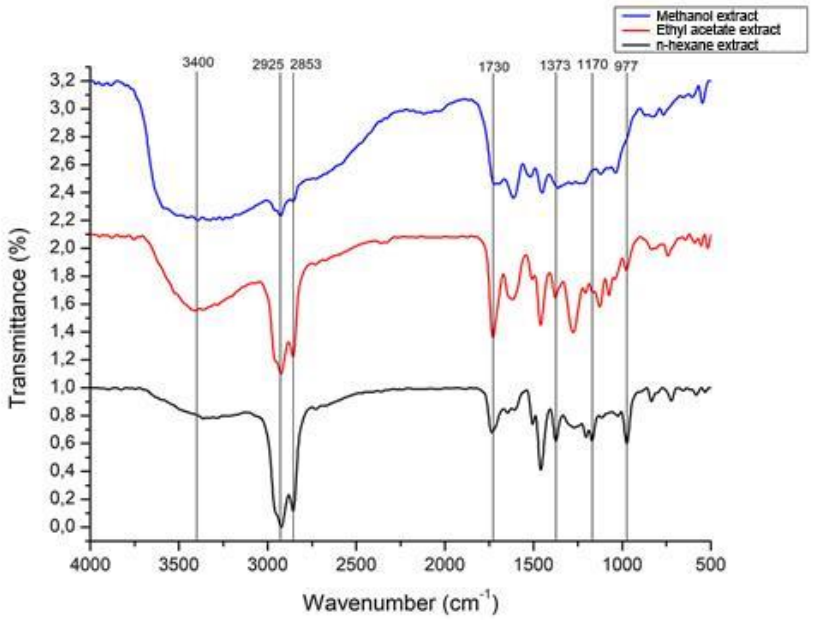

Figure 1. FTIR spectrum of Pelawan stem extract

C=C aromatic at 1500-1680 cm^{-1}, C-O at wave number 1060-1300 cm^{-1} as well as the results of the analysis of pelawan stem extract also contains phenolic as indicated by the presence of O-H groups found at wave numbers 3000-3600 cm^{-1}, C-H at wave numbers 2850-2960 cm^{-1}, C=C aromatic at 1500-1680 cm^{-1}, and C-O at wave numbers 1060-1300 cm^{-1}. Based on previous research on Pelawan leaves by Samsiar, Mahardika, & Roanisca, (2021) showed differences in the functional groups found in Pelawan, Pelawan leaves do not have C-H stretch groups which are in the range of wave numbers 2850-2960 cm^{-1}, and Pelawan stem extract does not there is a C-H group (CH_2 (methylene) which is present in wave number 1450 cm^{-1} and the aromatic C-H group in the methanol extract of pelawan stem which is present in the absorption wave number between 675-995 cm^{-1}.

Table 2. FTIR Spectrum Analysis Results of Pelawan Stem Extract

Fuctional Groups	Band shape	Wave Numbers (cm^{-1})			
		n-Hexane Extract	Ethyl Acetate Fraction	Methanol Fraction	Ethyl Acetate Fraction of Pelawan Leaves (Samsiar, Mahardika, & Roanisca, 2021)
O-H stretch	Broad	3364	3384	3379	3320
C-H stretch	Sharp	2925	2915	2926	2940
C-H stretch (aliphatic)	Sharp	2857	2855	2852	-
C=O stretch	Sharp	1735	1729	1720	1710
C=C stretch	Sharp	1610	1618	1612	1610
C-H (CH_2)	Sharp	-	-	-	1450
C-H (CH_3) bend	Sharp	1374	1376	1362	1320
C-O bend	Sharp	1023	1073	1037	1020
C-H (aromatic)	Sharp	973	976	-	750

Antioxidant activity

The antioxidant activity test was carried out quantitatively using a spectrophotometer by measuring the concentration inhibition by the sample's ability to scavenge radicals from DPPH. Antioxidant activity testing was carried out using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The reaction mechanism of DPPH with antioxidant compounds is radical. DPPH is a radical compound that reacts with antioxidant compounds, which can be phenolic compounds or flavonoids.

Antioxidant compounds will reduce DPPH and pair with hydrogen from antioxidant compounds to form reduced DPPH-H (Lee & Kim, 2022). The reaction between antioxidant compounds and DPPH will reduce the intensity of the purple color in DPPH, which can then be measured using a spectrophotometer. The decrease in the intensity of the purple color is due to the interaction of antioxidant compounds that donate electrons to the DPPH radicals. The more antioxidant compounds, the intensity of the purple color will decrease. Quantitative testing of antioxidant activity was carried out using a UV–Vis spectrophotometer at a wavelength of 515 nm to determine IC_{50}. IC_{50} states the concentration at which the extract can decompose 50% of free radicals, which in this test is DPPH. The results of the analysis of the antioxidant test can be seen in Table 3.

Based on Table 3. The antioxidant activity of Pelawan stem methanol extract of pelawan stem has a small IC_{50} value compared to another pelawan stem extract, namely 36.33 μg/mL. It shows a correlation between total phenolic and flavonoid levels with antioxidant activity. Based on the total phenolic test, the methanol extract had the highest total phenolic content, giving it a strong antioxidant activity. Pelawan stem ethyl acetate extract has antioxidant activity with an IC_{50} value of 387.43 μg/mL. At the same time, the pelawan stem n-hexane extract had very weak antioxidant activity giving an IC_{50} value of 759.88 μg/mL. The results of the antioxidant activity test showed that the methanol extract from pelawan stems had the highest antioxidant activity compared to the ethyl acetate and n-hexane extracts. It is due to the content of phytochemical compounds, especially phenolics which have more antioxidant activity extracted in polar solvents. The more phytochemical compounds that have antioxidant activity are extracted, the higher the antioxidant activity. Based on the results of the antioxidant activity test of the pelawan stem methanol extract, this is weaker when compared to the ethanol extract and acetone of pelawan leaves with consecutive IC_{50} values of 18.2772 μg/mL (Enggiwanto et al., 2018) and 22.1454 μg/mL (Roanisca et al., 2019). It is due to the presence of alkaloids in pelawan leaves, while in pelawan stems, no alkaloids were found.

Table 3. Antioxidant Activity of Pelawan

No.	Samples	Antioxidant activity (IC_{50})	References
1	Pelawan Stem Methanol Extract	36.33 μg/mL	This research
2	Pelawan stem ethyl acetate extract	387.43 μg/mL	This research
3	Pelawan stem ethyl n-hexane	759.88 μg/mL	This research
4	Ethanol Extract of Pelawan Leaves	18.2772 μg/mL	Enggiwanto et al., 2018
5	Acetone Extract of Pelawan Leaves	22.1454 μg/mL	Roanisca et al., 2019

CONCLUSION

Based on the results and discussion, it was concluded that qualitatively the phytochemical testing of Pelawan stem extract (T. merguensis) from the three solvent fractions, such as n-hexane, ethyl acetate, and methanol, was positive for containing secondary metabolites of flavonoids and phenolics. In comparison, the antioxidant activity in reducing DPPH radicals in the pelawan stem methanol extract fraction had a very strong activity with an IC_{50} value of 36.33 μg/mL. At the same time, the ethyl acetate and n-hexane extract fractions had a weak antioxidant activity with IC_{50} values, respectively, 387.43 μg/mL and 759.88 μg/mL.

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