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ANTIOXIDANT ACTIVITY OF LEAF AND STEM EXTRACTS OF PELAWAN PLANT (*Tristaniopsis obovata*) AND DETERMINATION OF TOTAL FLAVONOIDS, TOTAL PHENOLICS, AND TOTAL CAROTENOIDS

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ABSTRACT

The needs of antioxidants are increasing to prevent the effects of free radicals. Natural antioxidants can be developed from medicinal plants, one of them is Pelawan Plant. This study aims to determine antioxidant activity, total flavonoids, total phenolics, and total carotenoids of Pelawan plant extracts. The extraction was carried out by reflux method using various solvent polarity such as n-hexane, ethyl acetate, and ethanol. Antioxidant activity was qualitatively analyzed using thin layer chromatography and visible UV-ray spectrophotometry with DPPH free radical scavenging and CUPRAC methods. Determination of total flavonoids, total phenolics and total carotenoids was carried out using UV-Visible spectrophotometry. The results of antioxidant activity test by using a DPPH showed that ethanol extract of leaves of Pelawan plant had the strongest activity with IC50 values of 17.68 µg/mL. The results of antioxidant activity test by CUPRAC method showed that ethanol extract of leaves of Pelawan plant had the lowest capacity with EC50 value of 14.74 µg / mL. The highest total flavonoid content was found in ethyl acetate extract of stems of Pelawan plant (4.8237 mg QE/100 mg), the highest

total phenolic content was in ethanol extract of leaves of Pelawan plant (13.3625 mg GAE/100 mg) and the highest total carotenoid content was in n-hexane extract of leaves of (8.3297 mg BE/100 mg). In conclusion that the ethanol extract of leaves of Pelawan plant had very strong antioxidant activity.

Keywords: Antioxidant, Carotenoids, CUPRAC, DPPH, *Tristaniopsis obovata*

INTRODUCTION

Indonesian biodiversity is very potential in the discovery of new compounds as antioxidants (free radical). A free radical is an atom or group of atoms that have one or more unpaired electrons and as a consequence, it is highly reactive **[1]**. One of Indonesian forest plants that have not yet been explored for its potential is *Tristaniopsis obovata*, which is widely known as Pelawan plant. Studies on Pelawan plants as medicinal plants is still not widely investigated, so that further study is needed. This species is rich in essential oils that have been utilized in traditional medicines as antibacterial, antioxidant and antifungal.

Pelawan plant is plant species endemic to the island of Bangka and spread on the island of Sumatera as well. This plant is commonly used as fever and high blood pressure medications. Bangka's typical Pelawan plants are also useful as blood circulation and body fresheners. Pelawanplants (*Tristaniopsis obovata*) also have been used as new herbal plants for stroke treatments but it has not yet been tested scientifically. Several studies on antimicrobials from the plant which is the same species with Pelawan, namely White Pelawan (*Tristaniopsis whiteana*), showed that ethyl acetate extracts against Escherichia colli and Staphylococcus epidermidis bacteria had diameter of inhibition zone of 11 and 9 mm **[2]**.

The results of previous studies showed that n-hexane extracts from stem bark of White Pelawan plants were able to inhibit the activity of *S. aureus* and *E. coli* bacteria **[3]**. Extracts of stem bark of White Pelawan contained flavonoids, tannins, and terpenoids **[4]**. It is reported that stem bark of White Pelawan plants is used by Dayak people as antidiarrheal drugs **[5]**. The aim of this study is to conduct a preliminary screening including antioxidant activity test of leaves and stems of *Tristaniopsis obovata* and to determine total flavonoid contents, total phenolics and total carotenoids.

EXPERIMENITAL

Plant Materials

Fresh Pelawan leaves and stems was collected from the village of Cengkong

Abang, Bangka Induk, Bangka Belitung Islands. Next the sample was identified in the Herbarium Bogoreinse LIPI Bogor. The separated seed coat was shade dried and ground into powder for future use.

Chemicals and Reagents

All other chemicals and reagents used in this study were of analytical grade and experimental water was double distilled water.

Characterization and extraction

Characterization and extraction include the collection and determination of materials, and the making of simplicia including wet sorting, washing, drying, dry sorting, powder making, packaging and storage. Characterization of simplicia includes determination of total ash content, determination of acid insoluble ash content, determination of water insoluble ash content, determination of ethanol soluble extractives content, determination of water-soluble extract, determination of moisture content and drying shrinkage.

Phytochemical screening

Phytochemical screening includes examining alkaloids, flavonoids, saponins, quinones, steroids and triterpenoids **[6]**. Extraction was conducted by reflux method using various solvent polarity such as n-hexane, ethyl acetate, and ethanol. The extract is then concentrated using a vacuum rotary evaporator to obtain a concentrated extract.

Qualitative test of antioxidant activity

Qualitative test of antioxidant activity was carried out by thin layer chromatography, pre-coated silica gel GF254 as stationary phase and three solvents namely n-hexane-ethyl acetate (8: 2, v/v), ethyl acetate-chloroform (2:1, v/v), and ethyl acetate-chloroform-formic acid (9: 1: 1, v/v). A solution of 0.2% DPPH in methanol was used as a parameter of antioxidant activity which was characterized by the formation of yellow spots on a purple background **[7]**.

DPPH Radical Scavenging Activity

Quantitative test of antioxidant activity performed by DPPH method using a UV-visible spectrophotometry by reacting the extract sample with DPPH solution in a ratio of (1: 1) and incubated for 30 minutes at room temperature then measured its absorbance at a wavelength of 517 nm 7. While the CUPRAC method used bis (neocuproine) copper (II) ($Cu(NO)_2^{2+}$ as chromogenic. The blue colored $Cu(NO)_2^{2+}$ reagent was reduced to the yellow $Cu(NO)^{2+}$ and incubated for 30 minutes at room temperature where the absorbance was measured at a wavelength of 450 nm7. The DPPH radical scavenging activity was calculated using the following equation:

$$Scavengingrate(\%) = \frac{1-(A_i-A_j)}{A_0} \quad \text{.....(1)}$$

where A_i is the absorbance of the sample with the DPPH; A_j is the absorbance of the sample without DPPH; and A_0 is the absorbance of pure DPPH.

Determination of total phenolic

Determination of total phenolic content of the extract was carried out by adding FolinCiocalteu reagent with gallic acid as a standard for reacting Folin-Ciocalteu reagent samples which had been diluted first with distilled water (1:10) then incubated for five minutes and added 4 mL of 1M sodium carbonate then incubated for 15 minutes then measured by UV-Vis spectrophotometer at a wavelength of 765 nm **[8]**.

Determination of total flavonoid

Determination of total flavonoid contents from extracts used Ordon method, with AlCl3 reagent and quercetin as standard **[9]**. Whereas determination of total carotenoid contents of extracts used spectrophotometric method with β-carotene as standard **[10]**.

RESULTS AND DISCUSSION

Characterization and extraction

Phytochemical screening aims to determine class of compounds that contain in simplicia. Phytochemical screening includes examination of alkaloids, flavonoids, saponins, quinones, tannins, and steroids / triterpenoids. The results of phytochemical screening showed the absence of alkaloids in simplicia leaves and the absence of alkaloids and saponins in the stems. n-Hexane solvents were chosen because of their non-polar properties which are useful for dissolving non-polar compounds such as alkaloids, triterpenoids, steroids, pigments, and fats. Ethyl acetate solvents have semi polar properties so they can dissolve semi polar compounds such as chlorophyll, flavonoid aglycones, and free phenolic acids. Whereas 96% ethanol solvents have polar properties which are useful for dissolving polar compounds such as coumarins, flavonoids, tannins, glycosides, saponins, and other polar compounds. The results of extract rendement showed ethanol extract of leaves and stems of Pelawan had the highest rendement of 18.90% and 1.2%, respectively **(Table 1)**. This indicates that ethanol solvent dissolves more compounds contained in the simplicia samples, especially simplicia leaves.

***In Vitro* Antioxidant Activity Analysis (Qualitative)**

All extracts after reacting with 0.2% DPPH in methanol showed yellow spots on a purple background indicating antioxidant activity. The yellow color indicates the reaction between compounds that have conjugated double bonds with OH groups that react with DPPH free radicals **[7]**. Whereas, the

CUPRAC indicates antioxidant activity with the formation of yellow spots on a turquoise background. This indicates that there is a reduced reaction of CUPRAC by the compounds contained in the plants. Identification of flavonoid compounds on the plates sprayed with sitroborate resulted in fluorescent spots under UV lamp λ 366 nm. This indicates that the extract contains flavonoid compounds. On the plates sprayed by 10% $FeCl_3$ reagent showed black spots on a yellow background which indicates the extract contains phenolic compounds. Whereas the identification of carotenoid compounds on the plates sprayed by anisaldehyde-sulfuric acid reagent showed blue, orange and red purple spots which indicates the presence of carotenoid compounds. On the plates sprayed with sulfuric acid as a universal reagent, a greenish purple appeared after the plates were heated for several minutes **(Figure 1)**.

***In Vitro* Antioxidant Activity Analysis (Quantitative)**

The evaluation of antioxidant activity was carried out by reacting the sample with DPPH solution in a ratio of (1: 1) and the reaction mixture was incubated for 30 minutes in order to maximize the reaction between antioxidant compounds of extracts in various concentrations with free radicals. Evaluation of antioxidant activity was carried out in a dark room in order to avoid the degradation of DPPH solution which is easily oxidized. Previously, the reaction mixture was incubated for 30 minutes, at this time there was a proton donor reaction from extract sample that had antioxidant activity against DPPH free radicals. If the extract contains antioxidant active compounds, DPPH color will change from purple to pale yellow after reacting with the extract. It because the extract donates hydrogen atoms to reactive free radical compounds that have unpaired electrons, and in this case, DPPH is a stable free radical **[11] (Table 2)**.

The results of the evaluation of antioxidant activity with DPPH solution were expressed as IC_{50} values of the six extract samples. IC_{50} values for *n*-hexane, ethyl acetate and ethanol extracts of leaves were 130.17 µg / mL 39.91 µg / mL, leaves 17.68 µg / mL, respectively; whereas IC_{50} values for *n*-hexane, ethyl acetate and ethanol extracts of stems were 39.33 µg / mL, 43.78 µg / mL,and 51.91 µg / mL, respectively. The smaller the IC_{50} value, the higher the antioxidant activity. The antioxidant categorized as very strong if $IC_{50} \leq 50$µg / mL, strong antioxidant if IC_{50} values in the range of 50-100 µg / mL, medium antioxidant if IC_{50} values in the range 101-150 µg / mL, weak antioxidant if IC_{50} values

in the range 151-200 µg / mL and not active if the IC_{50} value is in the range of ≥ 200 µg / mL **[11]**.

In the CUPRAC method, bis (neocuproin) copper (II) ($Cu(NO)_2^{2+}$) will be reduced to form $Cu(NO)_2^{+}$). Visually the results of this reaction can be seen from the change in color of the solution from turquoise to yellow. If the sample is reduced ($Cu(NO)_2^{2+}$) to ($Cu(NO)_2^{+}$), oxidation occurs at that time, so the sample can be considered as an antioxidant. In the CUPRAC method, the sample will become an antioxidant if the reduction potential is lower than the reduction potential of Cu^{2+}/Cu^{+}which is 0.6 V, so that the sample can reduce $Cu(NO)_2^{2+}$ to $Cu(NO)_2^{+}$ **[12]**.

Antioxidant activity evaluation was carried out towards the samples and standard comparison of vitamin C. Vitamin C and samples were made in several concentrations where each of them was added with CUPRAC (1: 1) then incubated for 30 minutes at room temperature. After the incubation process was complete, the measurement was carried out using UV-visible spectrophotometry at a wavelength of 450 nm **(Table 3)**.

The results of the examination of antioxidant activity **(Table 3)** with the CUPRAC method were expressed with EC_{50} values of the six extract samples. EC_{50} values for *n*-hexane, ethyl acetate and ethanol extractsof leaves were 53.01 µg / mL, 44.35 µg / mL, and 14.74 µg / mL, respectively; while the EC_{50} value for *n*-hexane, ethyl acetate and ethanol extractsof stems were 36.05 µg / mL, 61.20 µg / mL, and 58.31 µg / mL, respectively. The smaller the EC_{50} value, the higher the antioxidant activity. The EC_{50} value obtained in the CUPRAC method is higher. This is due to the influence of potential energy values (E_0) of each sample so that the value obtained is relatively high. The principle of the CUPRAC method is the ability of a compound to reduce Cu^{2+} to Cu^{+}. Only compounds that have a reduction potential lower than Cu^{2+} / Cu^{+} have the ability to reduce.

Total flavonoid

Total flavonoids was measured using Ordon method based on the colorimetric principle using 2% aluminum chloride in 96% ethanol which incubated together with the samples (1: 1) for an hour will form a color complex. The color intensity was then measured using Uv-Vis spectrophotometer at a wavelength of 420 nm **[9]**.

The principle of determining the flavonoid content with aluminum chloride is the formation of complexes between aluminum chloride and keto groups on C-4 atom and

hydroxy groups on C-3 or C-5 atom **[13]**. The results obtainedshowed flavonoid contents of leaf extracts from the highest to the lowest levels were *n*-hexane 4.82 mg QE / 100 mg extract, ethyl acetate 3.07 mg QE / 100 mg extract, ethanol 2.47 mg QE / 100 mg extract, respectively. While, flavonoid contents of the stem extracts were *n*-hexane 0.69 mg QE / 100 mg extract, ethyl acetate 1.83 mg QE / 100 mg extract, ethanol 1.45 mg QE / 100 mg extract **(Table 4)**.

Total phenolic

Determination of total phenolic contents of leaf and stem extracts of Pelawan plants (*Tristaniopsiso bovata*) by reacting samples with Folin-Ciocalteu reagents **[8]**. The absorbance was read at wavelength 765 nm. Analysis was done in triplicate for each extract. Standard solution of gallic acid with concentration 30-180 µg/ml were used to obtain a standard curve. The total phenolic content was reported as percentage of total gallic acid equivalent per 100 g extract (mg GAE /100 mg) **[14]**. The results obtained showed phenolic contents of leaf extractsfrom the highest to the lowest levels were *n*-hexane 1.37 mg GAE / 100 mg extract, ethyl acetate 10.35 mg GAE / 100 mg extract, ethanol 13.36 mg GAE / 100 mg extract, respectively. While from the stem extracts, the phenolic contents were *n*-hexane 5.15 mg GAE / 100 mg extract, ethyl acetate 10.87 mg GAE / 100 mg extract, ethanol 10.29 mg GAE / 100 mg extract, respectively.

Determination of carotenoid contentsused spectrophotometry where the sample was dissolved in *n*-hexane solvent then the sample was measured at a wavelength of 470 nm and carotenoid contents were calculated from the calibration curve of β-carotene **[10]**. The results showed carotenoid contents of leaf extractsfrom the highest to the lowest level were *n*-hexane 8.33 mg BE / 100 mg extract, ethyl acetate 3.76 mg BE / 100 mg extract, ethanol 0.91 mg BE / 100 mg extract, respectively. Whereas carotenoid contents of stem extracts were*n*-hexane 2.84 mg BE / 100 mg extract, ethyl acetate 1.56 mg BE / 100 mg extract, ethanol 2.56 mg BE / 100 mg extract **(Table 5)**.

Of the tested extracts, the highest carotenoid compounds were *n*-hexane leaf extract because carotenoid components dissolved in non-polar solvents such as *n*-hexane and petroleum ether **[15]**. *n*-Hexane extract of Pelawan leaf contains the highest carotenoid compounds with levels of 8.3297 mg BE / 100 mg extract.

Table 1: Results of Extract Rendement

Simplicia	Weight sample (g)	Concentrated Extract (g)	Rendement (%)
Leaf (*n*-hexane)	400	6.961	1.740
Leaf (Ethyl Acetate)	400	71.15	17.79
Leaf (Ethanol,96%)	400	75.59	18.90
Stem (*n*-hexane)	500	3	0.6
Stem (Ethyl Acetate)	500	2.45	0.49
Stem (Ethanol,96%)	500	6	1.2

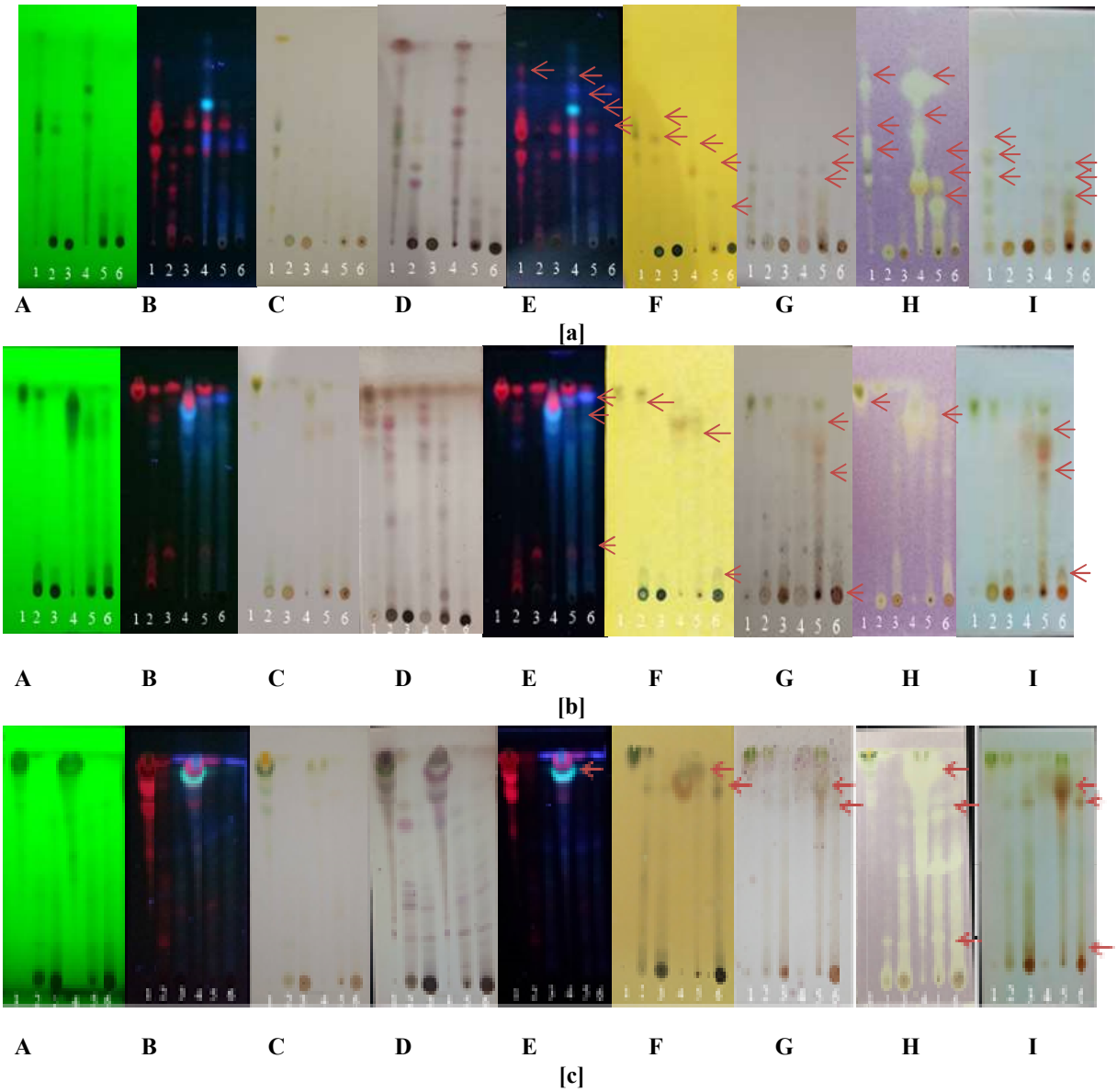

Figure 1: Qualitative monitoring of antioxidant activity using TLC silica gel GF_{254} as stationary phase and *n*-hexane-ethyl acetate (8: 2, v/v) [a], ethyl acetate-chloroform (2: 1, v/v) [b], and ethyl acetate-chloroform-formic acid (9: 1: 1, v/v) [c] as mobile phase. (A) UV 254 nm, (B) UV 366 nm, (C) Visual, (D) H_2SO_4, (E) Citroboric Acid UV 366 nm, (F) $FeCl_3$, (G) Anisaldehyde, (H) DPPH, (I) CUPRAC, (1) *n*-hexane leaf extract, (2) ethyl acetate leaf extract, (3) ethanol leaf extract, (4) *n*-hexane stem extract, (5) ethyl acetate stem extract, (6) ethanol stem extract

Table 2: DPPH radical scavenging activities at λ 517 nm

Extract	IC_{50} (µg/mL)	
	Leaf	Stem
n-Hexane	130.17 ±0.68	39.33 ± 0.26
Ethyl acetate	39.91 ± 0.05	43.78 ± 2.03
Ethanol 96%	17.68 ± 0.08	51.91 ± 1.19

Table 3: CUPRAC DPPH radical scavenging activities at λ 450 nm

Extract	EC_{50}(µg/mL)	
	Leaf	Stem
n-Hexane	53.01 ±0.02	36.05 ± 0.005
Ethyl acetate	44.35 ± 0.09	61.20 ± 0.08
Ethanol 96%	14.74 ± 0.02	58.31 ± 0.13

Table 4: Flavonoid contents

Extract		
	Leaf (mg QE/100 mg extract)	Stem (mg QE /100 mg extract)
n-Hexane	4.82	0.69
Ethyl acetate	3.07	1.83
Ethanol 96%	2.47	1.45

Table 5: Total phonolic contents

Extract		
	Leaf (mg GAE / 100 mg extract)	Stem (mg GAE / 100 mg extract)
n-Hexane	1.37	5.15
Ethyl acetate	10.35	10.87
Ethanol 96%	13.36	10.29

CONCLUSION

In conclusion, the strongest antioxidant activity was obtained from ethanol extract of Pelawan leaf with IC_{50} of 17.68 µg / mL and EC50 of 14.74 µg/mL. The main compound in the Pelawan extract is phenolic compound with a value of 13.3625 mg GAE / 100 mg extract.

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