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Antioxidant Activity Of Pelawan (*Tristaniopsis obovata* Benn.) Leaf Extracts By The Use Of DPPH Method.

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ABSTRACT

Pelawan (*Tristaniopsis obovata* Benn.) leaf extract from Myrtaceae family containing bioactive compounds such as flavonoids, alkoloid, tannin, phenol, and steroid. The objective of the study was to anayze antioxidant activity from ethanol extract and fraction extract of Pelawan leaf. Antioxidant activity was measured using DPPH (2,2-diphenyl-1-picryl-hydrazylhydrate) radical photometric assays in a process guided by the discoloration. Pelawan leaf simplicia was macerated with 70% ethanol to obtain ethanol extract, then were partitioned with n-Hexane, ethyl acetone and water solvents to obtain the fraction of hexane, ethyl acetate and water extracts. Antioxidant activity of ethanol and fraction Pelawan extract were analyzed, that using DPPH method. The results showed ethanolic Pelawan extract IC_{50} value lower than to other three extract fractions. Among the partitions, ethyl acetate and water fractions have higher antioxidant activity (AA) than hexane fraction. This study shows that ethanol, ethyl acetate and water fraction extracts have high antioxidant activity as a potential nutrient and treatment material.
Keywords : Antioxidant activity, DPPH, Myrtaceae, Tristaniopsis

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INTRODUCTION

Many research results have proven that herbal plants that are efficacious function as antioxidants. From the results of a study by Skrovankova *et al*.[16], has proven that high antioxidants in plant extracts can treat various diseases. High levels of antioxidants can delay or inhibit the oxidation of lipids or other molecules. The results of lipid oxidation products that interact with biological materials can cause cell damage. The oxidation process is always associated with chronic diseases. Antioxidants can neutralize free radicals that come from inside the body and outside the body.

Tristaniopsis obovata Benn. is a species of Opponent belonging to the family Mirtaceae which is a forest plant and timber plant. Pelawan tree trunks are used as building materials for poles and beams and their skin as paint material. Some previous studies have proven that several types of Pelawan plants contain antioxidant properties. One of them is methanol extract of bark powder *Tristania whitinia* Griffin containing tannins, flavonoids and terpenoids and the most effective as antibacterial against *Escherichia coli* bacteria in ethyl acetate fraction of 39.1% with a concentration of 2.5 ppm and 2.9 ppm concentration of *Staphylococcus aureus* bacteria [20]. Sugita [15] has proven the extract of methanol powder of *T. whitiana* Griff. in the fraction of methyl dichloride M1 with a concentration of 3.20 mg / mL and M5 at a concentration of 6.30 mg / mL effective as an antibacterial against *E.coli*. Other research results that natural extracts and ethanol of Pelawan *Tristaniopsis Whiteana* Griff. contains flavonoids, alkaloids, tannins, phenols and steroids [11, 18]. The results of research from Sartika [17], on natural extracts and ethanol of Pelawan *T. Whiteana* Griff. potential as an antiurolithiasis agent. In Riau Province in Siak Regency, the Talang Mamak tribe community has empirically used the natural extract of the Pelawan *T.obovata* Benn leaf. to treat women after childbirth.

DPPH method is a method that measures the capture of free radicals in a solution. DPPH is 1.1 diphenyl-2-pikrihydrazyl which is a stable free radical compound that is used as a reagent test material in the test of free radical capture in a solution or material. DPPH method is based on the reduction of DPPH free radical methanol solution which is colored and meets the electron donor material so the DPPH free radical methanol will give a fading color. From the color unggu turned yellow (11,12). Some antioxidant activity from plants can be tested using DPPH method. Some previous studies have carried out on several plants using the DPPH method for antioxidant activity. Research by Meena *et al*. [8], Astuti *et al*. [1], Indranila and Ulfah [5], and Stankovic *et al*. [19] has obtained antioxidant activity from plant extracts using DPPH method. The ingredients of Pelawan leaf extract which contain antioxidant ingredients have not been tested for antioxidant activity.

The aim of the study was to analyze antioxidant activity from ethanol extract and fractions extract of Pelawan leaf using DPPH method.

MATERIALS AND METHODS

T. obovata Benn. was collected from the Forest Park of Sultan Syarif Hasyim, Siak district, Pekanbaru City, Riau Province.The preparation of the Opponent extract fraction by making the simplicia dried leafs from 2, 3 and 4 leaf stalks that have been collected, sorted, washed, dried in an oven at 40^{o}C and crushed. The dried simplicia of Pelawan leafs was carried out by 600 mg of powdered Pelawan maceration with 70% of ethanol with 6 L. Then, the extract was deactivated by rotary vacum evaporator and cured in an oven at 40^{o}C, so that the initial ethanol extract or crude extract was obtained. Separation of the chemical components contained in Pelawan leaf extract by partitioning to obtain the fraction of the fighting extract. This separation uses different solvents, namely n-hexane (non-polar), ethyl acetate (semi-polar) and water (polar). The resulting layer was dried in an oven at 40^{o}C and hexane, ethyl acetate and water fraction were obtained. Pelawan extract fractions were tested for antioxidants using DPPH (1,1-diphenyl-2-pikrilhydrazyl) method [10].

Ethanol extract samples were tested with concentrations of 2,4,6,8 and 10 ppm. The n-hexane, ethyl acetate and leaf water fractions were tested with concentrations of 5, 10, 25, 50 and 100 ppm. Then, the manufacture of 0.4 M DPPH solution is as much as 4.8 mg DPPH (BM 394.32) with methanol analysis pro up to 30.0 ml. The solution is stored in a dark bottle. As much as 5 mg of ethanol extract, n-hexane, ethyl acetate and Pelawan leaf water fractions were dissolved in 10 ml of methanol PA (500 ppm) into the mother liquor. Then, the mother liquor as much as 50, 100, 250, 500 and 1000 µl were added 1000 µl of 0.4 mM DPPH solution and methanol PA to 5 ml.

Ethanol extract test solution of Opponent with a concentration of 2.4.6.8 and 10 ppm. In the fraction of n-hexane, ethyl acetate and leaf water of Pelawan with a concentration of 5, 10.25, 50 and 100 ppm. On positive control using vitamin C solution as much as 3 mg dissolved with methanol analysis pro to 10 ml (300 ppm) like induction solution. Then, the mother's vitamin C solution of 17, 50, 83, 117 and 150 µl was added with 1000 µl of 0.4 nM DPPH solution and analysia pro up to 5 ml of methanol.

Ethanol extract test solution opponent with a concentration of 2,4,6,8 and 10 ppm, and n-hexane, ethyl acetate and Pelawan leaf water fractions with a concentration of 5, 10,25, 50 and 100 ppm, and concentrated extract of vitamin C solution 1, 3, 5, 7 and 9 ppm were incubated in a 37°C water bath for 30 minutes. The uptake of the solution was measured at a maximum absorption wavelength of 515 nm with an Uv-vis spectrophotometer.

Analysis of DPPH method by looking at the color changes of the research samples that have been incubated with DPPH. DPPH electrons paired with electrons in the extract sample will produce a change in the color of the sample which is dark purple to bright yellow. Then, the color changes in the sample absorbance value measured by Uv-Vis spectrophotometer at a wavelength of 515 nm.

All data from two independent experiments are expressed as mean ± standard deviation. The results of the correlation coefficient and IC50 values obtained from the linear regression analysis in EXCEL program**.**

RESULTS

Table 1. Antioxidant activity test of ethanol Pelawan extract leafs, n-hexane, ethyl acetate, water extract fraction and vitamin C.

Sample	Concentration (ppm)	Antioxidant activity (%)	Probit Value	Probit Equations	IC_{50} Value
Etanol Extract	2	14.26	0.60	y = 10.45x + 0.34	4.82
	4	40.97	0.41	R^2 = 0.97	
	6	72.20	0.20		
	8	90.47	0.07		
	10	94.48	0.04		
n-Heksana Extract Fraction	5	4.04	0.76	y = 0.11x + 3.40	420.16
	10	5.24	0.75	R^2 = 0.98	
	25	5.56	0.75		
	50	8.40	0.73		
	100	14.85	0.67		
Water Extract Fraction	5	17.50	0.65	y=0.7269x + 28.058	30.19
	10	38.98	0.48	R^2 = 0.88	
	25	52.69	0.38		
	50	75.11	0.20		
	100	94.13	0.05		
Etil Asetat Extract Fraction	5	21.92	0.62	y = 0.72x + 29.14	28.93
	10	38.35	0.49	R^2 = 0.91	
	25	50.98	0.39		
	50	76.44	0.19		
	100	95.01	0.04		
Vitamin C	1	13.25	0.61	y = 11.57x + 3.68	4.00
	3	35.96	0.45	R^2 = 0.96	
	5	73.21	0.19		
	7	93.34	0.05		
	9	95.70	0.03		

Antioxidant activity test results on ethanol extract, n-hexane, ethyl acetate and water extract fraction can be seen in Table 1. In the fourth antioxidant activity test, the Pelawan leaf extract was compared with the antioxidant activity of ascorbic acid (vitamin C).

Antioxidant activity at IC_{50} value of Pelawan leafs ethanol extract was 4,818 µg / ml, n-hexane fraction extract was 420,161 µg / ml, ethyl acetate fraction was 28,932 µg / ml, water fraction was 30,188 µg / ml and vitamin C extract was 4,002 µg / ml. High IC50 values indicate low antioxidant activity. This can be seen in the IC50 value of the extract of the n-hexane fraction of the opponents who have low antioxidant activity.

Based on the results of the linear regression equation (Table 1) from this study which has the smallest IC50 value is ethanol extract, ethyl acetate and water extracts fraction when compared with n-hexane extract fraction. Low IC50 values indicate that the extracts have high antioxidant activity. The IC_{50} value of Pelawan ethanol extract is the lowest value of the fraction extract and its value is almost the same as the IC50 value vitamin C.

DISCUSSION

This can be seen in the IC_{50} value of ethanol extract, ethyl acetate fraction and water fraction have high antioxidant activity. Although, the extract of n-hexane fraction of the opponents have low antioxidant activity. The compound of Pelawan leaf fraction extract is more polar and semi polar. Plant extracts that have bioactive activity are strongly influenced by solvents. Abarca *et al*. [3] have examined the polarity of some solvents on *Bougainvillea x buttiana* Holttum and Standl, (var. Rose) extracts. Polarity values of these solvents are Ethyl acetate 4.4, Acetone 5.1, Ethanol 5.2, Dichloromethane 3.7, Methanol 6.6 and water 9.0. All of these solvents that showed high antioxidant activity were methanol solvents. The results of IC50 values of the solvents are dH2O 286.80 ± 0.01, MeOH 223.10 ± 0.02, EtOH 232.10 ± 0.02, DMK 300.90 ± 0.05, EtOAc 353.80 ± 0.01, DCM 1455.68 ± 0.19, Hex 373.70 ± 0.03. Polar properties of a solvent can affect antioxidant activity in a plant. However, different results of seeds and meals on *Camelina sativa* and flax with a low polarity solvents such as ethyl acetate were found to be inefficient in attracting phenolic amounts in a plant extract [14]. Also, the results of research conducted by Iloka-Assanga *et al*. [6] on *Bucida buceras*, *Phoradendron californicum-oak* and *Phoradendron californicum-mesquite* plants by using 4 types of solvents namely ethanol, water, methanol and acetone. Each plant extract showed different antioxidant activity results, such as low IC50 of *B. buceras* leafs with acetone solvents, *P. californicum-oak* and *P. californicum-mesquite* leafs having low IC_{50} with water solvents.

The IC_{50} value of Pelawan ethanol extract is almost the same as the IC_{50} value vitamin C. This is different from the results of Astuti *et al*. [1] on methanol extract of Paku Uban, namely IC_{50} of 78.45 µg / ml and ethanol extract of Karika leafs (*Carica pubescens*) of 30.790 µg / ml [5]. Kumar *et al*. [7], said that the value of antioxidant activity in a plant extract depends on the phenolic content of the plant. The results of research by Adebiyi *et al*. [2], on ethanol extract of stem and leaf *Grewia carpinifolia* which had phenolic content of 19.08 ± 1.21 mg GAE / g extract for leaf and 14.85 ± 1.09 mg GAE / g extract for steam, while flavonoids were 9.00 ± 0.13 mg quercetin / g extract for leaf and 13.22 ± 1.53 mg quercetin / g extract for steam. Antioxidant activity in steam and leaf *G. carpinifolia* is higher than vitamin C. The same research has also been carried out by Mahmoudi *et al*. [9], in extract leafs from ten *Algerian ficus carica* L. varieties have antioxidant activity values depending on the phenolic content of these plants. The higher phenolic content in plant extracts will reduce the IC50 value. High phenolic content shows that antioxidant content of the plant extracts is also high.

According to the results obatained by Barchan *et al*. [4], antioxidant activity of polar and non polar solvent of three *Metha* species extract showed activity as strong as BHT. The higher content of polyphenols on three *Metha* species extract was obtained with an increase in polarity of the solvent used. The total phenolics contents correlationed with reducing power and radical scavenging activity. Similar result were reported by Zazouli *et al*. [21], roots extract of *Caralluma europaea* have differents antioxidant activity in the test polar and non polar solvent used. Total polyphenol contents were significantly higher in methanol extract than cloroform and ethanol fraction.

CONCLUTION

From discussion above, we concluded that the research of antioxidant activity test on ethanol Pelawan extract and n-hexane, ethyl acetate and water Pelawan extract fraction has different antioxidant activity.

Allegedly, the phenolic content of the extract affects the antioxidant activity of ethanol extract, ethyl acetate and water extract fractions. The high antioxidan activity of ethanol, ethyl acetate fraction and water fraction of Pelawan extract which can potentially for nutrition and disease treatment are compared with the n-hexane extract fraction.

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