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Antioxidant Activity of Ethanol Extract of *Polygonum pulchrum* Blume

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Abstract

The use of antioxidants from natural resources has attracted increasing interest. One of the plant that was empirically used as an antioxidant dietary supplement was *Polygonum pulchrum* Blume (*P. pulchrum* Blume). This study aimed to investigate antioxidant activity of roots, stems, leaves and flowers ethanol extract of *P. pulchrum* Blume. The extract was obtained by maceration method using ethanol solvent. Antioxidant activity was determined with 1,1-diphenyl-picrylhydrazyl (DPPH) method. We found that ethanol extracts of *P. pulchrum* Blume roots and stems had strong antioxidant activity with IC_{50} values of 25.2 mg/l and 43.26 mg/l, respectively. Ethanol extract of flowers had the weakest antioxidant activity with IC_{50} value of 202.96 mg/l. Vitamin C had very strong antioxidant activity with IC_{50} value of 3.97 mg/l. In conclusion, this study revealed that ethanol extract of *P. pulchrum* Blume roots and stems had strong antioxidant activity, therefore, this plant might be potential as an excellent source for natural antioxidant agents for medical application.

Keywords: *Polygonum pulchrum* Blume, DPPH, antioxidant, maceration, ethanol

Introduction

The use of antioxidants derived from plants have widely improved around the world. Many plants contain various phytochemicals which possess antioxidant activity to protect cells from damaging effects of reactive oxygen species (ROS), such as superoxides, peroxyl radicals, hydroxyl radicals and peroxinitrins.[1,2]

In human, production of ROS as the result of biochemical process might increase with several factors, including toxins and chemicals in food, pollutants, radiations, *etc.* Antioxidants compounds are needed to tackle this problem. However, synthetic antioxidant compounds such as butylated hydroxytoluene and hydroxyanisole might cause various side effects.[3] This encourage researchers to find

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alternative treatments, particularly from natural source.

One of the plants that has a potential to be developed as a natural antioxidant is *Polygonum pulchrum* Blume (*P. pulchrum* Blume). This plant is widely spread around the Asian-African continents, including India, China, Malaysia, Myanmar, Srilanka, Thailand and Indonesia.[4] This plant is known as *ketapan* or *kumpai air* in Indonesia.

Previous studies showed that several plants from *Polygonum* family had antioxidant, antibacterial, and hepatoprotective activities.[5-7] However, limited information was available regarding biological activity of *P. pulchrum* Blume. Therefore, this study was performed to investigate antioxidant activity of root, stems, leaves and flowers ethanol extracts of *P. pulchrum* Blume.

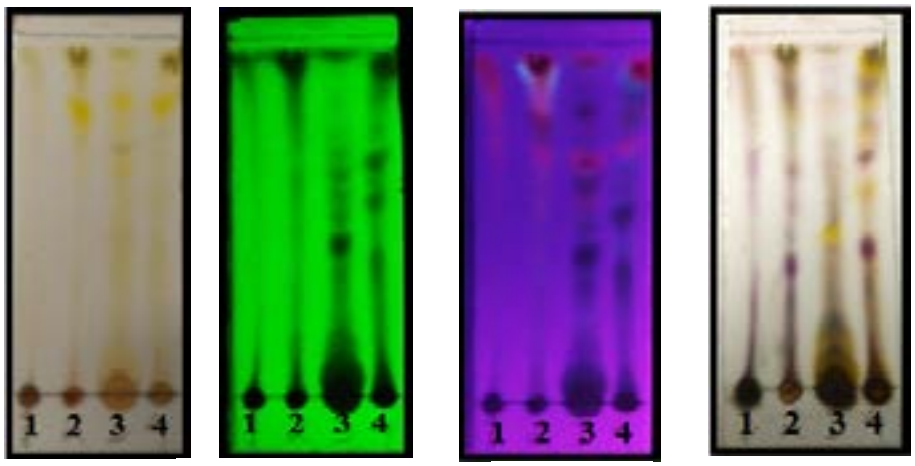
Methods

Instruments and materials

The instruments used in this study were rotary vacuum evaporator (Buchi®), hot plate (Stuart®), UV-Vis 20D (Thermo®) spectrophotometer, analytical scales (Precisa®), blender (Philips®), vortex (Stuart®), cuvette (Hellma analytics®), micropipette (Eppendorf®), stirring rod, vial, volumetric flask (Pyrex®), TLC plate GF254 (Merck®), UV lamp (Srahlen Germany®), chamber, capillary pipes. Materials used included ethanol 96% (Merck®), vitamin C (Braco®), and 1,1-diphenyl-picrylhydrazyl (DPPH) reagent. Roots, stems, leaves and flowers of *P. pulchrum* Blume was obtained

Table 1. Yield of extract

Sample		Powder sample (g)	Viscous extract (g)	Yield percentage (%)
P. pulchrum Blume	Roots	553	18	3.6
	Stems	800	25.5	5.1
	Leaves	700	16.2	3.24
	Flowers	750	11.5	2.3



(a) (b) (c) (d)

Figure 1. Phytochemical screening result (a) elution result (b) UV-254 nm (c) UV-366 nm (d) TLC with heating process. (1) roots (2) stems (3) leaves (4) flowers

solvent. Separation of residue and filtrate was performed every 1x24 hours accompanied by the same solvent replacement to obtain the filtrate. The filtrate was collected and concentrated using a rotary vacuum evaporator at a temperature of 58 °C to obtain a viscous extract.

Phytochemical screening using thin layer chromatography (TLC)

The phytochemical screening test with TLC was performed using silica gel plate GF254. The mobile phase used were chloroform and methanol (9:1). The developers and test reagents were as follows: the alkaloid using the Dragendorff reagent (orange), the flavonoid using the 0.1 N NaOH reagent (yellow) and sodium carbonate reagent (green, yellow), tannin using 1% $FeCl_3$ reagent (black/blue), saponins using H_2SO4 reagent (dark purple), violin triterpenoids using Lieberman-Burchard reagent (violet red).[8]

Antioxidant activity

Preparation of DPPH reagent 40 mg/ml

2 mg of DPPH were weighed and dissolved in ethanol until all dissolved. It was then added with 50 ml ethanol. The solution was shaken until homogeneous.

Preparation of blank solution

The preparation of the blank was performed by reacting 1ml of 40 mg/l DPPH and 3 ml ethanol. The solution was shaken until homogeneous and was incubated at 37 °C for 30 minutes.

Table 2. Phytochemical screening results

Sample		Phytochemical screening				
		Alkaloid	**Flavonoid**	**Tannin**	**Saponin**	**Triterpe-noid**
P. pulchrum Blume	Roots	+	-	-	++	++
	Stems	-	+	-	-	+
	Leaves	+	++	+	-	-
	Flowers	-	-	-	+	++

(a) (b1) (b2) (c) (d) (e)

Figure 2. Phytochemical screening using TLC

from the Faculty of Fisheries and Marine Sciences, Halu Oleo University, Indonesia.

Extraction

Maceration of plants was performed in a closed container for 3x24 hours using ethanol

Preparation of ethanol extract 1000mg/l

50 mg of roots, stems, leaves and flowers viscous extract were dissolved in 50 ml of ethanol. Dilution was performed on 1000 mg/l sample to obtain 12.5, 25, 50, 100, and 200 mg/l of samples. The solutions were shaken until homogeneous and were incubated at 37 °C for 30 minutes. The absorption test was performed at 515.5 nm.

Calculation

All of the inhibition concentrations (IC_{50}) of DPPH radical from each sample was calculated using this formula:

$$\% \text{ Inhibition} = \frac{\text{Blank Absorbance} - \text{Sample Absorbance}}{\text{Blank absorbance}} \times 100\%$$

Results and Discussion

Extraction

The extract was obtained using maceration with ethanol solvent. The yield of each extract was 3.6%, 5.1%, 3.24%, and 2.3% for roots,

Table 3. % inhibition of ethanol extract of *P. pulchrum* Blume

Sample	Absorbance		Concentration (mg/l)	% Inhibition
	Blank	Sample		
Roots	0.7528	0.3799	12.5	49.54
		0.3773	25	49.88
		0.3768	50	49.95
		0.3172	100	57.86
		0.2820	200	62.54
Stems	0.3319	0.1863	12.5	43.86
		0.1675	25	49.53
		0.1610	50	51.49
		0.1459	100	56.04
		0.0617	200	81.41
Leaves	0.3319	0.1913	12.5	42.36
		0.1904	25	42.63
		0.1859	50	43.98
		0.1462	100	55.95
		0.0813	200	75.50
Flowers	0.7528	0.4761	12.5	36.75
		0.4604	25	38.84
		0.4514	50	40.03
		0.4503	100	40.18
		0.3667	200	51.28
Vitamin C	0.4462	0.3811	1	14.58
		0.3101	2	30.50
		0.2653	3	40.54
		0.2142	4	51.99
		0.1832	5	58.94

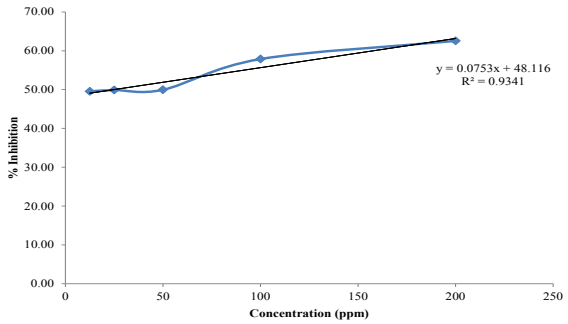


Figure 3. % inhibiton of *P. pulchrum* Blume roots extract

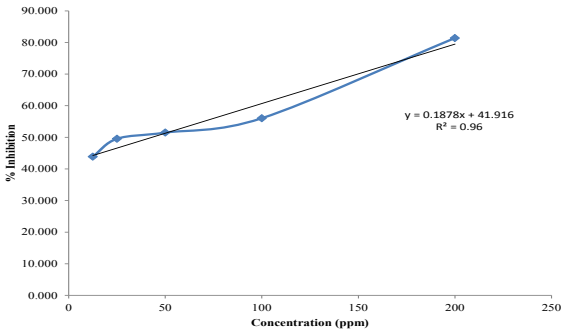


Figure 4. % inhibition of *P. pulchrum* Blume stems extract

stems, leaves, and flower extract, respectively (Table1).

Phytochemical screening

Phytochemical screening was performed to identify the secondary metabolites in the extract. We found that alkaloids, flavonoids, tannins, saponins, and triterpenoid were found in different parts of the plant (Figure 1, Table 1). Figure 2 shows the result of phytochemical screening indentification after reagent markers were sprayed into the TLC plate.

Antioxidant activity

Antioxidant activity testing aimed to determine the inhibiton concentration value (IC_{50}) of the extract using DPPH method. This method is based on the DPPH radicals color change which caused by the reaction between free radical of DPPH and one electron or hydrogen atom released from the compound contained in the extract to form a DPPH compound whose color is yellow. We compared the antioxidant activity of the extracts with vitamin C (Table 3).

Antioxidant activity increased in accordance with the rise of concentration. This was marked by the fading colour of DPPH and the greater percentage of inhibition value. After obtaining the inhibition percentages, the graphs between the sample concentration (x) and the percentage of inhibition (y) was made. The results can be found in Figure 3-7.

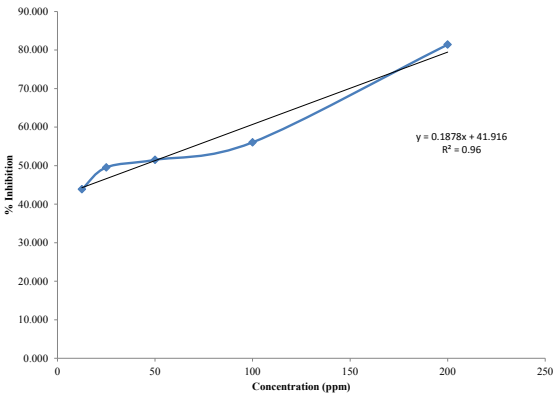


Figure 5. % inhibiton of *P. pulchrum* Blume leaves extract

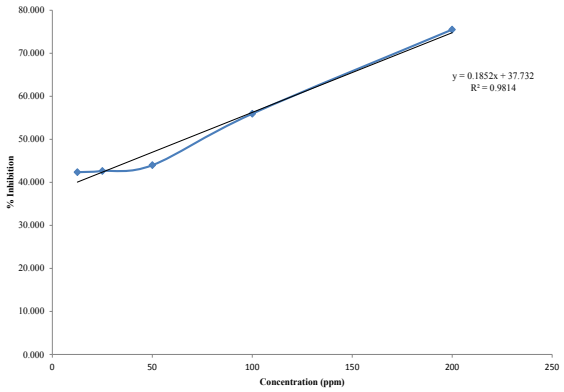


Figure 6. % inhibiton of *P. pulchrum* Blume flowers extract

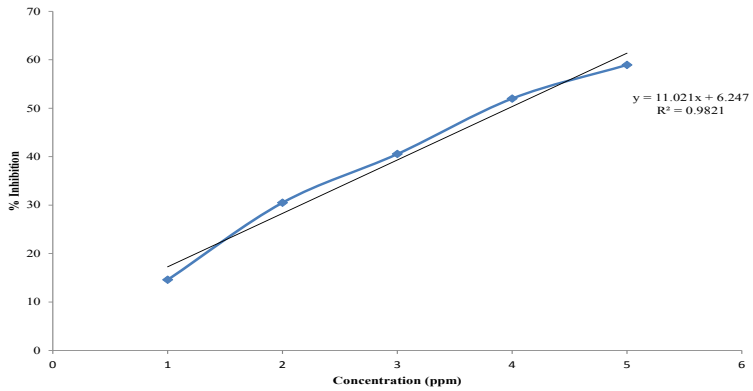


Figure 7. % inhibition of vitamin C

Table 4. IC_{50} values of ethanol extract

Sample	IC_{50} (mg/l)
Roots	25.2
Stems	43.26
Leaves	66.32
Flowers	202.96
Vitamin C	3.97

The IC_{50} values can be determined using the linear regression equations. The smaller IC_{50} value the greater the antioxidant activity. IC50 value extract of roots, stems, leaves, flowers *P. pulchrum* Blume and vitamin C can be seen in Table 4.

We found that ethanol extracts of *P. pulchrum* Blume roots and stems had strong antioxidant activity with IC_{50} values of 25.2 mg/l and 43.26 mg/l, respectively. Flowers ethanol extract had the weakest antioxidant activity with IC_{50} value of 202.96 mg/l. Vitamin C had very strong antioxidant activity with an IC_{50} value of 3.97 mg/l. A compound can be said to be a very powerful antioxidant if the IC_{50} is less than 50 mg/l, strong antioxidant if IC_{50} is between 50-100 mg/l, moderate antioxidant if IC_{50} is between 100-150 mg/l, weak antioxidant if IC_{50} value is between 150-200 mg/l, and very weak antioxidant effect if IC_{50} value is more than 200 mg/l.[9] High antioxidant activity in the ethanolic extract might be due to the presence of flavonoids, alkaloids, and triterpenoids.[10-12]

Conclusion

This study revealed that ethanol extract of *P. pulchrum* Blume roots and stems had strong antioxidant activity, therefore, this plant might be potential as an excellent source for natural antioxidant agents for medical application.

Acknowledgement

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None.

Conflict of Interest

None declared.

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