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Antioxidant Activity of Extract and Fractions of Dragon Scales (*Drymoglossum piloselloides* C. Presl).

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ABSTRACT

Antioxidants are compounds that can protect the body from damage which caused by free radicals that induced by oxidative stress. Empirically, herb of dragon scales (*Drymoglossum piloselloides* C. Presl) used as breast cancer drugs due to their antioxidant compounds. This study aims to determine the antioxidant activity of extract and fraction of dragon scales with 1,1-diphenyl-2-picrilhydrazyl (DPPH) method. Phytochemical screening showed that dragon scales contain flavonoids, tannins, polyphenols, saponins, steroids/triterpenoids, and essential oils. The IC_{50} value of extract, aqueous fraction, ethyl acetate fraction, and n-hexane fraction was 347.10, 114.48, 13.79, and 72.31 ppm, respectively. These antioxidant activities were compared with ascorbic acid and butylated hydroxytoluene as model antioxidants. The ethyl acetate fraction was potent antioxidant due to categorized as very strong antioxidant.

Keywords: Extract, aqueous fraction, ethyl acetate fraction, and n-hexane fraction, IC_{50} value

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INTRODUCTION

Herbal of dragon scales (*Drymoglossum piloselloides* C. Presl, Polypodiaceae) is spread in the tropical Asia region, including Indonesia. Characteristic of this plant is epiphyte, due to attach to other plants and make own food. Dragon scales grow wild in forests, fields, tea gardens, moist places, and old trees [1].

Empirically, dragon scales used for breast cancer treatment, due to antioxidant properties [2, 3], gum inflammation, sprue, bleeding, rheumatism in soft tissues, and pulmonary tuberculosis with blood coughing [3], mumps, jaundice, abdominal pain, constipation, vaginal discharge, and skin diseases, such as scabies [1]. The Indonesian people use 15-60 g of fresh herbal of dragon scales which boiled in three glasses of water until water is left halfway, then filtered after cold [1]. Ethanolic extract of dragon scales inhibit *Escherichia coli* growth, ethanolic and aqueous extract inhibit *Streptococcus aureus* growth [4]. Ethanolic extract of dragon scales has a cytotoxic effect on T47D cells [5].

Dragon scales contain flavonoids, saponins, sterols/triterpenes, polyphenols, tannins, volatile oils, and sugars [3]. These secondary metabolites are predicted to have antioxidant activity to free radicals by scavenging them or promoting their decomposition and suppressing such disorders [6-8]. The aim of this study was to determine the antioxidant activity of extract and fractions of herbal of dragon scales compared to ascorbic acid and butylated hydroxytoluene.

MATERIALS AND METHODS

Plant Materials

Herbal of dragon scales were obtained from Cilingga Village, Darangdan Subdistrict, Purwakarta Regency, West Java, Indonesia. The plants were identified at Herbarium Bandungense School of Biological Sciences and Technology, Bandung Institute of Technology with No. 1690/K01.14.2/PP.2.4.2/2010.

Chemical Materials

All chemical materials were pro analysis grade, which purchased from Merck, i.e. silica gel G60 F254-precoated TLC plates, 1,1-difenil-2-pikrilhidrazil (DPPH), ascorbic acid, butylated hydroxytoluene (BHT), ethanol, n-hexane, ethyl acetate, hydrochloride acid, petroleum ether, amyl alcohol, ferri chloride, gelatin, ethers, butane, acetic acid, chloroform, acetone, ammonia, magnesium powders, alkaloid reagents (Mayer, Dragendroff's, and Bouchardat), and Lieberman-Bouchard reagent.

Samples Preparation

Sorted herbs were washed and drained overnight, then dried without direct sunlight. Moisture contents were conducted with gravimetric method [9]. Simplicia was macerated with 70% ethanol for three days. The solvent was changed with the fresh one for each day. All extract were collected and vaporated with rotary rotavapor, then calculated the yield. Dissolve 10 g of ethanolic extract with warm aquadest to obtain 100 mL solution, then conducted the liquid liquid extraction with n-hexane and ethyl acetate, three times for each solvent. All fractions were collected and vaporated, then calculated the yields. Phytochemicals screening were determined in simplicia, extract, and fractions with Fransworth methods [10].

Confirmation of secondary metabolites were done by thin layer chromatography (TLC) with various mobile phase and detection with natural products reagent (NPR) with and without UV light (254 nm). The mobile phase was n-butanol: acetic acid: water (3: 1: 1) with a cross-section of ammonia as NPR for flavonoids [11], chloroform: acetone (4: 1) with vanillin-sulfuric acid as NPR for saponin, n-hexane: ethyl acetate (1: 1) with Lieberman-Burchard as NPR and heating at 85-95 °C for 15 min for sterols/triterpenoids, n-hexane : ethyl acetate (3: 7) with 1% $FeCl_3$ as NPR for tannins and polyphenols, 70% ethanol with vanilin-sulfuric acid as NPR and heating at 100-105 °C for essential oils [12]. The spots were light yellow for flavonoids [11], yellow for saponins, various colors for sterols/triterpenoids, green, purple, blue or black for tannins and polyphenols, green-blue, purple, brown color for volatile oils [12].

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Determination of Antioxidant Activity

The modified method of Okada and Okada (1998) was used [13] to determination of free radical-scavenging activity of the extracts and fractions. Five different concentrations of extracts, fractions, and standards (ascorbic acid and BHT) were dded with DPPH solution. All mixtures were incubated in a dark chamber for 30 min, then the absorbances were measured at 517 nm using spectrophotometer. The blank was 96% ethanol. Percentage scavenging activity was calculated using this formula:

$$\% \text{ of DPPH inhibition} = [(Ab-Aa)/Ab] \times 100$$

Aa and Ab are the absorbance values of the sample and the blank, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value.

RESULTS AND DISCUSSION

Sample Preparation

The leaves of dragon scales are 2-5 cm long, dark green color, round to oval shapes. The stems and roots are dark brown color (Fig 1). This study was used 7.5 kg of dragon scales leaves, then dried to produce 800 g of dried leaves. Water content of dried leaves was 4.3%, which met the criteria, i.e. 10% [14].

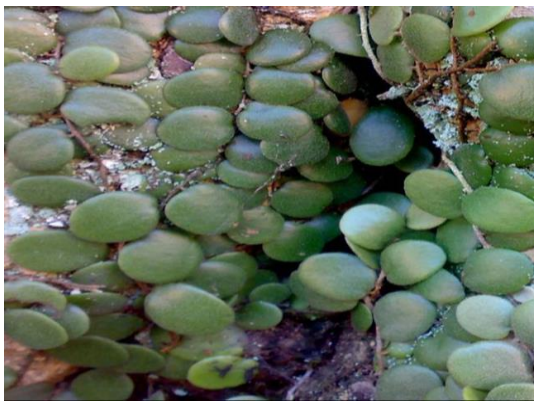

Fig 1: Herbal of dragon scales

Maceration is a soaking cold extraction. These methods was conducted to maximal extraction of secondary metabolites. The thick extract was dark brown in color and the yield was 18.4%. Liquid liquid extraction was conducted to seperate the secondary metabolites according to its polarity [15]. Aqueous fraction (63.25%) was higher than ethyl acetat fraction (12.55%) and n-hexane fraction (20.88%). We concluded that the majority of secondary metabolites were the polar secondary metabolites. All fraction color were dark brown.

Phytochemical screening with color reaction method was conducted to determine the group of secondary metabolites in the sample. Plants are synthesize the secondary metabolites to self defense from environmental stress and pathogens, which can be used to treatment several diseases. The phytochemical screening showed that dragon scales extract contains flavonoids, polyphenols, tannins, monoterpenoids, sesquiterpenoids, saponins, streroids, and triterpenoids. This result was accordance to the literature [3]. The fraction phytochemical screening results were different from extract. Aqueous and ethyl acetate fractions contain saponins and phenolic compounds, n-hexane fraction contains terpenoids. The phytochemical screening results were confirmed by TLC (Fig 2 and Table 1). The number of spots on UV 254 nm was more than visible light, due to interaction between UV light and fluorescence indicator in TLC plate thus increasing the sensitivity of qualitative analysis using TLC [16]. The results of phytochemical screening by color reaction same as chromatograms for extract and ethyl acetate fraction. Aqueous fraction have no steroids and triterpenoids, while n-hexane fraction have no saponins.

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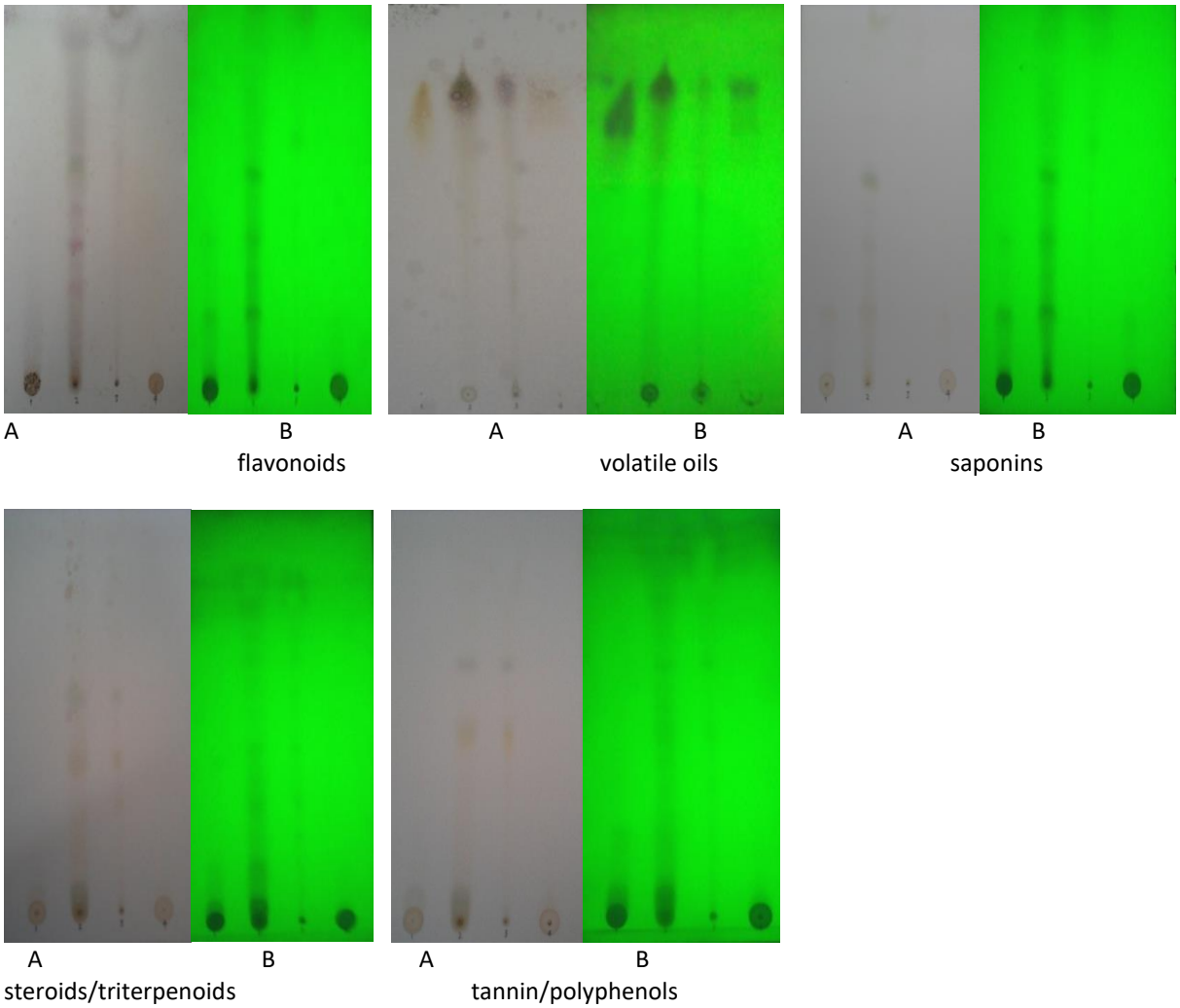

Fig 2: The chromatogram of the phytochemical screening confirmation on visible light (A) and UV 254 nm (B) after spraying with specific NPR

Table 1 Rf value of Extract and Fractions

Compound	Sample	After spraying with specific NPR		
		visible		UV 254 nm (quenching)
		Rf value	Spot color	Rf value
Flavonoids	Extract	0.14	Yellowish brown	0.14 0.27
	Ethyl acetat fraction	0.14 0.17 0.27 0.40	Yellowish brown Purplish red Purple Green	0.14 0.17 0.27 0.40
	n-hexane fraction	-	-	0.60
	Aqueous fraction	0.14 0.27	Yellowish brown Yellowish brown	0.14 0.27
Tannin and polyphenols	Extract	0.40 0.55	Orange Black	0.40 0.55
	Ethyl acetat fraction	0.40 0.55	Orange Black	0.40 0.55
	n-hexane fraction	0.40	Orange	0.40

		0.55	Black	0.55
	Aqueous fraction	-	-	-
Steroids and triterpenoids	Extract	0.28 0.40	Bluish green Blue	0.28 0.40
	Ethyl acetat fraction	0.28 0.40	Bluish green Blue	0.09 0.28 0.40 0.54
	n-hexane fraction	0.20 0.28 0.40	Green Bluish green Blue	0.20 0.28 0.40 0.48 0.54
	Aqueous fraction	-	-	-
Saponins	Extract	0.18	Orange	0.18 0.30
	Ethyl acetat fraction	0.18 0.30 0.40	Orange Orange Bluish yellow	0.18 0.23 0.34 0.60
	n-hexane fraction	-	-	-
	Aqueous fraction	0.18	Orange	0.18
Volatile oils	Extract	0.70	Orange	0.70
	Ethyl acetat fraction	0.80	Greenish orange	0.80
	n-hexane fraction	0.80	Reddish purple	0.80
	Aqueous fraction	0.70	Orange	0.70

Determination of Antioxidant Activity

The absorbance of 40 ppm DPPH radicals solution was 0.45 at at 517 nm due to color alteration from purple to yellow. The antioxidant potency is determine by degree of discolouration [17, 18]. Antioxidant activity were measured from reaction of the sample (extract and fractions) and ascorbic acid or BHT solutions with DPPH solution, then percentage inhibition were counted. The IC_{50} value of sample was counted from the linear regression equation of the curve of concentrations versus % inhibition (Table 2). Higher antioxidant activity have smaller IC_{50} value, which showed concentration dependent.

Table 2 Persentage of Inhibition Sample to DPPH Radicals

Sample	Concentration (ppm)	% inhibition	Linear Regression Equation	IC_{50} value
Extract	30 60 90 120 150	23.252 28.629 29.973 30.913 34.139	y =0.080x+22.16 R^2=0.912	347.10
Aqueous fraction	30 60 90 120 150	12.096 22.446 26.209 61.424 67.069	y =0.496x – 6.828 R^2=0.906	114.48
Ethyl acetate fraction	30 60 90 120 150	54.032 54.167 54.704 63.306 66.129	y = 0.111x + 48.460 R^2= 0.826	13.79

n-hexane fraction	30	42.876	y =0.139x+39.890 R^2=0.976	72.31
	60	49.193		
	90	53.225		
	120	57.258		
	150	59.811		
Ascorbic acid	20	61.156	y =0.840x+46.770 R^2=0.971	3.84
	30	73.521		
	40	81.989		
	50	90.591		
	60	94.623		
BHT	20	56.855	y=0.651x + 45.670 R^2=0.953	6.64
	30	66.129		
	40	72.715		
	50	81.048		
	60	81.989		

All secondary metabolites have the hydroxyl group which can donate hydrogen to interact with DPPH radical to produce the DPPH-H. Flavonoids is the potential antioxidants which inhibit the oxidation reaction through radical scavenging mechanisms by donating an electron to the unpaired electrons in free radicals. Molecular structure phenolic compounds determine the availability of antioxidant activity, the phenolic hydrogens will forming phenoxyl radicals due to hydrogen donation [19].

The IC_{50} value shows the potency of antioxidant activity [20]. The antioxidant activity of extract was categorized as very weak, aqueous fraction was categorized as moderate, the ethyl acetate fraction was categorized as very strong, and the n-hexane fraction categorized as weak.

CONCLUSION

The ethyl acetate fraction was categorized as very strong, so potential to be a source of natural antioxidants.

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