ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY TESTS OF DRAGON SCALES LEAVES CRUDE METHANOL EXTRACTS (*Drymoglossum piloselloides* (L.) Presl)

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ABSTRACT: Researches on the antioxidant and antibacterial activity of dragon scales leaves crude methanol extract (*Drymoglossum piloselloides* (L) Presl) had been done. This study aimed to determine the antioxidant activity of dragon scales leaves crude methanol extract by 0,5mM 40 ppm of free radical 1.1-diphenyl-2-picrylhydrazyl (DPPH) reduction method using UV Visibel Spectrophotometer at 515-517 nm wavelength obtained IC_{50} was 9.7 mg / L with the highest percent reduction in the amount of 99.0574% in 100 ppm at 517 nm wavelength which was a substance that has a very strong antioxidant activity. To test the antimicrobial activity of dragon scales leaves crude methanol extract (*Drymoglossum piloselloides* (L.) Presl) with Agar Diffusion Method in order to obtain inhibition zone in *S. mutans* extract concentration respectively 1%, 2% and 3% were 0.55; 0.56 and 0, 83 mm, the *E. coli* were 1.03; 1.30 and 1.34 mm, where the greater of extracts concentration increased the inhibition of *S. mutans and E. coli* so that effective to be developed as antibacterial agents.

KEYWORDS: antioxidant, antibacterial, *Drymoglossum piloselloides* (L) Presl, UV Visibel Spectrophotometer, Agar Diffusion Method

1. INTRODUCTION

Indonesia is known as a mega biodiversity Country because it has very high biodiversity of Plants. A number of studies conducted to investigate the potential of plants in Indonesia as a raw material for medicine. There are approximately 7000 species of plants including medicinal plants of ± 28 000 plant species that can be found in Indonesia. Medicinal plants are a group of plants that can be used as a drug. The utilization of medicinal plants is usually in the form of crude drugs from plant parts such as roots, stems, leaves, and fruit or seeds. (Fatmawati, 2008) With the diversity of medicinal plants there are some plants that have the same name even though a different kind. That is because some plants have not been identified completely yet. One is a dragon scales fern (*Drymoglossum piloselloides* (L.) Presl) that can be used as a remedy for sore gums, mouth ulcers, bleeding, in soft tissue rheumatism, tuberculosis, and for anticancer of breast (Hariana, 2003). Research has been conducted to investigate the potential of these plants include Simplisia Characterization, Phytochemical Screening and Antioxidant Activity of N-hexane, Ethyl Acetate and Ethanol Dragon Scales Leaf Extract (Mariani Sitorus, 2015) where Mariani Sitorus comparing the antioxidant activity of the solvent n-hexane, ethyl acetate and ethanol , from the third solvent ethanol extract had high value inhibitory concentration (IC_{50}) 42.62 ppm, which means a very strong inhibitory concentration compared with N-hexane extract and ethyl acetate. Therefore, researcher wanted to know and test the antioxidant and antibacterial activity of dragon scales leaves crude methanol extract (*Drymoglossum piloselloides* (L.) Presl) because methanol is the most polar solvent than it all.

2. RESEARCH METODOLOGY

2.1. EQUIPMENT

Pyrex Glassware, dark bottle, vial bottle, blender, Whatman paper, plat drops, knives, water bath, UV Visible Spectrophotometer, lamp, rotary evaporator, incubator, autoclave,bunsen, ose neddle, rubber pump, tube rack, hot plate.

2.2 MATERIALS

Dragon scales leaves, Methanol p.a Merck, Mayer reagent, Dragondorf reagent, Wagner reagent, Lieberman Bouchard reagent, Mg p.a Merck, HCl p.a Merck, Cerrium sulfuric, anhydride acetic acidp.a Merck, aquadest, DPPH p.a Sigma Aldrich, H_2SO_4p.a Merck, $FeCl_3$ p.a Merck, DMSO p.a Merck, Nutrient Agar (NA), Nutrient Broth (NB), *E. coli* Culture, *S. mutans* Culture, Mueller Hinton Agar (MHA).

2.3 RESEARCH PROCEDURES

2.3.1 Phytochemicals Tests

2.3.1.1 Sample Preparation of Fresh Dragon Scales Leaves

Taken ± 100 gram of fresh dragon scales leaves, cleaned and then cut into small pieces. Put it into a flask glass and then added 100 mL of Methanol p.a Merck, evaporated at water bath until it concentrated, cooled and taken the filtrate.

2.3.1.2 Alkaloids Test

A total of ± 5 drops of dragon scales leaves methanol extract respectively dropped on four test tubes. The first test tube was dropped of 2 drops of Mayer reagent (positive if forming a white precipitate or cloudy), the second tube was dropped of 2 drops of Dragendorf reagent (positive if it forms an orange precipitate), the third tube was dropped of 2 drops of Wagner reagent (positive if it forms a brown precipitate) and the fourth tube was droppedof 2 drops of Bouchardat reagent (positive if it forms a red precipitate). Observed changes (Harborne, 1987)

2.3.1.3 Phenolic Test

A total of ± 5 drops of dragon scales leaves methanol extract was dropped on a test tube and then added each with 3 drops of 1% $FeCl_3$ solution (positive if it formed a dark brown colour of solution). Observed change. (Harborne, 1987).

2.3.1.4 Flavonoids Test

A total of ± 5 drops of dragon scales leaves methanol extract was dropped on a test tube with ± 3 drops of ethyl acetate plus 2 drops of 1% $FeCl_3$ to form green to blackish brown color (positive form of green to blackish brown color). Observed change. (Harborne, 1987)

2.3.1.5 Test Terpenoids and Steroids

2.3.1.5.1 Liebermann Bouchard Test

20 mL of dragon scales leaves methanol extract put in a beaker glass and then evaporated and cooled. Dropped it with ± 5 drops of anhydride acetic acid and ± 5 drops strong sulfuric acid (positive terpenoids if formed red brown to violet and positive steroid if formed green to blue colour). Observed changes. (Harborne, 1987).

2.3.1.5.2 Thin Plate Test

3 mL of dragon scales leaves methanol extract spilled on a thin plate is heated on a hotplate then dropped ± 2 drops of concentrated cerium sulfuric (positive terpenoids if formed a reddish color). (Harborne, 1987)

2.3.1.6. Saponin Test

5 drops of dragon scales leaves methanol extract put in a test tube and then added 20 mL aquadest. The filtrate is cooled then shaked for 10 seconds and let it for 10 minutes (positive if the foam is formed). Observed change. (Harborne, 1987)

2.3.2. Sample Preparation

Collected the dragon scales leaves from false ashoka trees (*Polialthia longifolia*) growing around campus purposively (not compare with other areas) as a sample. Dragon scales leaves were cleaned and sliced thin and dried naturally (avoid sun light). Dried samples were crushed using a blender. Then about 70 g of it put into a glass flask with 700 mL of methanol pa Merck. Macerated for 1x24 hours at room temperature. Furthermore, taken the filtrate and added methanol again until translucent color and then collected filtrate. Evaporated with a rotary evaporator to get a thicky extract. Extract condensed in evaporating the solvent discharged until perfect and obtained the dragon scales leaves crude methanol extract that used to antioxidant and antimicrobial activity tests. (MOH, 2000)

2.3.3 Antioxidant Activity Test with DPPH

2.3.3.1 Sample Solutions Preparation

25 mg of dragon scales leaves crude methanol extract was dissolved in 25 ml volume flask with methanol p.a Merck until line sign to obtain 1000 mg / L extract (1000 ppm). 1000 ppm solution extracts made various concentration of 10, 30, 40 and 100ppm. (Mariani S, 2015)

2.3.3.2 Antioxidant Activity Test

Each of samples was added with 5 mL of 0,5mM DPPH 40 ppm (Mariani S, 2015 shaked until homogeneous and then incubated for 30 minutes in dark room. Measured absorbance at λ max 515-517 nm using a UV Visible Spectrophotometer. Noted the results. Did two repetitions. (Mariani S, 2015)

2.3.4. Antibacterial Test

2.3.4.1 Inoculum Bacteria Preparation

Bacterial colonies of *Streptococcus mutans* were taken from stock cultures using ose needle sterile then resuspended in 10 mL of sterile medium nutrient broth and incubated at 35 ± 2 ^{0}C to obtain the turbidity of the transmittance by means of UV wavelength of 580 nm (DG POM, 1995) , Do the same thing on *Escherichia coli*.

2.3.4.2 Test Solution Preparation

Prepared three test tubes for the test solution concentration of 1%, 2% and 3% with the addition of 20 µL of concentrated extract into 1980 µL DMSO solution, 40 µL of concentrated extract into 1960 µL DMSO and 60 µL of concentrated extract into 1940 µL DMSO.

2.3.4.3 Antibacterial Activity Test by Diffusion Method

Prepared 10 mL Mc. Farland Solution (10^8 CFU / mL) was taken *Streptococcus mutans* with a sterile ose neddle then put into 10 mL of aquadest in a test tube and then suspensed it with vortex until homogeneously and closed tube with cotton and seal wrap . Applied *Streptococcus mutans* in a petri dish containing MHA then perforated MHA in petri which had smeared it with holes uniformly using Cop Borer then inserted 50 mL of sample solution with a concentration of 1%, 2%, 3% and DMSO as a blank and then sealed with the seal wrap and incubated at temperature of 35 ± 2 ^{0}C for 24 hours. Furthermore, the diameter inhibitory measured regions surrounding the hole by using a caliper. Do the same thing on *Escherichia coli* . (DG POM, 1995)

3. RESULTS AND DISCUSSIONS

3.1 RESULTS

3.1.1 DETERMINATION OF DRAGON SCALES LEAVES CRUDE METHANOL EXTRACT

Fresh dragon scales leaves were cleaned then dried weighed as the initial weight is 900 g and then cut in small pieces and left to dry until the leaves could be crushed in the room (± 1 month) and weighed again as the final weight is 86.40 g or 9.6% and a water content of 90.4% was lost. 70 g powder dragon scales leaves were extracted by maceration method with methanol p.a Merck for 24 hours and then taken filtrate and maceration back until the filtrate sample was not colored or clear then evaporated with a rotary evaporator at 60 ^{0}C continued in a water bath obtained dragon scales methanol extract crude leaves as much as 8.59 g or by 12.27%.

3.1.2 PHYTOCHEMICAL SCREENING TEST

Table 1. Phytochemical Screening Results of Dragon Scales Leaves Methanol Extract

No	Groups	Reagents	Results
1	Alkaloid	Meyer	-
		Bouchardat	-
		Dragendorf	-
		Wagner	-
2	Phenolic	Flavonoid (Et.acetic extract with $FeCl_3$ 1%)	+++
		Phenolic (Methanol Extract with $FeCl_3$ 1%)	+++
3	Saponin	Aquadest	-
4	Terpenoid/Steroid	Lieberman Bouchad	+++
		CeSO4 1% in H_2SO4 with TLC Plat	+++

NB: - (Negative) and + (Positive)

3.1.3 ANTIOXIDANT ACTIVITIES TEST WITH DPPH

Table 2. Antioxidant Activity Test of Dragon Scales Leaves Crude Methanol Extract (*Drymoglossum piloselloides* (L.) Presl) with 0.5 mM DPPH 40 ppm at 515 nm wavelength

No	Concentration (ppm)	Absorbance (A)	% Scavenging
1	10	0,0540	88,4640
2	30	0,0092	98,0346
3	40	0,0061	98,6969
4	100	0,0055	98,8250
5	Blank	0,4681	0

From Table 2 , can be seen that scavenging by DPPH at 515 nm wavelength to the sample with 100 ppm concentration is very strong at 98.8250%, this illustrates that dragon scale leaves crude methanol extract (*Drymoglossum piloselloides* (L.) Presl) has very strong free radical activity inhibitory.

Table 3. Antioxidant Activity Test of Dragon Scales Leaves Methanol Extract Crude (*Drymoglossum piloselloides* (L.) Presl) with 0.5 mM DPPH 40 ppm at 516 nm wavelength.

No	Concentration (ppm)	Absorbance (A)	% Scavenging
1	10	0,0538	88,4871
2	30	0,0088	98,1168
3	40	0,0055	98,8230
4	100	0,0049	98,9514
5	Blank	0,4673	0

From Table 3 it can be seen that scavenging by DPPH at 516 nm wavelength to the sample with 100 ppm concentrati on is very strong at 98.9514 %, this illustrates that dragon scale leaves crude methanol extract (*Drymoglossum piloselloides* (L.) Presl) hasvery strong free radical activity inhibitory.

Table 4. Antioxidant Activity Test of Dragon Scales Leaves Crude Methanol Extract (*Drymoglossum piloselloides* (L.) Presl) with 0.5 mM DPPH 40 ppm at 517 nm wavelength.

No	Concentration (ppm)	Absorbance (A)	% Scavenging
1	10	0,0540	88,4318
2	30	0,0081	98,2648
3	40	0,0052	98,8860
4	100	0,0044	99,0574
5	Blank	0,4668	0

From Table 4 it can be seen that scavenging by DPPH at 517 nm wavelength to the sample with 100 ppm concentration is very strong at 99.0574 %, this illustrates that dragon scale leaves crude methanol extract (*Drymoglossum piloselloides* (L.) Presl)) has very strong free radical activity inhibitory.

Table 5. Regression Line Equations Data of Dragon Scales Leaves Crude Methanol Extract (*Drymoglossum piloselloides* (L.) Presl) Antioxidant Activity

No	X	Y1	Y2	Y3	Y
1	10	88,4640	88,4871	88,4318	88,4610
2	30	98,0346	98,1168	98,2648	98,1387
3	40	98,6969	98,8230	98,8860	98,8010
	ΣX=80				ΣY=285,4007

Information:
X = concentration of dragon scales leaves crude methanol extract (*Drymoglossum piloselloides* (L.) Presl)
Y1 =% scavenging of 515 nmwavelength
Y2 =% scavenging of 516 nmwavelength
Y3 =% scavenging of 517 nmwavelength
Y = % average scavenging of 515,516 and 517 nm wavelength

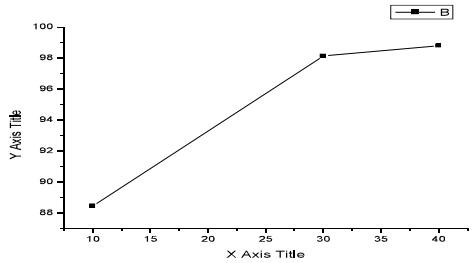
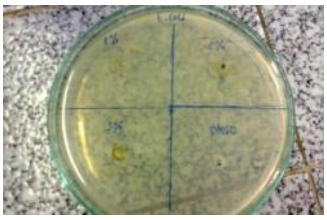


Figure 1. Graphic Antioxidant Activity as Sample Concentration and Y as the Average Scavenging Percent

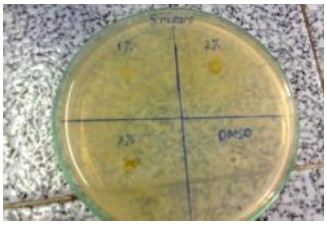
3.1.4 ANTIBACTERIAL ACTIVITY TEST OF DRAGON SCALES LEAVES CRUDE METHANOL EXTRACT (*Drymoglossum piloselloides* (L.) Presl)

Figure 2 Antibacterial Activity Test of *E. coli* with Dragon Scales Leaves CrudeMethanol Extracts (*Drymoglossum piloselloides* (L.) Presl)



From Figure 2 it can be seen negative bacteria of *E. coli* inhibitory zone to a concentration of 1%, 2% and 3% dragon scales leaves crude methanol extract (*Drymoglossum piloselloides* (L.) Presl) and DMSO as a blank.

Figure 3 Antibacterial Activity Test against *S. mutans* to Dragon Scales Leaves Crude Methanol Extracts (*Drymoglossum piloselloides* (L.) Presl).



Can be seen from Figure 3 positive bacteria of *S. mutans* inhibitory zone to a concentration of 1%, 2% and 3% dragon scales leaves crude methanol extract (*Drymoglossum piloselloides* (L.) Presl) and DMSO as a blank.

Table 6. Results of Inhibition Zone Diameter Measurement of Antimicrobial Activity Test Dragon Scales Leaves CrudeMethanol Extract (*Drymoglossum piloselloides* (L.) Presl)

No	Concentration (%)	Inhibitory Zone Diameter (mm)	
		S. mutans	*E. coli*
1	1	0,55	1,03
2	2	0,56	1,30
3	3	0,83	1,34
4	Blank	0,35	0

3.2 DISCUSSIONS

3.2.1 DETERMINATION OF DRY DRAGON SCALES LEAVES CONTENT

Fresh dragon scales leaves was 900 g. Then the sample was left to dry and reweighed as the final weight was 86.40 g or 9.6% and a water content of 90.4% was lost. Dragon scales leaves are leaf with thick cuticle and contains a lot of water content. Reproduction dragon scales are homospore / isospore which produces a kind of spore that is located in Sorrus under the leaves, spores that fell develop into prothalus containing organ sex male or female, so in fertilitation need water (wet environment) so that the sperm of ciliated toward the egg. Therefore ferns mostly live in wet habitats or damp (Hajjah, 2009). According to (Mehlltreter, 2010) dragon scales plant is a plant with Crassulacean Acid Metabolism (CAM) as it takes $C0_2$ predominantly at night.

3.2.2 DETERMINATION OF DRAGON SCALES LEAVES CRUDE METHANOL EXTRACT

The extract contains no residual solvent by drying shrinkage because it was identical to its water content, then extract produced in this research is viscous clay extract on room temperature, hard cast and percentage of water content of 5-30%. (RI Health Department, 2000). Samples leaf powder of dragon scales in this study was about 70 g and crude methanol extract of leaves as much as 8.59 g dragon scales or 12.27%.

3.2.3 PHYTOCHEMICAL SCREENING TEST

From phytochemical screening test of fresh dragon scales leaves methanol extract for Alkaloid tests with Meyer, Dragondorf, Wagner and Bouchardat reagents were not give a color changes (negative alkaloids) as same as saponins did not produce foam constantly (negative saponin) but with phenolic test, dragon scales leaves methanol extract with $FeCl_3$ 1% change blackish brown color (positive phenolic) and with ethyl acetate extract plus $FeCl_3$ 1% gave a blackish brown color (positive flavonoids) as well as test terpenoids with $CeSO_4$ and H_2SO4 gived red color on TLC plate and Lieberman Bouchad reagent gived a dark red color change was sharp colour. Can be seen from Table 1 that +++ Positive signs showed a strong color change and contrast showed that dragon scales leaves contained secondary metabolites were phenolic, flavonoid and terpenoid.

3.2.4 ANTIOXIDANT ACTIVITIES TEST WITH DPPH

From Table 2, table 3 and table 4, the percentage of scavenging by DPPH at a wavelength of 515,516, and 517 nm to the sample with a concentration of 100 ppm was very strong at 98.8250%, 98.9514% and 99.0574 %, this illustrated that the dragon scales leaves methanol extract (*Drymoglossum piloselloides* (L.) Presl) had very good inhibitory activity of free radical.
With the data in Table 5 regression line antioxidant activity of methanol extract of leaves of dragon scales (*Drymoglossum piloselloides* (L.) Presl) is obtained as follows:

Table 7. Regression Line Equations

No	X	Y	XY	X^2	Y^2
1	10	88,4610	884,6100	100	7825,3485
2	30	98,1387	2944,1610	900	9631,2044
3	40	98,8010	3952,0400	1600	9761,6376
	ΣX=80	ΣY=285,4007	ΣXY=7780,8110	ΣX^2 = 2600	ΣY^2=9072,7302

Regretion Line Equation

$$a = \frac{(\Sigma XY)-(\Sigma X)(\Sigma Y)/n}{(\Sigma X^2)-(\Sigma X)^2/n}$$

$$a = \frac{(7780,8116)-(80)(285,4007)/4}{(2600)-(80)^2/4}$$

$$a = \frac{(7780,8116)-(5708,0140)}{(2600)-(1600)}$$

a= 2,0728

To get the value of b is used the following formula:

b= Y-aX

b=71,3501-(2,0728)(20)

b=71,3501-41,4560

b=29,8941

To determine the inhibition using free radical DPPH used IC_{50} (Inhibit Concentration 50) with the following formula:

Y =aX + b

50 = 2,0728X + 29,8941

20,1059 = 2,0728X

X=9,6999

= 9,7 mg/L

The method most commonly used to test the antioxidant activity of medicinal plants is a test method using DPPH free radicals. The purpose of this method is to know the equivalent concentration parameter gives 50% effect of antioxidant activity (IC_{50}). This can be achieved by way of interpreting the experimental data of the method. DPPH is a free radical that can react with compounds that can donate a hydrogen atom, can be useful for testing the antioxidant activity of certain components in an extract. Because of the unpaired electron, DPPH provides strong absorption at a wavelength of 515 - 517 nm. When the electrons into pairs by the presence of free radicals catcher, then measure the absorbance decreases stoichiometric according to the number of electrons captured. The existence of antioxidant compounds can change the colour of DPPH solution from purple to yellow (Dehpour, Ebrahimzadeh, Fazel, and Mohammad, 2009). Absorbance changes as a result of this reaction has been used extensively to test the ability of some molecules as a catcher of free radicals. DPPH method is a method that is easy, fast, and sensitive to test the antioxidant activity of certain compounds or plant extracts (Koleva, van Beek, Linssen, de Groot, and Evstatieva, 2002; Prakash, Rigelhof, and Miller, 2010).

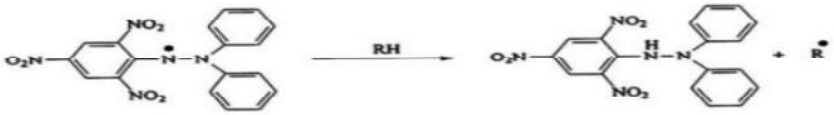


Figure 4. DPPH scavenging mechanism from free radical in to the stable form

From the data concluded that dragon scales leaves condensed methanol extract (*Drymoglossum piloselloides* (L.) Presl) has a very strong antioxidant activity of 9.7 mg / L. According Pokornya(2001), IC_{50} values are very strong if IC_{50}<50 mg / L, strong if IC_{50} is 50-100 mg / L, while if its IC_{50} value 101-150 mg / L and weak if IC_{50}> 150 mg / L.

3.2.6 ANTIBACTERIAL TEST

According to Davis, WW and Stout TR (1971) that the provisions of an extract antibacterial activity is based on inhibition zone where if <5 mm inhibitory power is weak, if 5-10 mm inhibitory power is moderate, 10-20 mm inhibition of both powerful and > 20 power is very strong inhibitory. In Table 6 explains that the inhibition of *S. mutans* and *E. coli* are greater with increasing of concentration of dragon scales leaves crude methanol extract, this proves that the effective antibacterial to be developed as antibacterial agents. Compounds which allegedly acted as antibacterial compounds are flavonoids and terpenoids. Flavonoids play a role in the inhibition of the synthesis of DNA-RNA by intercalation or hydrogen bonds with the built up of nucleic acid bases, as well as inhibit the metabolism of energy. These compounds disrupt energy metabolism by inhibiting respiration consistent because it takes energy to active absorption of various metabolites and for the biosynthesis of macromolecules. (Nuria, 2009). Terpenoids in antibacterial mechanism involves solving membrane by lipophilic components. (Bobbarala, 2010). In addition terpenoids have the main targets, namely the cytoplasmic membrane which refers to the natural hydrophobic properties. (Sari, FP, 2011)

4. CONCLUSIONS

From the results of research conducted on dragon scales leaves crude methanol extract (*Drymoglossum piloselloides* Presl) can be summarized as follows:

- Antioxidant activity tests of the dragon scales leaves crude methanol extract (*Drymoglossum piloselloides* Presl) with 0.5 µM DPPH 40 ppm which measured on a UV Visible Spectrometer at 515-517 nm wavelength were obtained IC_{50} of 9.7 mg / L and highest scavenging percent was 99.0574% at 100 ppm of 517 nm wavelength, which means a very strong antioxidant activity
- Antibacterial activity tests of the dragon scales leaves crude methanol extract (*Drymoglossum piloselloides* Presl) with Agar Diffusion Method in order obtained inhibition zone in *S. mutans* extract concentration respectively were 1%, 2% and 3% is 0.55; 0.56 and 0 , 83 mm and *E. coli*were 1.03; 1.30 and 1.34 mm where the greater extract concentration caused the inhibitory zone of *S. mutans* and *E. coli* were greater too, so effective to be developed as antibacterial agents.

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