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Antioxidant and Antibacterial Activity of Some Leaves Extracts (Methanol, Ethyl Acetate and *N*-Hexane) of *Scurrula fusca* G.Don

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Abstract: The purpose of this research was to test antioxidant and antibacterial activity of some leaves extracts of *Scurrula fusca* G.Don. The antioxidant activity of methanol, ethyl acetate and *n*-hexane extracts of *S. fusca* tested by Free Radical Scavenging method using DPPH (1,1-diphenyl-2-picrylhydrazil) are considered very strong since their IC_{50} value were less than 50 µg/ml (32.96; 27.35 and 40.31 µg/ml respectively). Antibacterial activity test by Disc Difusion method for the extracts showed that methanol extract had activity with category susceptible at concentration of 550 mg/ml against Gram Positive bacteria (*Streptococcus aureus* and *Bacillus cereus*) with inhibition zone 17.80 and 18.26 mm respectively, and at the same concentration showed activity with category intermediate against Gram Negative bacteria (*Pseudomonas aeruginosa* and *Escerichia coli*). Ethyl acetate extract showed activity with category susceptible at concentration of 450 mg/ml against same Gram Positive bacteria (18.28 mm and 18.05 mm) and activity with category intermediate against Gram Negative bacteria. *n*-Hexane extract showed activity with category resistant (9.34 - 10.10 mm) against all bacteria tested at concentration of 550 mg/ml.
Keywords : *Scurrula fusca* G.Don, antioxidant, antibacteria.

Introduction

One of the causes of certain diseases such as cancer, inflammation, atherosclerosis and early aging is reactive oxygen species (ROS) such as hydrogen peroxide, superoxide, hydroxyl radicals and other radical compounds. ROS will oxidize cells of the human body to make the growth of the cells disrupted and grow abnormally and finally induced degenerative diseases[1,2]. The antioxidant substances are needed to protected cells from those compounds.

Antioxidant is a compound that prevent or delay the oxidation process of molecules by terminating the chain reaction initiation and propagation[3]. Utility of synthetic antioxidant compounds such as butyl hydroxytoluene (BHT) and butyl hydroxyanisol (BHA) have carcinogenic side effects[4]. Peoples tend to find traditional medication to prevent side effects of synthetic medicine. According to World Health Organization (WHO), almost 70-80% of the total population in the world believe in herbal medicine[5].

Indonesia is rich in biodiversity that potential to be developed as drug or drug raw materials that function as antioxidant[6]. Based on their functions as medicinal concoction for cough, cancer, inflammation, wound, bacterial or mold infections, many researches have been done to plants used as herbal medicine ("Jamu"), spices, or plants lived on their host plant such as epiphyte or parasite [7].

A parasite (*Macrosolen cochinchinensis*) growing on a variety of Star fruits showed antioxidant activity (IC_{50} < 50 µg/ml) from all water extracts and ethanol extracts using DPPH free radical scavenging method[8]. Simanjuntak had isolated and identificated active anticancer compounds from water extract of tea parasite (*Scurrula oortina*) and obtained pure compounds catechin and phytol with IC_{50} of 82.4 and 88 ppm, 77 ppm respectively using DPPH free radical scavenging method[9]. Tripathy run antimicrobial activity test to water extracts of some hemi-parasite plants grown in southwestern forest of Bengal, India and found antibacterial activity on *Macrosolen cochichinensis* with clear inhibition zone 6-8 mm[10].

Scurrula fusca G.Don (*Loranthaceae*) is one of the parasite species grow on *Ficus riedelii* (*Moraceae*) that have been used by Central Sulawesi people as cancer traditional medicine[11]. *S. fusca* also grow on Orange tree (*Citrus sinensis*) and commonly found in Orange plantations in Karo, North Sumatra, Indonesia. This parasite is very detrimental because of their damaging effects to commercial crops. Local people never use the parasite because of their ignorance about this parasite advantage as herbal medicine. Research on secondary metabolites of this parasite also has not been done.

The structure and function of the parasite are different depend on their host[12]. This maybe because parasite get nutrients from their host plant to survive and prevent herbivore animals[13]. The antioxidant potency may also be different in different host plants[14].

Based on those background, it is necessary to run a research about the antioxidant and antibacterial acitvity of active compounds contained in *S.fusca*.

Material and Methods

Plant Materials

Leaves of *S.fusca* were collected from Orange plantations in Sukarame, Karo, North Sumatra, and identified in Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia.

Antioxidant Activity Test

Antioxidant activity test for methanol, ethyl acetate and *n*-hexane leaves extracts of *S. fusca* were done based on free radical scavenging method using DPPH (1,1-diphenyl-2-pikrylhydrazil) developed by Molyneux (2004). Inhibition percentage can be determine using equation formula (1) as follow[3] :

$$\text{InhibitionPercentage} = \left(\frac{\text{Blank Absorbance} - \text{Sample Absorbance}}{\text{Blank Absorbance}}\right) \text{x } 100\%$$

Antibacterial Activity Test

Antibacterial activity test for methanol, ethylacetate and *n*-hexane leaves extracts of *S. fusca* were done based on Disc Diffusion method by Bauer *et.al.*(1966) with concentration of 300, 400, 450, 500 and 550 mg/ml[15].

Phytochemical Screening

Phytochemical screening for methanol, ethylacetate and *n*-hexane leaves extracts of *S.fusca* were done based on method by Farnsworth (1996)[16].

Results and Discussion

Antioxidant Activity Test

Results of antioxidant activity test for methanol, ethyl acetate and *n*-hexane leaves extracts of *S. fusca* done based on free radical scavenging method using DPPH (1,1-diphenyl-2-pikrylhydrazil) and measured by UV-Visible Spectroscopy at λ 515 nm and ascorbic acid as positive control can be seen in Table 1.

Table 1. Free Radical Scavenging Percentage of Methanol, Ethyl acetate, *n*-Hexane Leaves Extracts of *S. fusca* and Ascorbic Acid.

No	Concentration (μg/ml)	Scavenging Percentage (%)			
		MeOH Ext.	EA Ext	*n*-Hex.Ext.	Ascorbic Acid
1	0	0	0	0	0
2	10	16.34	21.76	82.10	56.33
3	20	34.85	40.45	92.89	83.77
4	30	42.99	51.76	98.70	97.12

Based on the data, those three extracts showed activity as free radical scavenger. The higher sample concentration, the greater scavenging percentage. This is because free radical abstracted hydrogen radical from antioxidant compound and developed DPPHH (1,1-diphenil-2-pikrylhydrazin), a more stable compound. The reduction of free radical also can be seen by the solution color change from purple to light yellow[3].

High scavenging percentage showed that those samples have high antioxidant activity. The concentration of an antioxidant compound needed for scavenging 50% free radical DPPH at certain time (15-30 menit) is determined as IC_{50}. The antioxidant activity is expressed by IC_{50} value and this value can be obtained from regression equation with concentration as free variable and scavenging percentage as bonded variable[3].The regression equation, and IC_{50} value of methanol, ethyl acetate, and *n*-heksana leaves extracts of *S. fusca* and ascorbic acid as control can be seen in Table 2.

Table 2. The regression equation, and IC_{50} value of methanol, ethyl acetate, and *n*-hexane leaves extracts of *S .fusca* and ascorbic acid as control

No	Sample	Regression Line Equation	IC_{50} Value (μg/ml)
1	Methanol Extract	Y = 1.474x + 1.423	32.96
2	Ethyl acetate Extract	Y = 1.739x + 2.397	27.35
3	*n*-Hexane Extract	Y = 1.297x – 2.289	40.31
4	Ascorbic Acid	Y = 3.188x + 11.480	12.08

Based on data shown in Table 2, methanol, ethyl acetate, and *n*-hexane leaves extracts of *S. fusca* have IC_{50} value that greater (32.96; 27.35 and 40.31μg/ml) than IC_{50} value of ascorbic acid (12.08 (μg/ml), but the values are still considered as strong because their IC_{50}value are less than 50 μg/ml[17].

Those three extracts showed strong antioxidant activity because they contained secondary metabolite compounds that have power to scavenge free radicals, such as tanin, flavonoid and terpenoid [18]. Ethyl acetate extract showed highest antioxidant activity among all extracts. This may caused by flavonoid compounds contained with higher concentration in this extract than other extracts at the same concentration[19]. This is supported by phytochemical screening results. Based on phytochemical screening results, flavonoid compounds were not detected in *n*-hexane extract. This may result in poor antioxidant activity of this extract compared to other extracts. Flavonoids can easily give electron or proton to free radicals because flavonoid have ortho-dihydroxy structure on B ring, -2,3 double bond with 4-oxo group on C ring, and 3- and 5-hydroxyl on A ring [18]. Free radical DPPH scavenging by phenolic compound are shown in Figure 1.

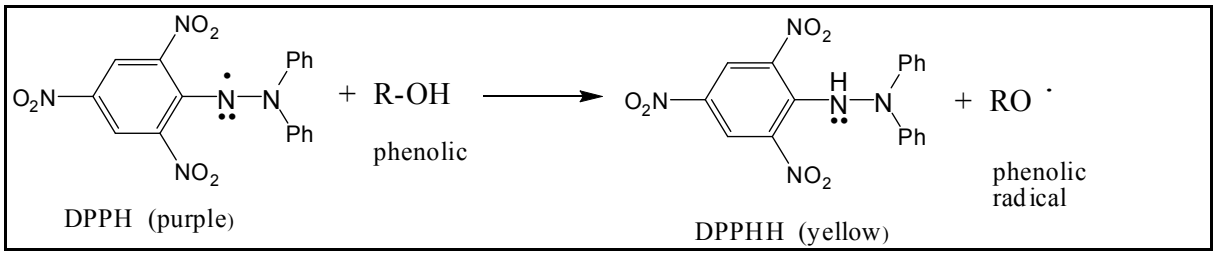

Figure1. Free radical DPPH scavenging by phenolic compound[18]

Antibacterial Activity Test for Methanol, Ethyl acetate and *n*-Hexane Leaves Extracts of *S. fusca*

Antibacterial activity test for leaves extracts of *S. fusca* were done by disc diffusion method using *B. cereus*, *S. aureus*, *P. Aeruginosa* and *E.coli* as tested bacteria. Inhibition activity of leaves extracts of *S. fusca* against bacterial growth were showed by clear zone formed around paper disc on petri disc spreaded with bacteria. The clear zone diameter formed as result can be seen in Table 3. Chloramphenicol was used as positive control and the results can be seen in Table 4.

Table 3.Inhibition zone diameter formed by methanol, ethyl acetate and *n*-hexane leaves extracts of *S.fusca* against Gram positive bacteria *S. aureus* (Sa), *B. cereus* (Bc), and Gram negative bacteria *E. coli* (Ec), *P. aeruginosa* (Pa).

No	Concentration (mg/ml)	Inhibition Zone Diameter Against Bacteria (mm)											
		Methanol extract				Ethylacetate extract				*n*-Hexane extract			
		Sa	Bc	Ec	Pa	Sa	Bc	Ec	Pa	Sa	Bc	Ec	Pa
1	300	16.07	16.30	12.93	12.93	16.92	16.40	13.10	12.56	8.50	8.45	8.33	8.63
2	400	16.30	16.98	13.67	14.20	17.50	17.35	15.10	14.40	9.10	9.03	8.63	8.90
3	450	17.00	17.20	13.95	14.88	18.25	18.08	16.00	15.10	9.25	9.35	8.89	9.14
4	500	17.40	17.80	14.33	15.12	18.84	18.90	16.22	16.34	9.33	9.90	9.23	9.40
5	550	17.95	18.26	14.87	16.20	19.23	19.30	16.40	16.68	9.70	10.10	9.34	9.60

Table 4. Inhibition zone diameter formed by Chloramphenicol against Gram positive bacteria *S.aureus* (Sa), *B.cereus* (Bc), and Gram negative bacteria *E. coli* (Ec), *P. aeruginosa* (Pa).

Concentration (mg/ml)	Inhibition Zone Diameter Against Bacteria (mm)			
	Sa	Bc	Ec	Pa
1	16.40	16.76	7.87	7.60
1.5	17.89	18.08	*	*
2	19.91	20.10	8.17	8.23
3	22.60	22.65	8.40	8.68
4	*	*	10.50	10.67
5	*	*	13.10	12.98
6	*	*	14.85	14.78
7	*	*	16.20	16.56
8	*	*	18.06	18.12
9	*	*	19.70	19.80
10	*	*	21.40	21.56

* : No test done.

Table 3 showed that methanol extract had antibacterial activity with category susceptible at concentration of 550 mg/ml against Gram positive bacteria *S. aureus* and *B.cereus* with inhibition zone 17.80 and 18.26 mm respectively, but at the same concentration had antibacterial activity with category intermediate against Gram negative bacteria. Ethy acetate extract had antibacterial activity with category susceptible at concentration of 450 mg/ml against the same bacteria (18.28 and 18.05 mm), but it had antibacterial activity

with category intermediate against Gram negative bacteria. On the contrary, n-hexane extract had antibacterial activity with catergory resistant against all bacteria with inhibition zone diameter 9.34-10.10 mm at concentration of 550 mg/ml. Ethyl acetate extract showed highest antibacterial activity and this may caused by high concentration of phenolic compounds contained in this extract compared to methanol extract at same concentration. *n*-Hexane extract that had no phenolic compound detected in phytochemical screening showed poor antibacterial activity against all bacteria in the test. Phenolic compound can damage cell membranes of the bacteria because cell membranes of bacteria are permeable to proton and easily get lysis [20].

According to Clinical Laboratory Standard Institute, Chloramphenicol is considered as susceptible against bacteria if it can form inhibition zone with diameter ≥ 18 mm, intermediate if inhibition zone diameter between 13-17 mm and resistant if inhibition zone diameter ≤ 12 mm [21]. In the test, chloramphenicol showed antibacterial activity with category susceptible at concentration of 1.5 mg/ml against Gram positive bacteria *S. aureus* and *B.cereus* with inhibition zone diameter 17.89 mm and 18.08 mm respectively, and against Gram negative bacteria *E. coli* and *P. aeruginosa* at concentration 8 mg/ml (18.06 mm and 18.12 mm).

Based on the data, we can get antibacterial activity equalization. Methanol extract at concentration of 550 mg/ml is equal with antibiotic activity of chloramphenicol at concentration of 1.5 mg/ml against Gram positive bacteria (*S. aureus* and *B. cereus*). Ethyl acetate extract at concentration of 450 mg/ml is equal with antibiotic activity of chloramphenicol at concentration of 1.5 mg/ml against Gram positive bacteria.

Methanol and ethyl acetate leaves extracts of *S. fusca* can act as antibacteria by their secondary metabolites contain such as terpenoid, saponin, and phenolic compounds. Phenolic compound will denaturate protein cells of microbe that caused biological activity reduction and lysis and cells mortality at the end[22, 23].

Phytochemical Screening for Leaves Extracts of *S. fusca*

Results of phytochemical screening for leaves extracts of *S.fusca* obtained from extraction process to discover composition of compounds contained in those extracts can be seen in Table 5.

Table 5. Phytochemical screening for leaves extracts of *Scurrula fusca*

No.	Phytochem. Compounds	Reagents	Phytochemical Screening Results		
			MeOH Ext.	EA Ext.	*n*-Hex Ext.
1.	Alkaloid	Wagner	-	-	-
		Meyer	-	-	-
		Boucherdat	-	-	-
		Dragendorff	-	-	-
2.	Flavonoid	$FeCl_3$ 5%	+	+	-
		NaOH 10% + HCl 2N	+	+	-
3.	Tanin	$FeCl_3$ 5%	+	-	-
4.	Terpenoid	$CeSO_4$ 1% dalam H_2SO_4 10%	+	+	+
5.	Saponin	Aquadest	+	-	-
6.	Steroid	H_2SO_4 (p)	-	-	-
8.	Carbohydrate	Benedict	-	-	-
		Molisch	-	-	-
9.	Protein	Biuret	-	-	-

Note : + = Detected; - = Undetected

Based on results of phytochemical screening, methanol extract contains flavonoid, tanin, saponin and terpenoid compounds, but alkaloid, carbohydrate, steroid and protein were undetected. Ethyl acetate extract does not contains carbohydrate, steroid, alkaloid, and protein compounds, but flavonoid dan terpenoid compounds were detected. In *n*-hexane extract we could only detect terpenoid compounds. This may caused by the nature of terpenoid as non-polar compound. Tanin was only detected in methanol extract because tanin is a polar compound that only dissolved in polar solvents like methanol and not dissolved in semi-polar and non-polar solvents. While flavonoid and terpenoid were detected in methanol and ethylacetate extracts because flavonoid is dissolved in polar and semipolar solvents.

Conclusion

Antioxidant activity of leaves extracts of *S. fusca* are considered strong because their IC_{50} values are less than 50 µg/ml (32.96; 27.35 and 40.31 µg/ml) although they still lower than antioxidant activity of ascorbic acid. Ascorbic acid as positive control showed IC_{50} value of 12.08µg/ml.

Methanol extract showed antibacterial activity with category susceptible at concentration of 550 mg/ml against Gram positive bacteria *S. aureus* (17.80 mm) and *B.cereus* (18.26 mm). At the same concentration, methanol extract showed antibacterial activity with category intermediate against Gram negative bacteria *P. aeruginosa* and *E. coli*. Ethyl acetate extract showed antibacterial activity with category susceptible at concentration of 450 mg/ml against the same Gram positive bacteria (18.28 dan 18.05 mm) and category intermediate against Gram negative bacteria. *n*-Hexane extract showed antibacterial activity with category resistant against all tested bacteria (9.34-10.10 mm) at concentration of 550 mg/ml.

Based on antibacterial activity equality test for chloramphenicol as positive control and all extracts, methanol extract at concentration of 550 mg/ml is equal with antibiotical activity of chloramphenicol at concentration of 1.5 mg/ml against Gram positive bacteria. Ethyl acetate extract at concentration of 450 mg/ml is equal witth chloramphenicol at concentration of 1.5 mg/ml against Gram positive bacteria.

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