

## THE ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL SCREENING OF ETHANOL EXTRACT, FRACTIONS OF WATER, ETHYL ACETATE, AND N-HEXANE FROM MISTLETOE TEA (*SCURRULA ATROPURPUREA* BL. DANS)

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### ABSTRACT

**Objective:** The aim of this study is to investigate antioxidant activity and phytochemical screening of ethanol extract, fractions of water, ethyl acetate, and n-hexane from mistletoe tea (*Scurrula atropurpurea* Bl. Dans).

**Methods:** Simplicia extracted using soxhlet equipment with 96% ethanol. Fractionation was conducted using liquid-liquid extraction using a solvent of water, ethyl acetate and n-hexane. Screening of phytochemical and antioxidant activity was performed against these fractions. Antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl method using ultraviolet-visible spectrophotometry with ascorbic acid as standard. Phytochemical screening was conducted based on the method of Farnsworth.

**Results:** The IC<sub>50</sub> values of ethanol extract, water fraction, fraction of ethyl acetate, and n-hexane fraction were 21.92 ppm, 89.57 ppm, 14.08 ppm, and 162.09 ppm, respectively, whereas for ascorbic acid was 4.41 ppm. The ethanol extract and ethyl acetate fraction contained compounds were the same group, polyphenolic, tannins, flavonoids, monoterpenoid, steroids, triterpenoids, and quinones. Fraction of water contained compounds such as polyphenolic group, flavonoids, monoterpenoids, sesquiterpenoids, steroids, and triterpenoids. n-hexane fraction compounds contained steroids and triterpenoids.

**Conclusion:** The ethanol extract, water fraction, ethyl acetate fraction, and n-hexane fraction showed antioxidant activities. The ethanol extract, fractions of water, and ethyl acetate fraction contained flavonoids and polyphenolic potential as antioxidants.

**Keywords:** Mistletoe tea, *Scurrula atropurpurea*, Antioxidant, 2,2-diphenyl-1-picrylhydrazyl, Ascorbic acid.

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### INTRODUCTION

On the one hand, mistletoe is a plant which is unique because it is a parasitic plant that can be used as medicine [1]. Ohashi *et al.* [2] reported that the alkynic fatty acid octadeca-8, 10, 12-triynoic acid found as chemical constituents of *Scurrula atropurpurea* (Loranthaceae), a parasitic plant of the tea plant *Thea sinensis* (Theaceae), exhibited potent inhibitory effect on cancer cell invasion *in vitro*. It said the *S. atropurpurea* included 16 bioactive materials comprising six fatty acid compounds, two xanthine, two flavonol glycosides, monoterpene glycosides, one lignan glycosides, and four flavones. The tea mistletoe also traditionally used to decrease high blood pressure [3], antimicrobial activities against four bacterial strains (*Bacillus subtilis*, *Klebsiella pneumoniae*, *Vibrio cholerae*, and *Escherichia coli*) [4]. Mistletoe preparations have been used medicinally in Europe for centuries to treat epilepsy, infertility, hypertension, and arthritis [5,6].

Degenerative disease and cancer can be inhibited when the body's own antioxidants as neutralizing free radicals [7]. There are two kinds of antioxidants: The natural and synthetic antioxidants. Natural antioxidant commonly found in plants such as fruits, grains, and vegetables. Some natural antioxidant compounds are derivatives of phenol, coumarin, hydroxycinnamic, tocopherol, diphenol, flavonoids, dihydroflavon, catechin, and ascorbic acid. Synthetic antioxidants include butyl hydroxyanisole, butyl hydroxytoluene, propyl gallate, ethoxyquin [8]. Activity mistletoe tea inhibit the oxidative damage caused by free radicals associated with the active ingredient in the leaves and stems of plants in the form of alkaloids, flavonoids, glycosides, triterpenoids, saponins and tannins [9].

This paper reports the activities of antioxidant and phytochemical screening of the ethanol extract, fractions of water, ethyl acetate and n-hexane from tea parasite (*S. atropurpurea*). *S. atropurpurea* used in this research was actually parasite tea as well as the methods used in the form of a modification of the existing methods with the application of different chemicals as well. It is worth mentioning here that in the Indonesian market there are two types of parasite tea is traded, namely *S. atropurpurea* and *Dendrophthoe pentandra*. *D. pentandra* is actually a parasite mango instead of tea parasite [10].

### METHODS

#### Plant material

The leaves of mistletoe tea (*S. atropurpurea*) which had been dried and obtained from the Manoko Experimental Farm, Lembang, West Java, and determined in Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran.

#### Extraction

The extraction procedure was soxhletation method [11-13] guided by Herbal Pharmacopoeia of Indonesia [14]. Extraction based on crude material weighed and then extracted by soxhletation until the liquid contained in the tube soxhlet clear. Then, the extract was concentrated using a rotary evaporator and freeze-dried. The viscous extract obtained was defined its water content. Determination of the water content of the extract was carried out by adding 200 mL of toluene into a flask which already contains 2 mg of the extract and then reheated.

### Fractionation

Fractionation of mistletoe tea was carried out by liquid-liquid extraction method (ECC). Certain amount extract obtained was dissolved in 200 mL of water and then put into a separating funnel and added n-hexane at an amount equal to the first solvent. Then, it was shaken with occasional air inside the funnel issued. Fraction of n-hexane and water fractions were separated. Extraction was repeated to obtain a fraction of n-hexane which was almost colorless. Into the layer of water was then added the same amount of ethyl acetate and shaken as above. A layer of n-hexane, ethyl acetate, and water that has been separated then evaporated with a rotary evaporator.

### Phytochemical screening

Phytochemical screening conducted by the Farnsworth method [15]. Screening was done on the ethanol extract, the water fraction, fraction of ethyl acetate and n-hexane to screen the secondary metabolites such as alkaloids, polyphenols, tannins, flavonoids, monoterpene and sesquiterpenoids, quinones, triterpenoids and steroids, and saponins.

### Thin layer chromatography (TLC)

TLC was carried out based on the modified method of Kumar *et al.* [16]. Extract and each fractions were spotted on a silica plate sized 4 cm×10 cm by the upper limit and lower limit of 1 cm so that the developer within 8 cm. A mixture of toluene:ethyl acetate:acetic acid (5:4:1) was prepared as a developer. Developers put in a chamber and allowed to saturate. Once saturated, silica plate inserted and allowed to touch the developer of the deadline. Plat silica observed in visible light, ultraviolet (UV) 254 nm and 366 nm, and with 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent.

### Determination of free radical DPPH scavenging

Antioxidant activity was carried out based on the modification of standard DPPH method [17,18], in which DPPH solution was prepared with a concentration of 40 ppm. DPPH maximum wavelength and specified operating time DPPH in ethanol was determined. DPPH:ethanol (3:2) its absorbance was measured using UV-Vis spectrophotometer every 5 minutes for 120 minutes. The ethanol extract, fractions of water, and the ethyl acetate fraction were made at a concentration of 2, 4, 6, 8, and 10 ppm. n-hexane fraction was made at a concentration of 20, 40, 60, 80, and 100 ppm. Vitamin C as a comparison was made with a concentration of 1, 2, 3, 4, and 5 ppm. Each test solution and reference mixed with DPPH, with a ratio of DPPH:sample (3:2). Once mixed, let the mixture stand for operating time DPPH in the test solutions, then the mixture was measured at UV-Vis spectrophotometry at wavelengths that has been obtained. From the absorbance obtained, percentage inhibition and regression curves were made, and the linear equation was applied to calculate the IC50.

## RESULTS AND DISCUSSION

### Extraction

The extraction of mistletoe tea (300 g) by soxhletation with 96% ethanol obtained viscous extract of 23.8 g with a yield of 11.90%. The water content in the mistletoe tea obtained at 4.98%. The water content was set to maintain the quality of the extract. Based on farmakope herbal Indonesia [19], the water content in the extract should not be more than 10% which aims to avoid the rapid growth of fungi and microorganisms in the extract.

### Fractionation

Fractionation ethanol extract of mistletoe tea with a liquid-liquid extraction method obtained three fractions of water fraction, fraction of ethyl acetate, and n-hexane fraction of the weight of each condensed fraction 4.51 g, 2.31 g, and 2.7 g. The percentage yield of the water fraction, the fraction of ethyl acetate, and n-hexane fraction were 2.255%, 1.155%, and 1.35%, respectively.

### Phytochemical screening

Phytochemical screening results are shown in Table 1. The *Scurrula* species has not been extensively studied in terms of its

phytochemistry [6]. Flavonols to be present in the *Scurrula* species reported by three articles [20-22]. Lohézic-Le Dévéhat *et al.* [23] successfully isolated three flavonols from *Scurrula ferruginea*: Quercetin, quercitrin, and 4''-O-acetylquercitrin, and the latter acetylated derivative was uncommon in higher plants. Priyanto *et al.* [24] declared an ethanol extract of *S. atropurpurea* consisting of flavonoids quercetin. Suspected quercetin was expressed using the color reagent magnesium powder, HCl, and added amyl alcohol and red, yellow, or orange forms on amyl alcohol showed flavonoids. This reaction is actually a pointer reaction for flavonoid in phytochemical screening.

This research revealed that the ethanol extract, fractions of water, and the ethyl acetate contained flavonoids and polyphenol potential as antioxidants. This assumption in-line with others that majority of antioxidant compounds present in plants are secondary metabolites-phenolic compounds (tannins, flavonoids, and phenolic acids) which exhibit their protective properties through scavenging free radicals [25-27]. In addition to phenolics, plants may also contain antioxidant volatile oils, carotenoids, and vitamins [28].

### TLC

Results of TLC using developer: Toluene: ethyl acetate: acetic acid (5:4:1) are shown in Fig. 1. Unlike this research, Krisnawati [29] used two-dimensional paper chromatography to isolate flavonoid from ethanolic extract with butanol: acetic acid: water (3:1:1). The partial structure of flavonoid was determined by spectroscopy UV-visible with different diagnostic reagent.

Fig. 1 shows that the ethanol extract, fraction of ethyl acetate, and n-hexane fraction had three spots while the water fraction had one spot. On the results of TLC with DPPH reagent, the color changes to yellow with purple background color indicated the presence of compounds that have antioxidant activity. In extracts of ethanol, water fraction, fraction of ethyl acetate, and n-hexane fraction, visible yellow color of the compound spotted with purple background meaning that extracts and fractions contain compounds that have antioxidant activity. Profile of each spots and Rf value of chromatography results is shown in Table 2.

### Antioxidant activity with DPPH method

The antioxidant activity of the extracts and fractions mistletoe tea was carried by 1,1-diphenyl-2 picrylhydrazyl using a UV-visible spectrophotometer. DPPH molecule is stable free radical molecules in the presence of electron delocalization around the molecule. Before testing the antioxidant activity, we carried out prior determination of the maximum wavelength of DPPH solution and determination of operating time DPPH solution in 96% ethanol. It aimed to determine the best time and stable work of DPPH solution. From the result of the determination of the maximum wavelength obtained, DPPH maximum

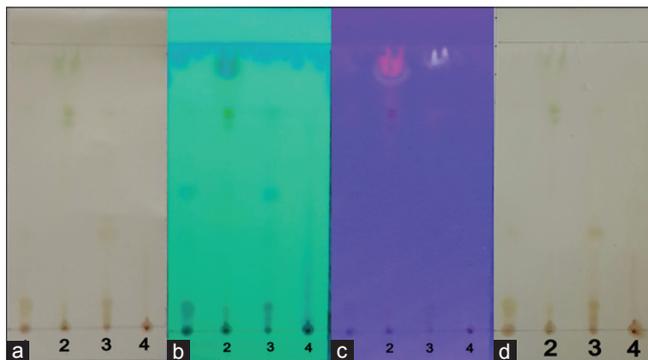


Fig. 1: Thin layer chromatography of mistletoe tea developer toluene:ethyl acetate:acetic acid (5:4:1). (1) Ethanol extract, (2) water fraction, (3) ethyl acetate fraction, (4) n-hexane fraction. (a) With visible light, (b) with ultraviolet (UV) 254 nm, (c) with UV 366 nm, (d) with 2, 2-diphenyl-1-picrylhydrazyl reagent

Table 1: Phytochemical screening of *S. atropurpurea*

S. No.	Secondary metabolite	Simplicia	Ethanol extract	Water fraction	Ethyl acetate fraction	n-hexane fraction
1	Alkaloids	-	-	-	-	-
2	Polyphenols	+	+	+	+	-
3	Tannins	+	+	-	+	-
4	Flavonoids	+	+	+	+	-
5	Monoterpenoid and sesquiterpenoids	+	+	+	+	-
6	Steroids	+	+	+	+	+
7	Triterpenoids	+	+	+	+	+
8	Quinones	+	+	-	+	-
9	Saponins	-	-	-	-	-

+: Detected, -: Not detected. *S. atropurpurea*: *Scurrula atropurpurea*

Table 2: TLC profile of *Scurrula atropurpurea*

Sample	Spotting number	Rf value	Visible light	UV 254 nm	UV 366 nm	DPPH reagent
Ethanol extract	1	0.08	Brown	Purple	-	Yellow
	2	0.5	-	Purple	-	Yellow
	3	0.93	-	Purple	Purple	Yellow
Water fraction	1	0.95	-	Purple	Purple	-
Ethyl acetate fraction	1	0.06	Brown	Purple	Green	Yellow
	2	0.75	-	Yellow	Red	Yellow
	3	0.93	-	Yellow	Red	Green
n-hexane fraction	1	0.94	Brown	Purple	-	Yellow
	2	0.75	Brown	Purple	Purple	Yellow
	3	0.95	-	Purple	Green	-

*S. atropurpurea*: *Scurrula atropurpurea*, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, UV: Ultraviolet, TLC: Thin layer chromatography

Table 3: Percentage of mistletoe tea extract, fractions and vitamin C

Sample	Concentration (ppm)	Absorbance	% inhibition
Ethanol extract	10	0.3997	21.29
	8	0.4245	16.4
	6	0.4465	12.07
	4	0.4677	7.857
	2	0.4997	1.595
Water fraction	80	0.3862	43.47
	40	0.5167	24.37
	20	0.6131	10.26
	10	0.6468	5.328
	5	0.6787	0.658
Ethyl acetate fraction	10	0.4365	34.38
	8	0.4755	28.52
	6	0.5146	22.64
	4	0.5673	14.71
	2	0.6279	5.607
n-hexane fraction	100	0.6347	31.56
	80	0.703	24.2
	60	0.769	17.08
	40	0.8074	12.94
	20	0.8695	6.243
Vitamin C	5	0.2274	57.27
	4	0.2924	45.06
	3	0.3531	33.65
	2	0.4191	21.25
	1	0.4698	11.72

wavelength was 517 nm. From the measurement of operating time, DPPH in ethanol 96% was obtained within DPPH best work from the 35<sup>th</sup> minute until the 50<sup>th</sup> minute. The curve of operating time DPPH solution in 96% ethanol is shown in Fig. 2.

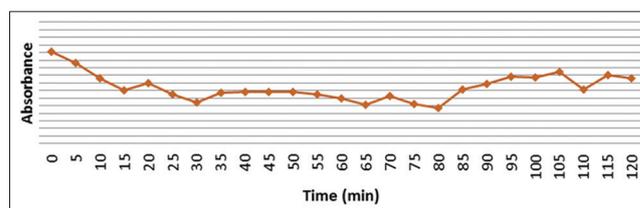


Fig. 2: Operating time of 2, 2-diphenyl-1-picrylhydrazyl solution in 96% ethanol

Before sample absorbance measurement, the test sample and the reference solution were incubated beforehand in DPPH solution of 40 ppm. The measurement results for the sample incubation time in the ethanol extract of DPPH solution was 30 minutes, the water fraction of 40 minutes, 30 minutes ethyl acetate fraction, n-hexane fraction of 45 minutes, and vitamin C 30 minutes. In testing the antioxidant activity with DPPH methods, parameters used for interpretation antioxidant activity was the IC50. IC50 values were calculated from the linear regression equation that states the relationship between the concentration of the sample (X-axis) and the percent inhibition (Y-axis) showing a 50% reduction activity DPPH concentration. Percentage inhibition was calculated from the reduction of arithmetic DPPH absorbance with the absorbance of the sample.

$$\%inhibition = \left[ 1 - \left( \frac{A_{sample, mean}}{ADPPH} \right) \right] \times 100$$

Data from % inhibition of the extract, fractions, and a comparison of vitamin C against DPPH solution are shown in Table 3.

Table 4 shows the calculated antioxidant activity of ethanol extract and the third fraction of mistletoe tea and vitamin C.

According to Jun *et al.*, as well as other text books [30,31] antioxidant activity is categorized as a very powerful when IC50 <50 ppm, strong if IC50 values of 50-100 ppm, moderate at 101-250 ppm, and

Table 4: Results of measurement of antioxidant activity

Sample	Concentration (ppm)	% inhibition	Linear regression equations	IC50 (ppm)
Ethanol extract	10	21.29	$y=2.3966x-2.5375$	21.92±0.044
	8	16.4		
	6	12.07		
	4	7.857		
	2	1.595		
Water fraction	80	43.47	$y=0.5665x-0.745$	89.57±0.078
	40	24.37		
	20	10.26		
	10	5.328		
	5	0.658		
Ethyl acetate fraction	10	34.38	$y=3.5678x-0.2354$	14.08±0.008
	8	28.52		
	6	22.64		
	4	14.71		
	2	5.607		
n-hexane fraction	100	31.56	$y=0.30947x-0.1636$	162.09±0.353
	80	24.2		
	60	17.08		
	40	12.94		
	20	6.234		
Vitamin C	5	57.27	$y=11.491x-0.683$	4.41±0.01
	4	45.06		
	3	33.65		
	2	21.25		
	1	11.72		

weak when IC50 250-500 ppm, and classified as inactive when the IC50 >500 ppm.

The antioxidant activity of the test results on mistletoe tea samples, obtained IC50 to extract ethanol, water fraction, fraction of ethyl acetate, n-hexane fraction was 21.92 ppm (category very strong), 89.57 ppm (strong category), 14.08 ppm (category very strong), and 162.09 ppm (medium category), while for the comparison of vitamin C obtained IC50 of 4.41 ppm (very strong category). It was found that the necessary concentration of ethyl acetate fraction small to capture 50% of free radicals than those of the ethanol extract, the water fraction, and the fraction of n-hexane. Ethyl acetate fraction which had the smallest IC50 was 14.08 ppm so that it could be said ethyl acetate fraction had the greatest antioxidant activity compared with other test samples because it required a concentration of about 14.08 ppm to dampen the activity of DPPH radicals by 50%, but still three times weaker than with vitamin C with IC50 of 4.41 ppm. The IC50 value was owned by a fraction of n-hexane with IC50 of 162.09 ppm, which meant the fraction of n-hexane had the smallest antioxidant activity compared with other test samples. In general, a compound having bioactivity as polyphenol antioxidant was a compound class that had a hydroxy group. Polyphenolic compounds inhibit free radicals by way of donating protons and form a stable radical. The formation of a stable radical was because free electrons contained in the radical stabilized by electron delocalization in the presence of resonance on the aromatic ring. In addition, a compound thought to have antioxidant activities such as polyphenolic compounds, flavonoids, and quinones. In studies of mistletoe tea, leaves have got the result that the highest antioxidant activity contained in ethyl acetate fraction. This is because in general polyphenols and flavonoids are polar, so it would be easy to dissolve in the fraction with a polar solvent or semi-polar, so that the fraction of n-hexane non polar compounds of flavonoids did not extract perfectly that cannot reduce the activity of radical DPPH maximally. Besides polyphenols and flavonoids, according Suhartono and Setiawan [32], quinone compounds and derivatives also have antioxidant activity due to its ability as an electron acceptor. This is consistent with the results of phytochemical screening the ethanol extract, fractions of water, and the ethyl acetate fraction, indicating that all three samples contain compounds that have antioxidant activity, namely, polyphenols, flavonoids, and quinones. Therefore, ethanol extract and ethyl acetate fraction by IC50 has antioxidant activity in the category very strong

and the water fraction have antioxidant activity with strong category, whereas the fraction of n-hexane has antioxidant activity in the category of weak due to phytochemical screening does not contain compounds polyphenol, flavonoids, or quinones and only contain steroids and triterpenoids. Athiroh and Sulistyowati [33], however, used different method in determination of antioxidant activity of tea parasite *S. atropurpurea* leaf extract claimed to get strong antioxidant with an IC50 of 11.64%. These researchers used methanol and 515 nm wavelength instead.

## CONCLUSIONS

The results showed that ethanol extract, the water fraction, fraction of ethyl acetate, and n-hexane fraction of mistletoe tea leaves have antioxidant activity. This is indicated by the IC50 value that is owned by the ethanol extract and each fraction. Best antioxidant activity contained in ethyl acetate fraction with IC50 value was 14.08 ppm (very strong), followed by the ethanol extract of IC50 value of 21.92 ppm (very strong), the fraction of water with IC50 value of 89.57 ppm (very strong), and the fraction of n-hexane with IC50 value of 162.09 ppm (average), while the IC50 value of vitamin C was 4.41 ppm (very strong). There is room for further research, that is to find a compound in the ethyl acetate fraction which is responsible for its antioxidant activity.

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