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Profiling secondary metabolites and antioxidant activity of tea mistletoe leaves (*Scurrula artopurpurea* (Bl.) Danser) in Nglinggo, Kulon Progo, Yogyakarta

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ABSTRACT. *Scurrula atropurpurea* is a hemiparasitic plant in its host. However, this mistletoe also has many health benefits. This type of mistletoe is found in tea (Camellia sinensis). This study aims to determine the group of secondary metabolites, antioxidant activity, and IC_{50} value of tea mistletoe that grows in Nglinggo, Kulon Progo, Yogyakarta. This research was divided into several stages: determination of hemiparasites, sample extraction, qualitative and quantitative tests, and antioxidant activity tests. The tea mistletoe leaf sample was prepared by the maceration method using 96% ethanol as solvent, with a tea mistletoe leaf extract yield of 28.8%. The phytochemical screening results showed that the mistletoe tea leaf ethanol extract positively contained flavonoids, tannins, saponins, steroids, and terpenoids. Tea mistletoe had a total flavonoid content of 36.70 mg QE/g extract and a total tannin content of 96.06 mg TAE/g extract with an IC_{50} value of 0.35 ppm. This value indicates that the ethanol extract of the leaves of tea mistletoe, *S. atropurpurea*, has the potential to be a potent antioxidant.

Keywords: *Camellia sinensis*; host plant; IC_{50} value; *Scurrula atropurpurea*; secondary metabolite

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INTRODUCTION

Tea is one of famous drink in Indonesia and the world. The consumption of tea in society is also high because of its health benefits as refreshing drinks other than just the tradition. Indonesian tea is known to contain the highest catechin in the world which contribute to the antioxidant activity of tea. Catechin is the largest group of tea leaf component, especially the flavanol catechin group. Tea leaf also contain amino acid and other compounds which give the slightly bitter, sweet, and sour taste. Tea compounds have anti-inflammatory, antioxidant, antiallergic and anti-obesity activity (Khan & Mukhtar, 2011).

Tea plantation is an important agricultural sector in Indonesia which play major role in the development of this country. However, in 2016, tea production in Indonesia decreased caused by the reduction of plantation land and poor plantation management. One of the facing problems in tea plantation is mistletoe infestation. Mistletoe is a hemiparasite plant which its nutrient and water was dependent to its host. The infestation of mistletoe causes many negative impacts to the growth of its host to the death of the host itself. In mistletoe, there is a physiological bridge, called haustorium between mistletoe and its host. Haustorium can build direct connections between the xylem and phloem of the host and mistletoe to provide a flow of water, mineral and macromolecules including mRNA and genetic material which possibly even genetic transfer (Kim & Westwood, 2015). The presence of mistletoe can directly damage host plants and increase the insect and disease attack (Kavosi *et al*., 2012).

Scurrula atropurpurea is one species of mistletoe which infest the tea plant. *S. atropurpurea* contains many secondary metabolites such as flavonoid (quercetin, chalcone, and flavone), c-glycoflavonol, and katekin. (Yuniwati *et al*., 2018) also showed the content of flavonoid (rutin, kaempferol, and epicatechin) and alkaloid (caffeine and theobromine) in *S. atropurpurea* which has potential as an anti-inflammatory substance to inhibit the endometriosis inflammation. Moreover, the content of monoterpene, flavonoid, tannin and phenol in this mitletoe exhibit its antifungal activity (Vidyawati *et al*., 2016). Based on previous studies, we find out various of pharmacological potential

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of tea mistletoe caused by its diversity of secondary metabolites. However, exploration and study about the pharmacological potential of tea mistletoe in Indonesia is still few and limited.

Nglinggo, Kulon Progo is one of tea plantations in Yogyakarta. In this area, there are several local tea plantations which was left and did not manage well. It caused there are many mistletoes can be found in this area. The local resident has been sold the dried leaf mistletoe without knowing its potential and biological activity. This study is performed to identify and explore the secondary metabolites of tea mistletoe in Nglinggo, Kulon Progo, Yogyakarta which can lead to its pharmacological activity such as its antioxidant activity. In this study, the content of secondary metabolites is analyzed qualitatively and quantitatively.

MATERIALS AND METHODS

Plant materials. Fresh leaves tea mistletoe *Scurrula atropurpurea* were collected in one of local tea plantation in Nglinggo, Kulon Progo, Yogyakarta. The sampling of mistletoe leaf was performed randomly but in the same area. The authenticity of the plant was confirmed in Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada. The collected fresh leaves of tea mistletoe were washed with normal tap water. Subsequently, the sample were air-dried for 4 days until the weight was constant then was mashed and sieved using 200 mesh to get its powder.

Extraction of tea mistletoe *S. atropurpurea.* The sieved sample, 122.60 g was then extracted by maceration method according to Mondong (2015) and Novia *et al*., (2020) using ethanol 96% with sample: solvent ratio 1:3 (m/v) for 2 x 24 hours. The extract filtrate obtained was then concentrated with a rotary evaporator (IKA HB 10) at a temperature of 40 ℃ with a speed of 50 rpm to become a concentrated extract. Then, the extract was dried at 40 ℃ inside the oven (Memmert) until they attained a constant weight.

Test for alkaloids. A total of 0.05 g of extract was blended with 1 mL of 2 N HCL and 9 mL of distilled water, then heated for 2 minutes. The solution is cooled and filtered. 3 test tubes were taken and then each was given 3 drops of sample and 2 drops of each reagent Mayer, Dragendroff or Wagner reagent. The presence of alkaloids is indicated by a reddish precipitate (Auwal *et al*., 2014).

Test for tannin. A total of 0.05 g of extract was blended with 2 mL of ethanol 96% and stirred, then added 3 drops of $FeCl_3$ 10% . The formation of blue-black, green or blackish green color a precipitate indicated the presence of tannin (Mojab *et al*., 2003).

Test for flavonoids. A total of 0.05 g of extract was blended with 2 mL of ethanol 96%, then added of magnesium chips and 1 mL of concentrated HCl solution. The presence of flavonoids is indicated by brick-red or orange color of solution (Sopianti & Sary, 2018).

Test for saponins. A total of 0.05 g extract was blended with 1 mL ethanol of 96% and stirred. Then, 10 mL of distilled water was added, and the sample was shaken vigorously and observed for 15-20 minutes. The formation of froth indicates the presence of saponins (Mojab *et al*., 2003).

Test for steroids and terpenoids. A total of 0.05 g extract was blended with 1 mL of chloroform, subsequently 0.5 mL of anhydrous acetic acid was added, and 2 mL of concentrated sulfuric acid was added through the tube wall. The presence of triterpenoids is indicated by the formation of a brownish or violet ring at the boundary of the solutions, while the presence of steroids is indicated by the formation of a blue-green ring (Padmasari *et al*., 2013).

Quantitative analysis of total flavonoids. Determination of total flavonoid content by colorimetric method which refers to the procedure Chang *et al*. (2002) and Ahmad & Elya (2014) in the presence of some modifications with quercetin as the standard solution.

Preparation of quercetin standard solution. A total of 10 mg of quercetin was dissolved in 5 mL of methanol for 1000 ppm. Then a various concentration of 20; 40; 60; 80 and 100 ppm were prepared by pipetting 0.1; 0.2; 0.3; 0.4 and 0.5 mL and then added with methanol until the final volume of 5 mL. From each concentration, 1 mL of sample was added 3 mL of 96% ethanol, 0.2 mL of 10% $AlCl_3$, 2 mL of 1 M potassium acetate, and distilled water until 5 mL for final volume. After

that, it was incubated for 30 minutes at room temperature in the dark. The absorbance was measured using UV-Vis spectrophotometer (Thermoscientific) at 431 nm wavelength.

Determination of Total Flavonoid content. This assay was conducted according to Chang *et al*. (2002) and Ahmad & Elya (2014)**.** Sample of 10 mg tea mistletoe extract was blended in methanol until up to 10 mL. A total of 1 mL of the extract was added with 3 mL of ethanol 96%, 0.2 mL of $AlCl_3$ 10%, 0.2 mL of 1 M potassium acetate and added with distilled water up to 10 mL. The solution is incubated for 30 minutes in a dark place at room temperature. The absorbance was measured using UV-Vis spectrophotometer (Thermoscientific) at 431 nm wavelength.

Quantitative analysis of total Tannin. Determination of total tannin content follows the procedure Malangngi *et al*. (2012) and Ahmad *et al*. (2015) in the presence of some modifications with tannic acid as the standard solution.

Preparation of tannic acid standard solution. A total of 10 mg of tannic acid was dissolved in ethanol 96% until up to 5 mL for 1000 ppm. Then, a various concentration of 60; 80; 100; 120 and 140 ppm were made by pipetting 0.3; 0.4; 0.5; 0.6 and 0.7 mL of the 1000 ppm and added with ethanol 96% until up to 5 mL. From each concentration 0,1 mL of sample and then added 2.8 mL of distilled water, 0.1 mL of 1 N Folin-Ciocalteu reagent and 2 mL of Na_2CO_3 10%. Then the samples were incubated in the dark at room temperature for 30 minutes. The absorbance was measured using UV-Vis spectrophotometer (Thermoscientific) at 760 wavelength (Malangngi *et al.*, 2012).

Determination of Total Tannin content. This assay was conducted according to Malangngi *et al.* (2012) and Ahmad *et al.* (2015)**.** Sample of 10 mg tea mistletoe extract was blended in ethanol 96% until up to 10 mL. A total of 0.1 mL of the extract sample was added with 0,.1 mL of 1 N Folin-Ciocalteu reagent, 2 mL of 10% Na_2CO_3 and added up to 10 mL of distilled water. Then the samples were incubated in the dark at room temperature for 30 minutes. The absorbance was measured using UV-Vis spectrophotometer (Thermoscientific) at 760 wavelength**.**

Determination of antioxidant activity. The antioxidant activity of the extract was determined using DPPH assay in Mondong (2015) and Phongpaichit *et al.*, (2007) with some modifications. DPPH solution (50 ppm) was prepared by dissolving 1.25 mg of DPPH in 25 mL of methanol and homogenized, then kept in a dark bottle. A total of 0.5 mL tea mistletoe extract from each concentration variation (0.2; 0.4; 0.6; 0.8; and 1 ppm), then added 3.5 mL of DPPH 50 ppm solution and mixed in a test tube. The same step with the ascorbic acid solution (2, 4, 6, 8, and 10 ppm). For the blank using methanol and for the control using DPPH 50 ppm as much as 3,5 mL. The test tubes were incubated in the dark for 30 minutes at room temperature. The absorbance was measured using UV-Vis spectrophotometer (Thermoscientific) at 517 wavelength. The data obtained during antioxidant testing on ascorbic acid and ethanol extract of mistletoe tea are presented in the form of a standard curve. The percentage of inhibition was then calculated as follows:

$$radical\ scavening\ (\%) = \frac{(A_{control} - A_{sampel})}{A_{control}} \times 100$$

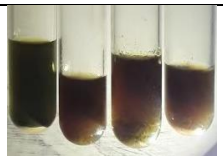
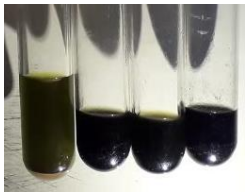
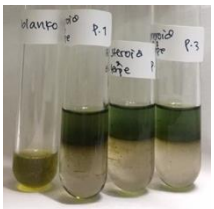
$A_{control}$ is the absorbance of the control (DPPH solution) and A_{sample} is the absorbance of the extracted sample with DPPH solution. The IC_{50} value is the concentration of tea mistletoe extract required to scavenge 50% of DPPH, then graphed the linear equation between the logarithm of the concentration of the solution or sample (x) and (y) inhibition % of the data, then the IC_{50} value of the extract was compared with the IC_{50} value of ascorbic acid.

Data analysis. Quantitative test sample data for total flavonoid, total tannin, and antioxidant activity with 3 technical replications. Each will be analyzed by descriptive statistical analysis using Microsoft Excel.

RESULTS AND DISCUSSION

Phytochemical of leaves tea mistletoe *Scurrula atropurpurea***.** The extraction of leaves tea mistletoe using ethanol 96% in maceration yield 28.83% of crude extract. In this study, ethanol 96% is used as solvent in the extraction process because ethanol 96% can attract most secondary metabolites and is generally safer than other solvent. According to Nirwana *et al*. (2014), ethanol is a non-toxic solvent and requires less heat in concentration process. The content of secondary metabolites of tea mistletoe was identified using phytochemical screening. The result of tea mistletoe phytochemical screening was represented in Table 1. The ethanolic extract of mistletoe gives positive result for flavonoid, tannin, saponin, steroid and terpenoid. But no alkaloid was detected in this extract.

Table 1. Phytochemical of leaves tea mistletoe *Scurrula atropurpurea* (BI) Danser

Secondary Metabolites	Result	Appearance	Description
Flavonoid	++		Brick-red or dark orange
Tannin	++		Blackish green
Saponin	++		Froth
Alkaloid	-		No precipitant
Steroid and Terpenoid	++		Brownish purple ring

Note: Annotation: ++ = very high; - = none

The result in this study provides the same result as the study of Werdyani *et al*. (2019) which show the content of flavonoid and tannin in rambutan mistletoe *S. atropurpurea.* The other research by Wirasti (2019) presented flavonoid, phenol, saponin and tannin in petai mistletoe *S. atropurpurea*, but also show the content of alkaloid. Similar to Mustarichie *et al*. (2017) for *S. atropurpurea* from tea, polyphenols, tannin, flavonoids, steroid, and terpenoids were also detected in this study. According to the result in this study, flavonoid and tannin are thought to be two secondary metabolites groups which can be produced by *S. atropurpurea* on various host plants. In plant, flavonoid plays an important role in plant development and defense mechanism from abiotic and biotic stress such as

UV radiation, pathogen infection and insect feeding (Liu *et al*., 2021). Then, tannin is an important class of polyphenol and an abundance secondary metabolite in plant which have a crucial role in plant defense mechanisms against pathogens, herbivore and changing environmental condition (Adamczyk *et al*., 2017).

In this study, we found no alkaloid detected in *S. atropurpurea* leaves extract from tea as host. It is similar to the simplicial and extract of *S. atropurpurea* from tea in Mustarichie *et al*. (2017). This result differs from Wirasti (2019) which uses mistletoe sample from petai as host. The difference result might be correlated with host nature and environmental condition, such as temperature and light intensity. *S. atropurpurea* connects to its host through haustorium to obtain nutrient and carbon for supporting its development. In this relationship, host nature might influence the performance of mistletoe in its growth and secondary metabolites production. However, the technical error from the number of samples, concentration and limitation of phytochemical assay must be considered. The low concentration of alkaloid in the sample can cause difficulty to identify it during phytochemical screening. It can also be caused by the type of alkaloid which fail to react whit the alkaloid precipitating agent, then give false negative result.

Metabolite compounds of *S. atropurpurea* **according to gas chromatography-mass spectrometry (GC-MS) analysis.** GC-MS analysis of leaves tea mistletoe *Scurrula atropurpurea* result 16 peaks in the chromatogram (Figure 1). Each peak represents a chemical compound or metabolite in leaves tea mistletoe which are further identify in mass spectrometry. The identified metabolites is presented in Table 2.

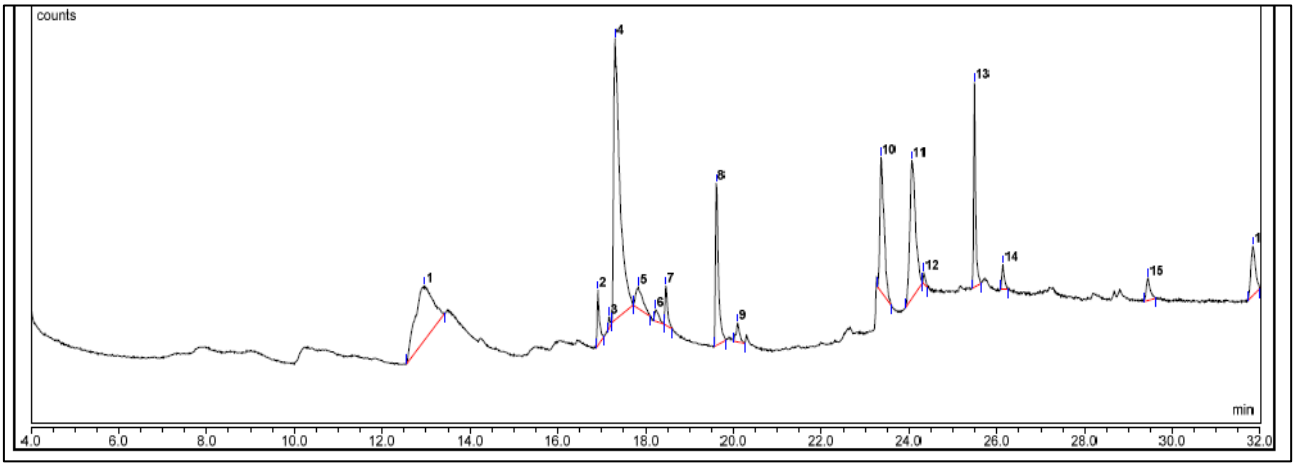


Fig. 1. Chromatogram of ethanolic tea *Scurrula atropurpurea* leaves extract

There are 12 compounds in tea mistletoe leaves extract with similarity index 624-752 which can be classified into 7 classes of compound. The primary metabolites consist of 4 compounds in fatty acid ester group and 3 compounds in sugar group. Then, the secondary metabolites are a compound in N-acyl amino acid (NAA), a compound in alkaloid, a compound in nicotinamide and a compound in acetylene. There are 5 compound that are not classified yet. The highest peak represents (2H) Pyrrole-2-carbonitrile, 5-amino-3,4-dihydro- from alkaloid group with 29.89% of relativity abundance. According to Janakiraman *et al*. (2012), (2H) Pyrrole-2-carbonitrile, 5-amino-3,4-dihydro- has biological activity such as antimicrobial and antiinflammation. GC-MS analysis result differs from phytochemical screening in which alkaloid compound is not detected. Candraningrat *et al*. (2021) stated that GC-MS has high sensitivity so it can separate compound that are mixed and detect the compound even in low level or concentration. It showed the limitation of phytochemical screening in detection of the secondary metabolites when was performed in low amount or concentration of compound of interest.

Table 2. Metabolites of ethanolic tea mistletoe leaves extract through GC-MS analysis

No. Peak	Retention time (min)	Chemical compound	Chemical formula	Relative abb (%)	SI	Cluster
1	12.97	Pyridine-3-carboxamide, 1,2-dihydro-4,6-dimethyl-2-thioxo-	$C_8H_{10}N_2OS$	16.63	625	Nicotinamide
2	16.91	3,6-Dimethyl-3,6-dihydro-pyran-2-one axime	$C_7H_{11}NO_2$	2.11	713	Other
3	17.16	3,6-Dimethyl-3,6-dihydro-pyran-2-one axime	$C_7H_{11}NO_2$	0.34	672	Other
4	17.31	(2H) Pyrrole-2-carbonitrile, 5-amino-3,4-dihydro-	$C_5H_7N_3$	29.89	705	Alkaloid
5	17.84	9-Tetradecen-1-ol, acetat, (E)-	$C_{16}H_{30}O_2$	2.60	677	Fatty acid ester
6	18.23	Deoxyspergualin	$C_{17}H_{37}N_7O_3$	0.84	624	N-Acyl amino acid
7	18.46	Mannosamine	$C_6H_{13}NO_5$	1.68	663	Sugar
8	19.61	2-Tridecen-1-ol, (E)-	$C_{13}H_{26}O$	8.21	728	Fatty acid ester
9	20.09	Imidazole, 2-amino-5-[(2-carboxy) vinyl]-	$C_6H_7N_{30}O_2$	1.14	698	Other
10	23.36	9,12,15-Octadecatrienal	$C_{18}H_{30}O$	9.65	751	Fatty acid ester
11	24.06	9,12,15-Octadecatrienal	$C_{18}H_{30}O$	13.53	714	Fatty acid ester
12	24.34	I-Gala-I-ido-octose	$C_8H_{16}O_8$	0.37	722	Sugar
13	25.49	10-Dodecyn-1-ol	$C_{12}H_{22}O$	6.54	698	Acetylene
14	26.14	3-(Prop-2-enoyloxy) dodecane	$C_{15}H_{28}O_2$	1.06	708	
15	29.44	I-Gala-I-ido-octose	$C_8H_{16}O_8$	1.49	699	Sugar
16	31.83	Z,Z,Z-4,6,9-Nonadecatriene	$C_{19}H_{34}$	3.92	693	Other

Note: Abb= abudance

In this GC-MS analysis, no compound in flavonoid, tannin, saponin, steroid or terpenoid was detected such as in phytochemical screening. It might be caused by the physical and chemical properties of these compounds which mostly involatile and complex structures. GC-MS analysis mostly is used to analyze volatile and semi-volatile compounds. For the complex compounds, the chemical derivatization step must be performed before GC-MS analysis. In this study, the derivatization step was not done, it might be the reason no flavonoid or tannin was analyzed. For further study, it is also better to separate or purify the compound in the extract based on its polarity using several solvents. Fiehn (2017) stated that GC-MS analysis is ideal for identifying and quantifying small molecular metabolites with molecular weight less than 650 daltons, but the chemical derivatization often uses to make this compound volatile enough for gas chromatography. Alkaloid in this study, (2H) Pyrrole-2-carbonitrile, 5-amino-3,4-dihydro-, has molecular weight of 109.13 g/mol which is lower than quercetin (302.236 g/mol) or tannic acid (1,701.19 g/mol).

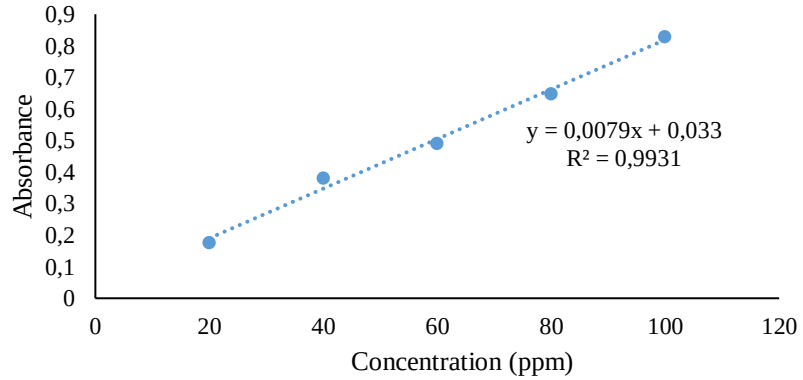


Fig. 2. Standard curve of quercetin

Total flavonoid content and total tannin content of *Scurrula atropurpurea*. Quantitative analysis of secondary metabolites in leaves tea mistletoe extract was conducted to flavonoid and tannin. The assay was iniatiated by creating the graph of standard curve for flavonoid and tannin using quercetin and tannic acid in sequential. The standard curve of quercetin and tannic acid were sequentially shown in Fig. 2 and Fig. 3. These standard curves were going to be used in calculation of total flavonoid content (TFC) and total tannin content (TTC). The equation, y=0.0079x +0.033 with R^2= 0.9931 in Fig. 2, was used in the calculation of total flavonoid content of ethanolic mistletoe leaves extract in this study.

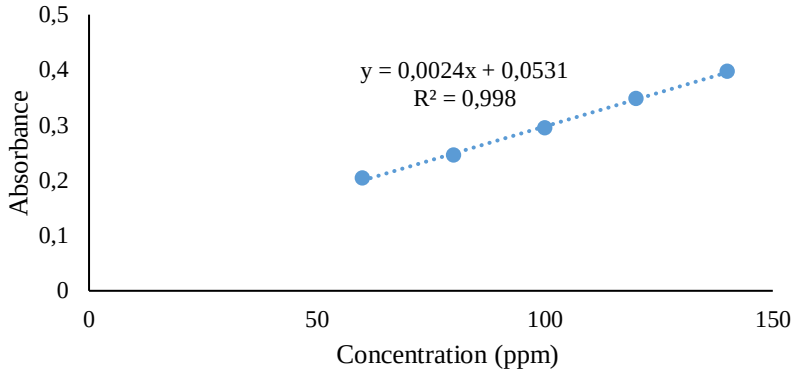


Fig. 3. Standard curve of tannic acid

The equation, y=0.0024x +0.0531 with R^2= 0.998 in Figure 3, was used in the calculation of total tannin content of mistletoe extract leaves in this study. The value of TFC and TTC were shown in Table 3. Total flavonoid content of *S. atropurpurea* is 36.70 ± 1.16 mg QE (Quercetin Equivalent)/g extract. Then, this study obtains the level of tannin content in *S. atropurpurea* is 96.06 ± 1.87 mg TAE (Tannic acid Equivalent)/g extract. The level of flavonoid in this study is lower than in Wirasti (2019) with 148.05 ± 4.44 mg QE/g extract in petai host. As previously explained, the flavonoid level of a mistletoe species can be affected by its host nature and environmental factors. It means that the same mistletoe species can produce different secondary compound in different levels when parasitizing different host. In this case, host conditions might cause a biotic stress for mistletoe. This stress changes the defense mechanisms of mistletoe which can be observed from the level of secondary metabolites. Apart from the biotic stress from host, the environmental condition and nutrient availability from its host also take part in abiotic stress which impact to defense mechanisms regulation. Zargoosh *et al.* (2019) states that biotic and abiotic stress can induce a variety of secondary metabolites mechanism in plant, thereby leading to a diversity of secondary metabolites.

Table 3. Total flavonoid content and total tannin content of *Scurrula atropurpurea*

Secondary metabolites	Value ± SD
Total flavonoid (mg QE/g extract)	36.70 ± 1.16
Total tannin total (mg TAE/g extract)	96.06 ± 1.87

Apart from flavonoid, plant also increase the production of phenolic compound as its defense response against stress. In this study, the level of phenolic compound is calculated from its tannin content. The tea mistletoe *Scurrula atropurpurea* leaves extract contains 96.06 ± 1.87 mg TAE/g extract (Table 3).

Antioxidant activity of *Scurrula atropurpurea*. Flavonoid and tannin are the compounds from the polyphenol groups which commonly have an antioxidant activity. Therefore, in this study, the antioxidant activity of tea mistletoe leaves extract was studied to obtain its potential as antioxidant. The positive control in this assay is ascorbic acid. The result is shown in Table 4. According to Phongpaichit *et al*. (2007) and Paudel *et al*., 2014), DPPH IC_{50} for very strong antixoxidant activity is around 0.45-5 µg/ mL, strong activity if DPPH IC_{50} 5-10 µg/ mL, moderate if DPPH IC_{50} 10-20

µg/ mL and weak if DPPH IC_{50} is more than 20 µg/ mL. DPPH IC_{50} value *Scurrula atropurpurea* extract is 0.35 ppm which can be categorized as very strong antioxidant activity. This value is lower than IC_{50} of ascorbic acid, 5.76 ppm. It showed the potential of *Scurrula atropurpurea* leaves extract as antioxidant. Compared to the previous study by Wirasti (2019) with DPPH IC_{50} 23.48 ppm and Mustarichie *et al*. (2017) with DPPH IC_{50} 21.92 ppm for *Scurrula atropurpurea* extract, tea mistletoe in this study has higher antioxidant activity than them. It is shown the potential of pharmacological activity of tea mistletoe *Scurrula atropurpurea* from Nglinggo, Kulonprogo, Yogyakarta.

Tabel 4. IC_{50} of ascorbic acid and tea mistletoe *Scurrula atropurpurea* leaves extract

Sample	IC_{50} (ppm)	Intensity (Paudel *et al*., 2014)
Ascorbic acid	5.76	Strong
Scurrula atropurpurea extract	0.35	Very strong

Antioxidant activity of extract comes from its diversity of secondary metabolites. In phytochemical screening, this extract shows the positive result of flavonoid, tannins, saponin, steroid and terpenoid. Then, in quantitative analysis, this extract contains flavonoid with 36.70 $\pm$ 1,16 mg QE/g extract and tannin total with 96.06 $\pm$ 1.87 mg TAE/g extract. Flavonoid and tannin are phenolic compound which can scavenge DPPH as free radicals in the assay. It is caused by the hydroxyl group such as ester, glycoside, and methyl ether group in phenolic compound structure (Kumar *et al*., 2022; Pratiwi *et al*., 2013). In addition, the extract also contains saponin which can scavenge radical superoxide by forming hydroperoxide intermediate so prevent the biomolecular damage (Syarif *et al*., 2016).

CONCLUSION

Tea mistletoe extract, *Scurrula atropurpurea* from Nglinggo Kulonprogo, Yogyakarta contains flavonoid, tannins, saponin, steroid and terpenoid with the flavonoid content 36.70 $\pm$ 1.16 mg QE/g extract and tannin content 96.06 $\pm$ 1.87 mg TAE/g extract. Tea mistletoe leaves also contains (2H) Pyrrole-2-carbonitrile, 5-amino-3,4-dihydro- (alkaloid group) with highest relative abundance 29.89% in GC-MS analysis. In GC-MS analysis, it also showed the diversity of metabolites in this sample both primary and secondary metabolites. This mistletoe is also potential to be developed as very potent antioxidant according to its DPPH IC_{50} value 0.35 ppm.

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