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Analysis of secondary metabolites and antioxidant activities of ethanol extract of *Dendrophthoe pentandra* (L.) Miq.) in Sapuran, Central Java

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ABSTRACT. *Dendrophthoe pentandra* (L.) Miq. are known to have the potential for health because they can be used as natural medicines to cure several diseases, including measles, coughs, diuretics, pain relievers, anti-inflammatory medications, as well as wound healing and infections. The local community in Indonesia has believed the health beneficial of tea mistletoe, but the information of its pharmacological activity is still limited. This research was conducted to explore the species and diversity of secondary metabolites of *D. pentandra* (L.) Miq. found in Sapuran, Wonosobo and its antioxidant activity. Extraction was performed by maceration in 96% ethanol. It is followed by phytochemical screening, determination of total secondary metabolite compounds, and antioxidant activity assay using DPPH. The results of this study confirmed that mistletoe is *D. pentandra* (L.). Its leaf extract has a yield of 8.76% that contains alkaloids, saponins, tannins, steroids, phenols, and terpenoids. Its total content of flavonoids, tannins, phenols and saponins was, respectively, 48.584 mg QE/g extract, 1.350 ± 0.140 mg TAE/g extract, 1.756 ± 0.171 g GAE/g extract, 4.665 ± 0.142 g SE/g extract. Its ethanolic extract has very high antioxidant activity with an IC50 value of 49.10 ppm. *D. pentandra* (L.) Miq. of Sapuran, Wonosobo, has great potential to be developed in the pharmacological field.

Keywords: antioxidant activity; *Dendrophthoe pentandra* (L.) Miq.; IC50 value; mistletoe; secondary metabolite compounds

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INTRODUCTION

Mistletoe is a parasitic plant that obtains nutrients and water from its host. As a parasitic plant, mistletoe has a negative impact on plants, such as reducing their productivity. However, mistletoe is also known to have a potential as a natural medicine to cure several diseases, including the treatment of measles, coughs, diuretics, pain relievers and anti-inflammatory drugs, as well as the healing of wounds and infections (Ang *et al*., 2014; Elsyana *et al*., 2016; Szurpnicka *et al*., 2020).

Mistletoe can be found in various countries, in a number of 4500 species of mistletoe belonging to 20 families (Lim *et al*., 2016; Watson, 2019). In Indonesia, 174 species of parasites can be found and grouped into 26 genera. Members of the Loranthaceae and Viscaceae families are typically characterized by the presence of chlorophyll-containing leaves, enabling them to conduct photosynthesis (Arumugam *et al*., 2015). While mistletoe exhibits parasitic behavior, its specific host range remains unclear, encompassing trees, horticultural plantings, and forest flora. Notably, heavy infestations on twigs and branches can lead to host mortality (Raftoyannis *et al*., 2015; Anselmo-Moreira *et al*., 2019).

There are several types of parasites that has been found in tea plants in Indonesia, such as *Scurrula atropurpurea*, *Scurrula oortina, Scurrula ferruginea, Dendrophthoe pentandra* (L.) Miq. (Parwati *et al*., 2015; Sammad *et al*., 2017; Mustarichie & Ramdhani, 2022). The tea mistletoe has been known to be beneficial for health as anticancer, antibacterial, antidiabetic, anti-inflammatory, and antioxidants. The type of mistletoe tea *S. atropurpurea* was found in the Nglinggo, Yogyakarta, and the active compounds identified were flavonoids, tannins, saponins, steroids and terpenoids and had high antioxidant activity because it had an IC50 value of 0.35 ppm and could be classified as very strong (Aditiyarini *et al*., 2022). Different studies found that the same type of mistletoe, but infected

different host plants produced different active compounds. The research conducted by Wirasti (2019) found *Scurrula atropurpurea* in banana plants in the Batang area, Central Java, and the active compounds identified were alkaloids, flavonoids, glycosides, triterpenes, saponins and tannins. The water and nutrient for supporting the growth of mistletoe comes from its host and surrounding environment which affected the production of phytochemical in mistletoe (Chen *et al*., 2013; Muche *et al*., 2022). *D. pentandra* (L.) Miq. is a type of parasite that can be found in forests, plantations, city parks, residential areas, and areas that are often exposed to rain. The spread of this type of mistletoe is carried out by seed-eating birds and *D. pentandra* (L.) Miq. It not only infects one type of host plant but can become a parasite for various types of host plants. The host plants that are usually infected by this type of mistletoe are bushes and trees, and they can be infected for years. Based on research Fitrilia *et al*. (2015), *D. pentandra* (L.) Miq. tea host plants contain secondary metabolite compounds in the form of tannins, flavonoids, saponins, and triterpenes. In other types of hosts, guava plants, *D. pentandra* (L.) Miq., produces other secondary metabolite compounds, including flavonoids, alkaloids, steroids, polyphenols, and quinones (Anita *et al*., 2014). Based on several studies, mistletoe has various secondary metabolites and has the potential to be developed in the pharmacological field, but the research conducted is still limited.

Another type of mistletoe was found in a tea garden owned by a resident of Sapuran, Wonosobo. The specific bioactive constituents of this mistletoe species remain uncharacterized, despite its traditional use in herbal remedies for various ailments by local residents. These products have been widely traded and distributed outside the region. This research was carried out with the aim of identifying types of mistletoe and exploring the diversity of secondary metabolites of mistletoe tea leaves found in Sapuran and Wonosobo, as well as the activity of their antioxidant content so that their pharmacological potential can be determined. The novel compound and/or activities can increase the potential of local mistletoe that indirectly drives the economy of local community.

MATERIALS AND METHODS

Leaf mistletoe tea *Dendrophthoe pentandra* (L.) Miq. Leaves were taken and collected from one of the plantations owned by residents in the Sapuran area, Wonosobo during the rainy season (March to June 2023). The mistletoe leaves taken as samples are young leaves with a green color on the leaf's upper surface, while the leaf's lower surface is brownish, and the part of the leaf used is the whole leaf. The plant's identity has been identified and confirmed at the Laboratory of Plant Systematics, Faculty of Biology, Universitas Gadjah Mada. The collected tea mistletoe leaf samples were washed with water until clean. Then, the leaf samples were air-dried for three days. To obtain simplicia powder, the sample is crushed and sieved.

Tea mistletoe extraction. A total of 250 g of simplicial powder has been obtained and continues at the extraction stage using the maceration method with a 96% ethanol solvent. The ratio between the sample and the solvent is 1:7 (m/v) for 3 × 24 h. The filtrate obtained was then evaporated using a rotary evaporator (IKA HB 10) at 40°C with a speed of 50 rpm until an extract with concentrated texture was obtained. To obtain a constant weight, the extract was dried in a 400°C oven (Memmert) (Akasia *et al*., 2021).

Qualitative analysis. For Alkaloid test, approximately 0.05 g of sample extract were dissolved in 1 mL of 2N HCl, 9 mL of distilled water was added, heated for 2 min and cooled and filtered. Three test tubes (Pyrex) were taken, and three drops of sample filtrate and two drops of Mayer, Wagner, and Dragendorf reagents were added. The positive results of the alkaloid test are indicated by the presence of white or yellow, brown to black, and orange precipitates. For saponin test, approximately 0.05 g of sample extract were dissolved in 10 mL of hot distilled water, cooled, and shaken until foam formed. A positive result of the saponin test was indicated by the formation of constant foam for 10 min when one drop of 2N HCl was added. For tannin test, a total of 0.05 g of sample extract was dissolved in 2 mL of 96% ethanol and three drops of 10% $FeCl_3$ were added. The positive results of the tannin test are indicated by the formation of black-blue or black-green colors.

For flavonoid test, as much as 0.05 g of sample extract was dissolved in 2 ml of 96% ethanol, then added a 1 cm long Mg band and 1 mL of concentrated HCl. The positive results of the flavanoid test are indicated by the formation of peacock, yellow, and orange colours. For phenol test, a total of 0.05 g of sample extract were dissolved in 0.2 mL of 70% ethanol and three drops of 5% $FeCl_3$ were added. The positive results of the phenol test are indicated by the formation of a green or blue green colour. For steroids and terpenoids test, a total of 0.05 g of sample extract was dissolved in chloroform, and then 0.05 mL anhydrous acid was added, then 2 mL H_2SO_4 was added through the tube wall. Positive results of the steroid and terpenoid tests are indicated by the formation of brownish or violet rings (Wirasti., 2019; Misfadhila *et al*., 2020; Wulandari *et al*., 2020; Akasia *et al*., 2021).

Quantitative analysis of total flavonoids. Total flavonoids were determined regarding the procedure by Hartati *et al*. (2016) and there were several modifications and the standard solution used was quercetin.

Preparation of the quercetin standard solution. A total of 10 mg was dissolved in 10 ml of methanol and diluted 25 times at 100 ppm. Dilution series were made with a concentration of 10, 20, 30, 40, 50 ppm, and 2 mL was taken for each dilution. Then 0.20 mL of 10% $AlCl_3$ 10% was added, then homogenized and allowed to stand for 3 min. Sodium acetate was added at 1.2 g/mL, and methanol was added until the solution volume reached 5 mL. The solution was incubated in a dark room for 40 min. The absorbance was measured using a UV-Vis (Thermoscientific) spectrophotometer at 380 nm (Hartati *et al*., 2016).

Determination of the total flavonoid content. 50 mg of the extracted sample was dissolved in 10 mL of methanol and diluted 25 times in 100 ppm. Dilution series were made with a concentration of 10, 20, 30, 40, 50 ppm, and 2 mL was taken for each dilution. Then 0.20 mL of 10% $AlCl_3$ 10% was added, then homogenized and allowed to stand for 3 min. Sodium acetate was added at 1.2 g/mL, and methanol was added until the solution volume reached 5 mL. The solution was incubated in a dark room for 40 min. Absorbance was measured using a UV-Vis spectrophotometer (Thermoscientific) at 380 nm (Spiridon *et al*., 2011; Kalita *et al*., 2013).

Quantitative analysis of total saponins. Total saponins were determined regarding the procedure conducted by Tuldjanah *et al.* (2022), and there were several modifications and the standard solution used was saponins.

Preparation of standard saponin solutions. A total of 10 mg of saponins were dissolved with 16 mL of methanol and 4 mL of distilled water was added to obtain a concentration of 1000 ppm and then homogenized. Dilution series were made with concentrations of 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 ppm. Pipette each concentration of 0.025; 0.05; 0.075; 0.1; 0.125; 0.15; 0.175; 0.2; 0.225; 0.25 mL in a new test tube and add 80% methanol until the volume of the solution reaches 0.25 mL. A total of 0.25 mL was taken from each concentration and 0.25 mL of vanillin reagent and 2.5 mL of 72% sulfuric acid were added through the wall. The solution was incubated in a 600°C water bath for 10 min, then cooled with ice water for 4 min and the absorbance of the solution was measured using a UV-Vis spectrophotometer with a wavelength of 544 nm.

Determination of the total saponin content. 2.5 mg of sample extract was dissolved with 4 mL of 100% methanol and 1 mL of distilled water and then homogenized. The sample solution was diluted 5 times with 1 mL of sample and 4 mL of distilled water. A total of 0.25 ml was taken from each dilution for three repetitions and then 0.25 mL vanillin and 2.5 mL sulfuric acid 72% were added and then homogenized. The solution was incubated in a 60°C water bath for 10 min and then cooled with ice water for 4 min. Absorbance was measured using a UV-Vis (Thermoscientific) spectrophotometer at 544 nm ((Tuldjanah *et al*., 2022)

Quantitative analysis of total phenols. Total phenol was determined with reference to the procedure (Tahir *et al*., 2017) and there were several modifications and the standard solution used was gallic acid.

Preparation of standard phenol solutions. Up to 10 mg of gallic acid was dissolved in 10 mL of distilled water. A series of dilutions were made with a concentration of 100, 200, 300, 400, 500,

600, 700 ppm and then pipetted each concentration of 0.5;1;1.5;2;2.5;3;3.5 mL then distilled water was added until the volume of the solution reached 5 mL. A total of 1 mL was taken from each concentration, and 0.4 mL folin ciocalteu was added and then incubated for 8 min. Then distilled water was added until the solution reached 10 mL and incubated again for 2 h at room temperature. The absorbance was measured using a UV-Vis (Thermoscientific) spectrophotometer at a wavelength of 662.85 nm.

Determination of the total phenolic content. A total of 10 mg of the extracted sample was dissolved with 10 mL of ethanol and then homogenized. Take 1 mL of the solution and add 0.4 mL of Folin Ciocalteau reagent, then vortex (Maximix II) and let stand for 8 min. A total of 4 mL of Na_2CO_3 20% was added, homogenized again, and distilled water was added until the volume of the solution reached 10 mL. The solution was incubated for 2 h at room temperature. Absorbance was measured using a UV-Vis spectrophotometer (Thermoscientific) at a wavelength of 662.85 nm (Tahir *et al*., 2017). Quantitative analysis of total tannins. Total tannins were determined regarding the procedure (Tuldjanah *et al*., 2022) and there were several modifications and the standard solution used was gallic acid.

Preparation of standard tannin solution. Up to 10 mg of tannic acid were dissolved with 10 mL of folin ciocalteu, then homogenized and allowed to stand for 5 min. 20% sodium carbonate is added until the solution reaches 100 mL. A series of dilutions is made with a concentration of 0.125; 0.25; 0.5; 1; 2; 4; 8; 16 ppm, then each concentration is pipetted as much as 0.0625; 0.125; 0.25; 0.5; 0.1; 0.2; 0.4; 0.8 mL and then add 96% ethanol until the volume reaches 5 ml. The solution was incubated for 30 min at room temperature. The absorbance was measured using a UV-Vis spectrophotometer (Thermoscientific) at a wavelength of 720 nm.

Determination of the total tannin content. 100 mg of the extracted sample was dissolved with 10 mL of diethyl ether and incubated for 20 h. The solution was poured into a Petri dish, evaporated in the room, and distilled water was added to 10 mL. A total of 1 mL of the solution was taken for three repetitions and then 0.1 mL of Folin ciocalteu was added, then homogenized and incubated for 5 min. A total of 2 mL of 20% Na_2CO_3 was added and homogenized, then incubated again for 5 min, then distilled water was added until the volume of the solution reached 10 mL and incubated again for 30 min. The absorbance was measured using a UV-Vis spectrophotometer (Thermoscientific) at a wavelength of 760 nm (Tuldjanah *et al*., 2022).

Measurement of antioxidant activity. The antioxidant activity of the extract was determined using the DPPH test (Wijaya *et al*., 2014). A total of 2.8 mg was dissolved with 56 mL methanol, homogenized to obtain a concentration of 50 ppm, and then stored in a dark bottle. Ascorbic acid 100 ppm was prepared by dissolving 1.5 mg of ascorbic acid into 15 mL of methanol. A dilution series was made with concentrations of 0.5, 10, 15, 20, and 25 ppm and repeated thrice at each concentration. Meanwhile, a stock solution of 1000 ppm mistletoe tea leaf extract was prepared by dissolving 5 mg of ethanolic mistletoe tea leaves extract into 5 mL of methanol. A dilution series was made for obtaining 0, 12.5, 25, 50, 100, and 200 ppm and repeated three times at each concentration. To test antioxidant activity with DPPH, mistletoe leaf ethanol extract samples were taken up to 1 mL at each concentration (0; 12.5; 25; 50; 100; 200 ppm) and 3 mL of 50 ppm DPPH solution were added at each concentration. To make a blank, 3 mL of methanol was used and for the negative control, 1 mL of methanol was used with 3 mL of DPPH, then incubated in a dark room for 30 min. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. The preparation of an ascorbic acid solution is carried out by the same method. The percentage of inhibition can be determined from the absorbance value obtained and to express antioxidant activity using the following formula that conducted by Nadri *et al*. (2014):

$$\%\text{inhibition} = \frac{A\ control - A\ sample}{A\ control}\ x\ 100$$

The calculation results are entered into the regression equation with the x-axis as the extract concentration and the y-axis as the percent inhibition value. The IC50 value is determined from the

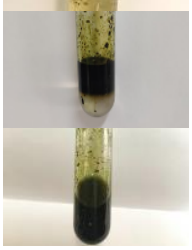
results of the regression equation. The IC50 value is the concentration of the ethanol extract of tea mistletoe required to inhibit free radicals by 50%.

Data analysis. Qualitative test data was collected and interpreted as positive or negative results which potentially influenced by measured environmental parameters. Quantitative test data for total flavonoids, tannins, phenols, saponins, and antioxidant activity were analyzed with three repetitions using Microsoft Excel.

RESULTS AND DISCUSSION

Phytochemicals of tea mistletoe leave *Dendrophthoe pentandra* (L.) Miq. Tea mistletoe leaves extracted using the maceration method with a 96% ethanol solvent produced a yield of 8.76%. The choice of 96% ethanol as a solvent in extraction is due to its advantages. Furthermore, 96% ethanol exhibits a potent extractive capacity (Lohvina *et al*., 2021; Wati *et al*., 2022). Consequently, a significant portion of secondary metabolite compounds present in plant materials can be efficiently extracted using this solvent. Phytochemical screening was carried out to identify secondary metabolites contained in the leaves of the tea mistletoe *D. pentandra* (L.) Miq. The positive parasite ethanol extract contains alkaloids, flavonoids, steroids, terpenoids, phenols and tannins (Table 1).

Table 1. Phytochemicals of tea mistletoe leaves *Dendrophthoe pentandra* (L.) Miq.

Secondary Metabolite	Results	Information	Picture
Alkaloid			
Wagner	+	Brown precipitate	
Dragendorf	+	Orange precipitate	
Mayer	+	Yellow precipitate	
Tanins	+	Blackish blue, green, or blackish green	
Flavonoids	+	Brick red or orange color	
Phenolic	+	Blackish blue colour	
Saponins	+	Constant foam	
Steroids dan Terpenoids	+	Brownish or violet ring at the boundary of the solution	

Environmental factors influence the production of secondary metabolites in plants. The data of the environmental parameters for the sampling point of D. *pentandra* (L.) Miq tea leaves are presented in Table 2. Environmental measurements showed that the air temperature at the sampling location was high, that is, 29°C. Elevated temperatures can induce abiotic stress, termed temperature stress, potentially suppressing secondary metabolite production in plants as an adaptive response, though the impact varies across species due to differential biosynthetic pathways (Akula *et al*., 2011; Balfagón *et al*., 2020). While elevated temperatures can indirectly influence secondary metabolite content through increased light intensity, a key driver of photosynthesis that provides precursors for these compounds, the direct effect of temperature on metabolite production remains species-specific due to diverse biosynthetic pathways (Li *et al*., 2017; Yang *et al*., 2018; Austen *et al*., 2019). The air humidity on the measurement results shows high results, while the air pressure shows low results. Differences in altitude will affect the metabolism in plants, and the content of secondary metabolite compounds produced will differ at each (Demasi *et al*., 2018; Hashim *et al*., 2020). Based on the results of parameter measurements, the location's altitude is 813 meters. Soil pH at the sampling point was as expected, but soil moisture at the location was low. The results of measuring environmental parameters are in line with the results of the diversity of secondary metabolites identified in the leaves of the tea mistletoe *D. pentandra* (L.) Miq in Sapuran, Wonosobo, which positively contain alkaloids, tannins, flavonoids, phenols, saponins, steroids, and terpenoids.

Table 2. Data for environmental parameter samples from the sampling point

Parameter	Value
Light Intensity	965 Lux
Air temperature	29°C
Air Humidity	72%
Air pressure	928,8 hPa
Soil moisture	5-10%
Soil pH	7
Location altitude	813 m

The results of this study provide positive results for the saponin test on the ethanolic extract of tea mistletoe *D. pentandra* (L.) Miq. This is different from the study by Tioline *et al*. (2021) which showed negative results of the saponin test on the cherry parasite *D. pentandra* (L.) Miq. Disparate host plants necessitate variations in the secondary metabolite profiles of their associated parasites due to the selective uptake of plant-derived nutrients, resulting in a host-dependent metabolite composition within the parasite (Heil *et al*., 2016; Hegenauer *et al*., 2017). Selection of the extraction solvent, based on its polarity, significantly influences the profile of extractable secondary metabolites from plant material (Bubalo *et al*., 2018; Lefebvre *et al*., 2021). The results of this study stated that the extract of *D. pentandra* (L.) Miq tea mistletoe extracted using the maceration method with 96% ethanol solvent could provide more diverse results in the detection of secondary metabolites, as seen in the positive results in all tests. when compared to the results of the extraction of only polar compounds. At the same time, 96% ethanol is a solvent that can dissolve all non-polar, semi-polar, and polar compounds.

The total flavonoids, tannins, phenols, and saponins content of *Dendrophthoe pentandra* (L.) Miq. Secondary metabolite compounds that have been identified in the qualitative test are followed by quantitative tests on flavonoids, tannins, phenols, and saponins. The quantitative test was initiated by sequentially graphing the standard curves of flavonoids, tannins, phenols, and saponins using standard solutions of quercetin, tannic acid, gallic acid, and saponins. The standard curve will be used in calculating the total levels of flavonoids, tannins, phenols, and saponins. The standard curve is the relationship between the concentration of the standard solution (x) and the absorbance value of the standard solution y). The standard curve equations for quercetin, tannic acid, gallic acid and saponins respectively are $y = 0.0309x-0.0475$ with $R^2 = 0.9971$, $y = 0.0035x – 0.0006$ with $R^2 = 0 .9715$, $y = 0.1139x + 0.006$ with $R^2 = 0.9785$, $y = 0.0012x - 0.0071$ with $R^2 = 0.981$.

Table 3. Total flavonoids, tannins, phenols, saponins content of *Dendrophthoe pentandra* (L.) Miq. extract

Secondary metabolites	Total content ± STD
Flavonoids	48.584 mg QE/g extract
Tanins	1.350 ± 0.140 mg TAE/g extract
Phenolic	1.755 ± 0.171 g GAE/g extract
Saponins	4.665 ± 0.142 g SE/g extract

The total content of flavonoids in the ethanolic extract of *D. pentandra* (L.) Miq tea leaves is 48.584 mg QE/g extract (Table 3). The total content of flavonoids in this study is greater than the results of the research conducted by Lekal & Watuguly (2017) with 134 mg QE/g of clove host methanol extract. Factors that affect the parasite's total flavonoids are the host type and environmental factors, which can be in the form of stress or stress on plants. The abiotic stress experienced by host plants will pressure host plants to produce secondary metabolites, as plants will produce a response of plants to the environment and more flavonoid compounds as a form of synergy in providing defense against a stress obtained from the environment. Nitrogen availability directly influences flavonoid accumulation as their biosynthetic pathways, linked via the shikimate pathway utilizing glycolytic and pentose phosphate pathway-derived carbohydrates, share precursors with aromatic amino acid synthesis (Deng *et al*., 2019; Li *et al*., 2021). The total tannin content of the leaves of *D. pentandra* (L.) Miq. was 1.350 ± 0.140 mg TAE /g of extract (Table 3). The total tannin content in this study was lower than a study conducted by Patria & Soegihardjo (2013) with 13.76 ± 0.92 mg TAE/g of ethanolic Kepel extract. The difference between the two is due to the different types of hosts that have grown on them, so the chemical compounds that they contain give different results. The total phenolic content of *D. pentandra* (L.) Miq. leaves are 1.755 ± 0.171 g GAE/g of extract (Table 3). Heavy parasite loads can induce stress in host plants by depleting nitrogen through the xylem, potentially leading to increased transpiration. This nitrogen depletion may trigger upregulation of the enzyme phenylalanine ammonia-lyase, promoting the synthesis of phenolic and flavonoid compounds as the plant attempts to mitigate stress (Griebel *et al*., 2017). The total content of saponins possessed by the leaves of the tea mistletoe *D. pentandra* (L.) Miq. was 4.665 ± 0.142 g SE/g extract. High levels of saponins have the potential to be antiviral, anticarcinogenic, and antioxidants.

The antioxidant activity of *Dendrophthoe pentandra* (L.) Miq. According to the result of phytochemical screening, ethanolic *D. pentandra* (L.) Miq. leaf extract has potential as an antioxidant. The extract showed positive result for phenolic compounds such as tannins and flavonoids, alkaloids and terpenoids, which are natural antioxidants. Measurement of antioxidant activity in this study using the DPPH method. In antioxidant activity assays, IC_{50} values less than 50 ppm indicate very strong activity, values between 51 and 100 ppm signify strong activity, 100-150 ppm suggest moderate activity, and 151-200 ppm denote weak activity (Clarkson *et al*., 2004). The IC_{50} value of mistletoe leaf ethanol extract shows a smaller value compared to the IC_{50} value of ascorbic acid, which is 49.10 ppm and 9.22 ppm, respectively. Based on the measurement results, the IC_{50} value of the ethanolic *D. pentandra* (L.) Miq. leaves extract and ascorbic acid is included in the robust category. Still, the results obtained are inversely proportional between the sample and the positive control, ascorbic acid, as a comparison because ascorbic acid is more potent in reducing free radicals when compared to the ethanolic extract of mistletoe leaves.

In general, the secondary metabolite compounds found and most abundant in plants are alkaloids, flavonoids, steroids, saponins, terpenoids, and tannins. These secondary metabolites represent a diverse pool of bioactive molecules with immense potential in the pharmacological field. They serve as crucial starting points for drug discovery and development across various therapeutic domains, including antioxidants, antibiotics, anticancer agents, blood anticoagulants, and even environmentally friendly pest control antigens (Leicach & Chludil, 2014; Wink, 2015). The total phenolic and flavonoid content of plant extracts significantly influences their antioxidant activity. A positive linear correlation exists between these factors, suggesting that higher levels of phenolics and flavonoids are associated with enhanced antioxidant capacity. The results of antioxidant activity in this study were

classified as very strong. This is in line with this study's total flavonoid and tannin content, which had high values, 48.584 mg QE/g extract and 1.350 ± 0.140 mg TAE/g extract, respectively. As phenolic compounds with aromatic rings and numerous hydroxyl groups, tannins and flavonoids contribute to antioxidant activity by donating hydrogen atoms to scavenge DPPH free radicals (Mbinda & Musangi, 2019; Gulcin, 2020). This study provides valuable preliminary data supporting the potential of *D. pentandra* (L.) Miq. from Indonesia as a source of natural medicine that could lead to the development of novel therapeutic drugs.

CONCLUSION

Dendrophthoe pentandra (L.) Miq. leaves extract from Sapuran, Wonosobo contains several secondary metabolites such as flavonoids, tannins, phenols, saponins, alkaloids, steroids and terpenoids. Total content of ethanol extract of flavonoids, tannins, phenols, and saponins *of Dendrophthoe pentandra* (L.) Miq. leaves successively are 48.584 mg QE/g extract, 1.350 ± 0.140 mg TAE/g extract, 1.755 ± 171.464 mg GAE/g extract, 4.665 ± 142.074 mg SE/g extract. *Dendrophthoe pentandra* (L.) Miq. has a high potential as an antioxidant with IC_{50} value of 49.10 ppm, indicating as strong antioxidant activity.

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