

The potential of *Dendrophthoe pentandra* and *Scurrula ferruginea* stem from *Syzygium aqueum* as source of natural antioxidant

Sri Kasmiyati & Elizabeth Betty Elok Kristiani*

Faculty of Biology, Universitas Kristen Satya Wacana, Jl. Diponegoro No.52-60, Salatiga, Kec. Sidorejo, Kota Salatiga, Jawa Tengah 50711, Indonesia

*Corresponding Author: betty.elok@uksw.edu

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Abstract. Many studies show the antioxidant ability of the parasite plant. This study aims to determine the capacity of *Dendrophthoe pentandra* (L.) Miq and *Scurrula ferruginea* stem from *Syzygium aqueum* to use as a natural antioxidant source. The sample was taken from the host *Syzygium aquarium*. The samples were extracted with ethanol using maceration. The parameters were determined spectrophotometrically using specific reagents that were antioxidant capacity using 1,1-diphenyl-2-picrylhydrazyl, phenolics compounds using Folin-Ciocalteu, flavonoids using AlCl₃, ascorbic acid using sulfosalicylic acid, while chlorophyll and carotenoid using dimethyl sulfoxide. The result of *D. pentandra* (L.) Miq and *S. ferruginea* extract showed that IC₅₀ were 15.09±1.02 and 20.53±1.77 mg/ml, the content of phenolics compounds were 557.45±19.77 and 433.99±20.89 mg GAE/ml extract, flavonoids were 9.72±0.23 and 3.02±0.71 mg QE/ml extract, chlorophylls-a were 2.67±0.15 and 2.77±0,08 mg/g extract, chlorophylls-b were 3.34±0.06 and 3.44±0.04 mg/g extract, total chlorophylls were 6.01±0.21 mg/g extract, carotenoids were 1.71±0.01 and 2.10±0.01 mg/g extract respectively. The stems of both *D. pentandra* (L.) Miq and *S. ferruginea* from *Syzygium aquarium* has very strong antioxidant abilities so they are the potential to use as a source of natural antioxidants. The compounds that support the antioxidant activity were phenolic, flavonoids, and ascorbic acids. The results of this study will open up opportunities to obtain new sources of antioxidants that can be utilized in human health management.

Key words: antioxidant capacity, *Dendrophthoe pentandra* (L.) Miq., *Scurrula ferruginea*, maceration, bioactive compounds

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INTRODUCTION

The attention of the whole world at this time is focused on various things related to the emergence of the COVID-19 pandemic. The body's immunity is an important issue in self-defense against exposure to a virus known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV2). Various efforts have been made by the government and the community to maintain or increase the body's immunity, one of which is the intake of antioxidant agents. The presence of antioxidants will play a role in counteracting free radicals in the human body so that the balance between oxidation and anti-oxidation is maintained. Antioxidants could be classified based on their sources into natural antioxidants, which are produced by living organisms, and synthetic antioxidants, which is produced in a laboratory (Ayoka et al., 2022).

Among the natural source of antioxidants, plants are natural sources that are widely used. The ability of plants to increase body immunity is related to the presence of antioxidant bioactive compounds in plants which are commonly called secondary metabolites. Several secondary metabolites are reported to have antioxidant abilities such as polyphenols (phenolic acids, flavonoids, anthocyanins, lignins, and stibenes), carotenoids (xanthophylls and carotenes), vitamins (vitamins C and E) (Xu et al., 2017), rutin and gallic acid (Girsang et al., 2020). Wafa et al. (2016) also reported antioxidant activity by flavonoid and tannin compounds. Gallic acid, ascorbic acid, and xanthone are important natural antioxidants (Naksuriya & Okonogi, 2015). Quercetin and curcumin are the important antioxidant and anti-inflammatory (Bidian et al., 2020).

Many parasitic plants are reported to have health benefits. Several researchers reported the antioxidant and immunostimulant activity of parasites. The ethyl acetate extract of stem bark and the leaf of *L. pulverulentus* showed the IC₅₀ value of $15.9 \pm 0.5 \mu\text{g}$, 14.5 ± 0.8 (Raza et al., 2013). Both water extracts and water-ethanol extract of leaves *L. parasiticus* Merr. were shown antioxidant activities stronger than ascorbic acid and quercetin standards (Thoa & Cuong, 2018). The aerial part extract of *L. europaeus* were immature berries, flowers, leaves, twigs, and stems extracted using ethanol and ethyl acetate had IC₅₀ value at about 36 -65 quercetin equivalents (Katsarou et al., 2012). At the concentration of 1000 ppm, the free radical scavenging of chloroform extract of *Loranthus acacia* Zucc was 88.3% while ascorbic acid was 90.7%, but the n-hexane extracts were 40.6% only (Noman et al., 2019). The purpose of this research was to determine the antioxidant capacity of *Dendrophthoe pentandra* (L.) Miq and *Scurrula ferruginea* stem from *Syzygium aqueum* extracted by ethanol and the antioxidant compounds which play a role in that capacity

In the current conditions, immunity is an indispensable factor for health. The results of this study will open up opportunities to obtain new sources of antioxidants that can be utilized in human health management.

METHODS

The research was conducted in September - December 2021 with a laboratory-experimental type of research. All of the laboratory activities are carried out at the Biochemistry and Molecular Biology Laboratory, Faculty of Biology, Satya Wacana Christian University, Salatiga Indonesia. There is no treatment or manipulation of the research variables during the study. The variables of research were the sample extracts and test parameters. The main materials were two types of parasites that grow on the host plant *Syzygium aqueum*, namely *Dendrophthoe pentandra* (L.) Miq. and *Scurrula ferruginea*. The host plant was growing in the Blotongan area of Salatiga, Central Java, Indonesia. Ethanol was used as a solvent for the extraction process. The organ part of the research sample was the stem, which was extracted using the maceration method. The parameters assay included the levels of phenolic acid, flavonoids, ascorbic acid, total chlorophyll, chlorophyll-a, chlorophyll-b, carotenoids, and free radical scavenging capacity of extracts at

various concentrations. Each parameter was calculated three times repeated. The stages of the research include sampling at a predetermined location, then sample preparation, and continued with the analysis of research parameters

Sample preparation

Each of the parasite plants was separated from the host plant and then the stems of the parasite were separated from other part plant samples. The stem dried without sunshine for 7 days. Furthermore, the samples were ground using a blender (Philips HR2071). The sample powder was ready for extraction.

Extract preparation

The extraction of sample using maceration technique with ethanol 96% solvent for 3 x 24 hours. In one maceration process, the dry sample was placed in a closed container and immersed in ethanol (1:3 m/v) for 24 hours while stirring occasionally. After 24 hours, the mixture was filtered, and the filtrate was set aside. The filtrate obtained from three times maceration was concentrated using a rotary evaporator (RE 300 bibli scientific) using a vacuum (Eyela A-1000S).

Free radical scavenging (ANTIOXIDANT) analysis

The antioxidant ability was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Chan et al., 2007). At the amount of 1.0 mg of the extract redissolved in methanol and added with 2.0 ml of DPPH in 50 ppm methanol and incubated in the dark for 30 minutes. The absorbance of the mixture was measured on an ultraviolet-visible (UV-Vis) spectrophotometer (Shimadzu UV mini1240) at 517 nm wavelength. Antioxidant capacity = $(1 - (\text{sample absorbance} / \text{blank absorbance})) \times 100\%$. Ascorbic acid is used as a standard antioxidant compound.

Phenolic acid analysis

The total phenolic acid content in the extract was measured using a colorimetric method with Folin-Ciocalteu reagent (Almey et al., 2010). In a volumetric flask, 25 ml mixed 1.0 ml of 100 ppm extract with 1 ml of Folin-Ciocalteu reagent and incubated for 5 minutes. After that added with 10 ml of 7%, Na₂CO₃ followed by added distilled water up to the mark of the volumetric flask, and then incubated for 90 minutes. The absorbance value of the mixture was measured using a UV-Vis spectrophotometer (Shimadzu UV mini1240) at a 550 nm wavelength. Gallic acid is used as a standard phenolic compound at a

concentration series of 20, 40, 60, 80, and 100 mg/l. Phenolic acid content (mg GAE/g extract) = c (V/m). c : gallic acid concentration based on gallic acid linear regression equation (mg/l); V = extract volume (l); m = mass of extract (g).

Flavonoid analysis

The flavonoid content in the extract was measured using the colorimetric method with an AlCl_3 reagent (John et al., 2014). In a volumetric flask, 10 ml and 1.0 ml of 500 ppm extract were added with 0.3 ml of 5% NaNO_2 and incubated for 5 minutes. Next, 0.3 ml of 10% AlCl_3 was added and incubated for 5 minutes. In the end, added 2 ml of 1 M NaOH followed by distilled water until exactly 10 ml. The absorbance value of the mixture was measured using a UV-Vis spectrophotometer (Shimadzu UV mini1240) at a wavelength of 510 nm. Quercetin was used as a standard compound with a concentration series of 20, 40, 60, 80, and 100 mg/l. Flavonoid content (mg QE/g extract) = c (V/m). c : flavonoid concentration based on quercetin linear regression equation (mg/l); V = extract volume (l); m = mass of extract (g)

Ascorbic acid analysis

The determination of ascorbic acid levels in the extract using colorimetric method with sulfosalicylic acid reagent (Balogh & Zsarka, 2016). A total of 1.0 ml of 500 ppm extract was added with 3.0 ml of sulfosalicylic acid, 2 ml of Na-molybdate, 2 ml of 0.15 N H_2SO_4 and 1 ml of 1.5 mM Na_2HPO_4 . The absorbance value of the mixture was measured using a UV-Vis spectrophotometer (Shimadzu UV mini1240) at a wavelength of 550 nm. Calculation of ascorbic acid content determined based on the linear regression equation of standard ascorbic acid.

Chlorophyll and carotenoid analysis

The chlorophyll and carotenoid content were measured from fresh plant samples using the colorimetric method with dimethyl sulfoxide (DMSO) reagent (Sumanta et al., 2014). At an amount of 50 mg of small pieces of fresh stem, samples were put into a black container and added with 5 ml of DMSO solution, then shaken and incubated in a dark room for 48 hours. After the incubation period, the samples were filtered, and the absorbance of the filter was measured using a UV-Vis spectrophotometer (Shimadzu UV mini1240) at wavelengths of 480 nm, 649 nm, and 665 nm. The content of chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoids (mg/g) was determined using the equation: $\text{Ca} =$

$12.19\text{A}665 - 3.45\text{A}649$, $\text{Cb} = 21.99\text{A}649 - 5.32\text{A}665$, $\text{Cc} = (1000\text{A}480 - 2, 14\text{Ca} - 70.16\text{Cb})/220$. Ca : chlorophyll-a, Cb : chlorophyll-b, Cc : carotenoids. A : sample absorbance.

Data analysis

The data were analyzed statistically using the analysis of variance test to determine the significant difference in values between extracts and test parameters and continued with the Tukey test to determine the correlation between the concentration of the test compound and the antioxidant ability of the sample.

RESULTS AND DISCUSSION

In this study, both samples (Figure 1.) were dried and powdered before being extracted. The extraction process of powdered samples uses the maceration method because it was good for thermolabile compounds and simple to process, although the length of time and the need for many volumes of solvent become the disadvantages of this method (Zhang et al., 2018).

The used of samples in powder form increase the chance of contact with the extraction solvent (Azwanida, 2015). Furthermore, it was also stated that ethanol, which was used as the extraction solvent in this study, is a universal solvent commonly used for phytochemical investigations.

Oxidation-reduction reactions are a pairs important processes that in occur all the time in the cell. Free radicals are one of caused of oxidation reaction. However, certain oxidation processes become harmful to cells because of its negative effects. Some compounds have the ability to bind free radicals so that oxidation reactions do not occur and prevent damage to healthy cells, known as antioxidants (Dontha, 2016). In assessing the antioxidant ability of natural ingredients, before carrying out the in vivo test, the researchers conducted an in vitro test first. It is assumed that compounds that have high antioxidant abilities in vitro will also have high abilities in vivo (Tukun et al., 2014). The test using DPPH is a non-enzymatic in vitro test that is widely used to measure the antioxidant capacity of a compound. The DPPH method is widely used because its implementation is very simple, fast using an ultraviolet-visible spectrophotometer, and low cost (Dontha, 2016).

Measurement in vitro of the antioxidant ability of the ethanolic extract of the parasite stem using



Figure 1. The samples of research. (A) *D. pentandra* (L.) Miq., (B) *S. ferruginea* from host plant *S. aqueum*. a. flower; b. leaf; c. stem; d. fruit

the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test (Figure 1). This test measures the free radical scavenging capacity of a compound based on a redox reaction.

The results showed that in all extracts, the antioxidant capacity was dose-dependent, namely the ability of the antioxidant increased with increasing extract concentration (Figure 2). The stem extract of *D. pentandra* (L.) Miq showed a higher antioxidant capacity than *S. ferruginea*. The antioxidant capacity of both extracts at the lowest

test concentration (5 mg/ml) was almost the same but on the increase of the concentration of the extract, the antioxidant capacity of the stem extract of *D. pentandra* (L.) Miq. higher than *S. ferruginea*. Several studies have shown that the reducing ability of *Loranthus* extracts exhibits dose-dependent properties, such as in *Loranthus begins's* L. (Oboh et al., 2016). *Scurrula ferruginea* (Marvibaigi et al., 2014), the combination of *Loranthus* with *Phyllanthus niruri* and *Euphorbia hirta* (Kristiani & Kasmiyati, 2021).

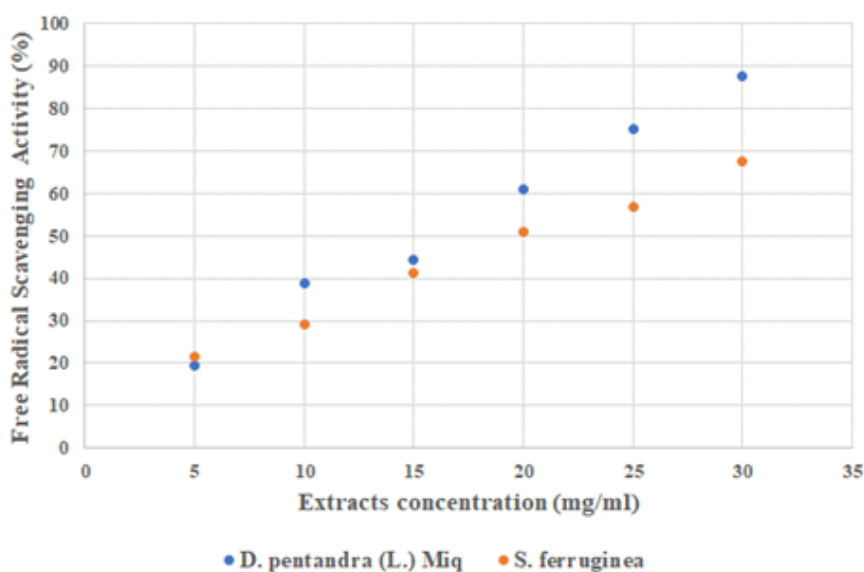


Figure 2. The graph of antioxidant activity of of the ethanol extract of the stem of *D. pentandra* (L.) Miq. and *S. ferruginea* from host plant *S. aqueum*

Table 1. IC₅₀ value and antioxidant ability of the ethanol extract of the stem of *D. pentandra* (L.) Miq. and *S. ferruginea* from host plant *S. aqueum*

Sample	IC ₅₀ value (mg/ml)	Category of antioxidant
<i>D. pentandra</i> (L.) Miq.	15.09 ± 1.02	Very strong
<i>S. ferruginea</i>	20.53 ± 1.77	Very strong
Ascorbic acid*	21.04 ± 0.10	Very strong

The same superscript letter which followed by the numbers in the same column indicate no significantly difference values based on analysis using Tukey test ($p < 0.05$). * (Kristiani & Kasmiyati, 2021)

Table 2. The bioactive content of the ethanol extract of the stem of *D. pentandra* (L.) Miq. and *S. ferruginea* from host plant *S. aqueum*

Bioactive compound	Unit	Ethanol extract of the stem	
		<i>D. pentandra</i> (L.) Miq.	<i>S. ferruginea</i>
Flavonoid	mg GAE/g extract	9.72 ± 0.23 ^a	3.02 ± 0.71 ^b
Phenolic acid	mg QE/g extract	557.45 ± 19.77 ^a	433.99 ± 20.89 ^b
Ascorbic acid	mg /g extract	8.53 ± 0.17 ^a	7.50 ± 0.16 ^b
Chlorophyll-a	mg /g extract	2.67 ± 0.15 ^a	2.77 ± 0.08 ^a
Chlorophyll -b	mg /g extract	3.34 ± 0.06 ^a	3.44 ± 0.04 ^a
Total Chlorophyll	mg /g extract	6.01 ± 0.21 ^a	6.21 ± 0.04 ^a
Carotenoid	mg /g extract	1.71 ± 0.01 ^b	2.10 ± 0.01 ^a

Note: The same superscript letter which follow the numbers in the same row indicate not significantly difference values based on analysis using Tukey test ($p < 0.05$).

The strength of antioxidant capacity of a material can be determine based on the IC₅₀ value (Molyneux, 2004). Table 1 present the IC₅₀ values and classification of the antioxidant power of the extracts. Ascorbic acid was used as a positive control because this compound is widely known as an antioxidant compound with very strong antioxidant power (Amna et al., 2021). Based on the IC₅₀ value, it appears that the ethanolic extract of the two types of parasite samples *D. pentandra* (L.) Miq. and *S. ferruginea* have very strong antioxidant capacity, equivalent to the strength of ascorbic acid. These results are in accordance with the report of Kristiani & Kasmiyati (2021) reported that the antioxidant ability of the ethanolic extract of *Loranthus* sp. strong with an IC₅₀ value of 97.2 g/ml.

In both *Loranthus* species, at the lowest test concentration (5 mg/ml) the antioxidant capacity of all extracts was almost the same but increasing the concentration of the extract, the antioxidant capacity of the stem extract of *D. pentandra* (L.) Miq. higher than stem extract of *S. ferruginea*. The increasing concentration of the extract means an increasing in the compounds contained in the extract, including an antioxidant compound. Non-enzymatic antioxidants can be obtained from food, including vitamins (vitamin A, vitamin C/ascorbic acid, vitamin E, vitamin K), uric acid, glutathione, polyphenolic compounds such as

flavonoids and phenolic acids, carotenoid pigments (β -carotene, lycopene, lutein), zeaxanthin) (Shalaby & Shanab, 2013), as well as chlorophyll pigments (Pérez-gálvez et al., 2020).

Natural antioxidants are widely distributed in food and medicinal plants (Xu, et al., 2017). Several of them are ascorbic acids, tocopherols, tocotrienols, carotenoids, glutathione, polyphenols, phenolic acids, and flavonoids (Yadav et al., 2016; Panche et al., 2016, Baiano & Nobile, 2016). In this study, the levels of phenolic acids, flavonoids, ascorbic acid, chlorophyll, and carotenoids were measured in samples of both types of *Loranthus* (Table 2).

CONCLUSION

The antioxidant activity of ethanol extract of the stem of *D. pentandra* (L.) Miq. and *S. ferruginea* from host plant *S. aqueum* were similar with the antioxidant standard ascorbic acid, in the very strong category. Ascorbic acids, flavonoids, and phenolics were supported the antioxidant activity of that extract. The further research needs to be design such as the specific antioxidant ability of phenolic and flavonoid compound extracts, as well as studies of antioxidant abilities using solvents with different polarities and detailed analysis of compounds in the extract.

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