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Research Article

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF VARIOUS EXTRACTS OF DUKU'S MISTLETOE LEAF (*DENDROPHTHOE PENTANDRA* (L.) *MIQ*) COLLECTED FROM MEDAN, INDONESIA

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ABSTRACT

Objective: The objective of this research is to evaluate the antioxidant and antibacterial activity of methanol, n-hexane, ethyl acetate, and flavonoid total of *Dendrophthoe pentandra* (L.) *Miq* extract.

Methods: The leaf of *D. pentandra* (L.) *Miq* was gradually extracted using methanol, n-hexane, and ethyl acetate. From that extraction process, various isolates were obtained such as isolate of methanol, n-hexane, ethyl acetate, and flavonoid total. The obtained flavonoid total was continued into separation process using column system with eluent chloroform:methanol in ratio (9:1, 8:2, 7:3, and 6:4) and from this process was obtained six fractions (F1-5).

Results: All isolates and fractions that obtained during the extraction and separation process were continued to the activity measurement, such as antioxidant activity using 1,1-diphenyl 2-picrylhydrazyl method and antibacterial activity using agar diffusion method to *Salmonella typhi*, *Pseudomonas sp.*, *Escherichia coli,* and *Staphylococcus aureus*. The antioxidant activity (IC_{50}) of the isolate of flavonoid total and fraction of F4 and F5 was 6.69, 5.98, and 5.95 µg/mL, respectively. On the antibacterial activity, measurement showed a linear correlation between the activity and concentration (10 and 100 µg/mL) of all isolates and fractions. The antibacterial activity was dominated by flavonoid total isolate.

Conclusions: The results obtained in this research work showed that the extracts and fractions of *D. pentandra* (L.) *Miq* have a potency as antioxidant and antibacterial, especially flavonoid total that acts as antioxidant and fractions F4 and F5 that act as antibacterial.

Keywords: *Dendrophthoe pentandra* (L.) *Miq*, Flavonoid total, Antibacterial, Antioxidant.

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INTRODUCTION

Mistletoe is a parasitic plant and it has been used for traditional medicine, and many species of mistletoe has been used in the research for evaluating their ability to cure degenerative illness by *in vivo* and *in vitro* methods, such as *Dendrophthoe falcata* (L.f) has been used on the treatment process of breast cancer [1], *Lorhantus bengwensis* L. has been used for treating diabetes mellitus [2], and *Viscum schimperi* Engl. has been used for antihyperglycemic [3].

Those activities of mistletoe are influenced by the presence of flavonoid and phenolic compounds that have the ability as antioxidant to reduce the pressure of free radical that can induce the degenerative illness in the human body [4,5].

Flavonoid has been reported in a lot of research work that has high activity as antioxidant and antibacterial [5-10], and those activities can be helpful properties for treating any kind of disease. Furthermore, in some research, flavonoid component of mistletoe extract is very effective to inhibit the growth of *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Serratia marcescens, Escherichia coli*, and *Salmonella typhi* [5,11]. In other work, the extract of mistletoe has a high antioxidant with the IC_{50} 27.35 µg/mL and antibacterial properties on value of *S. aureus* (21.67±0.06 mm) and *E. coli* (20±0.09 mm), respectively.

Antioxidant activity of any substrate can be measured by various methods, such as 1,1-diphenil 2-pichylhydazyl (DPPH) [12], 2,2-Azinobis 3-ethylbenzathiazoline 6-sulfonic acid [13], CUPRAC [14], and ferric reducing antioxidant power [15,16]. However, DPPH technique is the common method that chosen by the researcher. This method is marked by a change in color from purple to yellow [17]. Besides as antioxidant, flavonoid component also can be used for the antibacterial application.

Duku is a tropical fruit plant that quite common in Indonesia, and it is quite often overgrown with the mistletoe, especially from species of *Dendrophthoe pentandra* (L.) *Miq* and *Scurrula ferruginea* (Jack) Danser. As in the explanation at the previous paragraph, mistletoe has a lot of potency as the sources of antioxidant and antibacterial. On the other hand, the potency of the secondary metabolite of mistletoe, especially *D. pentandra* (L.) *Miq* of duku collected from Medan, Indonesia, has never been reported. As the impact, the objectivity of this research is to evaluate the antioxidant and antibacterial activity of *D. pentandra* (L.) *Miq*.

MATERIALS AND METHODS

Materials

The chemical that used in this research was methanol, n-hexane, ethyl acetate, chloroform, benzene, acetone, HCl, $FeCl_3$, and Mg powder. Those chemicals were obtained from Merck and used without further purification.

Collection and authentication of plant material

Duku's mistletoe leaf was collected from Medan Johor village, North Sumatera, Indonesia, in the months of May 2016. The taxonomy identification of plant was performed at the Herbarium Bogoriensis, LIPI, Cibinong, Indonesia.

Preparation of the extracts

Duku's mistletoe leaf was cleaned, dried for 7×24 h, and then were pulverized. The dried leaf powder was weighed, and then, it was continued with maceration process using methanol (48 h). The mixture of dried leaf powder was filtered to obtain the crude extract. The crude extract was concentrated using a rotary evaporator and water bath. The concentrated extract of methanol was dissolved with water and partitioned with ethyl acetate to separate the tannins. The tannin-free extract was dissolved with methanol and then partitioned with n-hexane to separate the nonpolar compounds to obtain the total flavonoids compounds [18].

Column chromatography

The separation process of flavonoid component was performed using a column chromatography with the ratio of sample and silica gel of 1:30. Silica gel was used as stationary phase, and the mobile phase for this process was the mixture of polar–non-polar solvent, chloroform, and methanol, with the ratio of 9:1, 8:2, 7:3, and 6:4, and the best mobile phase was determined using TLC analysis [18].

Antioxidant activity

The antioxidant activity of the extract was determined by the DPPH method. Seven point 9 mL of DPPH radical solution (50 mL in methanol) was added to 1 mL each of various concentrations of sample from 5, 10, 25, 50 to 100 µg/mL and standard ascorbic acid (concentrations 3, 6, 9, 12, and 15 µg/mL). The reaction mixtures with some concentration were incubated in a 37°C water bath for 30 min. The absorbance of all the resulting solutions was measured at 515 nm using a UV-visible spectrophotometer. Percentage inhibition was calculated by the following equation [19]:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control solution} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100\%$$

Antibacterial activity

The antibacterial activity was performed with agar diffusion method. A total of 0.1 mL of each inoculum was applied using a cotton bud into an agar nutrient medium. The disc paper was immersed in various concentration test solutions, yhen placed on a medium that had been rubbed by bacteria to be incubated in an incubator at 35±2°C for 18–24 h, and measured that dragline diameter [20].

RESULTS AND DISCUSSION

Antioxidant activity

The antioxidant activity of isolate and fraction that obtained from *D. pentandra* (L.) *Miq*) by extraction and separation process is shown in Fig. 1.

The antioxidant activity that measured using DPPH method uses Vitamin C as the standard of antioxidant. The activity of each antioxidant in this research was stated in IC_{50}. That value is a correlation between doses of chemical/compound and the response that obtained during the experiment as much as 50% [21].

The result in Fig. 1 showed that the methanol extract [22] and Vitamin C has the lowest and highest value of antioxidant activity, respectively. The data can be divided into two groups, extract and fraction.The antioxidant activity of all extract showed that total flavonoid extract [22] has the highest value, followed by an extract of ethyl acetate and n-hexane, and the last is methanol. The antioxidant activity of all fractions showed that F4 has the highest value, followed by F5, F2, F1, and F3. The antioxidant activity of fractions is higher than the extract. The IC_{50} that obtained from this research is in the range of 13.21–2.89 µg/mL.

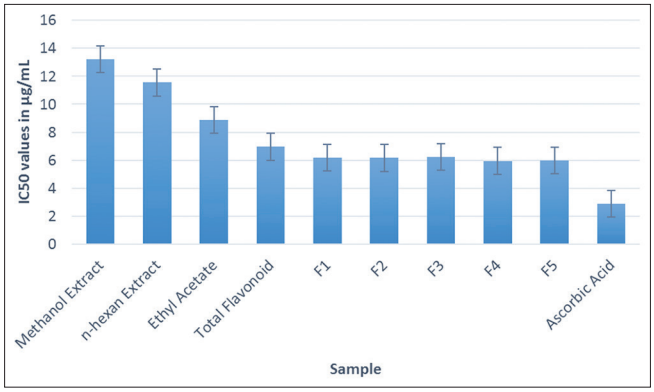

Fig. 1: The IC_{50} values of methanol extract, n-hexane extract, ethyl acetate extract, total flavonoid, F1, F2, F3, F4, and F5, and ascorbic acid on 1,1-diphenil 2-pichylhydazyl

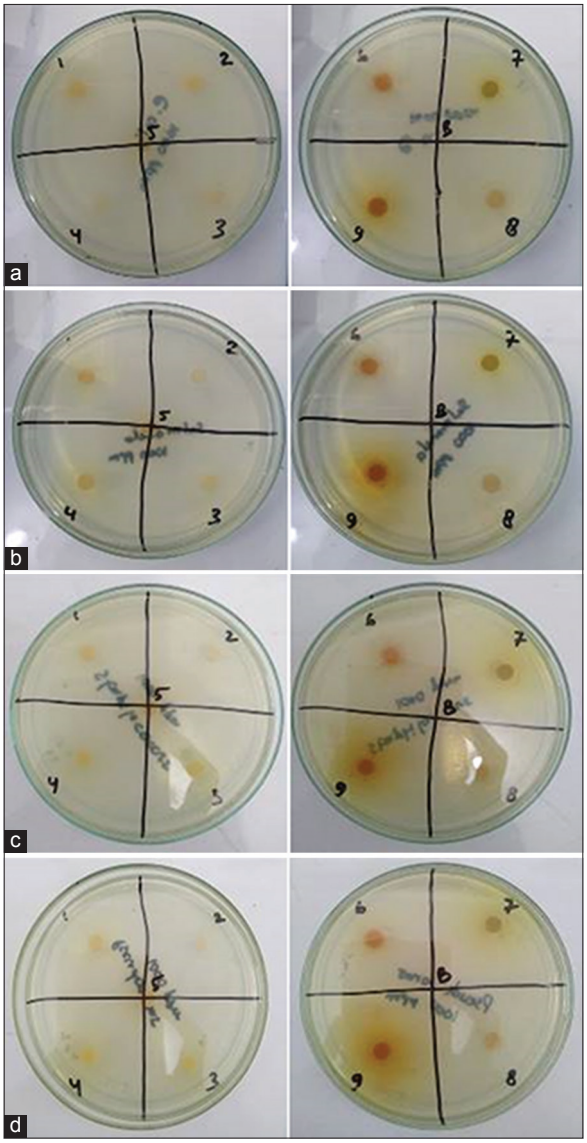

Fig. 2: Antibacterial activity of extracts on (a) *Escherichia coli* (1000 µg/mL), (b) *Salmonella typhi* (1000 µg/mL), (c) *Staphylococcus aureus* (1000 µg/mL) and (d) *Pseudomonas sp.* (1000 µg/mL)

AQ5

All extracts and fractions that obtained from *D. pentandra* (L.) *Miq* showed a lower IC_{50} value than Vitamin C. However, the IC_{50} value of

Table 1: Antibacterial activity of the obtained extract of *Dendrophthoe pentandra* (L.) *Miq*

Name of bacteria	Concentration (µg/mL)	Zone of inhibition (mm)				
		Methanol extract	n-hexane extract	Ethyl acetate extract	Total flavonoid	DMSO
Escherichia coli	10	8.22±0.13	6.75±0.08	7.11±0.07	6.33±0.05	NZ*
	100	8.45±0.14	6.98±0.13	7.15±0.10	9.00±0.04	NZ*
	1000	8.70±0.28	8.68±0.14	7.75±0.04	9.68±0.04	NZ*
Salmonella typhi	10	7.08±0.10	7.23±0.04	5.70±0.07	7.07±0.05	NZ*
	100	7.47±0.05	7.85±0.14	7.70±0.16	8.03±0.15	NZ*
	1000	9.00±0.04	8.12±0.14	8.73±0.12	9.32±0.06	NZ*
Staphylococcus aureus	10	5.75±0.04	8.32±0.06	8.23±0.30	8.38±0.11	NZ*
	100	7.92±0.07	8.65±0.13	8.66±0.08	8.45±0.08	NZ*
	1000	9.12±0.09	8.97±0.08	9.00±0.04	9.92±0.02	NZ*
Pseudomonas	10	6.28±0.11	7.23±0.16	6.76±0.17	7.57±0.06	NZ*
	100	6.53±0.06	7.33±0.17	7.12±0.03	7.85±0.06	NZ*
	1000	8.13±0.17	7.85±0.11	8.10±0.06	8.77±0.16	NZ*

*NZ: No zone of inhibition, Results represent the means±SD (standard deviation) from at least two separate experiments

Table 2: Antibacterial activity of the obtained fraction of *D. pentandra* (L.) *Miq*

Bacteria	Concentration (µg/mL)	Sample				
		F1	F2	F3	F4	F5
Escherichia coli	10	6.15±0.05	7.05±0.06	6.03±0.07	6.22±0.07	NZ*
	100	6.98±0.15	7.13±0.13	6.78±0.08	6.95±0.11	7.15±0.13
	1000	8.13±0.11	7.73±0.07	8.45±0.05	8.03±0.13	7.75±0.13
Salmonella typhi	10	5.83±0.23	6.57±0.10	6.43±0.07	6.42±0.14	5.35±0.11
	100	6.02±0.03	6.98±0.14	6.92±0.06	6.80±0.11	7.15±0.16
	1000	7.75±0.19	9.72±0.08	6.98±0.09	7.27±0.18	7.42±0.13
Staphylococcus aureus	10	6.05±0.11	7.10±0.04	9.38±0.20	8.20±0.09	8.17±0.20
	100	7.32±0.03	7.52±0.07	9.58±0.22	8.58±0.11	8.35±0.14
	1000	7.65±0.05	7.83±0.07	9.67±0.16	8.87±0.17	8.38±0.14
Pseudomonas	10	NZ*	NZ*	6.92±0.04	6.38±0.08	NZ*
	100	6.57±0.07	6.90±0.11	7.45±0.05	7.93±0.14	NZ*
	1000	7.13±0.17	7.33±0.18	8.25±0.08	8.48±0.17	6.80±0.06

*NZ: No zone of inhibition, Results represent the means±SD (standard deviation) from at least two separate experiments, F1: Fraction 1, F2: Fraction 2, F3: Fraction 3, F4: Fraction 4, F5: Fraction 5

all the extracts and fractions can be categorized to be a very strong group. It is explained in some literatures; the antioxidant activity can be classified to be several classes based on the IC_{50} value, such as IC_{50} <50 µg/mL is categorized to be a very strong antioxidant, 50–100 µg/mL is strong, 100–150 µg/mL is medium, and 151–200 is low antioxidant.

On the extraction process, the IC_{50} value of flavonoid total showed a higher antioxidant activity than the other extract. This can be assumed as the synergy effect that come from the flavonoid compounds in the flavonoid total extract [5]. However, after the separation process, the flavonoid total showed a lower antioxidant activity than F4 and F5 fractions, it is assumed on the column chromatography process, and those fractions (F4 and F5) contained some flavonoid component that has synergy activity. As a report in Chibi *et al*. [23], there is a correlation between the phenolic content of extract and the antioxidant activity. This data also supported with a study of Alidadi *et al*. [24] that the activity of material to interact with free radical was influenced by the phenolic content.

Antibacterial activity

The antibacterial activity of extract and fraction of *D. pentandra* (L.) *Miq* is shown in Tables 1 and 2. Also the inhibition zone that formed during the treatment can be seen in Fig. 2.

Antibacterial activity of extract and fraction of *D. pentandra* (L.) *Miq* (Tables 1 and 2) showed a trend that the activity was increased with the increase of concentration (10–1000 µg/mL). On the extract part, in general, the flavonoid total showed a higher antibacterial activity than methanol, n-hexane, and ethyl acetate extract. This effect can be caused by the present of a lot of flavonoid compounds in that extract [5]. Furthermore, it supports by the presence of hydroxyl group on the aromatic structure of flavonoid which can increase the activity as antibacterial. The mechanism of flavonoid to inhibit the growth of bacterial supposed through the inhibition of nucleic acid synthesis, inhibition of cytoplasm function, the formation of biofilm, etc. [25]. However, there is no linearity between the antibacterial and antioxidant activities. The fractions F1–F5 have low activity compared to the total flavonoid in almost of all bacterial that used in this research. Foroughi *et al*. [26] described that, during the *in vitro* evaluation, the flavonoid content is able to act as antibacterial.

CONCLUSIONS

Flavonoid total and some fractions have been successfully isolated from *D. pentandra* (L.) *Miq*. The fractions F1–F5 showed higher antioxidant activity than flavonoid total extract. However, in the antibacterial activity, flavonoid total showed a higher activity than the fractions F1–F5.

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AUTHORS' CONTRIBUTION

Rini Hardiyanti and Lamek Marpaung performed the experiments and wrote the paper; I Ketut Adnyana and Partomuan Simanjuntak analyzed the data.

CONFLICTS OF INTEREST

All authors have none to declare.

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