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Variation of antioxidant activity profile of *Dendrophthoe pentandra* (L.) Miq. leaves extract from two varieties of mangoes (*Mangifera indica*)

Variasi profil aktivitas antioksidan ekstrak daun *Dendrophthoe pentandra* (L.) Miq. dari dua varietas mangga (*Mangifera indica*)

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ABSTRACT

Mistletoe is a hemiparasites plant that interfere the growth of their host plant, However, mistletoe itself can be used in the herbal medicine. One of the cultivated plants in Indonesia which is affected by this mistletoe is mango. Mangoes is rich in antioxidant, so that its mistletoe is also thought to rich in antioxidant. This study is aimed to analyze the secondary metabolites and antioxidant activity of mango mistletoe from two varieties of mangoes, namely Harumanis and Madu. This study has been carried out experimentally. Samples of mistletoe were collected from Caturtunggal, Sleman, Yogyakarta. Extraction was carried by maceration in methanol. Phytochemical screening, quantitative analysis of phenolics compound and quercetin were performed using HPLC. Antioxidant activity was determined using DPPH method. Data was analyzed through descriptive statistisc. The result showed that the quercetin level and total phenolic compound of *D.pentandra* leaves extract from *M.indica* var. Madu are higher than var. Harumanis, which are 257.917 ppm and 399.195 ± 12.352 mg GAE/g extract. Antioxidant activity of *D.pentandra* extract from *M.indica* var. Madu is also higher than var. Harumanis, with IC50 37.19 ppm. In this study, environmental factor did not affect the secondary metabolites of mistletoe. Through this data, the difference of secondary metabolites profile and the antioxidant activity of mistletoe from different host varieties were found, particularly in quercetin and its phenolic compounds.

Keywords: antioxidant, mango, mistletoe, phenolics, quercetin

INTISARI

Benalu merupakan tanaman hemiparasit yang menganggu pertumbuhan tanaman inangnya. Meskipun demikian, benalu sendiri dapat dimanfaatkan dalam pengobatan herbal. Salah satu tanaman budidaya di Indonesia yang terserang benalu adalah mangga. Mangga diketahui kaya antioksidan sehingga benalu yang menyerangnya diduga juga kaya antioksidan. Studi ini bertujuan untuk menganalisis metabolit sekunder dan aktivitas antioksidan benalu mangga dari dua varietas mangga, yaitu Harumanis dan Madu. Studi ini dilaksanakan secara eksperimental. Sampel benalu diperoleh dari Caturtunggal, Sleman, Yogyakarta. Ekstraksi dilakukan melalui maserasi dalam metanol. Skrining fitokimia, analisis kuantitatif senyawa fenolik dan kuersetin dilakukan menggunakan HPLC. Aktivitas antioksidan diukur menggunakan metode DPPH. Data dianalisis secara statistik deskriptif. Hasil menunjukkan bahwa kadar kuersetin dan kadar total fenolik ekstrak daun of *D.pentandra* dari *M.indica* var. Madu lebih tinggi dibandingkan var. Harumanis, yaitu 257,917 ppm and 399,195 ± 12,352 mg GAE/g ekstrak. Aktivitas antioksidan ekstrak *D.pentandra* dari *M.indica* var. Madu juga lebih tinggi dibandingkan var. Harumanis, dengan IC50 37,19 ppm. Dalam studi ini, faktor lingkungan tidak berpengaruh terhadap metabolit sekunder benalu. Melalui data ini, perbedaan profil metabolit sekuder dan aktivitas

antioksidan benalu dari varietas tanaman inang berbeda diketahui, terkhsus pada kadar kuersetin dan senyawa fenolik.

Kata kunci: antioksidan, mangga, benalu, fenolik, kuersetin

INTRODUCTION

Dendropthoe pentandra (L) Miq. is a hemiparasitic plant from Loranthaceae family which lives depending on its host plants. This species mistletoe can be found in many different species of trees such as duku, orange, cocoa, coffee, and mango. The nutrient for its growth is obtained from its host through haustorium. Haustorium is a specialized organ that enable the exchange of water, mineral, nutrient, and genetic information (Teixeira-Costa, 2021). Hemiparasite plant can perform photosynthesis to fulfill its organic carbon. Infestation of mistletoe can cause stress for host which lead to the death of plant. The infestation of mistletoe is a biotic stress which can promote the biosynthesis of secondary metabolites in its host as self-defense. In the other side, mistletoe also undergoes stress from host and environment. To overcome these stresses, mistletoe develops itself defense system which increases production of secondary metabolites. Many studies have proven the pharmacological activity of secondary metabolites from many plants.

In Indonesia and many countries, mistletoe has been used in traditional and folk medicine for treating several diseases, such as, diarrhea, diabetes, hypertension, cancer, diuretic, smallpox, ulcer, and skin infection (Szurpnicka et al., 2020). According to Hardiyanti et al. (2019), quercitrin from *Dendrophtoe pentandra* (L.) Miq. leaves have antioxidant activity with IC50 3.59 ppm and antibacterial property to *Salmonella thyphi*, *Pseudomonas* sp., *Escherichia coli* and *Staphylococcus aureus*. Information about its host is not provided. Elsyana et al. (2016) showed the inhibition activity of clove mistletoe n-hexane fraction was 38.69% at 125 ug/mL on K562 cancer cell lines and 41.5% on MCM-B2. The biological activity of the mistletoe is due to the content of secondary metabolites. The pharmacological activity of mistletoe can also be affected by their host. Artanti et al. (2012) in their studies found that antioxidant activity of methanol extract benalu teh (*D. pentandra* grew on *Camellia sinensis*) is higher than others (*D. pentandra* grew on *Stelechocarpus burahol* (kepel)*, Spondias dulcis* (kedondong)*, Annona squamosa* (srikaya)). This data has suggested that different host probably affected the mistletoe activity. Further study needs to be performed to understand the effect of different host to the diversity of bioactive compound.

According to Elsyana et al. (2016), the presence of flavonoid and triterpenoid has been reported in n-hexane fraction of clove mistletoe. These two compounds are known to have an important role in cancer chemoprevention and chemotherapy because of its antioxidant activity. Radical oxygen species (ROS) have been known to be cytotoxic causing various disease such as cancer and neurodegenerative diseases. Therefore, secondary metabolites of mistletoe are high potential to be developed as medicine.

Mango *or Mangifera indica* is a tropical plant which is popular in Indonesia that having many varieties. Its fruit is rich of vitamin C which have high antioxidant activity. Based on Noratto et al. (2010), polyphenolic compounds from several mango varieties have a high potential as chemo preventive agents. In its cultivation, this plant is vulnerable to the infestation of mistletoe *Dendrophtoe pentandra* (L.) Miq, or commonly called as benalu mangga. According to the phytochemical screening by Kurniasih et al. (2015), benalu mangga leaves contained flavonoid, tannin, polyphenol and saponin. It had high

antioxidant activity with IC50 33.31 ppm (Kurniasih et al., 2015) and 25.40 µg/mL (Yulianti R et al., 2016). However, it is not clear about the varieties of mango as host for *D. pentandra.* To understand the relation between varieties of mango and the antioxidant activity of its mistletoe, we conducted the study about antioxidant activity of *Dendropthoe pentandra* which infects *Mangifera indica* in two mangoes with varieties Harumanis and Madu.

MATERIALS AND METHOD

Sample preparation and extraction

The leaves of *D. pentandra* from *M. indica* var. Harumanis and Madu were collected from Caturtunggal, Sleman, Yogyakarta and identified at the Plant Systematics Laboratory, Faculty of Biology, UGM. Separately, the green leaves of *D. pentandra* were washed thoroughly. Samples is dried for 3 days in an oven at 40ºC. The dried leaves of *D. pentandra* were ground. Two hundred grams of sample were macerated using 1 L methanol for 2 times in 48 hours. Methanol extract was evaporated with a rotary evaporator at 60 rpm, 40ºC, then dried in an oven at 40ºC until it became pasta form. The yield of extract was calculated quantitatively by the following formula below.

$$\%\ \text{yield} = \frac{\text{The weight of extract that obtained (g)}}{\text{The weight of simplicia that extracted (g)}} \times 100\%$$

Phytochemical analysis

Phytochemical assay was performed qualitatively, following the procedure of Harborne (1984). In flavonoid assay, 0.05 g of each extract was mixed with 5 mL of 96% ethanol. Then, 1 mL of concentrated HCl and a pinch of Mg powder is added. Positive result is indicated by the formation of orange or red solution. In tannin assay, 0.05 g of each extract was added with aquadest until the solution was colorless. Subsequently, 2 mL of the solution was added with 1-2 drops of $FeCl_3$ 1%. Positive result was showed with blue or blackish green color solution. In saponin assay, 0.05 g of each extract was added with 10 ml of hot water and then cooled. After that, it is shaken vigorously for about 10 seconds. Positive sign of saponin is indicated by the formation of 1-10 cm foam for 10 min. Next, in terpenes and steroids assay, 0.05 g of each extract was added with 2 drops of CH_3COOH anhydrous and 1 drop of conc. H_2SO_4. The positive result for steroids was getting green color in the solution, whereas for terpenes the results were getting red or purple color. For alkaloids assay, 0.05 g each of extract was added with 1 ml of HCl 2 N and 9 ml of aquadest. Then, samples are heated for 2 min. The mixture was filtered and then divided into three parts: (1) 3 drops of the filtrate were added with 2 drops of Mayer reagent. The positive result was getting a yellow-white precipitate. (2) three drops of the filtrate were added with 2 drops of Dragendorff reagent. The positive result was getting a brick-red precipitate. (3) three drops of the filtrate were added with 2 drops of Bouchard reagent. The positive result was getting a brown to black precipitant.

Detection of quercetin using thin-layer chromatography (TLC)

TLC analysis was performed qualitatively according to Skorek et al. (2016). Three milligrams mango mistletoe extracts are diluted in 1 mL of methanol. Standard is quercetin 1 mg/mL in methanol. Each sample is attached to a TLC plate. Elution was carried out on the mobile phase of methanol: aquadest (7:3). Then, the TLC plate was dried and sprayed with a 1% $AlCl_3$ color reagent.

Observations were made under UV light and the retention factor (Rf) value was calculated by the following formula:

$$\text{Rf} = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by the eluent}}$$

Determination of quercetin using high-performance liquid chromatography (HPLC)

The determination concentration of quercetin from mango mistletoe extract was carried out quantitatively using HPLC (Shimadzu LC 2030) using column C-18 and mobile phase methanol: aquadest (59:41) with a flow rate of 1.2 mL/min and measured with a UV detector at λ 371 nm. Twenty-five milligrams extract of mango mistletoe was dissolved with 10 mL methanol. Meanwhile, a standard curve of quercetin was prepared from 0.1-2.4 ppm. After that, the sample and standard were sonicated for 15 min and filtered with a 0.22 µm membrane filter. Each filtrate of sample and standard 20 µL was injected into HPLC. The linear equation of quercetin y = a ± b was used to calculate the total quercetin in the sample.

Determination of total phenolic content (TPC)

Total phenolic content of mistletoe mango extract was carried out quantitatively according to Alharits et al. (2019). The stock solution of mango mistletoe extract was made at a concentration of 1000 ppm in MeOH. Each 0,2 mL of mistletoe extract and gallic acid were taken and then added 1.8 mL of aquadest and 0,1 mL of Folin ciocalteu reagent, then incubated for 5 min in the dark. 1 mL of Na_2CO_3 5% and 1.9 mL of aquadest were added and incubated for 1 h in the dark. Measurements were made at l 760 nm by spectrophotometry UV-Vis. Gallic acid 12.5- 200 ppm was used as a standard curve. The linear equation is obtained from standard curve, y = a ± b. Total phenolic content in the sample was calculated with TPC = (C x V x fp)/ m. C: concentration (ppm), V : volume (mL), fp : dilution factor, m : weight of extract (g), and TPC : total phenolic content (mg GAE/ g). Three technical replicates were carried out in this assay.

Determination of antioxidant activity (Inhibition Concentration 50, IC_{50})

Antioxidant activity of mistletoe extract was performed by following the procedure of Zhang et al. (2006). Each extract of mango mistletoe was prepared at 15.625-250 ppm. Samples is mixed with DPPH 100 ppm in the volume comparison of 1:2. Total volume per each well is 150 µL. Methanol is used as blank, then DPPH 100 ppm and 50 µL methanol as control. The mixtures are incubated for 30 min in the dark, then measured with a microplate reader at λ 517 nm. Same procedure is performed for quercetin standard 3.90625-31.25 ppm. Three technical replicates were carried out in this assay. The percentage of inhibition was calculated using following formula:

$$\%\ inhibition = \frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance} \times 100\%$$

IC_{50} is calculated by graphing a linear equation between the concentration of sample (x) and the percentage of inhibition (y) to obtain the equation y = a ± b.

RESULT

During the sampling, the measurement of environmental parameter was conducted. The result was presented in Table 1. Difference of soil humidity, air temperature and air humidity between two samples were observed in this study.

Table 1. Environmental data of *D. pentandra* leaves from *M. indica* var. Harumanis and Madu

Host of *D.pentandra*	Soil pH	Soil temperature (°C)	Soil humidity (%)	Air temperature (°C)	Air humidity (%)
Harumanis	6.5	34	25.67	40	29
Madu	6.5	32	40	31	41

This study was started by the extraction of mistletoe samples from two hosts using maceration method in methanol as solvent. Methanol can solve polar, semi-polar and non-polar compound which increase the efficiency of extraction process. From the extraction, the yield of methanol extract from *D. pentandra* (L.) Miq leaves. from *Mangifera indica* var. Harumanis and Madu are 21.65% and 18.25% respectively. Then, it was followed by detection of flavonoid and phenolic compound. In Shinoda test, the presence of flavonoids was indicated by the appearance of red to pink color after few minutes (Saptarini et al., 2016). In this study, we obtained positive result for both extracts, *D. pentandra* (L.) Miq leaves. var. Harumanis and Madu. The other qualitative assay used in this study is TLC with quercetin as standard. Quercetin is a compound in flavonoid group and contained in *D. pentandra.* TLC result showed that there is a yellow spot with a height equal to quercetin standard. Rf quercetin is 0.94, then Rf *D. pentandra* var. Harumanis and Madu is 0.93 and 0.94 respectively. Same Rf value indicated that extract of *D.pentandra* contains quercetin. It is similar with the study by Dewi et al. (2019) that *D.pentandra* obtains quercetin. Moreover, alkaloid, saponin, tannin and steroids are also detected in both *D.pentandra* leaves from both *M.indica* through phytochemical screening in this study.

According to the phytochemical screening, the extract was known to contain flavonoid compound. From HPLC analysis, quercetin was detected in both samples of mistletoe in *M. indica* var. Madu dan var. Harumanis. The chromatogram was shown in Figure 1.

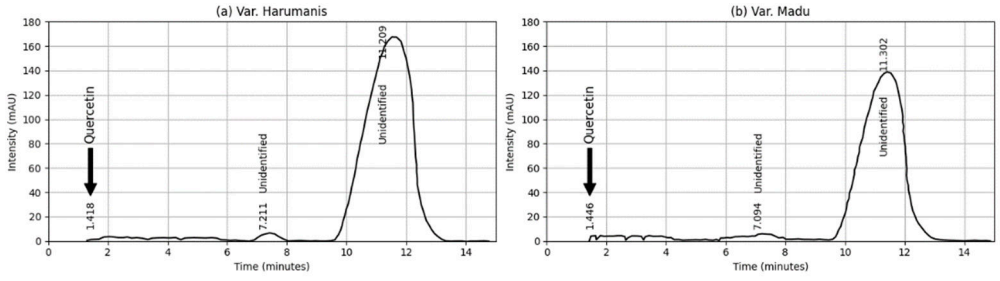

Figure 1. Chromatogram of (a) *D.pentandra* leaves from *M. indica* var. Harumanis and (b) var. Madu at 371 nm

Peak of quercetin in *D.pentandra* leaves extract var. Harumanis was identified at 1.418 min with area 18226 AUC, then var. Madu at 1.446 min with area 43689 AUC. From chromatogram, the highest peak was observed at 11.209

min and 11.032 min for *D.pentandra* extract. It indicates that there is main compound in these extracts besides quercetin, but it is still not identified.

To quantify quercetin in the *D.pentandra* extract, the analysis was begun with standard curve of quercetin. Regression linear equation of quercetin is y= 156.8x + 3247.6 with correlation coefficient value (R^2) 0.9984. From this equation, the calculated quercetin in *D.pentandra* leaves extract var. Harumanis is 95.781 ppm and from var. Madu is 257.917 ppm (Table 2). Data showed that quercetin level of *D.pentandra* extract var. Madu is higher than from var. Harumanis. In addition to quercetin, total phenolic content of both samples was also analyzed. The result was presented in Table 2. In TPC assay, gallic acid was used as standard to produce standard curve. Gallic acid is a simple phenolic acid with high stability and purity. The regression linear equation of gallic acid is y = 0.0045x – 0.0157 with R^2 = 0.9998. TPC value of *D. pentandra* leaves extract from *M.indica* var. Madu was higher than from var. Harumanis.

Table 2. Quercetin level and TPC of *D. pentandra* leaves from *M.indica* var. Harumanis and Madu

Varieties of *M.indica*	Quercetin Level (ppm)	Total Phenolic Compound (mg GAE/g)
Harumanis	95.781	208.822 ± 7.424
Madu	257.917	399.195 ± 12.352

Flavonoid and phenolic compound was contributed to its antioxidant activity. The antioxidant activity of each mistletoe was presented in Figure 2.

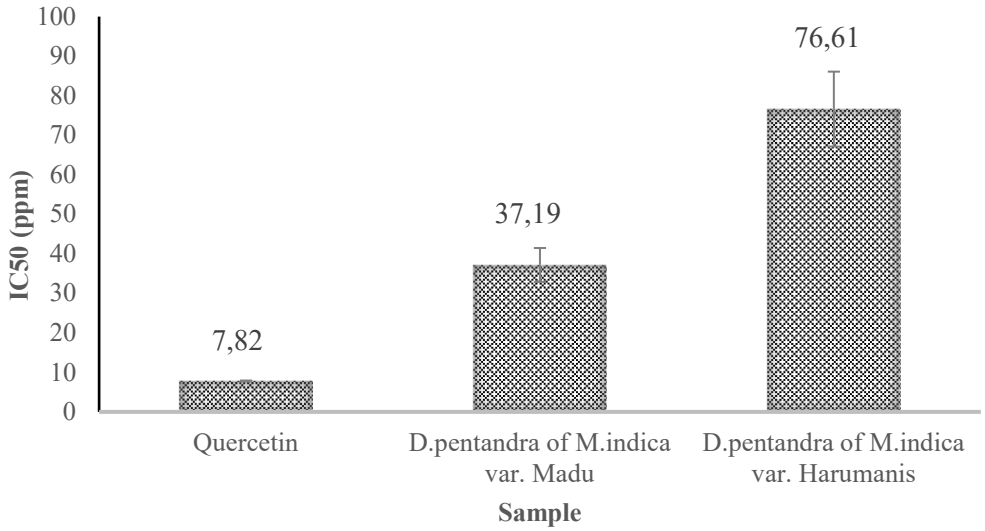

Figure 2. IC50 of quercetin, *D.pentandra* leaves extract from *M.indica* var. madu and harumanis.

Based on the data, IC50 value of *D. pentandra* leaves extract from var. Harumanis and Madu is 76.61± 9.485 ppm and 37.19 ± 4.275 ppm ppm respectively. This data resulted that the antioxidant activity of *D. pentandra* leaves extract from *M. indica* var. Madu is higher than var. Harumanis. However, the antioxidant activity of both mistletoes was lower than quercetin with IC50 7.82 ± 0.086 ppm. The variance between samples was presented in Table 3. F-ratio between *D. pentandra* from var. Harumanis and var. Madu (F= 4.92) is higher than 1. This data was also shown between quercetin and *D. pentandra*

from var. Harumanis or var. Madu (F= 12265.23 or F = 2491.83). The value of F-ratio is greater than 1 suggests that there are significant differences between the group means. According to Badarinath et al. (2010), the antioxidant activity of quercetin can be categorized as very strong activity. Similar to quercetin, *D. pentandra* extract from *M. indica* var. Madu also has a very strong antioxidant activity, whereas *D. pentandra* extract from *M. indica* var. Harumanis was strong.

Table 3. F-ratio of IC50 samples from F-test for variances

F-Ratio	*D.pentandra* from var. Harumanis	*D.pentandra* from var. Madu	Quercetin
D.pentandra from var. Harumanis	-	4.92	12265.23
D.pentandra from var. Madu	4.92	-	2491.83
Quercetin	12265.23	2491.83	-

Production of secondary metabolites was affected by abiotic factor. In this study, statistic calculation was performed between environmental parameter, TPC and IC50 from samples using Pearson correlation. The result was presented in Table 4.

Table 4. Significance value of Pearson correlation statistatic assay between environmental parameter, TPC, and IC50 of *D. pentandra* leaves from *M.indica* var. Harumanis and Madu

Parameter	*D.pentandra* from var. Harumanis		*D.pentandra* from var. Madu	
	TPC	IC50	TPC	IC50
Soil pH	0.766	0.253	0.861	0.215
Soil temperature (°C)	0.099	0.414	0.740	0.094
Soil humidity (%)	0.857	0.344	0.407	0.240

DISCUSSION

Mistletoe is a parasitic plant which depends on its host and environment for its growth and metabolism. In this study, *D. pentandra* was identified as mango mistletoe from *M. indica* var Harumanis and var. Madu. Difference of host probably affects the secondary metabolites of mistletoe leading to its antioxidant activity. According to qualitative analysis, the phytochemical of *D. pentandra* from both hosts contains flavonoids, phenolics, alkaloids, saponins, tannins, and steroids. It indicated the potency of pharmacological activity of *D.pentandra* leaves extract. No difference of secondary metabolites diversity of *D.pentandra* is observed from different host. However, the quercetin content and total phenolic content of *D.pentandra* from M.indica var. Harumanis is lower than from var. Madu. Data shows that varieties of *M. indica* (host) has an impact to quercetin and phenolic content of *D. pentandra* (mistletoe). From statistical analysis, p-value of total phenolic compound from T-test is 2.16e-05. It is less than 0.05 indicating a significant difference between the two groups. Phenolic compound is a secondary metabolite that highly produced during stress in plants.

According to Kumar et al. (2020), phenolics compounds play important role for plant in their defense mechanisms to pathogens and abiotic stress, such as drought, salinity, temperature, and nutrient deprivation. Although Madu and Harumanis mangoes grow in the same areas, they own their form of defense mechanisms that could be different. This situation brings impact to secondary metabolites of mistletoe *D.pentandra* which take nutrient from its host. Özcan et al. (2018) in his study also stated that the differences of total phenolics compounds in bulbs can be caused by plant species, harvest time, genetic factor, and environmental conditions. This shows that the physiological adaptive responses were correlated with the plant taxonomics group to cope the stress and stimulate defensive mechanism (Isah, 2019).

The number of phenolic and quercetin compounds is also influenced the antioxidant activity. In this study, data represents that high total phenolic content and quercetin content leads to high antioxidant activity. IC50 value of *D.pentandra* from *M.indica* var. Madu is lower than from var. Harumanis. IC50 represents the ability of extract or sample to scavenge 50% free radical population. It means that IC50 value is inverse proportion with antioxidant activity. The antioxidant activity of extract comes from the phenolic and quercetin which react with a variety of free radicals. It involved hydrogen atom transfer, transfer of single electron, sequential proton, loss electron transfer and chelation of transition metals (Zeb, 2020).

In this study, the influence of host to the phenolic and quercetin content, and antioxidant activity of mistletoe was observed. The high phenolic and quercetin in the extract leads to its high antioxidant activity. The difference variety of host between *M.indica* var. Harumanis and var. Madu represents their physiological defense response that affect the production of secondary metabolite and antioxidant capacity. But, it still need to be observed more in detail. In addition, the production of secondary metabolites is also influenced by abiotic stress from environment. According to data in Table 1, these two mangoes grow in similar condition. However, soil humidity and air humidity in *M.indica* var. Madu is higher than var. Harumanis. The air temperature of *M.indica* var. Madu is lower than var. Harumanis. According to Pearson correlation assay, data Table 3 shows no impact from the environmental condition to antioxidant activity of mistletoe statistically ($p > 0.05$). Soil pH and soil temperature of both samples are same. Difference of soil humidity, air temperature and air humidity between two samples were observed in this study, but it did not impact directly and significantly to total phenolic content and antioxidant activity of samples ($p = 0.094$-0.861, $p > 0.05$ in Pearson correlation assay). But, each mistletoe samples gives different pattern in secondary metabolite content and antioxidant activity.

According to Assanga et al. (2020), the phenolic composition and antioxidant activity of parasitic plant depend on its host. In our observations, we found that infestation of mistletoe in *M.indica* var. Madu is higher than *M.indica* var. Harumanis (no data provided). It is a possible reason why the phenolic compound and antioxidant of *D.pentandra* in *M. indica* var.Madu is higher than var.Harumanis. Mistletoe is a hemiparasitic plant that take nitrogen in its high transpiration rate from its host through xylem (Hosseini et al., 2008). The higher infestation of mistletoe could increase stress on the host plant which promote a high production of phenolic compounds caused by increasing the PAL (Phenylalanine ammonium lyase) activity. Besides that, a direct connection between mistletoe and xylem and/or phloem of the host through haustorium contributes to the exchange of a stress-responsive molecules (Zagorchev et al.,

2021). This phenomenon causes the increase of stress oxidative in mistletoe. Moghadamtousi et al. (2014) in his study found that several lactones compounds in *Loranthus parasiticus* were transported directly from its host plants because these compounds are not native to the *Scurulla* species. Different level of phenolic and antioxidant activity of *Viscum album* mistletoe was also observed when infesting different host species (Vicaş et al., 2011). Based on the result, the variety of host and environmental condition needs to be considered in the utilization of mistletoe as herbal medicine. The qualitative and quantitative assay of phytochemicals must be done in the beginning of the development of herbal medicine from mistletoe. Extract of mistletoe must meet the requirement as medical substance to optimize its pharmacological activity.

CONCLUSION

D. pentandra leaves from *M. indica* var. Madu and Harumanis are potential to be developed as traditional medicine ingredients. *D. pentandra* leaves from these two varieties of mangoes contain quercetin and phenolic compounds. However, there is a slight difference of quercetin level and total phenolic compound from both. *D. pentandra* leaves from var. Madu has a higher level of quercetin and total phenolic compound compared to var. Harumanis. These two values have implications for its antioxidant activity. The antioxidant activity of *D. pentandra* leaves from var. Madu (IC50 37.09 ppm) is higher than var. Harumanis. There are different antioxidant quantity and activity between mistletoes that grow in two mango varieties which might reflect the different secondary metabolism of the hosts.

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