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**Antioxidant compounds and activity from the leaf of the mistletoe
(*Dendrophthoe pentandra* (L.) Miq.) on Duku plant
(*Lansium domesticum* Corr.)**

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ABSTRACT

Mistletoe, known in Indonesia as benalu, is a parasitic plant that can thrive on a variety of hosts. This study determined that *Dendrophthoe pentaphylla* (L.) Miq. was accountable for the antioxidant activity of mistletoes growing on Duku (*Lansium domesticum* Corr.). This study aims to assess the antioxidant potency in the fractions of n-hexane, ethyl acetate, and methanol-water, as well as to identify the chemical groups and IC₅₀ values. Extraction by maceration, fractionation by liquid fractionation method, isolation by vacuum liquid chromatography (VLC) and column chromatography, and DPPH assay for antioxidant activity were employed in this study. The n-hexane and ethyl acetate fractions exhibited antioxidant activity, but the methanol fraction showed minuscule activity. NH.1 (compounds from the N-hexane fraction followed by VLC were found in eluate 1) and NH.3 (compounds from the N-hexane fraction followed by VLC were found in eluate 3) were successfully isolated from the n-hexane fraction. The former was identified as terpenoids, while the latter belonged to flavonoids. The ethyl acetate fraction yielded the flavonoid EA.3 (compounds from the ethyl acetate fraction followed by VLC were found in eluate 3), which was isolated from the ethyl acetate fraction. Compound NH.1 showed weak antioxidant activity indicated by IC₅₀ value of 151.35 g/mL. Meanwhile, NH.3 and EA.3 were categorized as moderate, proven by IC₅₀ values consecutively of 96.64 and 78.37 g/mL.

Keywords: antioxidants, *Dendrophthoe pentandra* (L.) Miq, flavonoids, terpenoids

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INTRODUCTION

Reactive Oxygen Species (ROS) contribute to the pathological progression of several human diseases, including cardiovascular problems, neurological disorders, and diabetes, among others. The most promising technique for preventing oxidative damage induced by these reactive species is the employment of antioxidant molecules, which can operate by scavenging free radicals as direct antioxidants and improving antioxidant status (enzymatic and non-enzymatic) as indirect antioxidants (Gonzalez-Burgos & Gomez-Serranillos, 2012). Plants naturally produce antioxidants as a part of their defense system against environmental stressors, such as UV radiation and pests.

Dendrophthoe pentandra (L.) Miq shows potential as a plant source for antioxidant compounds. *Dendrophthoe pentandra* is a parasitic plant capable of surviving on a variety of hosts. (Artanti et al., 2009) conducted research on the antioxidant activity of *Dendrophthoe pentandra* living in a variety of hosts. They discovered that *Dendrophthoe pentandra* living on duku (*Lansium domesticum* Corr.) had the most significant antioxidant activity after mango, as proven by IC₅₀ values of 9.6 ppm in the ethanolic extract and 51.8 ppm in the aqueous extract.

The phytochemical examination of the extracts of *Dendrophthoe pentandra* leaf discovered the presence of terpenoids, alkaloids, tannins, saponins, and flavonoids. The quantitative analysis indicates that the floral extract contained a higher concentration of flavonoids and phenols than the leaf extract. The DPPH test confirmed that the floral extract exhibited a greater IC₅₀ concentration than the leaf extract. These findings suggest that the compounds played a role as antioxidants in *Dendrophthoe pentandra*. However, additional research is required to discover whether the types of phenols and flavonoids in the leaves differ from flowers in *Dendrophthoe pentandra* (Alharits et al., 2019).

The methanolic extract of *Dendrophthoe pentandra*, mistletoes on cocoa, contains saponins, tannins, flavonoids, and terpenoids, but the ethyl acetate extract only contains flavonoids, terpenoids, and saponins. IC₅₀ values for total flavonoids, flavonoids aglycones, methanolic extract, and ethyl acetate extracts are 24.07, 18.22, 30.31, and 36.23 mg/mL, respectively. As a control, ascorbic acid exhibited an IC₅₀ value of 12.08 mg/mL. Flavonoids included in the parasite leaves of cocoa can serve as antioxidants, albeit with less potency than ascorbic acid (Sembiring et al., 2016).

Dendrophthoe pentandra can also be seen growing as a parasite on duku trees in South Sumatra, Indonesia. Farmers simply discard the parasite plant since it is regarded as useless. This study aims to identify antioxidant activity in the duku parasite (*Dendrophthoe pentandra*), calculate the IC₅₀ of the compounds discovered, and identify the class of obtained antioxidant compounds.

MATERIALS AND METHODS

Materials

The primary materials used were the parasite *Dendrophthoe pentandra* leaves on duku. Leaves sample was obtained from duku trees in the Keromongan Village, OKU Timur Regency, Sumatra Selatan. The other material used in this study was N-Hexane p.a (Merck), Ethyl acetate p.a (Merck), methanol p.a (Merck), silica gel 60 GF 254 (Merck), 5% H₂SO₄ solution. The equipment utilized in this study is TLC plate silica gel GF 254 20x20 mm (Merck), DPPH (Sigma-Aldrich), ascorbic acid and DMSO solution (Merck), rotary evaporator (Buchi-B480), UV-Vis spectrophotometer (Shimadzu 1280), vacuum chromatography, and column chromatography.

Methods

Extraction using methanol solvent and fractionation with organic solvents

Leaves sample from the parasite *Dendrophthoe pentandra* was washed and dried naturally in the sun. The dried leaves were then pulverized in a blender before being extracted via maceration. The simplicia was soaked in 2 L of methanol solvent for 15 minutes, agitated for 15 minutes, let to stand for 2x24 hours, then filtered. The leftovers were immersed in methanol once more until the filtrate was clear. To obtain a thick methanolic extract, the liquid extract was concentrated using a rotary evaporator.

The liquid-liquid fractionation (LLF) method was used for fractionation. The thick methanolic extract was dissolved in a 1:1 mixture of methanol and water. The extract was then placed in a separating flask

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with 250 mL of n-hexane, slowly shaken, and left to stand until two layers formed. The N-hexane and water-methanol fractions were separated in a separating flask. The water-methanol fraction was placed in a separatory flask and n-hexane was added until the top layer turned transparent. The fractionation process was repeated using ethyl acetate solvent in the same manner as with n-hexane solvent. The liquid fraction was then evaporated with a rotary evaporator to yield the viscous fraction.

Antioxidant activity of fractions

Thin-layer chromatography (TLC-DPPH) was used to assess the antioxidant activity of the n-hexane, ethyl acetate, and water-methanol fractions. A capillary tube is employed to stain the TLC plate with a fraction. The stained TLC plate was eluted with n-hexane and ethyl acetate (8:2) and sprayed with DPPH solution. The TLC plate was left for one minute before the spots were examined. The antioxidant chemicals were then isolated using the VLC method and then further purified using column chromatography.

Vacuum liquid chromatography (VLC)

The VLC process was carried out in the following manner. The VLC column was packed with silica gel to a height of 4 cm. Silica gel was added to the active fraction. The active portion was then mixed with silica gel and placed on top of the silica gel. The fraction was then eluted using solvents with gradient polarity characteristics, starting from low and changing toward higher polarity by varying the solvent ratio of n-hexane, ethyl acetate, and methanol. The VLC yield was collected and evaporated in a beaker glass. The antioxidant activity of the VLC yield was determined using a TLC plate coated with DPPH.

Column chromatography

The process of purification or isolation of active compounds was carried out using gravity column chromatography. Silica gel was mixed with the eluent and then put into the column. The active fraction was dissolved with a solvent, put into a silica gel column, and eluted using the gradient method. The eluate was collected in a 10 mL vial. The eluate from column chromatography was analyzed using thin-layer chromatography. The eluates with the same stain on thin-layer chromatography were combined, and the solvent was evaporated.

Identification of compound group

The silica gel-TLC plate with the sample was inserted into the chamber and then eluted. The TLC plate was sprayed with a 2% H₂SO₄ detection reagent. The TLC plate was then heated. Simultaneously, the color reaction was observed. Then, the value of the retention factor (R_f) was calculated.

Antioxidant activity test

DPPH solution was prepared with a concentration of 0.1 mM by dissolving 3.94 mg of DPPH in 100 mL of methanol. The active compound and ascorbic acid were prepared at 2000 µg/mL concentration by dissolving 8 mg in 4 mL DMSO. Subsequently, solutions were prepared with a concentration of 100, 500, 250, 125, and 62.5 µg/mL by taking 2 mL of sample and adding 2 mL of DPPH solution. The solution mixture was homogenized and left for 30 minutes in the dark. Absorption was measured by UV-Vis spectrophotometer at a max λ of 517 nm.

Data Analysis

The sample will be assessed for its antioxidant activity by determining DPPH radical absorption inhibition. The inhibition is calculated as a percentage, as the formula suggests.

$$\text{Inhibition percentage} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \times 100\% \quad (1)$$

IC₅₀ value denotes the sample concentration mandatory to scavenge 50% DPPH free radicals. The samples are categorized as active only if the IC₅₀ is lower than 100 µg/mL.

RESULT AND DISCUSSION

Extraction

The leaves of *Dendrophthoe pentandra* living on the duku tree were extracted. From 500 g of *Simplicia*, the extraction yielded 104 g of methanolic extract, with an extract yield of 20.8%. The extract obtained was thick and colored blackish-green. The extract yield value showed the number of compounds obtained from the *simplicia*. According to (Prawira, 2015), the yield value obtained indicates the number of components a solvent can extract from a sample. The greater the extract yield value, the greater the component or compound obtained. The extraction process in this study used methanol as a solvent due to its polar properties so that it can dissolve all groups of compounds that exist in the *simplicia*. (Febrina et al., 2015) said that methanol could dissolve almost all compounds, both polar and non-polar. The results of research conducted by (Yulian & Safrijal, 2018) obtained alkaloids, flavonoids, terpenoids, and tannins from the ethanolic extract of the parasite of coffee plants.

Dendrophthoe pentandra leaves were extracted from the duku tree. The extraction yielded 104 g of methanolic extract from 500 g of *Simplicia*, with an extract yield of 20.8%. The resulting extract was thick and blackish-green in color. According to (Prawira, 2015), the extract yield value indicated how many compounds were extracted from the *simplicia*. The higher the extract yield value, the more components or compounds are obtained. (Febrina et al., 2015) implied that methanol could dissolve almost any compound, both polar and non-polar. Thus, due to its polarity characteristic, methanol was used as a solvent in this study to dissolve all groups of compounds in the *simplicia*. (Yulian & Safrijal, 2018) utilized ethanol to extract *Dendrophthoe pentandra* living in a coffee plant and managed to identify alkaloids, flavonoids, terpenoids, and tannins within the extract.

Fractions of methanolic extract

The fractionation goal is to separate the compounds in the extract based on the level of polarity. The results of the fractionation of methanolic extract using the LLF method obtained fractions with different weights, as depicted in Table 1.

Table 1. The fraction weight and yield of methanolic extract from mistletoe leaves

| Fraction | Fraction Weight (g) | Fraction Yield (%) |
|----------------|---------------------|--------------------|
| N-hexane | 6.98 | 6.69 |
| Ethyl Acetate | 79.63 | 76.39 |
| Water-methanol | 17.66 | 16.94 |

According to Table 1, 104.24 g of methanolic extract provided the highest yield, namely the ethyl acetate fraction of 76.39 %, followed by the water-methanol fraction of 16.94 % and the n-hexane fraction of 6.69 %. These results show that the chemical compounds in *Dendrophthoe pentandra* leaves are predominantly semipolar, followed by polar chemicals and, lastly, non-polar compounds. To separate the chemicals based on their polarity, fractionation was performed using polar, semipolar, and non-polar solvents. Non-polar chemicals are dissolved by N-hexane, while semipolar and polar compounds are dissolved by ethyl acetate and water-methanol. The fractionation results in the form of fractions of n-hexane, ethyl acetate, and water-methanol were examined for antioxidant activity using TLC and sprayed with DPPH. Table 2 and Figure 1 show the results of the antioxidant activity test on the fractions.

The intensity of the yellow spots on the TLC plate following spraying with DPPH was used to classify antioxidant activity. An intense yellow spot on the TLC plate indicates significant antioxidant activity. The n-hexane and ethyl acetate fractions in Table 2 and Figure 1 exhibit high antioxidant activity, but the water-methanol fraction has low antioxidant activity. The study's results are consistent with findings on the mistletoe of duku Medan (*Dendrophthoe pentandra*) (Hardiyanti et al., 2018). The study suggested that ethyl acetate and n-hexane fractions had more powerful antioxidant capabilities than water-methanol ones.

Table 2. The antioxidant activity of fractions from mistletoe leaf methanol extract following DPPH treatment

| Fraction | Rf | Category | Criteria |
|----------------|----------|----------|-------------------------------|
| N-hexane | 0.7; 0.4 | +++ | strong antioxidant activity |
| Ethyl Acetate | 0.6 | ++ | moderate antioxidant activity |
| Water-methanol | 0.4 | + | weak antioxidant activity |

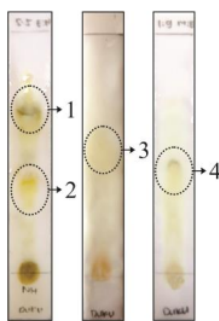


Figure 1. Chromatogram showing the results of the antioxidant activity test using DPPH of the methanol extract fractions of mistletoe leaves, with the stationary phase of GF-254 and the mobile phase of n-hexane: ethyl acetate (8:2). Number 1 and 2 shows the antioxidant compound of the n-hexane fraction, Number 3 shows The antioxidant compound of ethyl acetate fraction, Number 4 shows the antioxidant compound of the water-methanol fraction

According to the findings shown in Figure 1, the n-hexane and ethyl acetate fractions have strong antioxidant activity, whereas the water-methanol fraction has weak antioxidant activity. The variation in the intensity of the yellow spots on the TLC plate indicates the outcome. (Rumengan & Mantiri, 2015) suggested that the presence of antioxidant chemicals can lower the intensity of the original purple color of DPPH and turn it yellow. The intensity of the color indicates the efficacy of antioxidant activity. A strong antioxidant activity will decrease the purple color intensity and increase the yellow color intensity simultaneously. Consequently, the higher the overall antioxidant activity, the higher the concentration of the compounds responsible for the fraction. Nonetheless, tailings may still be seen in the chromatogram. The parameters that cause tailings are excessive sample volume applied to the TLC plate, TLC plate activity, developer distance, and chamber saturation (Sherma & Fried, 2003).

The occurrence of yellow spots on the TLC plate is due to the antioxidant compound donating its atoms to DPPH free radicals. Antioxidant compounds could respond to DPPH radicals through the mechanism of hydrogen atom contribution, causing color degradation from purple to yellow. Therefore, the spots that gave a yellow color change indicated the presence of a reaction between free radicals and antioxidants (Handayani et al., 2014).

Vacuum liquid chromatography of fractions

The n-hexane and ethyl acetate fractions were analyzed by liquid chromatography under vacuum (VLC). Chromatograms were sprayed with DPPH in order to evaluate their antioxidant activity. Figure 2 depicts the assessment of antioxidant activity within fractions of VLC results.

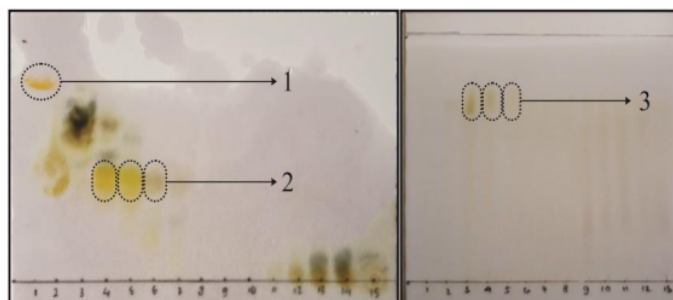


Figure 2. Chromatogram of testing the antioxidant activity of subfraction of mistletoe leaves by Vacuum liquid chromatography, with the stationary phase GF-254 and the mobile phase of n-hexane: ethyl acetate (8:2) and detection by DPPH

According to Figure 2, the chromatogram of the subfraction that was eluted and sprayed with DPPH produced the active antioxidant from the n-hexane fraction in eluates 1 and 4. Likewise, eluates 5 and 6 of ethyl acetate fraction possess antioxidant properties. Similarly, eluates 3, 4, and 5 exhibit antioxidant activity indicated by the occurrence of yellow dots on a purple background.

The TLC result of the n-hexane and ethyl acetate fractions produced six active subfractions, including four from the n-hexane subfraction and three from the ethyl acetate subfraction. Since each had the same Rf value, subfractions 3, 4, and 5 of the n-hexane fraction were combined in the same vial. A similar occurrence was discovered in antioxidant subfractions 3, 4, and 5 of the ethyl acetate fraction. (Sopiah et al., 2019) suggested that the similar Rf value in compounds suggests the comparable properties of the compounds. Compounds with higher Rf value have low polarity and vice versa.

Isolation and identification of antioxidant compounds

Chromatographing the active subfraction allowed the isolation of antioxidant chemicals. Compound NH.1 denotes pure antioxidant compounds from subfraction 1 of n-hexane. Antioxidant compounds isolated from subfractions 4, 5, and 6 of the n-hexane fraction were designated as NH.3 compounds. Compound EA.3 was assigned to antioxidant compounds from subfractions 3, 4, and 5 of the ethyl acetate fraction. The obtained antioxidant compounds were then determined. Table 3 presents the results of the chromatogram of antioxidant compounds.

The antioxidant compound was identified using a 2% H₂SO₄ spray and heating treatment. The compound NH.1 was placed on the TLC plate, sprayed with 2% H₂SO₄, and heated until purple spots appeared. The presence of terpenoids was shown by the change in color of the compound NH.1. The results obtained in this study are consistent with those of (Lau & Wuru, 2018), that discovered terpenoids within the methanolic extract of *M. iasa* (*Melochiaum bellata*) leaves utilizing the similar method. Several terpenoids found in plants have been reported to be responsible for their antioxidant activity. Likewise, NH.3 and EA.3 compounds were also identified. The results showed that NH.3 and EA.3 show yellow spots with comparable Rf values of 0.51 and 0.62, consecutively. According to (Lisi et al., 2017), flavonoids were identified by the formation of yellow spots on the TLC plate after being sprayed with 2% H₂SO₄ and heated.

Table 3 summarizes the result of isolation and identification. NH.1 is a terpenoid, whereas NH.3 and EA.3 are flavonoids. The presence of terpenoids and flavonoids in the mistletoe parasite of duku is consistent with the reports of various studies on mistletoes. Research by (Fahmi & Bulan, 2018) discovered terpenoids in glodokan mistletoes. In another study, Lekal and (Lekal & Watuguly, 2017) discovered flavonoids in tea mistletoes.

Table 3. Rf values, spot color, and the compound group from mistletoe leaves

| Compound | Rf | Color | Group of compounds |
|----------|------|--------|--------------------|
| NH.1 | 0.69 | Purple | Terpenoid |
| NH.3 | 0.51 | Yellow | Flavonoid |
| EA.3 | 0.62 | Yellow | Flavonoid |

Terpenoids are one of the most abundant and diversified substances in nature. Terpenoids are one example of antioxidants that can be found in plants. They display an extensive array of biological and pharmacological activities. Their antioxidant properties have significantly protected against oxidative stress in numerous diseases, including liver, renal, neurological, and cardiovascular disorders, cancer, diabetes, and aging (Guilherme et al., 2013).

Terpenoids naturally serve multiple essential functions in both plants and humans. Carotenoids, a subclass of terpenoids, are crucial to eyesight health together with provitamin A. Three main mechanisms of carotenoid antioxidant activity have been identified to date, i.e., singlet oxygen quenching, hydrogen transfer, or electron transfer. Another subclass of terpenoids, Limonene, is a promising substance in cancer therapy when utilized with perillyl alcohol. In addition, antioxidant compounds affect the human immune system. It is considered that the antioxidant capacity of terpenoids accounts for the health-promoting characteristics of fruits and vegetables (Graßmann, 2005).

Flavonoids are a group of small-molecular-weight compounds linked by a polyphenol skeleton. Flavonoids are classified into flavonols, flavones, flavanones, isoflavones, flavonols, and flavanols by distinctions of oxidation and saturation located in the chemical structure, specifically the heterocyclic C-ring. Using natural flavonoids, numerous in vitro and in vivo studies have confirmed the correlation between the structure within the flavonoid group and antioxidant activity. (Lisi et al., 2017) investigated particular chemical structures in flavonoids that are responsible as an antioxidant. The research showed that hydroxyl groups, ortho-dihydroxy arrangement in ring B, C2-C3 unsaturated bonds paired with C-4 carbonyl groups in the C-frame, and O-methylation are associated with the antioxidant activity of flavonoids.

As antioxidants, flavonoids delay, prevent, and eliminate oxidative damage to target molecules. The detailed antioxidant mechanism of flavonoids consists of quenching free radicals, chelating metals, inhibiting enzymes involved in free radical formation, and stimulating the activity of antioxidant enzymes. The primary antioxidant capabilities of flavonoids are derived from their capability to directly scavenge reactive oxygen species. By donating hydrogen atoms or transferring a single electron, flavonoids can chelate free radicals (Procházková et al., 2011). The impact of flavonoid hydroxyl structure can stimulate the production of antioxidant enzymes (Guilherme et al., 2013). The free hydroxyl structure can stabilize radical molecules by donating their hydrogen atom, generating a comparatively stable radical called flavonoid phenoxyl. Not only is it more stable, but the radical can also react with the next radical to form a favorable quinone structure (Amic et al., 2007). The position and the number of hydroxyl groups significantly impact the antioxidant potency (Dugas et al., 2000).

In addition, the B-ring hydroxyl structure plays an important role as a scavenger of free radicals that oxygen and nitrogen center (Santos & Mira, 2004). The hydroxyl groups in these nuclei contribute hydrogen and electrons to radicals such as hydroxyl, peroxy, and peroxy nitrite. The process stabilizes the radical and generates fairly stable flavonoid radicals. The study revealed that the incidence of hydroxyl groups in ring B and the number of hydroxyl groups were proportional to the antioxidant activity. According to (Moalin et al., 2011), quercetin and its derivatives have the highest antioxidant activity compared to other compounds. (Celik & Arinç, 2010) revealed that quercetin has a greater antioxidant capacity than its flavonoid derivatives, such as rutin and naringenin. Moreover, the hydroxyl group simultaneously is a potent attractant for ROS and RNS.

Antioxidant activity test

The pure compounds obtained using the DPPH method are then tested for antioxidant activity by calculating IC_{50} . The results of IC_{50} are reported in Table 4 and Figure 3. The classification of antioxidant activity is based on research conducted by (Phongpaichit et al., 2007), which stated that antioxidant compounds with IC_{50} values $<10 \mu\text{g/mL}$ belonged to the group of very strong antioxidant activity, those with $10\text{-}50 \mu\text{g/mL}$ are strong, those with $50\text{-}100 \mu\text{g/mL}$ are moderate, those with $1000\text{-}250 \mu\text{g/mL}$ are weak, and those with $>250 \mu\text{g/mL}$ are inactive. Both table and figure show that ascorbic acid as a positive control has an IC_{50} value of $8.9 \mu\text{g/mL}$, thus classified as having very strong antioxidant activity. The compound NH.1 had a weak antioxidant activity with IC_{50} values of $151.35 \mu\text{g/mL}$, while NH.3 and EA.3 had a moderate antioxidant activity with IC_{50} values of 96.64 and $78.37 \mu\text{g/mL}$, respectively. (Matheos, 2014) highlighted that the smaller the IC_{50} value, the more effective the compound can act as a radical scavenger.

Table 4. Antioxidant activity test of compounds from *Dendrophthoe pentandra*, by DPPH method

| Compound | Concentration ($\mu\text{g/mL}$) | Inhibition (%) | IC_{50} ($\mu\text{g/mL}$) | Antioxidant Activity (Phongpaichit et al., 2007) |
|-----------------|------------------------------------|----------------|--------------------------------|--|
| Ascorbic Acid | 1000 | 75.52 | 8.9 | Very strong |
| | 500 | 71.29 | | |
| | 250 | 70.23 | | |
| | 125 | 67.98 | | |
| | 62.5 | 66.66 | | |
| NH ₁ | 1000 | 67.06 | 151.35 | Weak |
| | 500 | 59.92 | | |
| | 250 | 55.42 | | |
| | 125 | 52.11 | | |
| | 62.5 | 41.00 | | |
| NH ₃ | 1000 | 77.11 | 96.64 | Moderate |
| | 500 | 67.19 | | |
| | 250 | 63.35 | | |
| | 125 | 51.98 | | |
| | 62.5 | 47.35 | | |
| EA ₃ | 1000 | 89.41 | 78.37 | Moderate |
| | 500 | 82.8 | | |
| | 250 | 63.49 | | |
| | 125 | 59.52 | | |
| | 62.5 | 50.79 | | |

The compound NH.1 was classified as a terpenoid, had an IC_{50} value of $151.35 \mu\text{g/mL}$, and was categorized as a weak antioxidant. The IC_{50} value of the compound NH.1 from the parasite's leaves of duku is stronger than the IC_{50} value of the terpenoid compound from the fruit of *Clidemia hirta* (L.) D.Don with a value of $327.01 \mu\text{g/mL}$ (Fadhli et al., 2020). According to (Setiawan & Amalia, 2017), terpenoids work as antioxidants by donating hydrogen atoms so that they inhibit the occurrence of lipid peroxidation or LPO (damage to lipids in cell membranes by free radicals), which have the potential as free radicals.

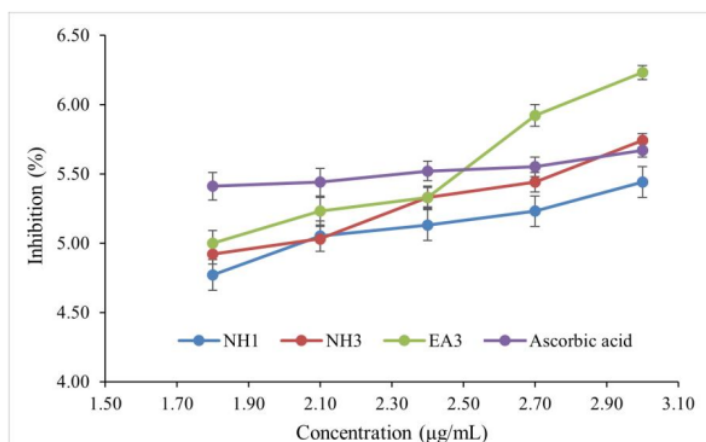


Figure 3. Graph inhibition (%) for standard ascorbic acid and various concentrations of mistletoe leaf-derived compounds

The terpenoid contained in NH.1 exhibited an IC_{50} value of 151.35 $\mu\text{g/mL}$ and was classified as a weak antioxidant. However, when compared to the terpenoid compound from the fruit of *Clidemia hirta* (L.) D.Do, which is 327.01 $\mu\text{g/mL}$ (Fadhli et al., 2020), The duku mistletoe shows more significant antioxidant activity. (Setiawan & Amalia, 2017) investigated that terpenoids functioned as antioxidants by donating hydrogen atoms, inhibiting the incidence of lipid peroxidation (LPO), which is damage to lipids in cell membranes caused by free radicals.

The compounds NH.3 and EA.3 are flavonoids having moderate antioxidant activity with IC_{50} values of 96.64 and 78.37 $\mu\text{g/mL}$, respectively. The potency is indeed mediocre compared to other studies of parasite-derived flavonoids. Flavonoids from the methanolic extract of the stem of the parasite *Scurrula ferruginea* indicated an IC_{50} value of 27.81 $\mu\text{g/mL}$ (Marvibaigi et al., 2016). Similarly, the results of flavonoids from the leaves of *Dendrophthoe pentandra* have strong antioxidant activity with an IC_{50} value of 36.23 $\mu\text{g/mL}$ (Sembiring et al., 2016). However, compared to more general plant-derived flavonoids, flavonoids in this study performed adequately. NH.3 and EA.3 showed stronger antioxidant activity than johar leaf-derived flavonoids with an IC_{50} value of 139.84 $\mu\text{g/mL}$ (Ningrum et al., 2017). Therefore, the compounds of NH.1, NH.3, and EA.3 from the *Dendrophthoe pentandra* (L.) Miq has the potential as an antioxidant and needs further investigation for its characterization and efficacy.

CONCLUSION

Several fractions derived from the parasite leaf of duku (*Dendrophthoe pentandra*) exhibit various antioxidant properties. The n-hexane fraction possessed strong antioxidant activity, the ethyl acetate fraction showed moderate activity, and the water-methanol fraction showed weak activity. NH.1 and NH.3 were isolated from the n-hexane fraction and classified as a terpenoid and flavonoid, respectively. EA.3 obtained after the ethyl acetate fraction was classified as a flavonoid. NH.1 had an IC_{50} value of 151.35 $\mu\text{g/mL}$, indicating weak antioxidant activity. Meanwhile, NH.3 and EA.3 had a moderate antioxidant activity, proven with IC_{50} values of 96.64 and 78.37 $\mu\text{g/mL}$.

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