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ANTIOXIDANT TESTING OF MANGGO CONSTRAINT LEAVES EXTRACT (*DENDROPHTHOE PENTANDRA*) DEKOKTA RESULTS

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ABSTRACT

Mango mistletoe leaves have been identified as a natural source of antioxidants because they contain the quarcetin. The aim of this research is to determine whether mango mistletoe *Dendrophthoe pentandra* leaf extract extracted using the dekokta method has antioxidant activity if tested using the DPPH method. This research was carried out by extracting samples of mango mistletoe leaf powder *Dendrophthoe pentandra* using the decoction method, the extract obtained was tested for antioxidant activity using the DPPH method using UV-Vis spectrophotometry with a comparison in the form of vitamin C. The results of the mango mistletoe leaf extract was tested at a wavelength of λ 521.5 nm obtained with an IC_{50} value of 322.80 ppm, with SD ± 107.08 and RSD 33.17%. while for ascorbic acid the IC_{50} value was 53.89 ppm, with SD ± 6.39 and RSD 11.86%. This research can be concluded that the dekokta mango mistletoe leaf extract has very weak antioxidant activity while ascorbic acidhas relatively strong antioxidant activity. It would be advisable to measure the levels of secondary metabolites contained in the extract of mango mistletoe and it is necessary to carry out research on the antioxidant activity of mistletoe mango leaf extract using methods other than the DPPH method.

Keywords: *Antioxidants; Decoction; Dendrophthoe pentandr; DPPH.*

I. INTRODUCTION

The body possesses natural defence mechanisms to protect itself from the harmful effects of free radicals and thus safeguard health. However, environmental factors and unhealthy habits such as exposure to ultraviolet radiation, pollution, consumption of fast food, and smoking can weaken the body's defence system against excessive free radicals. Free radicals are reactive molecules characterised by

unpaired electrons in their outer shell, formed when molecules lose electrons and become unstable. Additionally, free radicals are natural by-products of cellular metabolism. Their presence in the human body contributes to the development of various degenerative diseases, including cancer, atherosclerosis, rheumatoid arthritis, coronary heart disease, cataracts, and neurodegenerative conditions such as Parkinson's disease (Rizkayanti et al., 2017). These free radicals are significant factors in the aetiology of degenerative diseases and the ageing process. Reactive oxygen species can induce oxidative stress, which is typically counterbalanced by certain antioxidant enzymes (Brito et al., 2012). Antioxidants actively neutralise excess free radicals by capturing them and preventing damaging chain reactions.

Antioxidants are categorised into two types based on their source, natural and synthetic. Natural antioxidants produced within the body include enzymes such as superoxide dismutase, glutathione, and catalase, whereas natural antioxidants obtained externally include vitamin C, E, β-carotene, xanthophyll, and flavonoids. Conversely, synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ), despite their benefits, may cause serious side effects if used continuously, including an increased risk of carcinogenesis (Rahmi et al., 2021). Therefore, there is a need for readily available natural antioxidant sources that are abundant in nature and have fewer side effects compared to synthetic antioxidants. One promising natural antioxidant source is the mango mistletoe leaf (*Dendrophthoe pentandra*).

Dendrophthoe pentandra is commonly found in Indonesia, as much of the country's terrain consists of lowlands suitable for the growth of mango trees (Nurfaat et al., 2016). This parasitic plant possesses antiproliferative and androgenic properties and contains various bioactive compounds such as flavonoids, tannins, amino acids, carbohydrates, alkaloids, and saponins. The flavonoids in mango mistletoe function by inhibiting key phases in the biosynthesis of prostaglandins, targeting enzymes including cyclooxygenase, aldose reductase, monoamine oxidase, protein kinase, DNA polymerase, and lipoxygenase. The primary phytochemical compounds identified in *Dendrophthoe pentandra* include flavonoids, tannins, alkaloids, saponins, steroids, phenols, terpenoids, and quercetin. Quersetin is the principal flavonoid component and exhibits notable antioxidant activity (Setyawardana *et al.*, 2013). Furthermore, mango mistletoe has demonstrated benefits as an antiplasmodium agent, a colon cancer inhibitor, and in reducing cholesterol and Low Density Lipoprotein (LDL) levels (Nurfaat et al., 2016). *Dendrophthoe pentandra* is also utilised to alleviate inflammation caused by breast cancer (Mustarichie et al., 2015), and exhibits antibacterial (Lazuardi et al., 2022) and anti-ulcer properties (Fitrya et al., 2023). Mango mistletoe leaves are extracted using the decoction method with distilled water as the solvent. This decoction technique is a simple water-based extraction process that does not require complex laboratory or industrial equipment (Lestari et al., 2016). Distilled water is preferred due to its affordability, accessibility, and relative safety for use (Novita et al., 2016).

A commonly employed free radical model for assessing radical scavenging activity is 1,1-diphenyl-2-picrylhydrazyl (DPPH). DPPH is a stable free radical with a 515 and 520 nm absorption peak. The DPPH assay is based on reducing the purple-coloured methanolic DPPH solution by an electron-donating substance. Upon interaction with an electron donor, the purple colour fades and changes to yellow due to the formation of the picryl group (Tristantini et al., 2016). These findings support the use of medicinal plants as alternatives to synthetic antioxidants and encourage the adoption of healthier lifestyles. This study aims to

determine the constituents and evaluate the antioxidant activity of decoction extracts from mango mistletoe leaves (*Dendrophthoe pentandra*) collected from the Akademi Farmasi Surabaya.

II. METHOD

Materials and Equipment

The equipment includes an analytical balance (Ohaus, USA), a UV-Vis spectrophotometer (Thermo, USA), a blender, micropipettes, and standard laboratory glassware. The material utilised was leaves *Dendrophthoe pentandra* sourced from the campus of the Akademi Farmasi Surabaya and authenticated by the Indonesian Biological Generation Foundation BT-022516. The chemicals used comprised p.a. methanol (Fulltime), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Tokyo Chemical Industry, Japan), ascorbic acid, and distilled water.

Preparation of Mango Mistletoe Leaf Extract

Dried mango mistletoe leaves was ground finely, then weighed to 100 g and extracted by decoction using 400 ml of distilled water as the solvent. The decoction extraction method is similar to infusion, differing mainly in the heating duration; decoction requires a longer heating time of 30 minutes once the temperature reaches 90°C (Awang et al. 2023).

Determination of Antioxidant Activity

A DPPH solution was prepared by dissolving 4 mg of DPPH powder in 100 ml of p.a. methanol to obtain a 40 ppm concentration. The blank solution was made by pipetting 2 ml of the DPPH solution and 1 ml of distilled water into a test tube, mixing thoroughly, and incubating for 30 minutes. The absorbance of the 40 ppm DPPH solution was measured using a UV-Vis spectrophotometer over the wavelength range of 400–800 nm to determine the optimum wavelength. Ten milligrams of ascorbic acidwere weighed and dissolved in 100 ml of distilled water, then mixed until homogeneous. A series of ascorbic acidsolutions at concentrations of 1, 2, 3, 4, and 5 ppm were prepared by pipetting 0.01, 0.02, 0.03, 0.04, and 0.05 ml of the stock solution into separate 10 ml volumetric flasks, topped up with distilled water, and mixed thoroughly. Similarly, 10 mg of the decoction extract was dissolved in 10 ml of distilled water and mixed well. A series of mango mistletoe leaf extract solutions at concentrations of 10, 20, 30, 40, and 50 ppm were prepared by pipetting 0.1, 0.2, 0.3, 0.4, and 0.5 ml of the stock extract solution into 10 ml volumetric flasks, topped up with distilled water, and mixed thoroughly. One millilitre of each standard and extract solution was pipetted into test tubes, followed by the addition of 2 ml of DPPH solution. The mixtures were incubated for 30 minutes before measuring absorbance at the maximum wavelength using the UV-Vis spectrophotometer. Antioxidant activity was calculated by determining the inhibitory concentration (IC_{50}), which is the concentration of extract or ascorbic acidthat provides 50% antioxidant activity compared to the control, based on a linear regression equation between concentration and percentage radical scavenging (Andriani et al., 2020).

The percentage of free radical inhibition can be calculated following the method described by Umarudin et al. (2020). The calculated values are then plotted into the regression equation $Y = a + bx$, where the extract concentration (ppm) serves as the independent variable (X-axis) and the percentage inhibition (% antioxidant activity) as the dependent variable (Y-axis). The IC_{50} value is determined from this calculation as the concentration at which 50% inhibition occurs.

III. RESULTS AND DISCUSSION

The research utilised mango mistletoe leaves (*Dendrophthoe pentandra*) collected from Ketintang Madya No. 81, Surabaya. The samples were authenticated to ensure their accuracy and avoid selection errors. Based on the identification test, the plant used in this study was confirmed to be mango mistletoe,

scientifically named *Dendrophthoe pentandra*. This species belongs to the family Loranthaceae. Following identification, the mango mistletoe leaves were ground to increase the surface area for extraction. The resulting simplicia was then extracted using the decoction method. This method was chosen due to its simplicity and the fact that it does not require complex laboratory or industrial equipment (Lestari et al., 2016). Distilled water was selected as the solvent because it is inexpensive, readily available, and relatively safe to use (Novita et al., 2016). The extraction yielded 107.4 ml of liquid extract, exhibiting a dark greenish-brown colour. The results of the decoction extraction of mango mistletoe leaves are illustrated in Figure 1.

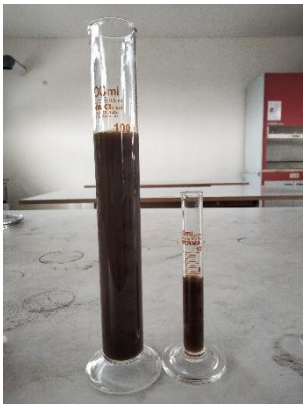

Figure 1. Extract of Mango Mistletoe Leaves

The DPPH assay in this study was conducted by determining the maximum wavelength within the 400–800 nm range. Subsequently, the optimised maximum wavelength was obtained as follows:

Table 1. Results of Maximum Wavelength and Blank Absorbance

Blank	λ Maximum (nm)	Absorbance (A)
Ascorbic acid testing	521,5	0,671
Mango Mistletoe Leaf Extract Testing	521,5	0,699

Table 1 shows that the ascorbic acid assay's maximum wavelength (λ max) was 521.5 nm, producing an absorbance of 0.671 A. Similarly, the mango mistletoe leaf extract exhibited a maximum wavelength of 521.5 nm with an absorbance of 0.699 A. The absorbance results for the ascorbic acid assay are presented in Table 2.

Table 2 shows that as the concentration of ascorbic acid increases, the absorbance value decreases. The percentage of inhibition (% scavenging) was subsequently calculated based on the obtained absorbance data. The % inhibition value is a key parameter indicating the ability of an antioxidant to neutralise free radicals (Pratiwi et al., 2023).

The results of the % inhibition calculations for the series of ascorbic acid standard solutions are presented below.

Table 2. Absorbance Results of Ascorbic Acid Standard Solution Series

Tretment	Concentration (ppm)	Absorbance
Replication 1	1	0,605
	2	0,599
	3	0,595
	4	0,593
	5	0,583
Replication 2	1	0,606
	2	0,600
	3	0,598
	4	0,593
	5	0,580
Replication 3	1	0,592
	2	0,579
	3	0,578
	4	0,576
	5	0,572

Table 3. Percentage Inhibition Results of the Ascorbic Acid Standard Solution Series

Concentration (ppm)	% Damping For Replication To-			Average % Inhibition
	1	2	3	
1	9,836	9,687	11,773	10,432
2	10,730	10,581	13,711	11,674
3	11,326	10,879	13,860	12,022
4	11,624	11,624	14,158	12,469
5	13,115	13,562	14,754	13,810

Table 3 shows that as the concentration increases, the percentage of inhibition also rises. This finding is supported by previous research which stated that higher concentrations of the test solution correspond to greater percentage inhibition (Islami et al., 2022). The obtained inhibition percentages were subsequently used to derive a linear regression equation, with the concentration of ascorbic acidas the independent variable (X) and the percentage inhibition as the dependent variable (Y). Here are the results of the IC_{50} calculations using the linear regression equations obtained from each replication. The IC_{50} value was then determined by substituting Y with 50 as follows:

Table 4. IC_{50} Calculation Results for the Ascorbic Acid Standard Solution Series

Replication 1	y = 0,7452x + 9,0906 50 = 0,7452x + 9,0906 50 - 9,0906 = 0,7452x x = 54,8972 ppm
Replication 2	y = 0,8793x + 8,6287 50 = 0,8793x + 8,6287 50 - 8,6287 = 0,8793x x = 47,0503 ppm
Replication 3	y = 0,6409x + 11,729 50 = 0,6409x + 11,729 50 - 11,729 = 0,6409x x = 59,7145 ppm

After calculating the IC_{50} values using the linear regression equations for each replication, the average IC_{50}, standard deviation (SD), and relative standard deviation (RSD) were determined. The IC_{50}, SD, and RSD values for the series of ascorbic acid standard solutions are presented in Table 5.

Table 5. IC_{50}, SD, and RSD Values for the Ascorbic Acid Standard Solution Series

Replication	Linear Regression Equation	IC_{50} (ppm)	Average (ppm)	SD	RSD
1	y = 0,7452x + 9,0906 r = 0,9731	54,8972	53,89	± 6,39	11,86
2	y = 0,8793x + 8,6287 r = 0,9530	47,0503			
3	y = 0,6409x + 11,729 r = 0,9020	59,7145			

Table 5 shows that the average IC_{50} value of ascorbic acid is 53.89 ppm, with a standard deviation (SD) of ±6.39 and a relative standard deviation (RSD) of 11.86%. The obtained IC_{50} value falls within the strong category. Ascorbic acid is an antioxidant that donates one or two hydrogen atoms to bind with superoxide radicals. When a single hydrogen atom is donated, the superoxide radical is reduced to a hydroperoxyl radical, while ascorbic acidbecomes negatively charged. If two hydrogen atoms are donated, the superoxide radical forms hydrogen peroxide and an ascorbate radical (Rahmadi et al., 2018). The absorbance results for the mango mistletoe leaf extract assay are presented in Table 6.

Table 6. Absorbance Results of the Mango Mistletoe Leaf Extract Standard Solution Series

Mango Mistletoe Leaf Extract	Concentration (ppm)	Absorbance
Replication 1	10	0,599
	20	0,588
	30	0,580
	40	0,571
	50	0,544
Replication 2	10	0,596
	20	0,590
	30	0,583
	40	0,580
	50	0,571
Replication 3	10	0,589
	20	0,585
	30	0,572
	40	0,566
	50	0,562

Table 6 shows that as the mango mistletoe leaf extract concentration increases, the absorbance value decreases. The percentage of inhibition (% scavenging) was subsequently calculated based on the obtained absorbance data. The % inhibition value is a key parameter indicating the ability of an antioxidant to neutralise free radicals (Pratiwi et al., 2023). The results of the % inhibition calculations for the series of standard solutions of *Dendrophthoe pentandra* leaf extract are presented below.

Table 7. Percentage Inhibition Results of the *Dendrophthoe pentandra* Leaf Extract Standard Solution Series

Concentration (ppm)	% Damping For Replication To-			Average % Inhibition
	1	2	3	
10	14,306	14,735	15,737	14,926
20	15,880	15,594	16,309	15,928
30	17,024	16,595	18,169	17,263
40	18,312	17,024	19,027	18,121
50	22,174	18,312	19,599	20,028

Table 7 shows that as the concentration increases, the percentage of inhibition also rises. This finding is supported by previous research, which stated that higher test solution concentrations correspond to greater percentage inhibition (Islami et al., 2022). The obtained inhibition percentages were then used to derive a linear regression equation, with the concentration of *Dendrophthoe pentandra* extract as the independent variable (X) and the percentage of inhibition as the dependent variable (Y). The results of the IC_{50} calculations using the linear regression equations obtained from each replication.

Table 8. IC_{50} Calculation Results for the *Dendrophthoe pentandra* Extract Standard Solution Series

Replication 1	y = 0,1817x + 12,089 50 = 0,1817x + 12,089 50 - 12,089 = 0,1817x x = 208,6461 ppm
Replication 2	y = 0,0858x + 13,877 50 = 0,0858x + 13,877 50 - 13,877 = 0,0858x x = 421,0140 ppm
Replication 3	y = 0,1044x + 14,636 50 = 0,1044x + 14,636 50 - 14,636 = 0,1044x x = 338,7356 ppm

The results of the IC_{50} calculations using the linear regression equations obtained from each replication.

Table 9. IC_{50} Calculation Results for the *Dendrophthoe pentandra* Extract Standard Solution Series

Replication	Linear Regression Equation	IC_{50} (ppm)	Average (ppm)	SD	RSD
1	y = 0,1817x + 12,089 r = 0,9636	208,6461	322,80	± 107,08	33,17
2	y = 0,0858x + 13,877 r = 0,9918	421,0140			
3	y = 0,1044x + 14,636 r = 0.9800	338,7356			

Table 9 shows that the average IC_{50} value of the *Dendrophthoe pentandra* extract is 322.80 ppm, with a standard deviation (SD) of ±107.08 and a relative standard deviation (RSD) of 33.17%. This IC_{50} value falls within the very weak category. The IC_{50} represents the concentration of the sample solution required to inhibit 50% of the DPPH free radicals. The lower the IC_{50} value, the stronger the antioxidant's ability to neutralise free radicals, indicating higher antioxidant activity. Specifically, a compound is considered a powerful antioxidant if the IC_{50} is less than 50 ppm, strong between 50 and 100 ppm, moderate between 100 and 150 ppm, and weak if between 151 and 200 ppm (Islami et al., 2022).

The IC_{50} value obtained from the antioxidant activity test using the DPPH method on the *Dendrophthoe pentandra* extract was 322.80 ppm, indicating weak activity. This result shows that the IC_{50} value of *Dendrophthoe pentandra* leaves is higher than that of ascorbac acid. This study is compared with the research conducted by Kristiningrum et al. (2018), which reported that the water fraction, with an IC_{50} value of 29.46 ± 0.99 μg/mL, showed better antioxidant activity. This difference may be due to the decoction method, which extracts fewer compounds when using distilled water as the solvent. Additionally, it is possible that some secondary metabolites present in the leaves of *Dendrophthoe pentandra* extracted by the water solvent do not possess antioxidant activity. This may be due to the heating process during decoction extraction, which increases the risk of degradation of active compounds, thereby reducing their potential activity (Purnomo *et al.*, 2022). As a parasitic plant, the mistletoe may absorb nutrients suboptimally from its host. Mistletoe grows by extracting nutrients from its host plant (Artanti et al., 2012; Werdyani et al., 2019).

Mango mistletoe leaves contain flavonoids and quercetin, which are believed to function as antioxidants (Afiqah et al., 2016; Permatasari et al., 2019; Mustarichie et al., 2012). The bioactive flavonoid compounds, including quercetin, act as antioxidant agents and cofactors in plant photosynthesis (Lazuardi et al., 2022). According to Mustarichie et al. (2015), the aqueous fraction of Dendrophthoe pentandra contains flavonoids, polyphenols, tannins, and quinones. The mechanism by which flavonoids scavenge free radicals begins with releasing hydrogen atoms, resulting in reactive flavonoid radicals. These flavonoid radicals then bind to free radicals, reducing or eliminating their reactivity (Lestari et al., 2023).

IV. CONCLUSION

The decoction extract of *Dendrophthoe pentandra* leaves exhibits antioxidant activity with an average IC_{50} value of 322.80 ± 107.08 ppm, which is categorised as very weak antioxidant activity. In contrast, ascorbic aciddemonstrated strong antioxidant activity with an average IC_{50} value of 53.89 ± 6.39 ppm.

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