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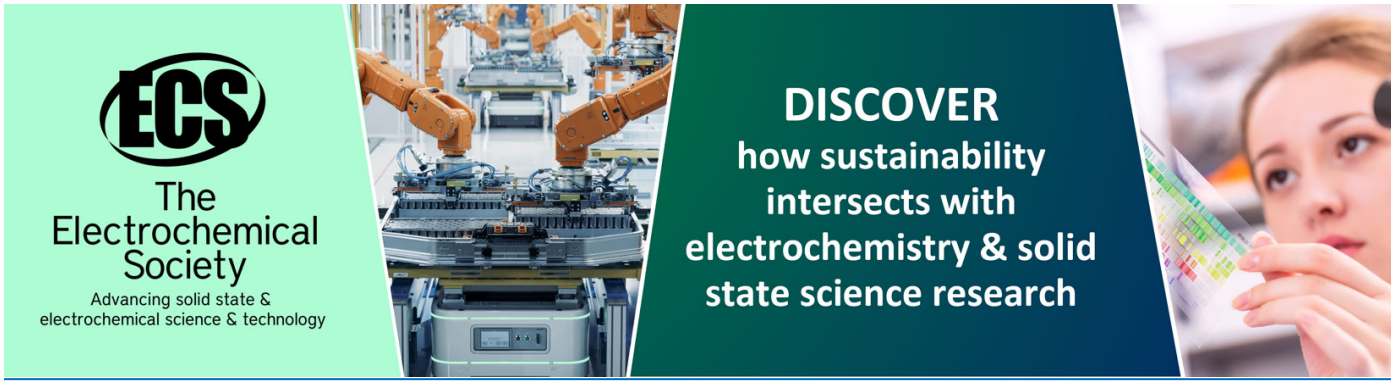

Phytochemical and antioxidant evaluation of ethanol extract leaves of *dendrophthoe falcata* (loranthaceae) hemiparasitic on *melia azedarach* host tree

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Abstract: The aim of the study was to identify the content of total phenolic and flavonoids, and to test the antioxidant activity of leaves extract of *Dendrophthoe falcata* hemiparasite on *Melia azedarach* plants. The research method was carried out by extracting dry powder from the leaves of *D. falcata* with ethanol. Ethanol extract was partitioned using consecutive *n*-hexane, chloroform, and ethyl acetate solvents. Each fraction was then analyzed for the content of total phenolic and total flavonoids by spectroscopic methods. The method for testing antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) by in vitro. The results showed that leaves of *D. falcata* were rich in phenolic compounds and flavonoid. Furthermore, in the antioxidant test showed ethyl acetate fraction had the highest activity, with IC_{50} 6.66 ± 0.11 µg /ml. This study shows that *D. falcata* leaves have potential as natural antioxidants.

1. Introduction

Dendrophthoe falcata is one of the hemiparasitic plant species found in tropical and sub-tropical regions. This plant is usually attached to the host *Mangifera indica* (Anacardiaceae), *Melia azedarach* (Meliaceae) and *Psidium guajava* (Myrtaceae). The *D. falcata* plant is used in the original treatment system as cooling, bitter, substance, aphrodisiac, narcotics and diuretics and is also useful for lung disease, asthma, menstrual disorders, and antifertilitas [1-2]. Methanolic extract and water of leaf *D. falcata* in animal models has a potent anti-inflammatory effect and antioxidant [3]. Several studies have shown that loranthus plants are also known to be a polio virus prevention [4], antimicrobial and anti-inflammatory [5], neuroprotective [6], antioxidant [7], and antidiabetic [8].

D. falcata is one of the parasitic plant species (Loranthaceae) which is widely found and used for traditional medicine in some countries such as China and India. The content of chemical compounds from parasites is generally influenced by the host plants [9]. A study comparing the chemical compounds found in the parasitic *Loranthus longiflorus* plant parasite *Casuarina equisetifolia* and *Ficus religiosa* showed different compound components. *L longiflorus* found in *C. equisetifolia* shows the main compound of myristic acid, vinyl ester (82.81%) which also has antimicrobial and anticancer activity. While *L. longiflorus* contained in *F. religiosa* plant is a major component of vinyl dekanoate (71.7%) and showed no antimicrobial and anticancer activity [10]. Similarly, the parasitic *Loranthus micranthus* in various plant stems such as *Persian americana*, *Baphia nitida*, *Kola acuminata*, *Penthaclethra macrophylla*, and *Azadiracta indica* show variation in various major component types depending on the host plant [11]. Previous research the leaves of *D. falcata* reported to contain compounds identified as lupeol, 3-β-acetoxy-12-ene-11-one and β-sitosterol [12]. In this article we will

report the phytochemical and antioxidant evaluation of ethanol extrat from leaves of *D. falcata* plant that hemiparasite on *Melia azedarach*.

2. Experimental Section

2.1. Apparatus and reagent
The tools used include the evaporator Buchi Rotavapor R-114, spectrophotometer, vortex mixers, and glassware. The materials used include organic solvents such as ethanol, methanol, *n*-hexane, ethyl acetate, and acetone, 2,2-diphenyl-1-picrilhidrazil (DPPH, Aldrich), ascorbic acid (Aldrich), folin-ciocalteu reagent, sodium carbonate, gallic acid (Sigma), rutine (Sigma), aluminum nitrate, potassium acetate, acetic acid, ammonia, sulfuric acid, wagner reagent, iron (III) chloride, acetic acid, leaf of *D. falcata* hemiparasite on *Melia azedarach*, and distilled water.

2.2. Preparation of extracts from leaves of D. falcata
The leaves of *D. falcata* hemiparasite on *Melia azedarach* were collected in October 2017 and determined by botanists. Voucher specimens (DF-01-2017) are stored in the Organic Laboratory. The dried powder leaves of *D. falcata* (2.5 kg) were extracted using ethanol for 24 hours. The extract was then evaporated so that the concentrated extract was obtained and produced brown residues for about 140 g. Ethanol extract of *D. falcata* (120 g) was partitioned using *n*-hexane, chloroform, and ethyl acetate, respectively. Each separated fraction was then concentrated and dried, so the concentrated fraction was obtained of *n*-hexane (11 g), chloroform fraction (19.8 g), ethyl acetate fraction (65.1 g), and residual ethanol fraction (10 g).

2.3. Phytochemical screening
The qualitative phytochemical screening was performed using reagent for terpenoid test, alkaloid test, phenolic test, and saponin test. Terpenoid and steroid test with Salkowski reagent**,** alkaloid test with Wagner reagents, phenolic test with with ferrous (III) chloride test, and foam test for saponin [13].

2.3.1. Terpenoid and steroid test. 2 ml of each *D. falcata* fractions (1%) were added with 10 drops of CH_3COOH and H_2SO_4 concentrated in 2 drops. The solution is shaken gently and left for several minutes. Positive test of steroids shows producing blue or green colour, while triterpenoid produces red or purple colour.

2.3.2. Alkaloid test. The alkaloid test was carried out by adding 2 mL of each sample to the test tube (1%) plus 2 mL of chloroform, and 2 mL of ammonia. The mixture is stirred and then filtered. The filtrate is then added to 3-5 drops of concentrated H_2SO_4 and then shaken to form two layers. The top layer is added with Wagner reagent 4-5 drops. Orange red sediment formation indicates that the sample contains alkaloids.

2.3.3. Phenolic test. The sample was dissolved in a suitable solvent, as much as 2 ml was introduced into the test tube and added a few drops of $FeCl_3$ solution, when a purple color was shown to be positive against phenolic.

2.3.4. Saponin test. Saponin test was done by foam formation method. In the test tube, 0.5 mL sample and 5 mL of distilled water were added. The mixture was shaken vigorously and the foam formed was observed. The foam then mixed with 3 drops of olive oil and shaken vigorously after being observed for emulsion formation.

2.4. Determination of total phenolic
Analysis of total phenolic content from samples was carried out using folin-ciocalteu reagents [14]. 200 µL of sample were mixed with 0.4 mL of folin-ciocalteu reagent, 0.4 mL of aquadest, and 2 mL of sodium carbonate (15% b/v). The mixture was incubated at 50^{o}C for 10 minutes. Then the absorbance was measured at 760 nm. The total phenolic content was calculated according to the standard curve

determined for gallic acid as a reference. Total phenolic content is expressed as mg Gallic Acid Equivalent (GAE) per g of plant extract.

2.5. Determination of total flavonoids

Total flavonoid content was determined by the spectrophotometric method, with slight modifications [15]. In the test tube, 0.5 mL sample was diluted in ethanol (80%), then add 0.1 mL of aluminum nitrate (10%), 0.1 mL of potassium acetate (1M), and 4.3 mL of ethanol (80%). The mixture was incubated at room temperature for 40 minutes. The absorbance of the solution was then measured by a spectrophotometer at 415 nm. The total flavonoid content was calculated based on the standard curve determined by rutine as a reference. The total flavonoid content is expressed as mg Equivalent Rutine (RE) per g of plant extract.

2.6. Determination Antioxidant Activity Test by DPPH Method

The antioxidant activity of each sample was tested using the DPPH (2,2-diphenyl-1-pikrilhidrazil) method, with a slight modification [16]. About 5 mL of the sample in various concentrations was mixed with 5 mL of DPPH solution in methanol (0.12 mM) and incubated at room temperature for 30 minutes. Absorption of each sample was determined using spectrophotometer at 516 nm. As a positive control, ascorbic acid was used. Each sample was tested with three repetitions. The inhibitory activity was calculated as the percentage of DPPH decrease compared to the control. Furthermore, from the calculation of inhibitory activity can be used to calculate antioxidant activity expressed as IC_{50} (concentration of samples that have a 50% inhibitory activity).

3. Results and discussion

Phytochemical studies of plant leaves of *D. falcata* hemiparasites on *Melia azedarach* plant (figure 1). The results of qualitative analysis which include the content of terpenoids, alkaloids, phenolics, and saponins are listed in table 1.

Figure 1. Leaves of *D. falcata*

Table 1. Qualitative phytochemical screening of the extracts and fractions from leaves of *D. Falcata*

Qualitative phytochemical screening	Extract ethanol total	*n*-hexane fraction	Chloroform fraction	Ethyl acetate fraction	Residual fraction of ethanol extract
Terpenoid & Steroid (Salkowski Test)	+++	+++	++	-	-
Alkaloid (Wagner Test)	+	-	+	-	-
Phenolic ($FeCl_3$ test)	+++	-	+++	+++	+++
Saponin (Foam test)	+++	-	-	-	+++

Note: Note: - = absent, + = present, ++ = present a lot; +++ = present very large number

In this study, quantitative analysis of total phenolic levels was used by spectrophotometric methods. Each sample was added with Folin-Ciocalteu reagent. Absorption of each mixture is measured at a wavelength of 760 nm. As standard phenolic compounds are used gallic acid at various concentrations (50-200 µg /mL) and absorbance is measured at the same wavelength. The measurement results obtained a calibration curve with a linear regression equation $y = 0.004x - 0.032$ ($R^2 = 0.985$). The total phenolic content is expressed as mg of Gallic Acid Equivalents (GAE) per g of plant extract. Meanwhile, to determine the total flavonoid content, rutin standard compounds were used at various concentrations (50-200 µg / ml) and absorbance was measured at a wavelength of 415 nm. The measurement results obtained by the regression equation as a calibration curve with a linear regression equation $y = 0.003x + 0.099$ ($R^2 = 0.963$). The total flavonoid content is expressed as mg Rutin Equivalents (RE) per g of plant extract. The results of the phenolic content and total flavonoids from the extract and each fraction were collected in table 2.

Table 2. Results of analysis of total phenolic content and flavonoids from leaves of *D. falcata*

Sample	Total phenolic content (TPC) (mg GAE/g)	Total flavonoid content (TFC) (mg RE/g)
Crude ethanol extract of *D.falcata*	670.0± 2.83	386.4± 1.1
n-hexane fraction	0	0
Chloroform fraction	1363.3 ±3.21	347.5± 1.9
Ethyl acetate fraction	1675.0± 2.70	731.4± 2.8
Residual of ethanol extract fraction	1072.5±5.40	354.4± 3.8

This study indicate the suitability between qualitative and quantitative data. Qualitative data showed phenolic compound content of total ethanol extract, chloroform fraction, ethyl acetate fraction, and ethanol extract residue, while n-hexane fraction showed negative test. The phenolic compound is a relatively polar compound, so it will dissolve in a relatively polar solvent as well. Terpenoid compound is a compound that is relatively less polar, so there are many in the fraction of *n*-hexane and a little on chloroform. The crude ethanol extract, chloroform fraction, etyl acetate fraction, and residual ethanol fraction leaves of *D. falcata* showed a total phenolic content as much as 670.0± 2.83; 1363.3 ±3.21; 1675.0± 2.70; 1072.5±5.40 mg Gallic Acid Equivalen (GAE)/g) respectively, while flavonoid total content 386.4± 1.1; 347.5± 1.9; 731.4± 2.8; and 354.4± 3.8 mg Rutin Equivalent (RE)/g) respectively. This data is consistent with the results of previous studies showing the presence of phenolic compounds from leaves *Loranthus micranthus* Linn, such as epicatechin, epicatechin-gallate, rutin, and peltatoside [17]. While in *Scurrula ferruginea* Danser (Loranthaceae) plants found phenol compounds, among others quercetin, quercitrin, and flavonol glycoside 4-O-acetylquercitrin [18].

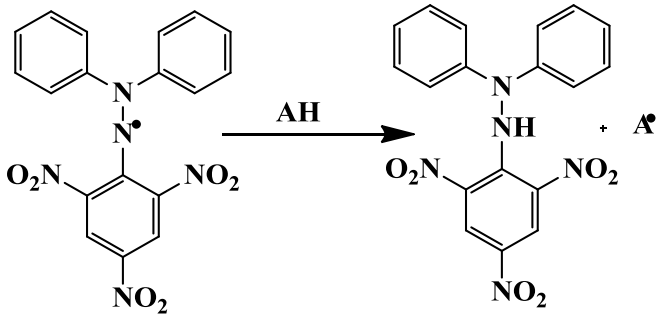

Figure 2. DPPH radical reaction with antioxidant (AH)

Test of antioxidant activity from plant samples can be done by various methods. One of the easiest methods to do is the DPPH method. The antioxidant activity test with the DPPH method is based on radical capture reactions by antioxidant compounds with the mechanism of transfer of oxygen atoms, which will produce a stable DPPH-H molecule [19]. Reduction of DPPH concentration is indicated by

a decrease in the intensity of purple solution compared to blank. Absorbance of each solution was measured using spectrophotometry at a wavelength of 516 nm. Antioxidant capacity is expressed as IC_{50} (μg/mL) from DPPH capture activity at various concentrations. The absorbance data of each sample was then made into a linear regression equation. From the regression equation used to calculate the IC_{50}. The reaction captures DPPH radicals by the antioxidants shown in the figure 2. The inhibitory activity of each sample and calculation of antioxidant activity data expressed as IC_{50} are shown in table 3.

Table 3. Antioxidant activity (IC_{50}) test leaves of *D. Falcata*

Sample	Data of antioxidant test			
	Concentration (μg/mL)	Inhibition activity (%)	The regression equation	Antioxidant activity (IC_{50} μg/mL)
Crude ethanol extract of *D.falcata*	25	86.63	y = 3.230x + 9.239 R^2 = 0.967	12.62
	12.5	58.25		
	6.25	27.11		
	3.125	16.39		
n-Hexane fraction of leaves *D. falcata*	100	19.25	y = 0.107x + 8.692 R^2 = 0.925	392.07
	50	13.74		
	25	12.98		
	12.5	8.82		
Chloroform fraction of leaves *D. falcata*	25	81.21	y = 2.531x + 18.91 R^2 = 0.995	12.28
	12.5	52.83		
	6.25	34.80		
	3.125	25.47		
Ethyl acetate fraction of leaves *D. falcata*	25	91.04	y = 2.315x + 34.58 R^2 = 0.975	6.66
	12.5	65.69		
	6.25	52.58		
	3.125	3.125		
Residual ethanol extract of leaves *D. falcata*	25	92.3	y = 3.198x + 19.13 R^2 = 0.883	9.65
	12.5	75.28		
	6.25	38.21		
	3.125	20.68		
Ascorbic acid (positive control)	5.00	95.59	y = 13.76x + 32.51 R^2 = 0.883	1.27
	2.50	79.82		
	1.25	51.45		
	0.625	32.28		

The high content of phenol and flavonoid compounds from each extract and fraction is associated with high antioxidant activity as well. The IC_{50} less than 10 μg/mL indicate very active antioxidant activity, such as positive controls used ascorbic acid. Ascorbic acid is a widely known antioxidant compound, exhibiting very high antioxidant activity and is commonly used as a positive control. The results showed that IC_{50} of ethyl acetate and chloroform fraction from leaves *D. falcata* were below than 10 μg/mL so that antioxidants were very active. The previous studies also showed the stem of *D. falcata* plant contained high phenolic and flavonoid compounds and showed very high antioxidant activity and medium antimicrobial activity [20].

4. Conclusion

From this study it can be concluded that leaves from *D. falcata* extract contains high phenolic and flavonoid. The most phenolic and flavonoids content is found in ethyl acetate fractions. The antioxidant test results showed total ethanol extract, *n*-hexane fraction; chloroform fraction, ethyl acetate fraction, and residues of ethanol fraction leaves of *D. falcata* with IC_{50} values 12.62 ± 0.15, 392.07 ± 4.22, 12.28 ± 0.42, 6.66 ± 0.11, and 9.65 ± 0.52 μg/mL respectively. The leaves of *D. falcata* can be developed as natural antioxidants.

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