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Sri handayani <handayani@uny.ac.id>

Fwd: Confirmation of paper

- sriatun <sriatun@uny.ac.id> 26 Maret 2019 17.14
Kepada: Sri handayani <handayani@uny.ac.id>

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From: <info@orientjchem.org>
Date: Wed, 4 Apr 2018, 12:49
Subject: Confirmation of paper
To: <sriatun@uny.ac.id>

Dear,

Dr.

I thankfully acknowledge the receipt of your valuable research paper entitled Study Potential Phenolic Compounds from Stem of Dendrophthoe falcata (Loranthaceae) Plant as Antioxidant and Antimicrobial Agents**(OJC-12003-18)**

We are processing your article for detail review and we will inform you accordingly.

INITIAL REVIEW

Make the initials corrections written as under otherwise the paper may be delay in the first phase.

1) In the abstract there must be one or two lines about the introduction and the importance of the work.

2) It should describe in brief the work actually done by the authors.

3) In few lines the abstract must describe the conclusion of the work & future applications.

In your abstract you have described major portion of the material and methods as well as the result is given in the form of data also, which is not required. So rewrite the abstract and reduce its matter to half (1/2)

Please send us your ORCID ID, along with the co-authors ORCID ID

The deadline for receiving your initial corrections is one week.

Thanks

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Sri handayani <handayani@uny.ac.id>

Fwd: Review Reports 12003

- sriatun <sriatun@uny.ac.id> 26 Maret 2019 17.11
Kepada: Sri handayani <handayani@uny.ac.id>

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From: <info@orientjchem.org>
Date: Mon, 10 Sep 2018, 11:48
Subject: Review Reports 12003
To: <sriatun@uny.ac.id>

Dear Dr. Sri Atun ,

Hope you are doing well, as per our guidelines we get two reviews done for each article separately to refine the content quality of our journal. Please find two separate review reports by both the reviewers attached (Review1 & Review2) and author response form 1 & 2 also we request you to go through both the reports and send us a final revised file including all the corrections suggested by both the reviewers. Also kindly send us filled author response form 1 & 2 within seven days.

Best Regards

Dr. S. A. Iqbal, Ph. D., FICS, FICC, FIAEM, MNASc.

Chief Editor

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Review Report of Manuscript

(Final Review Part A)

Title of the Journal : **Oriental Journal of Chemistry**

Title of the Manuscript : **Study Potential Phenolic Compounds from Stem of Dendrophthoe falcata (Loranthaceae) Plant as Antioxidant and Antimicrobial Agents**

Ref. No. of Manuscript and Corresponding Author Name :

Abstract : ☑ **(i) Appropriate** ☐ **(ii) Requires modification**
☐ **(iii) Too Long Requires Brevity** ☐ **(iv) Lacks clarity**

Keywords : ☑ **Sufficient** ☐ **Lacking** ☐ **Require modification**

Introduction : ☑ **Appropriate** ☐ **Not related to the work**
☐ **Ambiguous** ☐ **Too detailed, requires brevity**

Experimental (Materials and Methods) : ☐ **Incomplete** ☑ **Detailed and clear**
☐ **Requires improvement** ☐ **Not clearly explained**

Tables : ☐ **Title of table(s) missing** ☐ **Caption not appropriate**
☐ **Requires correction** ☐ **Tables not in corrected form**

Graphs, Figures, Structures and Equations : ☑ **Appropriate** ☐ **Labeling not clear**
☐ **Not clear, requires redrawing** ☐ **Not self explanatory**

Result and Discussion : ☑ **Convincing** ☐ **Not Convincing**
☐ **Not supported by relevant references.**

Language and Write-up : ☐ **Lucid** ☐ **Ambiguous** ☐ **Non-coherent**

References : ☐ **Not according to our format, modify, See example**
☐ **Ref.(s) Nos. …… are incomplete**
☐ **Ref.(s) Nos. …… require correction**
☐ **No uniformity maintained Not in chronological order**
☐ **Don't use *et al.;(write names of all the authors)in references***

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Continue to page 2nd

(Final Review Part B)

Read the instructions in the box carefully

(It is mandatory for every reviewer to complete both the parts of final review i.e. Review Part A & Review Part B.

If a reviewer only completes the Part A while ignores the Part B, his review will not be accepted.)

To write your views about the work and the suitability of manuscript for publication is very important.

It is a good manuscript with beneficial results; however, a revision is required before the manuscript is accepted for publication.

1- There are a few instances of slightly odd choice of word and some minor grammatical errors - I would suggest a thorough proofreading. The language at present does not affect the understanding of the article but it could read better with some minor editing.

2- Formatting seems appropriate and in accordance with the journal's requirements.

3- The full botanical plant names, including authorities, must be mentioned besides the local names and if existing English names. Please check also if your plant name corresponds to the latest revision in "The Plant List" (www.theplantlist.org).

4- Add the bibliographic support for the extraction procedure.

5- Discuss the main results with reference to previous research (add more references). Discuss policy and practice implications of the results. Analyse the strengths and limitations of the study. Offer perspectives for future work.

Review Decision :

☐ **The paper is accepted without modification.**

☑ **The paper is accepted after minor modification.**

☐ **The paper is accepted after major modification.**

☐ **Rewrite the paper and send us at your earliest.**

☐ **The paper is not acceptable, hence we are reluctantly returning to you.**

Reply on this email: info@orientjchem.org

Reviewer's Name & Address **Signature of the Editor**

Reviewer : Anonymous

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Sri handayani <handayani@uny.ac.id>

Fwd: Review Reports 12003

- sriatun <sriatun@uny.ac.id> 26 Maret 2019 17.11
Kepada: Sri handayani <handayani@uny.ac.id>

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From: **- sriatun** <sriatun@uny.ac.id>
Date: Mon, 10 Sep 2018, 15:34
Subject: Re: Review Reports 12003
To: <info@orientjchem.org>

Dear Editor
Thank you for your review my article, I will revised for any coment from reviewers as soon as posible.

Best Reagard
Sri Atun
[Kutipan teks disembunyikan]

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Sri handayani <handayani@uny.ac.id>

Fwd: Review Reports 12003

- sriatun <sriatun@uny.ac.id> 26 Maret 2019 17.12
Kepada: Sri handayani <handayani@uny.ac.id>

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From: **- sriatun** <sriatun@uny.ac.id>
Date: Mon, 17 Sep 2018, 10:38
Subject: Re: Review Reports 12003
To: <info@orientjchem.org>

Dear Editor
Herewith I submit a revision of the article (OJC-12003-18),and author's response to reviewer's comments 1 and 2.
Thank you for your attention

Sri Atun
[Kutipan teks disembunyikan]

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Study of Potential Phenolic Compounds from Stems of *Dendrophthoe falcata* (Loranthaceae) Plant as Antioxidant and Antimicrobial Agents

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Abstract

Dendrophthoe falcata is a hemiparasitic plant that has been used in traditional medicine.The study was conducted to identify the total phenolic and flavonoid compounds, to test antioxidant, and antimicrobial activity of ethanol extract stems of *D. falcata* parasite on *Melia azedarach* host tree. The dry powder stems of *D. falcata* was extracted with ethanol. The ethanol extract was subsequently partitioned successively using *n*-hexane, chloroform, and ethyl acetate. Each fraction was analyzed by a quantitative phenolic and flavonoid content with spectrophotometer method, and tested as antioxidant and antimicrobial activites. Antioxidant activity was performed by 2,2-diphenyl-1-picrilhidrazil (DPPH) method, while antimicrobial assay used pathogenic bacteria by disk-diffusion method. The results concluded that the stem of *D. falcata* plant showed a high content of phenolic and flavonoid compounds, very high antioxidant and moderate antimicrobial activities. It was also found that stems of *D. falcata* contain potential phenolic compounds that can be used as natural antioxidants and the treatment of various infections caused by microbes.

Keyword: Dendrophthoe falcata; antioxidant; antimicrobial; phenolic compound; flavonoids

INTRODUCTION

Loranthacea is a parasitic plant widely used in traditional Chinese medicine systems. One of this species is *L. parasiticus* which is known as Sang Ji Sheng (in Chinese), parasites (in Malay) and baso-Kisei (in Japanese). The plants are commonly found as parasitic plants that attach to other plant stems. Parasites can be found in tropical and sub-tropical regions, but with different species diversity.[1-4] *L. parasiticus* known as traditional medicine is widely used

as a diuretic, high blood pressure, polio virus prevention[5], antimicrobial and anti-inflammatory[6], and neuroprotective[7], antioxidant[8], and antidiabetic[9].

Dendrophthoe falcata (*D. falcata*) is one of the hemiparasitic plants commonly attached to host plants such as *Mangifera indica* (Anacardiaceae), *Melia azedarach* (Meliaceae) and *Psidium guajava* (Myrtaceae). The plant is widely distributed in tropical and sub-tropical regions. The whole plant of *D. falcata* is used in native treatment systems such as astringent, aphrodisiac, diuretics, and treat menstrual disorders, and antifertility agent[10]. It has been reported that methanol extract and water decoction of *D. falcata* leaves have anti-inflammatory effect on experimental animals and showed strong antioxidant activity by *in-vitro*[11].

A study conducted by Chandrakasan[2] comparing the ethanol extract stems of *Loranthus longiflorus* plants that are parasitic to the plant stems of *Casuarina equisetifolia* and *Ficus religiosa* by GC-MS showed different compounds and biological activity. A comparative study of the phytochemical and anti-microbial properties from leaf of *Loranthus micranthus* harvested from six host trees, showed marked variations in phytochemical constituents and anti-microbial activities[12]. The work reported by Chandrakasan[13] showed the presence of cumarin compounds from leaf of *L. longiflorus* plant, whereas in fresh flowers *Loranthus acaciae* was reported to contain antimicrobial quercetin 3-O- glucoside. Research on chemical and biological studies of leaf extract of *D. falcata* showed the crude extracts and the fractions have potential biological activities as cytotoxic, antioxidant, and thrombolytic activities. The leaves of *D. falcata* have also been reported to contain compounds identified as lupeol, 3-β-acetoxy-12-ene-11-one and β-sitosterol[14]. In this work we report the potential of phenolic compounds from stems of *D. falcata* plant that hemiparasite on *Melia azedarach* host tree as antioxidant and antimicrobial agents.

EXPERIMENTAL

Apparatus and reagents

Evaporator Buchi Rotavapor R-114, spectronic 20, incubator, autoclave, LAF (Laminatory Air Flow), coloni counter, forceps, micro pipettes, shaker, water bath, deep freezer, ruler (millimeter scale), petri plate, and analytical balance were used in this work. Ethanol, methanol, *n*-hexane, ethyl acetate, acetone, DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, folin-ciocalteu, sodium carbonate, gallic acid, rutin, aluminum nitrate, potassium acetate, chloramphenicol, Mueller-Hinton agar (MHA, OXOID), Nutrient Broth (NB), Nutrient Agar (NA), paperdisk, DMSO (dimethyl sulfoxide), plastic wrap, aluminium foil, and aquadest were used in this work without particular treatment.

Plant Materials

The sample used in this study was a parasitic plant attached to the stem of the *Melia azedarach* host tree, collected in October 2017. The sample was then determined by Biology Laboratory as specimen (DF-01-2017) and scientifically known as *Dendropthoe falcata* (L.f.) Ettingsh, the local name of “benalu” or “kemadealan”.

Microorganism

This study used four pathogenic bacterial isolates, three isolates of *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC25923, and *Staphylococcus epidermidis* FNCC 0048 were obtained from the Microbiology laboratory, while *Streptococcus mutans* was obtained from Dentistry laboratory. The microorganism were sub-cultured and stored in a semisolid medium (Mueller Hinton agar plates) at 4^0C until needed.

Preparation of samples

The milled dried from stem of *D. falcata* (4 kg) was extracted using 95% ethanol as the solvent for 24 h and repeated 3 times. The extracts were then concentrated using a vacuum rotatory evaporator and yielded brown residue for about 72g. The ethanol extract of *D. falcata*

(60 g) was successively partitioned using *n*-hexane, chloroform, and ethyl acetate. Each fraction was evaporated to dryness under vacuum resulting in a fraction of *n*-hexane (12 g), chloroform fraction (11.3 g), ethyl acetate fraction (10.62 g), and residual ethanol fraction (22 g). The procedure of sample preparation can be seen in Figure 1.

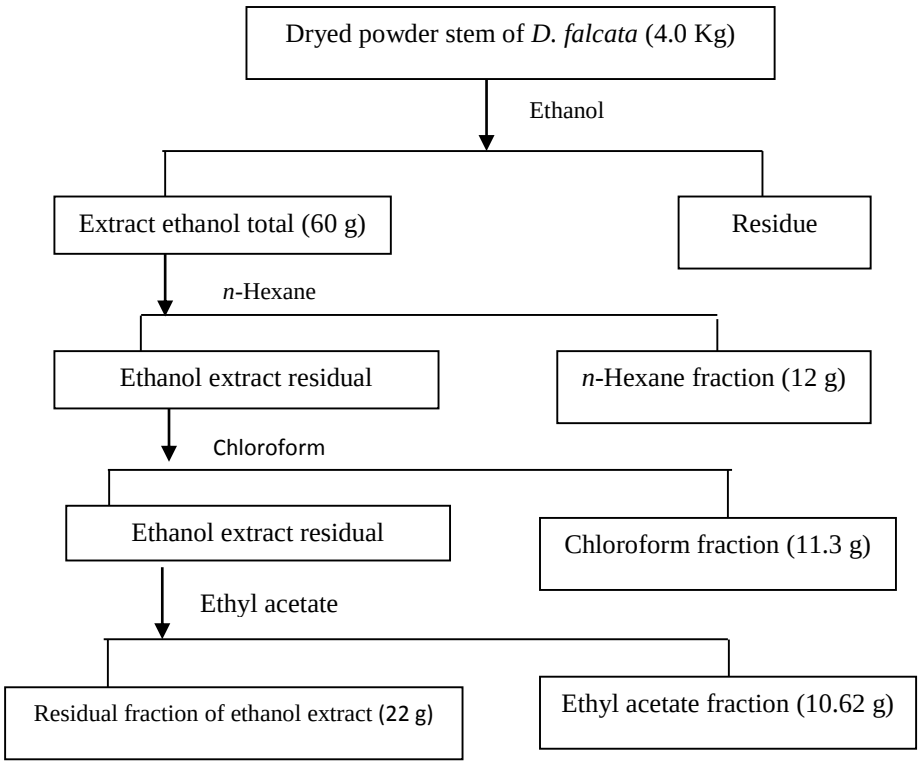

Fig. 1. The procedure of sample preparation

Phytochemical screening

The qualitative phytochemical screening was performed using reagent for terpenoid test, alkaloid test, phenolic test, and saponin test. Terpenoids test was performed using Salkowski reagent**,** alkaloid test was done with Wagner reagents, phenolic test was done with iron(III) chloride test, and foam test was for saponin[15].

Quantitative determination of total phenolic and flavonoid content

The quantitative analysis of the total phenolic content of each extract and fractions were determined using the folin-ciocalteu reagent[16]. About 200 µL of sample (500 µg) was mixed with folin-ciocalteu reagent (0.4 mL), aquadest (4 mL) and sodium carbonate (2mL, 15% w/v). The mixture was subsequently incubated at 50 °C for 10 minutes and measured its absorbance at 760 nm. The total phenolic content was calculated according to the standard curve specified for the gallic acid as a reference. Each measurement was performed three replicates. The total phenolic content is expressed as mg of Gallic Acid Equivalent (GAE) per g of plant extract.

The quantitative analysis of total flavonoid content was determined by spectrophotometric method. This method was adopted with a slight modification of Liu TC[17]. The samples of each extract were diluted with 80% ethanolic-aqueous solution (0.9 mL). A total of 0.5 mL of the sample was added to the reaction tube containing 0.1 mL 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 mins and its absorbance was determined at 415 nm with a UV spectrophotometer. Total flavonoid content was calculated using standard curves with rutin as references. The total flavonoid content is expressed as mg Rutin Equivalents (RE) per g of plant extract.

Determination of antioxidant activity test

The antioxidant activity test of each sample was done by free radical scavenger method. This method used DPPH (2,2-diphenyl-1-picrylhydrazyl) as a source of free radicals[18]. A total of 5 mL of sample (in various concentrations between 100-3,125 µg/mL) and positive control (ascorbic acid in various concentrations between 6.25-0.390 µg/mL) were mixed with 5 mL of DPPH solution in methanol (0.12 mM) and incubated at room temperature for 30 minutes. Absorbance was measured at 516 nm using spectronic 20 (Genesys). The absorption of DPPH from the sample was compared to the blank solution. Each sample was tested in three

replications. Antioxidant activity was calculated as a reduced percentage of DPPH compared with control and inhibitory activity can be calculated to determine IC_{50}.

Determination of activity as an antimicrobial agent

The antimicrobial activity test of the crude extract and each fraction were performed by agar diffusion method. This method was adopted with slight modifications of Bisnu[19]. In this work four bacteria i.e *Escherichia coli* ATCC-11229, *Staphylococcus aureus* ATCC-25923, *Staphylococcus epidermidis* FNCC-0048, and *Streptococcus mutans* were used. As a positive control chloramphenicol was applied, while DMSO (dimethyl sulfoxide) 10% was used as a solvent and also as a negative control.

The test bacteria of 100 μL was inoculated onto the Muller Hinton agar plate, and flattened with drigalsky. The paperdisk was immersed in the solution of each sample for five minutes and was dipped into the sample solution which was placed on top of the agar medium, and incubated at 37^0C for 12 h. After 12 h incubation, the zone of inhibition around the disks was measured in millimeter scale. A four serial dilution (0.01; 0.1; 0.5 and 1.0 % w/v) was carried out for each sample. The measurement was done in three repetitions.

RESULTS AND DISCUSSION

This study used stem bark of *D. falcate* that hemiparasite on *Melia azedarach* host tree (Figure 2). After being dried and mashed the stem bark was extracted by maceration using 95% of ethanol solvent. Total concentrated ethanol extract obtained was about 72 g. A portion of the extract (60 g) was partitioned using *n*-hexane, chloroform, and then ethyl acetate solvents. Each subsequent fraction was evaporated to dryness under vacuum. Each of the extracts and fractions were identified to phytochemical test and its activity as antioxidant and antimicrobial. The results of qualitative phytochemical test including terpenoid, alkaloid, phenolic, and saponin are listed in Table 1.

Fig 2. Stem of *D. falcata*

Table 1. Qualitative phytochemical screening of the extracts and fractions from stem of *D. falcata*

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

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

Quantitative analysis of total phenolic content was performed by spectrophotometer following the Folin-Ciocalteu method[17]. The absorbance of each sample was recorded at 760 nm. In this work gallic acid was used as a reference at various concentrations and recorded at the same wavelength. The result of calibration curve follows the linear regression equation of $y = 0{,}004x - 0{,}032$ ($R^2 = 0{,}985$). The total phenolic content is, therefore, expressed as mg Gallic Acid Equivalents (GAE) per g of plant extract.

Total flavonoid content was obtained by using rutin as standard reference at various concentrations[17]. The absorbance of each sample was recorded at its maximum wavelength, which was obtained at 415 nm. The calibration curve follows the linear regression equation of

y = 0,003x + 0,099 (R^2 = 0,963). The total flavonoids content is, therefore, expressed as mg Rutin Equivalents (RE) per g of plant extract[17]. The total phenolic and flavonoids content of the extract and each fraction were collected in Table 2.

Antioxidant capacity was expressed as IC_{50} (µg/ml) of DPPH scavenging activity[18]. The concentrations of each sample used in the antioxidant activity test were 25.00, 12.50, 6.25, and 3.125 µg/mL, except for ascorbic acid in various concentrations of 10.00, 5.00, 2.50, 1.25, and 0.625 µg/mL. From the absorbance of each sample, a linear regression for the corresponding IC_{50} equations was made to calculate (concentration samples having 50% inhibitory activity). The inhibition activity (IC_{50}) of each sample, and the result is presented in Table 2.

Table 2. Quantitative of total phenolic and flavonoids content, and antioxidant activity of the crude ethanol extract and each fraction from stem of *D. falcata*

Sample	Total phenolic content (TPC) (mg GAE/g)	Total flavanoids content (TFC) (mg RE/g)	Antioxidant activity by DPPH method (IC_{50} µg/ml)
Crude ethanol extract	1290.8 ± 7.63	195.6 ±1.3	11.02± 0.12
n-hexane fraction	0	0	65.96±1.22
Chloroform fraction	954.2 ± 5.13	315.6 ±1.6	20.33±0.32
Ethyl acetate fraction	2079.2 ± 5.10	980.0 ± 1.7	7.56±0.41
Residu fraction of ethanol extract	261.7 ± 3.37	0	27.54±0.62
Ascorbic acid (Positive control)	-	-	1.14±0.11

*Data are mean ± SD for triplicate measurement

Screening of antimicrobial activity of crude ethanol extract and the fraction of stem bark from *D. falcata* was done by using agar disk-diffusion method activities againts pathogenic bacteria i.e. *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* FNCC 0048, and *Streptococcus mutans* through the disk-diffusion method[19]. The zones of inhibitions of each compound against pathogenic bacteria are presented at Table 3.

Table 3: Zones of inhibitions as shown by crude ethanol extract and its fraction of stem bark from *D. falcata* at different concentrations against selected microorganisms

No	Sampel	Concentration (%)	Diameter Zona of Inhibition (Mean±SD) mm after 12 h incubations			
			Streptococcus mutans	*Staphylococcus epidermidis* FNCC 0048	*Staphylococcus aureus* ATCC25923	*Escherichia coli* ATCC 11229
1	DMSO (10%)		6.17±0.14	6.17±0.22	6.57±0.80	6.17±0.84
2	Crude ethanol extract of *D. falcata*	0.01	10.36±0.13	10.12±0.87	8.00±0.53	8.92±0.71
		0.1	9.67±0.53	10.12±0.87	8.45±0.05	9.18±0.03
		0.5	9.68±0.82	10.06± 0.87	8.28± 0.68	9.23± 0.58
		1.0	7.75± 0.29	10.46±0.63	8.06±0.59	8.72±0.22
3	*n*-Hexane fraction of *D. falcata*	0.01	9.37±0.50	9.14±0.19	8.77±0.07	7.48±0.39
		0.1	9.41±0.53	8.46±0.16	8.03±0.31	8.33±0.33
		0.5	9.54±0.51	7.97±0.62	8.12±0.74	8.64±0.87
		1.0	8.59±0.85	8.14± 0.56	8.31± 0.51	8.35± 0.33
4	Chloroform fraction of *D. falcata*	0.01	9.14± 0.30	9.23±0.68	8.13±0.28	8.12±0.85
		0.1	9.83±0.61	9.02± 0.98	7.32± 0.82	8.33±0.33
		0.5	10.49± 0.32	8.82±0.18	9.59±0.29	8.67±0.48
		1.0	8.54±0.39	8.36± 0.77	10.09± 0.36	8.44± 0.37
5	Ethyl acetate fraction of *D. falcata*	0.01	9.83± 0.41	7.95±0.48	7.95±0.48	8.84±0.63
		0.1	8.90±0.42	7.43± 0.43	7.43± 0.43	8.09±0.08
		0.5	8.67± 0.50	7.20±0.53	7.20±0.53	8.33±0.47
		1.0	7.75±0.29	7.54± 0.35	7.54± 0.35	8.09± 0.49
6	Residual of ethanolic fraction of *D. falcata*	0.01	10.36± 0.13	8.73±0.38	7.44±0.29	8.41±0.40
		0.1	9.67±0.53	8.12± 0.65	7.81± 0.43	8.77±0.47
		0.5	9.68± 0.82	8.98±0.06	6.98±0.50	7.92±0.54
		1.0	7.75±0.29	8.15± 1.13	7.28± 0.52	7.95± 1.02
7	Chloramphenicol (positive controle)	0.01	12.26± 0.72	12.57± 1.88	12.37± 1.38	17.41±1.88
		0.1	17.05± 0.93	20.58±1.09	11.25±1.67	22.38±1.11
		0.5	21.20±0.41	24.05± 1.52	13.70± 2.90	28.26±1.68
		1.0	22.52± 0.72	24.98± 1.06	17.35± 1.07	32.57± 1.88

From this study it could be shown the suitability between qualitative and quantitative data. The total ethanol extract still contains a mixture of both non-polar and relatively polar compounds. After successive fractionation using *n*-hexane, chloroform, and ethyl acetate solvents, groups of soluble compounds according to the polarity of each solvent should result. *n*-Hexane should dissolve the relatively non-polar compounds, chloroform should dissolve the relatively semipolar compounds, while ethyl acetate should dissolve the relatively polar compounds. The results of this study showed that between the crude extract and the fractions, the ethyl acetate fraction showed the highest total phenolic content (2079.2 ± 5.10 mg GAE/g)

followed by crude ethanol extract (1290.8 ± 7.63 mg GAE /g), chloroform fraction (954.2 ± 5.13 mg GAE/g) and then residual fraction of ethanol extract (261.7 ± 3.37 mg GAE/g).

The total flavonoid contents of crude ethanol extract and this fractions determined by spectrophotometry method were reported as rutine equivalents (RE). Flavonoid content was observed on crude ethanol extract (195.6 ±1.3 mg RE/g), chloroform fraction (315.6 ±1.6 mg RE/g), and ethyl acetate (980.0 ± 1.7 mg RE/g). In general, phenolic compounds and including flavonoids can dissolve in chloroform, ethyl acetate, and ethanol, but in different concentrations depending on their structure and polarity. The more polar phenolic compounds are more easily dissolved in more polar solvents. Phenolic compounds generally exhibit antioxidant activity, therefore the antioxidant activity of chloroform and ethyl acetate fractions with IC_{50} shows very active, although relatively less active than ascorbic acid (positive control). Table 2 shows that the total phenolic and flavonoid content in the ethyl acetate fraction stem of *D. falcata* plant is much higher than that in the chloroform fraction, thus indicating the highest antioxidant activity compared to the other fractions. Several studies have shown that many plants contain phenolic compounds. Flavonoids, are one of the natural phenolic compounds found in fruits, vegetables, grains, barks, roots, stems, flowers, tea and wine[20]. The phenolic compound includes a number of compounds generally having an aromatic ring with one or more hydroxyl groups[21]. The antioxidant potential is usually associated with the content of phenolic compounds due to the conjugated π-electron system which facilitates electron donation from hydroxyl groups to oxidizing radicals. The antioxidant activity test by DPPH method is based on a radical capture reaction by an antioxidant compound with an oxygen atom transfer mechanism, which will produce stable DPPH-H molecules in a non-radical form[22]. The IC_{50} of each extract and fraction stems of *D. falcata* in this study indicate the presence of phenolic compounds which have very high antioxidant activity. The activity is caused by the content of polyphenol compounds such as flavonoids, tannins, terpenoids, phenols, and

saponins from each extract and the fraction stem of the *D. falcata*. Previous research evaluating the antioxidant activity of methanol extract from the stems of the *D. falcata* parasite on mangoes, *Mangifera indica* (Anacardiaceae) showed the presence of compounds that have significant antioxidant activity[23]. Several studies have shown the presence of phenolic compounds in the Loranthaceae family, including the leaves of *Loranthus micranthus* Linn showed 3-O- (3,4,5-trimethoxybenzoyl) - (-) - epicatechin (TMECG), (-) -epicatechin-3 -O- (3 ″ -O-methyl) -gallate (ECG3 ″ Me), rutine, and peltatoside[24]. Whereas in *Scurrula ferruginea* Durrer (Loranthaceae) found phenol compounds, quercetin and quercitrin, and 4-O-acetylquercitrin flavonol glycosides[25]. These phenolic compounds show high antioxidant activity.

Table 3 shows that the activity of crude ethanol extract from stem of *D. falcata* has a larger zone diameter than the negative control (DMSO 10%), but much smaller than the positive control (chloramphenicol). This indicates that the extract and its fractions exhibit activity as antimicrobial agent, although not as good as chloramphenicol. The classification of zone inhibition response of bacterial growth based on clear zone diameter includes weak response (5 mm), medium response (5-10 mm), strong response (10-20 mm), and very strong response (diameter> 20 mm)[26]. Several previous studies have also shown that some methanol extracts of *loranthus sp* plants have significant antimicrobial activity against different gram-positive and gram-negative bacterial, and fungi being tested[27]. The mechanism of antibacterial action may be through various means, including inhibition of cell wall synthesis, inhibition of cell wall permeability, inhibition of cell wall proteins, inhibition of nucleic acid synthesis, and inhibition of microbial metabolisme[28]. The maximum zone of inhibition was 10.46±3.63 mm at the maximum concentration used 1% of crude ethanol extract stems of *D. falcata* against *Staphylococcus epidermidis* FNCC 0048.

Plant phenolic compounds are main contributors of antioxidant activity and are also responsible for anti-inflammatory, antiviral and anticancer and antimicrobial activities[29]. The presence of terpenoids and saponin have already shown as antimicrobial and antiinflammatory activity[30]. The results of this study show that the stem bark of *D. falcata* plant contains a high number of phenolic compounds, has antioxidant activity, and exhibits moderate antimicrobial activity. Isolation and identification of the compound structure of the *D. falcata* plant is being analyzed in our laboratory.

CONCLUSION

The study showed the presence of total phenolic content and flavonoids from extracts and fractions from stems of *D. falcata*. Ethyl acetate fraction from stems of *D. falcata* showed the highest phenolic and flavonoid levels, as well as very high antioxidant activity. Zones of inhibition of extracts and each fraction showed moderate activity against *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* FNCC 0048, and *Streptococcus mutans*. This study shows that stems of *D. falcata* contain potential phenolic compounds that can be suitable as natural antioxidants and treatment of various infections caused by microbes.

Acknowledgements

This work was supported by the Ministry of Research and Technology of the Directorate of Higher Education, Indonesia for funding of competency research grants (PBK, Number: 0045 / E3 / LL / 2018, January 16, 2018) and foreign seminar aid program (*BSLN, MENRISTEK DIKTI*) Indonesia has provided financial assistance to attend the ISTEC 2018 international seminar. The author also thanks to Prof.K. H. Sugiyarto from Dept. Chem. Ed. Yogyakarta State University which helps review the manuscript.

CONFLICTS INTEREST

None

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Author's Response to Reviewer's Comments (1)

Paper number: OJC-12003-18

Paper title: **Study Potential Phenolic Compounds from Stem of *Dendrophthoe falcata* (Loranthaceae) Plant as Antioxidant and Antimicrobial Agents**

Title	Reviewers Comments	Authors Response
	1- There are a few instances of slightly odd choice of word and some minor grammatical errors - I would suggest a thorough proofreading. The language at present does not affect the understanding of the article but it could read better with some minor editing.	ok, I fixed it and I discussed it with a linguist
	2- Formatting seems appropriate and in accordance with the journal's requirements.	Ok, thankyou
	3- The full botanical plant names, including authorities, must be mentioned besides the local names and if existing English names. Please check also if your plant name corresponds to the latest revision in "The Plant List" (www.theplantlist.org).	I checked on "The Plant List" (www.theplantlist.org), with the full name *Dendrophthoe falcata* (L.f.) Ettingsh
	4- Add the bibliographic support for the extraction procedure.	ok , I'll add
	5- Discuss the main results with reference to previous research (add more references). Discuss policy and practice implications of the results. Analyse the strengths and limitations of the study. Offer perspectives for future work.	I have added an explanation and reference to the discussion section
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Keywords		

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Paper number: OJC-12003-18

Paper title: **Study Potential Phenolic Compounds from Stem of *Dendrophthoe falcata* (Loranthaceae) Plant as Antioxidant and Antimicrobial Agents**

Title	Reviewers Comments	Authors Response
	1. Reiteration: The introductory paragraphs at results and discussion are reiterative. They are duplication of the same paragraph written at the experimental section. Those paragraphs should be deleted and the manuscript should be reduced. The very first two paragraphs in the introduction are also reiterative. Table 3 and Figure 2 are also reiterative. Authors should reduce the manuscript. Some brief is better than tedious and boring.	ok, I've fixed it
	English should be refined. Change flavanoids by flavonoids	I checked and fixed it

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	References are not completed. Some important references are missing. I recommend author read Flavonoids: an overview, A. N. Panche, A. D. Diwan, and S. R. Chandra, J Nut Sci, (2016), vol. 5, e47, 1-15 doi:10.1017 or Chemical and Biological Studies of Leaf Extract of Dendrophthoe falcata Linn. *Md Rajdoula Rafe, Monira Ahsan, Choudhury Mahmood Hasan, Mohammad Mehedi Masud,* Dhaka Univ. J. Pharm. Sci. 16(2): 215-219, 2017 (December). On the other hand, reference numbering should be checked. For instance, Chandrakasan is not ref. [12]	I checked and fixed it
	Some methods should be referenced, including determination of phenols, flavonoids and antioxidant activity. Related to flavonoids, absorbance at 415 nm is not correct. The absorbance of different types of flavonoids at that wavenumber differ a lot.	I checked and fixed it. Flavonoids react with reagents (aluminum nitrate; potassium acetate; ethanol 80%) giving a yellow color. The maximum wavelength obtained in this experiment is 415 nm, according to the reference (Liu C.T, Wu Ching-Yi, WengYih-Ming, Tseng Chin-Yin. *J. Ethnopharmacol.* **2005,** 99:293–300.

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	Some Tables are too qualitative. For instance, criteria for (+), (++) or (+++) at Table 1 should be indicated	I checked and fixed it - = absent, + = present, ++ = present a lot; +++ = present very large number
	It is very doubtful that ethyl acetate solubilize polar compounds, as stated in the manuscript. Flavonoids should be added to keywords.	Ethyl acetate relative more polar than chloroform, some phenolic compounds can be soluble in ethyl acetate. I checked and fixed it
	The percentage of vinyl esters (miristate or decanoate) are hard to believe. They seems to be very high. Please check it.	I checked and fixed it
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Abstract		
Keywords		
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Methodology		

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Study of Potential Phenolic Compounds from Stems of *Dendrophthoe falcata* (Loranthaceae) Plant as Antioxidant and Antimicrobial Agents

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(Received: April 04, 2018; Accepted: October 15, 2018)

ABSTRACT

Dendrophthoe falcata is a hemiparasitic plant that has been used in traditional medicine. The study was conducted to identify the total phenolic and flavonoid compounds, to test antioxidant, and antimicrobial activity of ethanol extract stems of *D. falcata* parasite on Melia azedarach host tree. The dry powder stems of *D. falcata* was extracted with ethanol. The ethanol extract was subsequently partitioned successively using n-hexane, chloroform, and ethyl acetate. Each fraction was analyzed by a quantitative phenolic and flavonoid content with spectrophotometer method, and tested as antioxidant and antimicrobial activites. Antioxidant activity was performed by 2,2-diphenyl-1-picrilhidrazil (DPPH) method, while antimicrobial assay used pathogenic bacteria by disk-diffusion method. The results concluded that the stem of *D. falcata* plant showed a high content of phenolic and flavonoid compounds, very high antioxidant and moderate antimicrobial activities. It was also found that stems of *D. falcata* contain potential phenolic compounds that can be used as natural antioxidants and the treatment of various infections caused by microbes.

Keyword: *Dendrophthoe falcata*, Antioxidant. Antimicrobial. Phenolic compound, Flavonoids.

INTRODUCTION

Loranthacea is a parasitic plant widely used in traditional Chinese medicine systems. One of this species is *L. parasiticus* which is known as Sang Ji Sheng (in Chinese), parasites (in Malay) and baso-Kisei (in Japanese). The plants are commonly found as parasitic plants that attach to other plant stems. Parasites can be found in tropical and sub-tropical regions, but with different species diversity.[1-4] *L. parasiticus* known as traditional medicine is widely used as a diuretic, high blood pressure,

polio virus prevention5, antimicrobial and anti-inflammatory[6], and neuroprotective[7], antioxidant[8], and antidiabetic[9].

Dendrophthoe falcata (*D. falcata*) is one of the hemiparasitic plants commonly attached to host plants such as *Mangifera indica* (Anacardiaceae), *Melia azedarach* (Meliaceae) and *Psidium guajava* (Myrtaceae). The plant is widely distributed in tropical and sub-tropical regions. The whole plant of *D. falcata* is used in native treatment systems such as astringent, aphrodisiac, diuretics, and treat menstrual disorders, and antifertility agent[10]. It has been reported that methanol extract and water decoction of *D. falcata* leaves have anti-inflammatory effect on experimental animals and showed strong antioxidant activity by *in-vitro*[11].

A study conducted by Chandrakasan[2] comparing the ethanol extract stems of Loranthus longiflorus plants that are parasitic to the plant stems of Casuarina equisetifolia and Ficus religiosa by GC-MS showed different compounds and biological activity. A comparative study of the phytochemical and anti-microbial properties from leaf of Loranthus micranthus harvested from six host trees, showed marked variations in phytochemical constituents and anti-microbial activities[12]. The work reported by Chandrakasan13 showed the presence of cumarin compounds from leaf of *L. longiflorus* plant, whereas in fresh flowers Loranthus acaciae was reported to contain antimicrobial quercetin 3-O-glucoside. Research on chemical and biological studies of leaf extract of *D. falcata* showed the crude extracts and the fractions have potential biological activities as cytotoxic, antioxidant, and thrombolytic activities. The leaves of *D. falcata* have also been reported to contain compounds identified as lupeol, 3-β-acetoxy-12-ene-11-one and β-sitosterol[14]. In this work we report the potential of phenolic compounds from stems of D. falcata plant that hemiparasite on Melia azedarach host tree as antioxidant and antimicrobial agents.

EXPERIMENTAL

Apparatus and reagents

Evaporator Buchi Rotavapor R-114, spectronic [20], incubator, autoclave, LAF (Laminatory Air Flow), coloni counter, forceps, micro pipettes, shaker, water bath, deep freezer, ruler (millimeter scale), petri plate, and analytical balance were used in this work. Ethanol, methanol, n-hexane, ethyl acetate, acetone, DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, folin-ciocalteu, sodium carbonate, gallic acid, rutin, aluminum nitrate, potassium acetate, chloramphenicol, Mueller-Hinton agar (MHA, OXOID), Nutrient Broth (NB), Nutrient Agar (NA), paperdisk, DMSO (dimethyl sulfoxide), plastic wrap, aluminium foil, and aquadest were used in this work without particular treatment.

Plant Materials

The sample used in this study was a parasitic plant attached to the stem of the Melia azedarach host tree, collected in October 2017. The sample was then determined by Biology Laboratory as specimen (DF-01-2017) and scientifically known as Dendropthoe falcata (L.f.) Ettingsh, the local name of “benalu” or “kemadealan”.

Microorganism

This study used four pathogenic bacterial isolates, three isolates of Escherichia coli ATCC 11229, *Staphylococcus aureus* ATCC25923, and *Staphylococcus epidermidis* FNCC 0048 were obtained from the Microbiology laboratory, while *Streptococcus mutans* was obtained from Dentistry laboratory. The microorganism were sub-cultured and stored in a semisolid medium (Mueller Hinton agar plates) at 4^0C until needed.

Preparation of samples

The milled dried from stem of *D. falcata* (4 kg) was extracted using 95% ethanol as the solvent for 24 h and repeated 3 times. The extracts were then concentrated using a vacuum rotatory evaporator and yielded brown residue for about 72 g. The ethanol extract of *D. falcata* (60 g) was successively partitioned using n-hexane, chloroform, and ethyl acetate. Each fraction was evaporated to dryness under vacuum resulting in a fraction of n-hexane (12 g), chloroform fraction (11.3 g), ethyl acetate fraction (10.62 g), and residual ethanol fraction (22 g). The procedure of sample preparation can be seen in Figure 1.

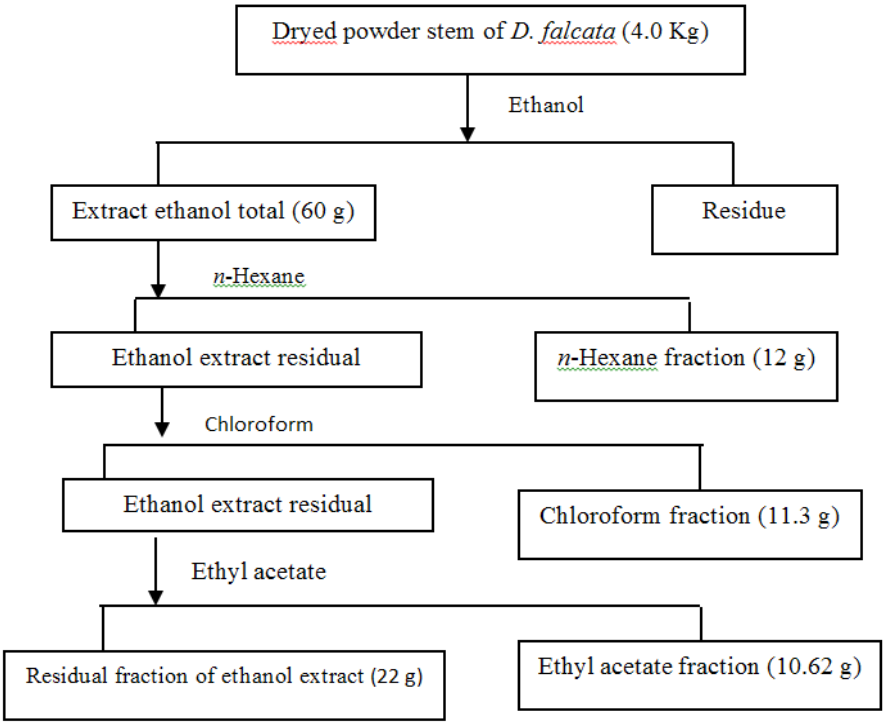

Fig. 1. The procedure of sample preparation

Phytochemical screening

The qualitative phytochemical screening was performed using reagent for terpenoid test, alkaloid test, phenolic test, and saponin test. Terpenoids test was performed using Salkowski reagent, alkaloid test was done with Wagner reagents, phenolic test was done with iron(III) chloride test, and foam test was for saponin[15].

Quantitative determination of total phenolic and flavonoid content

The quantitative analysis of the total phenolic content of each extract and fractions were determined using the folin-ciocalteu reagent[16]. About 200 µL of sample (500 µg) was mixed with folin-ciocalteu reagent (0.4 mL), aquadest (4 mL) and sodium carbonate (2mL, 15% w/v). The mixture was subsequently incubated at 50°C for 10 min. and measured its absorbance at 760 nm. The total phenolic content was calculated according to the standard curve specified for the gallic acid as a reference. Each measurement was performed three replicates. The total phenolic content is expressed as mg of Gallic Acid Equivalent (GAE) per g of plant extract.

The quantitative analysis of total flavonoid content was determined by spectrophotometric method. This method was adopted with a slight modification of Liu TC[17]. The samples of each extract were diluted with 80% ethanolic-aqueous solution (0.9 mL). A total of 0.5 mL of the sample was added to the reaction tube containing 0.1 mL 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 min. and its absorbance was determined at 415 nm with a UV spectrophotometer. Total flavonoid content was calculated using standard curves with rutin as references. The total flavonoid content is expressed as mg Rutin Equivalents (RE) per g of plant extract.

Determination of antioxidant activity test

The antioxidant activity test of each sample was done by free radical scavenger method. This method used DPPH (2,2-diphenyl-1-picrylhydrazyl) as a source of free radicals[18]. A total of 5 mL of sample (in various concentrations between 100-3,125 µg/mL) and positive control (ascorbic acid in various concentrations between 6.25-0.390 µg/mL) were mixed with 5 mL of DPPH solution in methanol

(0.12 mM) and incubated at room temperature for 30 minutes. Absorbance was measured at 516 nm using spectronic[20] (Genesys). The absorption of DPPH from the sample was compared to the blank solution. Each sample was tested in three replications. Antioxidant activity was calculated as a reduced percentage of DPPH compared with control and inhibitory activity can be calculated to determine IC_{50}.

Determination of activity as an antimicrobial agent

The antimicrobial activity test of the crude extract and each fraction were performed by agar diffusion method. This method was adopted with slight modifications of Bisnu[19]. In this work four bacteria i.e *Escherichia coli* ATCC-11229, *Staphylococcus aureus* ATCC-25923, *Staphylococcus epidermidis* FNCC-0048, and *Streptococcus mutans* were used. As a positive control chloramphenicol was applied, while DMSO (dimethyl sulfoxide) 10% was used as a solvent and also as a negative control.

The test bacteria of 100 µL was inoculated onto the Muller Hinton agar plate, and flattened with drigalsky. The paperdisk was immersed in the solution of each sample for five minutes and was dipped into the sample solution which was placed on top of the agar medium, and incubated at 37^{0}C for 12 h. After 12 h incubation, the zone of inhibition around the disks was measured in millimeter scale. A four serial dilution (0.01; 0.1; 0.5 and 1.0 % w/v) was carried out for each sample. The measurement was done in three repetitions.

RESULTS AND DISCUSSION

This study used stem bark of *D. falcate* that hemiparasite on Melia azedarach host tree (Fig. 2). After being dried and mashed the stem bark was extracted by maceration using 95% of ethanol solvent. Total concentrated ethanol extract obtained was about 72 g. A portion of the extract (60 g) was partitioned using n-hexane, chloroform, and then ethyl acetate solvents. Each subsequent fraction was evaporated to dryness under vacuum. Each of the extracts and fractions were identified to phytochemical test and its activity as antioxidant and antimicrobial. The results of qualitative phytochemical test including terpenoid, alkaloid, phenolic, and saponin are listed in Table 1.

Fig. 2. Stem of *D. falcata*

Table 1: Qualitative phytochemical screening of the extracts and fractions from stem of *D. falcata*

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

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

Quantitative analysis of total phenolic content was performed by spectrophotometer following the Folin-Ciocalteu method[17]. The absorbance of each sample was recorded at 760 nm. In this work gallic acid was used as a reference at various concentrations and recorded at

the same wavelength. The result of calibration curve follows the linear regression equation of y = 0,004x - 0,032 (R^2 = 0,985). The total phenolic content is, therefore, expressed as mg Gallic Acid Equivalents (GAE) per g of plant extract.

Total flavonoid content was obtained by using rutin as standard reference at various concentrations[17]. The absorbance of each sample was recorded at its maximum wavelength, which was obtained at 415 nm. The calibration curve follows the linear regression equation of y = 0,003x + 0,099 (R^2 = 0,963). The total flavonoids content is, therefore, expressed as mg Rutin Equivalents (RE) per g of plant extract[17]. The total phenolic and flavonoids content of the extract and each fraction were collected in Table 2.

Antioxidant capacity was expressed as IC_{50} (µg/ml) of DPPH scaveng¬ing activity[18]. The concentrations of each sample used in the antioxidant activity test were 25.00, 12.50, 6.25, and 3.125 µg/mL, except for ascorbic acid in various concentrations of 10.00, 5.00, 2.50, 1.25, and 0.625 µg/mL. From the absorbance of each sample, a linear regression for the corresponding IC_{50} equations was made to calculate (concentration samples having 50% inhibitory activity). The inhibition activity (IC_{50}) of each sample, and the result is presented in Table 2.

Table 2. Quantitative of total phenolic and flavonoids content, and antioxidant activity of the crude ethanol extract and each fraction from stem of *D. falcata*

Sample	Total phenolic content (TPC) (mg GAE/g)	Total flavanoids content (TFC) (mg RE/g)	Antioxidant activity by DPPH method (IC_{50} µg/ml)
Crude ethanol extract	1290.8 ± 7.63	195.6 ±1.3	11.02± 0.12
n-hexane fraction	0	0	65.96±1.22
Chloroform fraction	954.2 ± 5.13	315.6 ±1.6	20.33±0.32
Ethyl acetate fraction	2079.2 ± 5.10	980.0 ± 1.7	7.56±0.41
Residu fraction of ethanol extract	261.7 ± 3.37	0	27.54±0.62
Ascorbic acid (Positive control)	-	-	1.14±0.11

*Data are mean ± SD for triplicate measurement

Screening of antimicrobial activity of crude ethanol extract and the fraction of stem bark from *D. falcata* was done by using agar disk-diffusion method activities againts pathogenic bacteria i.e. *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* FNCC 0048, and *Streptococcus mutans* through the disk-diffusion method[19]. The zones of inhibitions of each compound against pathogenic bacteria are presented at Table 3.

From this study it could be shown the suitability between qualitative and quantitative data. The total ethanol extract still contains a mixture of both non-polar and relatively polar compounds. After successive fractionation using n-hexane, chloroform, and ethyl acetate solvents, groups of soluble compounds according to the polarity of each solvent should result. n-Hexane should dissolve the relatively non-polar compounds, chloroform should dissolve the relatively semipolar compounds, while ethyl acetate should dissolve the relatively polar compounds. The results of this study showed that between the crude extract and the fractions, the ethyl acetate fraction showed the highest total phenolic content (2079.2 ± 5.10 mg GAE/g) followed by crude ethanol extract (1290.8 ± 7.63 mg GAE /g), chloroform fraction (954.2 ± 5.13 mg GAE/g) and then residual fraction of ethanol extract (261.7 ± 3.37 mg GAE/g).

The total flavonoid contents of crude ethanol extract and this fractions determined by spectrophotometry method were reported as rutine equivalents (RE). Flavonoid content was observed on crude ethanol extract (195.6 ±1.3 mg RE/g),

chloroform fraction (315.6 ±1.6 mg RE/g), and ethyl acetate (980.0 ± 1.7 mg RE/g). In general, phenolic compounds and including flavonoids can dissolve in chloroform, ethyl acetate, and ethanol, but in different concentrations depending on their structure and polarity. The more polar phenolic compounds are more easily dissolved in more polar solvents. Phenolic compounds generally exhibit antioxidant activity, therefore the antioxidant activity of chloroform and ethyl acetate fractions with IC_{50} shows very active, although relatively less active than ascorbic acid (positive control). Table 2 shows that the total phenolic and flavonoid content in the ethyl acetate fraction stem of *D. falcata* plant is much higher than that in the chloroform fraction, thus indicating the highest antioxidant activity compared to the other fractions. Several studies have shown that many plants contain phenolic compounds. Flavonoids, are one of the natural phenolic compounds found in fruits, vegetables, grains, barks, roots, stems, flowers, tea and wine[20]. The phenolic compound includes a number of compounds generally having an aromatic ring with one or more hydroxyl groups[21]. The antioxidant potential is usually associated with the content of phenolic compounds due to the conjugated π-electron system which facilitates electron donation from hydroxyl groups to oxidizing radicals. The antioxidant activity test by DPPH method is based on a radical capture reaction by an antioxidant compound with an oxygen atom transfer mechanism, which will produce stable DPPH-H molecules in a non-radical form[22]. The IC50 of each extract and fraction stems of D. falcata in this study indicate the presence of phenolic compounds which have very high antioxidant activity. The activity is caused by the content of polyphenol compounds such as flavonoids, tannins, terpenoids, phenols, and saponins from each extract and the fraction stem of the *D. falcata*. Previous research evaluating the antioxidant activity of methanol extract from the stems of the *D. falcata* parasite on mangoes, *Mangifera indica* (Anacardiaceae) showed the presence of compounds that have significant antioxidant activity[23]. Several studies have shown the presence of phenolic compounds in the Loranthaceae family, including the leaves of Loranthus micranthus Linn showed 3-O- (3,4,5-trimethoxybenzoyl)-(-) -epicatechin (TMECG), (-)-epicatechin-3-O-(3″-O-methyl)-gallate (ECG_3 ″ Me), rutine, and peltatoside[24]. Whereas in Scurrula ferruginea Durrer (Loranthaceae) found phenol compounds, quercetin and quercitrin, and 4-O-acetylquercitrin flavonol glycosides[25]. These phenolic compounds show high antioxidant activity.

Table 3 shows that the activity of crude ethanol extract from stem of *D. falcata* has a larger zone diameter than the negative control (DMSO 10%), but much smaller than the positive control (chloramphenicol). This indicates that the extract and its fractions exhibit activity as antimicrobial agent, although not as good as chloramphenicol. The classification of zone inhibition response of bacterial growth based on clear zone diameter includes weak response (5 mm), medium response (5-10 mm), strong response (10-20 mm), and very strong response (diameter> 20 mm)[26]. Several previous studies have also shown that some methanol extracts of loranthus sp plants have significant antimicrobial activity against different *Gram-positive* and *Gram-negativ*e bacterial, and fungi being tested[27]. The mechanism of antibacterial action may be through various means, including inhibition of cell wall synthesis, inhibition of cell wall permeability, inhibition of cell wall proteins, inhibition of nucleic acid synthesis, and inhibition of microbial metabolisme[28]. The maximum zone of inhibition was 10.46±3.63 mm at the maximum concentration used 1% of crude ethanol extract stems of *D. falcata* against *Staphylococcus epidermidis* FNCC 0048.

Plant phenolic compounds are main contributors of antioxidant activity and are also responsible for anti-inflammatory, antiviral and anticancer and antimicrobial activities[29]. The presence of terpenoids and saponin have already shown as antimicrobial and antiinflammatory activity[30]. The results of this study show that the stem bark of *D. falcata* plant contains a high number of phenolic compounds, has antioxidant activity, and exhibits moderate antimicrobial activity. Isolation and identification of the compound structure of the *D. falcata* plant is being analyzed in our laboratory.

Table 3: Zones of inhibitions as shown by crude ethanol extract and its fraction of stem bark from *D. falcata* at different concentrations against selected microorganisms

No	Sampel	Concentration (%)	Diameter Zona of Inhibition (Mean±SD)mm after 12 h incubations			
			Streptococcus mutans	*Staphylococc-us epidermidis* FNCC 0048	*Staphylococcus aureus* ATCC25923	*Escherichia coli* ATCC 11229
1	DMSO (10%)		6.17±0.14	6.17±0.22	6.57±0.80	6.17±0.84
2	Crude ethanol extract of *D. falcata*	0.01	10.36±0.13	10.12±0.87	8.00±0.53	8.92±0.71
		0.1	9.67±0.53	10.12±0.87	8.45±0.05	9.18±0.03
		0.5	9.68±0.82	10.06± 0.87	8.28± 0.68	9.23± 0.58
		1	7.75± 0.29	10.46±0.63	8.06±0.59	8.72±0.22
3	n-Hexane fraction of *D. falcata*	0.01	9.37±0.50	9.14±0.19	8.77±0.07	7.48±0.39
		0.1	9.41±0.53	8.46±0.16	8.03±0.31	8.33±0.33
		0.5	9.54±0.51	7.97±0.62	8.12±0.74	8.64±0.87
		1	8.59±0.85	8.14± 0.56	8.31± 0.51	8.35± 0.33
4	Chloroform fraction of *D. falcata*	0.01	9.14± 0.30	9.23±0.68	8.13±0.28	8.12±0.85
		0.1	9.83±0.61	9.02± 0.98	7.32± 0.82	8.33±0.33
		0.5	10.49± 0.32	8.82±0.18	9.59±0.29	8.67±0.48
		1	8.54±0.39	8.36± 0.77	10.09± 0.36	8.44± 0.37
5	Ethyl acetate fraction of *D. falcata*	0.01	9.83± 0.41	7.95±0.48	7.95±0.48	8.84±0.63
		0.1	8.90±0.42	7.43± 0.43	7.43± 0.43	8.09±0.08
		0.5	8.67± 0.50	7.20±0.53	7.20±0.53	8.33±0.47
		1	7.75±0.29	7.54± 0.35	7.54± 0.35	8.09± 0.49
6	Residual of ethanolic fraction of *D. falcata*	0.01	10.36± 0.13	8.73±0.38	7.44±0.29	8.41±0.40
		0.1	9.67±0.53	8.12± 0.65	7.81± 0.43	8.77±0.47
		0.5	9.68± 0.82	8.98±0.06	6.98±0.50	7.92±0.54
		1	7.75±0.29	8.15± 1.13	7.28± 0.52	7.95± 1.02
7	Chlorampheni-col (positive controle)	0.01	12.26± 0.72	12.57± 1.88	12.37± 1.38	17.41±1.88
		0.1	17.05± 0.93	20.58±1.09	11.25±1.67	22.38±1.11
		0.5	21.20±0.41	24.05± 1.52	13.70± 2.90	28.26±1.68
		1	22.52± 0.72	24.98± 1.06	17.35± 1.07	32.57± 1.88

CONCLUSION

The study showed the presence of total phenolic content and flavonoids from extracts and fractions from stems of *D. falcata*. Ethyl acetate fraction from stems of *D. falcata* showed the highest phenolic and flavonoid levels, as well as very high antioxidant activity. Zones of inhibition of extracts and each fraction showed moderate activity against *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* FNCC 0048, and *Streptococcus mutans*. This study shows that stems of *D. falcata* contain potential phenolic compounds that can be suitable as natural antioxidants and treatment of various infections caused by microbes.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Research and Technology of the Directorate of Higher Education, Indonesia for funding of competency research grants (PBK, Number: 0045 / E3/ LL/ 2018, January 16, 2018) and foreign seminar aid program (BSLN, MENRISTEK DIKTI) Indonesia has provided financial assistance to attend the ISTEC 2018 international seminar. The author also thanks to Prof.K. H. Sugiyarto from Department Chemistry Ed. Yogyakarta State University which helps review the manuscript.

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ISSN: 0970-020 X
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2018, Vol. 34, No.(5):
Pg.

Study of Potential Phenolic Compounds from Stems of *Dendrophthoe falcata* (Loranthaceae) Plant as Antioxidant and Antimicrobial Agents

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(Received: April 04, 2018; Accepted: October 15, 2018)

ABSTRACT

Dendrophthoe falcata is a hemiparasitic plant that has been used in traditional medicine. The study was conducted to identify the total phenolic and flavonoid compounds, to test antioxidant, and antimicrobial activity of ethanol extract stems of *D. falcata* parasite on *Melia azedarach* host tree. The dry powder stems of *D. falcata* was extracted with ethanol. The ethanol extract was subsequently partitioned successively using n-hexane, chloroform, and ethyl acetate. Each fraction was analyzed by a quantitative phenolic and flavonoid content with spectrophotometer method, and tested as antioxidant and antimicrobial activites. Antioxidant activity was performed by 2,2-diphenyl-1-picrilhidrazil (DPPH) method, while antimicrobial assay used pathogenic bacteria by disk-diffusion method. The results concluded that the stem of *D. falcata* plant showed a high content of phenolic and flavonoid compounds, very high antioxidant and moderate antimicrobial activities. It was also found that stems of *D. falcata* contain potential phenolic compounds that can be used as natural antioxidants and the treatment of various infections caused by microbes.

Keyword: *Dendrophthoe falcata*, Antioxidant. Antimicrobial. Phenolic compound, Flavonoids.

INTRODUCTION

Loranthacea is a parasitic plant widely used in traditional Chinese medicine systems. One of this species is *L. parasiticus* which is known as Sang Ji Sheng (in Chinese), parasites (in Malay) and baso-Kisei (in Japanese). The plants are commonly found as parasitic plants that attach to other plant stems. Parasites can be found in tropical and sub-tropical regions, but with different species diversity.[1-4] *L. parasiticus* known as traditional medicine is widely used as a diuretic, high blood pressure,

polio virus prevention5, antimicrobial and anti-inflammatory[6], and neuroprotective[7], antioxidant[8], and antidiabetic[9].

Dendrophthoe falcata (*D. falcata*) is one of the hemiparasitic plants commonly attached to host plants such as *Mangifera indica* (Anacardiaceae), *Melia azedarach* (Meliaceae) and *Psidium guajava* (Myrtaceae). The plant is widely distributed in tropical and sub-tropical regions. The whole plant of *D. falcata* is used in native treatment systems such as astringent, aphrodisiac, diuretics, and treat menstrual disorders, and antifertility agent[10]. It has been reported that methanol extract and water decoction of *D. falcata* leaves have anti-inflammatory effect on experimental animals and showed strong antioxidant activity by *in-vitro*[11].

A study conducted by Chandrakasan[2] comparing the ethanol extract stems of Loranthus longiflorus plants that are parasitic to the plant stems of Casuarina equisetifolia and Ficus religiosa by GC-MS showed different compounds and biological activity. A comparative study of the phytochemical and anti-microbial properties from leaf of Loranthus micranthus harvested from six host trees, showed marked variations in phytochemical constituents and anti-microbial activities[12]. The work reported by Chandrakasan13 showed the presence of cumarin compounds from leaf of *L. longiflorus* plant, whereas in fresh flowers Loranthus acaciae was reported to contain antimicrobial quercetin 3-O-glucoside. Research on chemical and biological studies of leaf extract of *D. falcata* showed the crude extracts and the fractions have potential biological activities as cytotoxic, antioxidant, and thrombolytic activities. The leaves of *D. falcata* have also been reported to contain compounds identified as lupeol, 3-β-acetoxy-12-ene-11-one and β-sitosterol[14]. In this work we report the potential of phenolic compounds from stems of D. falcata plant that hemiparasite on Melia azedarach host tree as antioxidant and antimicrobial agents.

EXPERIMENTAL

Apparatus and reagents

Evaporator Buchi Rotavapor R-114, spectronic 20, incubator, autoclave, LAF (Laminatory Air Flow), coloni counter, forceps, micro pipettes, shaker, water bath, deep freezer, ruler (millimeter scale), petri plate, and analytical balance were used in this work. Ethanol, methanol, n-hexane, ethyl acetate, acetone, DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, folin-ciocalteu, sodium carbonate, gallic acid, rutin, aluminum nitrate, potassium acetate, chloramphenicol, Mueller-Hinton agar (MHA, OXOID), Nutrient Broth (NB), Nutrient Agar (NA), paperdisk, DMSO (dimethyl sulfoxide), plastic wrap, aluminium foil, and aquadest were used in this work without particular treatment.

Plant Materials

The sample used in this study was a parasitic plant attached to the stem of the Melia azedarach host tree, collected in October 2017. The sample was then determined by Biology Laboratory as specimen (DF-01-2017) and scientifically known as Dendropthoe falcata (L.f.) Ettingsh, the local name of “benalu” or “kemadealan”.

Microorganism

This study used four pathogenic bacterial isolates, three isolates of Escherichia coli ATCC 11229, *Staphylococcus aureus* ATCC25923, and *Staphylococcus epidermidis* FNCC 0048 were obtained from the Microbiology laboratory, while *Streptococcus mutans* was obtained from Dentistry laboratory. The microorganism were sub-cultured and stored in a semisolid medium (Mueller Hinton agar plates) at 4^0C until needed.

Preparation of samples

The milled dried from stem of *D. falcata* (4 kg) was extracted using 95% ethanol as the solvent for 24 h and repeated 3 times. The extracts were then concentrated using a vacuum rotatory evaporator and yielded brown residue for about 72 g. The ethanol extract of *D. falcata* (60 g) was successively partitioned using n-hexane, chloroform, and ethyl acetate. Each fraction was evaporated to dryness under vacuum resulting in a fraction of n-hexane (12 g), chloroform fraction (11.3 g), ethyl acetate fraction (10.62 g), and residual ethanol fraction (22 g). The procedure of sample preparation can be seen in Figure 1.

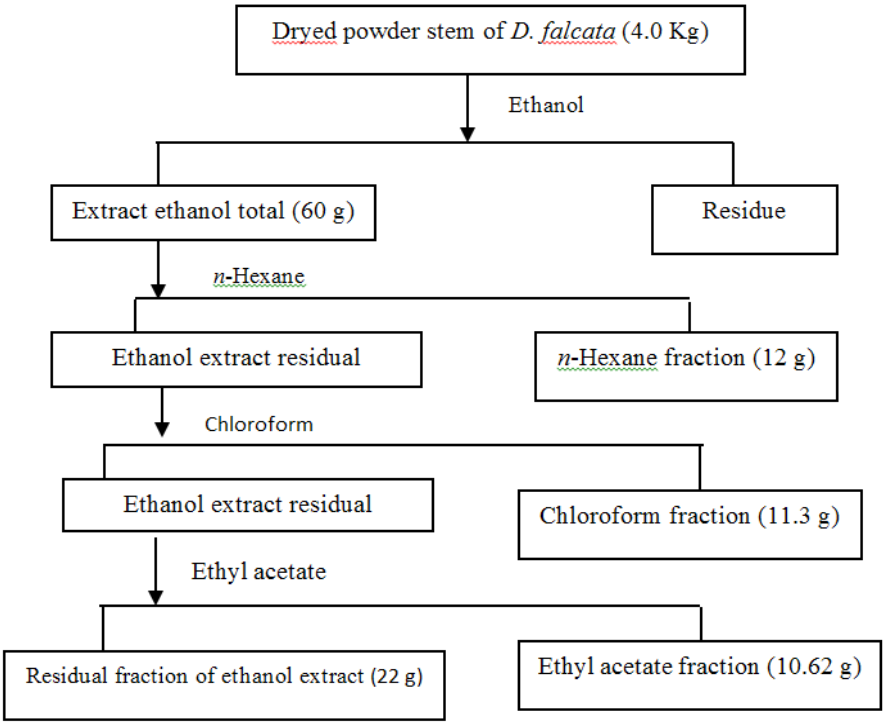

Fig. 1. The procedure of sample preparation

Phytochemical screening

The qualitative phytochemical screening was performed using reagent for terpenoid test, alkaloid test, phenolic test, and saponin test. Terpenoids test was performed using Salkowski reagent, alkaloid test was done with Wagner reagents, phenolic test was done with iron(III) chloride test, and foam test was for saponin[15].

Quantitative determination of total phenolic and flavonoid content

The quantitative analysis of the total phenolic content of each extract and fractions were determined using the folin-ciocalteu reagent[16]. About 200 µL of sample (500 µg) was mixed with folin-ciocalteu reagent (0.4 mL), aquadest (4 mL) and sodium carbonate (2mL, 15% w/v). The mixture was subsequently incubated at 50°C for 10 min. and measured its absorbance at 760 nm. The total phenolic content was calculated according to the standard curve specified for the gallic acid as a reference. Each measurement was performed three replicates. The total phenolic content is expressed as mg of Gallic Acid Equivalent (GAE) per g of plant extract.

The quantitative analysis of total flavonoid content was determined by spectrophotometric method. This method was adopted with a slight modification of Liu TC[17]. The samples of each extract were diluted with 80% ethanolic-aqueous solution (0.9 mL). A total of 0.5 mL of the sample was added to the reaction tube containing 0.1 mL 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 min. and its absorbance was determined at 415 nm with a UV spectrophotometer. Total flavonoid content was calculated using standard curves with rutin as references. The total flavonoid content is expressed as mg Rutin Equivalents (RE) per g of plant extract.

Determination of antioxidant activity test

The antioxidant activity test of each sample was done by free radical scavenger method. This method used DPPH (2,2-diphenyl-1-picrylhydrazyl) as a source of free radicals[18]. A total of 5 mL of sample (in various concentrations between 100-3,125 µg/mL) and positive control (ascorbic acid in various concentrations between 6.25-0.390 µg/mL) were mixed with 5 mL of DPPH solution in methanol

(0.12 mM) and incubated at room temperature for 30 minutes. Absorbance was measured at 516 nm using spectronic[20] (Genesys). The absorption of DPPH from the sample was compared to the blank solution. Each sample was tested in three replications. Antioxidant activity was calculated as a reduced percentage of DPPH compared with control and inhibitory activity can be calculated to determine IC_{50}.

Determination of activity as an antimicrobial agent

The antimicrobial activity test of the crude extract and each fraction were performed by agar diffusion method. This method was adopted with slight modifications of Bisnu[19]. In this work four bacteria i.e *Escherichia coli* ATCC-11229, *Staphylococcus aureus* ATCC-25923, *Staphylococcus epidermidis* FNCC-0048, and *Streptococcus mutans* were used. As a positive control chloramphenicol was applied, while DMSO (dimethyl sulfoxide) 10% was used as a solvent and also as a negative control.

The test bacteria of 100 µL was inoculated onto the Muller Hinton agar plate, and flattened with drigalsky. The paperdisk was immersed in the solution of each sample for five minutes and was dipped into the sample solution which was placed on top of the agar medium, and incubated at 37^{0}C for 12 h. After 12 h incubation, the zone of inhibition around the disks was measured in millimeter scale. A four serial dilution (0.01; 0.1; 0.5 and 1.0 % w/v) was carried out for each sample. The measurement was done in three repetitions.

RESULTS AND DISCUSSION

This study used stem bark of *D. falcate* that hemiparasite on Melia azedarach host tree (Fig. 2). After being dried and mashed the stem bark was extracted by maceration using 95% of ethanol solvent. Total concentrated ethanol extract obtained was about 72 g. A portion of the extract (60 g) was partitioned using n-hexane, chloroform, and then ethyl acetate solvents. Each subsequent fraction was evaporated to dryness under vacuum. Each of the extracts and fractions were identified to phytochemical test and its activity as antioxidant and antimicrobial. The results of qualitative phytochemical test including terpenoid, alkaloid, phenolic, and saponin are listed in Table 1.

Fig. 2. Stem of *D. falcata*

Table 1: Qualitative phytochemical screening of the extracts and fractions from stem of *D. falcata*

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

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

Quantitative analysis of total phenolic content was performed by spectrophotometer following the Folin-Ciocalteu method[17]. The absorbance of each sample was recorded at 760 nm. In this work gallic acid was used as a reference at various concentrations and recorded at

the same wavelength. The result of calibration curve follows the linear regression equation of y = 0,004x - 0,032 (R^2 = 0,985). The total phenolic content is, therefore, expressed as mg Gallic Acid Equivalents (GAE) per g of plant extract.

Total flavonoid content was obtained by using rutin as standard reference at various concentrations[17]. The absorbance of each sample was recorded at its maximum wavelength, which was obtained at 415 nm. The calibration curve follows the linear regression equation of y = 0,003x + 0,099 (R^2 = 0,963). The total flavonoids content is, therefore, expressed as mg Rutin Equivalents (RE) per g of plant extract[17]. The total phenolic and flavonoids content of the extract and each fraction were collected in Table 2.

Antioxidant capacity was expressed as IC_{50} (µg/ml) of DPPH scaveng¬ing activity[18]. The concentrations of each sample used in the antioxidant activity test were 25.00, 12.50, 6.25, and 3.125 µg/mL, except for ascorbic acid in various concentrations of 10.00, 5.00, 2.50, 1.25, and 0.625 µg/mL. From the absorbance of each sample, a linear regression for the corresponding IC_{50} equations was made to calculate (concentration samples having 50% inhibitory activity). The inhibition activity (IC_{50}) of each sample, and the result is presented in Table 2.

Table 2. Quantitative of total phenolic and flavonoids content, and antioxidant activity of the crude ethanol extract and each fraction from stem of *D. falcata*

Sample	Total phenolic content (TPC) (mg GAE/g)	Total flavanoids content (TFC) (mg RE/g)	Antioxidant activity by DPPH method (IC_{50} µg/ml)
Crude ethanol extract	1290.8 ± 7.63	195.6 ±1.3	11.02± 0.12
n-hexane fraction	0	0	65.96±1.22
Chloroform fraction	954.2 ± 5.13	315.6 ±1.6	20.33±0.32
Ethyl acetate fraction	2079.2 ± 5.10	980.0 ± 1.7	7.56±0.41
Residu fraction of ethanol extract	261.7 ± 3.37	0	27.54±0.62
Ascorbic acid (Positive control)	-	-	1.14±0.11

*Data are mean ± SD for triplicate measurement

Screening of antimicrobial activity of crude ethanol extract and the fraction of stem bark from *D. falcata* was done by using agar disk-diffusion method activities againts pathogenic bacteria i.e. *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* FNCC 0048, and *Streptococcus mutans* through the disk-diffusion method[19]. The zones of inhibitions of each compound against pathogenic bacteria are presented at Table 3.

From this study it could be shown the suitability between qualitative and quantitative data. The total ethanol extract still contains a mixture of both non-polar and relatively polar compounds. After successive fractionation using n-hexane, chloroform, and ethyl acetate solvents, groups of soluble compounds according to the polarity of each solvent should result. n-Hexane should dissolve the relatively non-polar compounds, chloroform should dissolve the relatively semipolar compounds, while ethyl acetate should dissolve the relatively polar compounds. The results of this study showed that between the crude extract and the fractions, the ethyl acetate fraction showed the highest total phenolic content (2079.2 ± 5.10 mg GAE/g) followed by crude ethanol extract (1290.8 ± 7.63 mg GAE /g), chloroform fraction (954.2 ± 5.13 mg GAE/g) and then residual fraction of ethanol extract (261.7 ± 3.37 mg GAE/g).

The total flavonoid contents of crude ethanol extract and this fractions determined by spectrophotometry method were reported as rutine equivalents (RE). Flavonoid content was observed on crude ethanol extract (195.6 ±1.3 mg RE/g),

chloroform fraction (315.6 ±1.6 mg RE/g), and ethyl acetate (980.0 ± 1.7 mg RE/g). In general, phenolic compounds and including flavonoids can dissolve in chloroform, ethyl acetate, and ethanol, but in different concentrations depending on their structure and polarity. The more polar phenolic compounds are more easily dissolved in more polar solvents. Phenolic compounds generally exhibit antioxidant activity, therefore the antioxidant activity of chloroform and ethyl acetate fractions with IC_{50} shows very active, although relatively less active than ascorbic acid (positive control). Table 2 shows that the total phenolic and flavonoid content in the ethyl acetate fraction stem of *D. falcata* plant is much higher than that in the chloroform fraction, thus indicating the highest antioxidant activity compared to the other fractions. Several studies have shown that many plants contain phenolic compounds. Flavonoids, are one of the natural phenolic compounds found in fruits, vegetables, grains, barks, roots, stems, flowers, tea and wine[20]. The phenolic compound includes a number of compounds generally having an aromatic ring with one or more hydroxyl groups[21]. The antioxidant potential is usually associated with the content of phenolic compounds due to the conjugated π-electron system which facilitates electron donation from hydroxyl groups to oxidizing radicals. The antioxidant activity test by DPPH method is based on a radical capture reaction by an antioxidant compound with an oxygen atom transfer mechanism, which will produce stable DPPH-H molecules in a non-radical form[22]. The IC50 of each extract and fraction stems of D. falcata in this study indicate the presence of phenolic compounds which have very high antioxidant activity. The activity is caused by the content of polyphenol compounds such as flavonoids, tannins, ~~terpenoids~~, ~~phenols~~, ~~and saponins~~ from each extract and the fraction stem of the *D. falcata*. Previous research evaluating the antioxidant activity of methanol extract from the stems of the *D. falcata* parasite on mangoes, *Mangifera indica* (Anacardiaceae) showed the presence of compounds that have significant antioxidant activity[23]. Several studies have shown the presence of phenolic compounds in the Loranthaceae family, including the leaves of Loranthus micranthus Linn showed 3-O- (3,4,5-trimethoxybenzoyl)-(-) -epicatechin (TMECG), (-)-epicatechin-3-O-(3″-O-methyl)-gallate (ECG_3 ″ Me), rutine, and peltatoside[24]. Whereas in Scurrula ferruginea Durrer (Loranthaceae) found phenol compounds, quercetin and quercitrin, and 4-O-acetylquercitrin flavonol glycosides[25]. These phenolic compounds show high antioxidant activity.

Table 3 shows that the activity of crude ethanol extract from stem of *D. falcata* has a larger zone diameter than the negative control (DMSO 10%), but much smaller than the positive control (chloramphenicol). This indicates that the extract and its fractions exhibit activity as antimicrobial agent, although not as good as chloramphenicol. The classification of zone inhibition response of bacterial growth based on clear zone diameter includes weak response (5 mm), medium response (5-10 mm), strong response (10-20 mm), and very strong response (diameter> 20 mm)[26]. Several previous studies have also shown that some methanol extracts of loranthus sp plants have significant antimicrobial activity against different *Gram-positive* and *Gram-negative* bacterial, and fungi being tested[27]. The mechanism of antibacterial action may be through various means, including inhibition of cell wall synthesis, inhibition of cell wall permeability, inhibition of cell wall proteins, inhibition of nucleic acid synthesis, and inhibition of microbial metabolisme[28]. The maximum zone of inhibition was 10.46±3.63 mm at the maximum concentration used 1% of crude ethanol extract stems of *D. falcata* against *Staphylococcus epidermidis* FNCC 0048.

Plant phenolic compounds are main contributors of antioxidant activity and are also responsible for anti-inflammatory, antiviral and anticancer and antimicrobial activities[29]. The presence of terpenoids and saponin have already shown as antimicrobial and antiinflammatory activity[30]. The results of this study show that the stem bark of *D. falcata* plant contains a high number of phenolic compounds, has antioxidant activity, and exhibits moderate antimicrobial activity. Isolation and identification of the compound structure of the *D. falcata* plant is being analyzed in our laboratory.

Table 3: Zones of inhibitions as shown by crude ethanol extract and its fraction of stem bark from *D. falcata* at different concentrations against selected microorganisms

No	Sampel	Concentration (%)	Diameter Zona of Inhibition (Mean±SD)mm after 12 h incubations			
			Streptococcus mutans	*Staphylococc-us epidermidis* FNCC 0048	*Staphylococcus aureus* ATCC25923	*Escherichia coli* ATCC 11229
1	DMSO (10%)		6.17±0.14	6.17±0.22	6.57±0.80	6.17±0.84
2	Crude ethanol extract of *D. falcata*	0.01	10.36±0.13	10.12±0.87	8.00±0.53	8.92±0.71
		0.1	9.67±0.53	10.12±0.87	8.45±0.05	9.18±0.03
		0.5	9.68±0.82	10.06± 0.87	8.28± 0.68	9.23± 0.58
		1	7.75± 0.29	10.46±0.63	8.06±0.59	8.72±0.22
3	n-Hexane fraction of *D. falcata*	0.01	9.37±0.50	9.14±0.19	8.77±0.07	7.48±0.39
		0.1	9.41±0.53	8.46±0.16	8.03±0.31	8.33±0.33
		0.5	9.54±0.51	7.97±0.62	8.12±0.74	8.64±0.87
		1	8.59±0.85	8.14± 0.56	8.31± 0.51	8.35± 0.33
4	Chloroform fraction of *D. falcata*	0.01	9.14± 0.30	9.23±0.68	8.13±0.28	8.12±0.85
		0.1	9.83±0.61	9.02± 0.98	7.32± 0.82	8.33±0.33
		0.5	10.49± 0.32	8.82±0.18	9.59±0.29	8.67±0.48
		1	8.54±0.39	8.36± 0.77	10.09± 0.36	8.44± 0.37
5	Ethyl acetate fraction of *D. falcata*	0.01	9.83± 0.41	7.95±0.48	7.95±0.48	8.84±0.63
		0.1	8.90±0.42	7.43± 0.43	7.43± 0.43	8.09±0.08
		0.5	8.67± 0.50	7.20±0.53	7.20±0.53	8.33±0.47
		1	7.75±0.29	7.54± 0.35	7.54± 0.35	8.09± 0.49
6	Residual of ethanolic fraction of *D. falcata*	0.01	10.36± 0.13	8.73±0.38	7.44±0.29	8.41±0.40
		0.1	9.67±0.53	8.12± 0.65	7.81± 0.43	8.77±0.47
		0.5	9.68± 0.82	8.98±0.06	6.98±0.50	7.92±0.54
		1	7.75±0.29	8.15± 1.13	7.28± 0.52	7.95± 1.02
7	Chlorampheni-col (positive controle)	0.01	12.26± 0.72	12.57± 1.88	12.37± 1.38	17.41±1.88
		0.1	17.05± 0.93	20.58±1.09	11.25±1.67	22.38±1.11
		0.5	21.20±0.41	24.05± 1.52	13.70± 2.90	28.26±1.68
		1	22.52± 0.72	24.98± 1.06	17.35± 1.07	32.57± 1.88

CONCLUSION

The study showed the presence of total phenolic content and flavonoids from extracts and fractions from stems of *D. falcata*. Ethyl acetate fraction from stems of *D. falcata* showed the highest phenolic and flavonoid levels, as well as very high antioxidant activity. Zones of inhibition of extracts and each fraction showed moderate activity against *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* FNCC 0048, and *Streptococcus mutans*. This study shows that stems of *D. falcata* contain potential phenolic compounds that can be suitable as natural antioxidants and treatment of various infections caused by microbes.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Research and Technology of the Directorate of Higher Education, Indonesia for funding of competency research grants (PBK, Number: 0045 / E3/ LL/ 2018, January 16, 2018) and foreign seminar aid program (BSLN, MENRISTEK DIKTI) Indonesia has provided financial assistance to attend the ISTEC 2018 international seminar. The author also thanks to Prof.K. H. Sugiyarto from Department Chemistry Ed. Yogyakarta State University which helps review the manuscript.

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