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Phytochemical screening and antioxidant activity of clove mistletoe leaf extracts (*Dendrophthoe pentandra* (L.) Miq)

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Abstract : *Clove mistletoe (Dendrophthoe pentandra (L.) Miq) is one of the semi-parasitic plants belonging to the Loranthaceae family. Clove mistletoe leaf extracts have many biological activities such as antibacterial, antioxidant and antidiabetes. The purpose of this study was to determine the content of secondary metabolites in clove mistletoe leaf extracts through phytochemical screening and determine its antioxidant activity through DPPH free radical scavenging. Samples were tested include water and ethanol 70 % extracts, as well as n-hexane, ethyl acetate and ethanol fractions. Phytochemical screening showed that all samples containing tannins and flavonoids but no alkaloids. The highest total phenol contents was ethyl acetate fraction namely 358.4 mg GAE/ g. The best antioxidant activity was water extract, ethanol 70 % extract and ethyl acetate fraction. Therefore, clove mistletoe leaf extracts are potential source for antioxidant.*

Keywords – *Antioxidant, clove mistletoe leaf, DPPH, phytochemical*

I. INTRODUCTION

Mistletoe is semi-parasitic plants widely spread in Indonesia. Usually people recognize mistletoe based on host plant where it lives. Its spread occurs through seed-eating birds. Beside is known as destroyer of host plants, it also is known has ability as medicinal plants [1] such as cough medicine, diabetes, hypertension, cancer, ulcers and skin infection [2-3]. One kind of mistletoe is not widely known yet biological activity is clove mistletoe (*Dendrophthoe pentandra* (L.) Miq). This plant is included into Loranthaceae family, i.e. semi-parasitic plants bound the branches of their hosts via a sucker called haustorium [4].

Previous study showed that clove mistletoe phenol extract in roots, stems, leaves and flowers contain phenol compound that act as antioxidant agent [5]. Antioxidant is substance that can inhibit oxidation process by pressing the cell damage caused by free radical oxidation. Free radical is unstable because it has unpaired electron and usually look for pairs of electrons in biological macromolecules [6]. Presence of free radicals in the body can lead to various degenerative diseases such as cancer, diabetes and heart [7-8]. Using of the natural antioxidants in free radicals scavenging have also some advantages as the examples are being obtained easily, economically and have slight or no side effects [9].

One of the method to determine antioxidant activity from natural substance extract is DPPH (1,1-diphenyl-2-pycrilhydrazyl) method [9-11]. DPPH is stable free radical in room temperature and the result reaction with antioxidant involves a colour change from violet to yellow [9]. In this study, clove mistletoe leaf was extracted with different solvent that used to evaluate secondary metabolite compound through phytochemical screening and antioxidant activity through DPPH free radical scavenging.

II. MATERIALS AND METHODS

2.1 Plant Materials

Fresh leaf of *Dendrophthoe pentandra* (L.) Miq was collected from Curup, Bengkulu Province, Indonesia. Taxonomic authentication of the sample was conducted by a botanist in the Herbarium Bogoriense, Research Centre for Biology-Indonesia Institut of Sciences, Bogor, Indonesia.

2.2 Extraction and Fractination

Extractions were conducted by boiling and maceration. Comparison of the clove mistletoe leaf powder with solvent is 1:10. Boiling was conducted by boil the leaf powder into the water solvent for 2 hours while maceration by it immersing into ethanol 70 % for 24 hours with a shaker in speed of 150 rpm. The mixture was separated and evaporated using an evaporator to obtain water and ethanol extracts. The ethanol extract further fractionated with a solvent which has a different polarity, i.e. n-hexane, ethyl acetate and ethanol. The test samples in this study consist of water and ethanol extracts as well as n-hexane, ethyl acetate and ethanol fractions.

2.3 Phytochemical Screening

Phytochemical screening is qualitative assay consists of test for alkaloids, tannins, flavonoids, saponins and triterpenoids.

2.3.1 Test for alkaloids

10 mg sample was mixed with 1 mL of 2 N HCl and 9 mL of distilled water. The mixture was heated for 2 minutes and cooled. The filtrate was filtered and used for following test (i.e. Meyer, Bouchardart and Dragendorf tests).

2.3.2 Test for tannins

10 mg sample was boiled in 50 mL of distilled water and then filtered. A quantity (5 mL) of test solution was added into a test tube followed some drop of $FeCl_3$. Formation of brownish green or blue black colouration indicates presence of tannins.

2.3.3 Test for flavonoids

10 mg sample was mixed with 10 mL of distilled water. The mixture was heated for 5 minutes and filtered. The filtrate was mixed with Mg powder, 1 mL of strong HCl and 1 mL of amyl alcohol. Formation of colour in amyl alcohol layer indicates flavonoids.

2.3.4 Test for saponins

10 mg sample was added into test tube and 10 mL of boiling water was added and than cooled. The mixture was agitated vertically for 10 seconds. For 10 minutes formation of foam indicates saponins.

2.3.5 Test for triterpenoids

10 mg sample was mixed with 5 mL of eter solution and evaporated. Test solution was mixed with anhydrate acetate acid and strong H_2SO_4 (2:1). Formation of red-green colour indicates triterpenoids.

2.4 Total Content Phenol Determination

Three hundred microliter of samples were added into test tubes followed by 1.5 mL of Folin-Ciocalteu reagent (1:10) and 1.2 mLof Na_2CO_3 (7.5 %). The mixture was incubated at room temperature for 30 minutes and then the absorbance was measured using a spectrophotometer at 765 nm. Total phenol content of samples were expressed as mg gallic acid equivalents (GAE)/ g fresh sample.

2.5 Antioxidant Assay

100 µL sample solution (5, 10, 30, 50, 100 and 200 µg/mL) were added into wellplate and than mixed with 100 µL of ethanol solution and 100 µL of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals solution. The mixture was incubated for 30 minutes in room temperature, and than the absorbance was measured using microplate reader at 517 nm. α-tocopherol was used as a positive control. The capacity to scavenge the DPPH radical was calculated by the equation : [(A-B)/A] × 100 %; whereas A is the absorbance of the negative control (DPPH plus ethanol) and B is the absorbance of the sample. The activity was expressed as IC_{50} (the concentration of sample required to scavenge 50 % of DPPH free radicals).

2.6 Statistical Analysis

The statistical significance was assessed using Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). Significance was accepted at $P < 0.05$.

III. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Phytochemical screening is a preliminary analysis to determine the content of secondary metabolites in plants. Naturally produced by plants to interact with biotic and abiotic environment [12]. Presence of phytochemical compounds can be used to develop new drugs [13]. The result showed that all test samples containing flavonoids and triterpenoids, but no alkaloids. While tannins was not contained in the n-hexane fraction and also all samples contained saponin except n-hexane and ethyl acetate fractions. Methanol extract of *Viscum album* mistletoe contained tannins and flavonoids but did not contain alkaloids and saponins [3]. Differences of secondary metabolites in plants are influenced by kinds of host mistletoe and solvents used for extraction [2,14]. Phytochemical compounds of test samples can be seen in Table 1.

3.1.1 Tannins

Tannins are plant polyphenols which have many biological activities such as antitumor, anti-plasmin inhibitors, antioxidants and can bind to the protein [15-17]. Tannins can be found in every part of the plant that serves to help the regulation of tissue growth and as a pesticide [17-18]. $FeCl_3$ was known can be used to detect the presence of tannins in the leaf methanol extract of *F. thonningii* [19], ethanol and acetone extract of guava leaf [20]. Presence of tannins compound can be react with Fe^{3+} ions form complex green or blue-black [20]. Tannins have a high solubility in polar solvents and other organic solvents. Ethanol is a good solvent to extract tannin because it has OH groups which can react with functional groups of tannin [20].

In general, tannins can be classified into two groups, namely hydrolysable tannins and condensed tannins. Tannin compounds that have been isolated and have antioxidant activity were epicatechin on *C. equisetilofia* [21], epigallocatechin, gallocatechin and epigallocatechin- (4β → 8) -gallocathecin on Stryphnodendron obovatum Benth [22]. In addition, the antioxidant activity *of Castanea mollissima* Blume characterized as hydrolysable tannin [23].

3.1.2 Alkaloids

Alkaloids are alkaline compound, having one or more nitrogen atom those are part of a cyclic system. Alkaloids are widely distributed in the plant kingdom [24]. Alkaloids can be detected by extracting plant with water solvent added acid. In addition, alkaloids also can be detected by adding alkaline directly to plants sample powder, then extracted with organic solvents [24]. Alkaloids can be used as therapeutic and pharmacological agents. They have biological activities such as anti-inflammatory, antidiabetes, antioxidant and antibacterial [25-27].

3.1.3 Flavonoids

Flavonoids are secondary metabolites of plants and represent a large group of polyphenol compounds [28]. Many plants containing flavonoids such as ethanol extract of *Entada scandens* [29], ethanol extract of *Ocimun basilicum* L. (Basil) [30] and water extract of *Dendrophthoe falcata* mistletoe leaf [31]. Flavonoid compounds are relatively polar so that can be extracted with solvent polar such as water, methanol and ethanol [32]. Presence of flavonoids can be detected by adding a small piece of magnesium ribbon into alcohol solvent and mixed with HCl. The formation of color in the alcohol layer indicates a flavonoid compound. Type of color formed depends by the type of flavonoid compounds found in plants [24].

Flavonoids were known have biological activities such as antioxidant, anticancer and antimicrobial [33-36]. The kinds of flavonoid that have been isolated and have antioxidant activity were rutin and quercetin of mulberry leaves [37] and flavonol glycosides in the ethanol extract of *Dendrophthoe pentandra* (L.) Miq mistletoe [38]. Antioxidant activity of flavonoid shown by the basic structure in the form of flavan nucleus, which consists of 15 carbon atoms arranged in three rings (C_6-C_3-C_8), labeled A, B and C (Fig. 1) [34]. In the basic structure of flavonoids, flavan nucleus, number, position and types of substititions effect in radical scavenging and chelating activity [39].

3.1.4 Saponins

Saponins are glycoside which consist of steroidal or terpenoid aglycone and associated to one or more sugar chain [40]. They can be found in many species of plants. They have biological activities such as antioxidants and antiglycation [40-41].

3.1.5 Triterpenoids

Triterpenoids are organic compounds which formed naturally in plants. They have a C30 carbon skeleton and biosynthesized from squalene, a precursor of steroid [42]. They are classified as tetracyclic triterpenoids and pentacyclic triterpenoids [43]. Several triterpenoid have biological activities such as antioxidant from methanol extracts of *Salvia macrochlamys* and stems of *Momordica charantia*, antyglycation from *Aralia taibaiensis* and antidiabetes from bitter melon [44-47]

3.2 Total Phenol Compounds

Total phenol contents was determined by the Folin-Ciocalteau method. It was stated as Gallic Acid Equivalent (GAE). Regression similarity was obtained from measuring of gallic acid standard namely y= 0.018x + 0.113 with R^2= 0.990. The result measuring of total phenol contents showed that water extract, ethanol extract, n-hexane fraction, ethyl acetate fraction and ethanol fraction were significantly different ($p<0.05$) namely 85.6, 109.8, 26.4, 358.4 dan 37.4 mg GAE/g sample, respectively (Fig. 2).

In this study, the highest of total phenol contents was showed by ethyl acetate fraction and the smallest was showed by n-hexane fraction. This suggest that ethanol 70 % extract was more containing phenol compound which has the nature of semipolar. According to research conducted by The *et al* [14] n-hexane solvent has small ability to extract of hydrophilic phenol compounds, but it was strong to extract phosphatides, lipid and other compounds that soluble in fat. Thus, total phenol content depends on the type of solvent that used during extraction [14].

3.3 Antioxidant Activity

Antioxidants act as free radical scavenging that derived from oxidation process. Its activity based on IC_{50} value (Table 2). The result in this study showed that water extract, ethanol 70 % extract and fraction of ethyl acetate were not significantly different, but they were significantly different with n-hexane and ethanol

fractions. They depend on phenol derivate compounds that obtained from extraction. Phenol compounds are essential constituen in plants and extract that containing much phenol has free radical scavenging activity is higher than other extracts [48-49]. In addition, based on phytochemical screening showed that all samples containing flavonoids which known act as antioxidant agent [30].

Antioxidant compounds in this study were more soluble in polar and semipolar solvents than nonpolar like n-hexane. High-polarity solvents have good ability in free radical scavenging [14]. α-tocopherol was used as comparator in DPPH free radical scavenging. Its antioxidant activity was not significantly different with water extract, ethanol 70 % extract and ethyl acetate fraction. This was caused by presence of OH group in aromatic ring from α-tocopherol and phenol compounds [50]. In addition, α-tocopherol or phenol compounds has one site of electron arrest so that their stoichiometric be 1:1, it means that one mocelule of DPPH can be reducted by one molecule of α-tocopherol or phenol compound.

IV. FIGURES AND TABLES

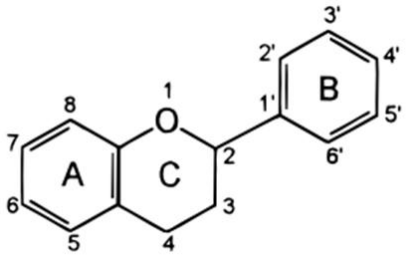

Fig 1: Basic structure of flavonoid

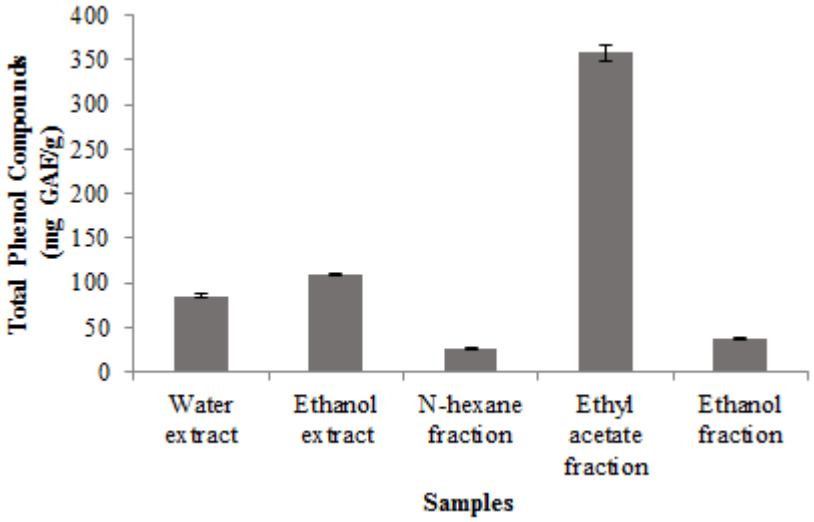

Fig 2: Comparison of total phenol contents in the samples

Table 1: Phytochemical compound of clove mistletoe leaf samples

Phytochemical	Water extract	Ethanol extract	N-hexane fraction	Ethyl acetate fraction	Ethanol fraction
Alkaloids					
Mayer	-	-	-	-	-
Dragendorf	-	-	-	-	-
Bouchardat	-	-	-	-	-
Tanins	+	+	-	+	+
Flavonoids	+	+	+	+	+
Saponins	+	+	-	-	+
Triterpenoids	+	+	+	+	+

Table 2: Antioxidant activity of clove mistletoe leaf samples

Samples	IC_{50} (µg/mL)
Water extract	11.4[a]
Ethanol extract	6.8[a]
N-hexane fraction	936.6[c]
Ethyl acetate fraction	6.4[a]
Ethanol fraction	217.8[b]
α-tocopherol	6.1[a]

Note: Superscrift characters (a,b,c) showed the result significantly different ($p<0.05$)

V. CONCLUSION

The study showed that clove mistletoe leaf extracts (Dendrophthoe pentandra (L.) Miq) contained of secondary metabolites which act as antioxidant agent. Phytochemical compounds that obtained depend on the kind of solvent that used for extraction. All the test samples had different of activity antioxidant. The highest IC_{50} in free radical scavenging was water extract, ethanol extract and ethyl acetate fraction with the value 11.4 µg/mL, 6.8 µg/mL and 6.4 µg/mL, respectively.

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