Isolation and Antioxidant Activity of Phenolic Compound from Leaves Extract of *Clidemia hirta* D. Don

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Abstract: Phenolic compounds have been extracted and isolated to its purity from the leaves extract of *Clidemia hirta* D.Don. Extraction procedure included maceration, fractionation, and chromatographic separation of numerous compounds. Elucidation of purified compound was performed using spectroscopy technique included UV-Vis, and H-NMR. The results showed that the phenolic compound was categorized as phenolic acid. Antioxidant activity (IC_{50}) of purified phenolic acid based on DPPH assay was 49.9 μg/mL.

1 INTRODUCTION

Wild plants are sources of bioactive compounds commonly neglected due to the absence of profounding health and farmacological information. However, in some region of Indonesia, local people have already utilized some wild plant species as a part of ingredient of their traditional remedies (Tuttolomondo et al, 2014).

Clidemia hirta (L.) D. Don is a wild plant species native to South America and also distributed in Australia, South Asia, Sri Lanka, India, East Africa and other Pacific islands. This herb is widely used in traditional medicine. In some examples in Malaysia, the leaves of *senduduk bulu* are mixed with saliva and applied as wound-dressing to prevent bleeding. Moreover, the root decoction of *Clidemia hirta* is used by local Malaysian tribes for the treatment of fever, diarrhea, irritation and bacterial infections. The boiled water of the leaves and roots can also treat stomachache and heart disease. In Brazil, this species is used to treat skin infections (Lopez et al, 2016).

Two studies highlighted the antibacterial activity of the leves extract by agar diffusion method, however no report so far on the bioactive compounds in the extract. Although the plant has been described as wild and native plant, they are also known as invasive shrubs used in folk medicine to treat several bacterial infections.

Clidermia hirta has antimicrobial activity which is potential as a source of preservatives in cosmetic applications. (Abdellaoui et al, 2014). *Clidemia hirta* has been extracted in various organic solvents, such as ethanol, petroleum ether and chloroform which yielded crude extracts with antiproliferative and antioxidant activity (Narasimham et al, 2017).

One of the factors causing disease is oxidative stress which results in cell or tissue damage. Currently, a viable and safe alternative to synthetic antioxidants is being developed which are known for their ability to prevent oxidation (Marianne et al, 2017). Phenolic compounds including phenolic acids are secondary metabolites found in plants and fungi. These compounds are produced for protection against UV rays, insects, viruses and bacterial infections as well as inhibiting the growth of other competing plants (allelopathy).

Phenolic acid can be divided into two major groups, hydroxybenzoic acid and hydroxyinamic acid (Haleno, 2015; Sousa, 2018). Phenolic acid is a polyphenol compound that has bioactivity as an antioxidant, related to the hydroxyl group attached to the ring structure. These molecules can act in various roles, i.e. reducing agents, hydrogen donors, radical scavengers metal chelating superoxides, superoxide anions and peroxynitrites (Terpinc et al., 2011).

Other phenolic compounds, namely gallic acid, apart from having astringent and styptic uses, also have several bioactivities such as anti-neoplastic, bacteriostatic, anti-melanogenic and antioxidant properties (Kim, 2007). This research was conducted to isolate the phenolic compounds and test the

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antioxidant activity of the leaves extract of *Clidemia hirta* (L.) D. Don.

2 MANUSCRIPT PREPARATION

2.1 Materials

Chemicals used for separation and purification consist of methanol, ethyl acetate, n-hexane, 60 G (70-230 mesh) silica gel, $FeCl_3$, leaves of *Clidemia hirta* (L.) D. Don. Tools in this study were glasswares, column chromatography, TLC GF254 plate 20×20 cm, IR spectroscopy, UV spectroscopy, ^{1}H-NMR spectroscopy.

2.2 Extraction and Isolation of Phenolic Compound

Dried powder of *Clidemia hirta* leaves as much as 1.7 kg was macerated using MeOH to yield crude MeOH extract (124.64 g). The crude MeOH extract was further fractionated using hexane and EtOAc to yield each extract for 37.5 and 10.36 g respectively. The EtOAc extract was purified under column chromatography using silica gel as stationary phase and solvent system using chloroform:MeOH following gradient of polarity. Each fraction was checked for its purity on thin layer chromatography (TLC) by spraying $FeCl_3$ to indicate any phenolic compounds. Based on this procedure, we obtained 5 fractions with Fraction-1 (510.5 mg) containing the highest yield of compound. F1 was further purified using preparative TLC apparatus. Purified compound was elucidated using spectroscopy technique such as UV-Vis, IR, and H –NMR. The phenolic compound was described following the description by previous reports.

2.3 Antioxidant Assay

Antioxidative properties of phenolic compound of *Clidemia hirta* was assayed based on DPPH radical scavenging test. Standard solution (DPPH) was prepared by dissolving 9 mg of compound into 450 mL to obtain a 50 µM solution. The standard solution was diluted to obtain various concentration of 100, 50, 25, 12.5, 6.25, and 3.175. Antioxidant assay was prepared by reacting 0.2 mL of sample solution into 3.8 mL of 50µM DPPH solution. Mixture was homogenized for 30 min in dark room. Absorbance of the solution was checked using UV-Vis at λ = 516 nm. Antioxidant activity of sample and positive control was measured using following formula:

$$Inhibition\ (\%) = \frac{Absorbance\ (Control) - Absorbance\ (Sample)}{Absorbance\ (Control)}\ x\ 100\%$$

3 RESULTS AND DISCUSSION

3.1 Spectral Analysis of Compound

Purified EtOAc fraction of the leaves extract of *Clidemia hirta* (L.) D. Don was yielded as much as 7.4 mg in the form of yellowish white solids. UV-Vis spectrum (CH_3OH) showed a peak at 275 nm, whereas 270–290 nm was determined as the detection region for phenolic acid compound (Vijayalakshmi, 2012). FT-IR spectra showed absorption for hydroxyl group at 3410 cm^{-1}, =C-H group at 2962 cm^{-1}, -C-H group at 2924 and 2854 cm^{-1}, carbonyl group at 1712 cm^{-1}, and aromatic C=C group at 1612 cm^{-1}.

The ^{1}H-NMR spectrum gave a proton signal with chemical shift at 7.0 ppm (2H, s, H-2,6) and 3.8 ppm (3H, s, OCH_3). These spectrums displayed two signals at chemical shift δ 7,0 ppm (2H, s) which is the signal of two aromatic protons at H-2 and H-6 while the chemical shift of δ 3,8 ppm (3H, s) was the signal of methoxy group. The chemical shift based on ^{1}H-NMR resemble the methyl gallate compound (Ekaprasada, 2009).

3.2 Antioxidant Activity

Determination of antioxidant activity was performed by measuring the absorbance of remaining DPPH radical using UV-VIS instrument at 516 nm. The antioxidant activity was expressed as the percentage of inhibition of a sample in reacting with radical solution. Minami et al (1998) grouped the capacity of antioxidant activity based on IC_{50} value. An antioxidant compound may be classified as very active, active, and inactive compound if the IC_{50} value falls between <10, <100, and >100 respectively. The antioxidant activity of phenolic acid compound from *C. hirta* leaves was shown in Figure 1.

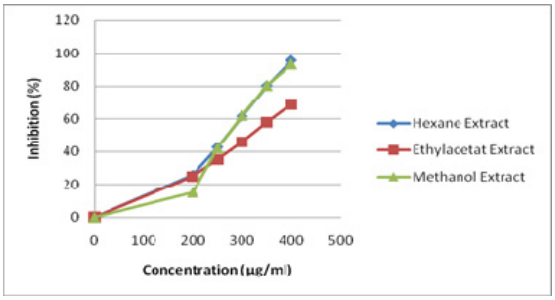

Figure 1. Percentage of DPPH inhibition by various extracts of Clidemia hirta leaves.

DPPH is a stable free radical as common standard in determining the antioxidant properties of a compound or crude extracts. Based on Figure 1, the IC_{50} of MeOH, EtOAc, and hexane extract was 50.8, 49.9, and 88.9 µg/mL. Leaves extract of *C. hirta* was classified as an active antioxidant since its IC_{50} < 100. Meanwhile, the EtOAc extract was regarded as the most prominent antioxidant in this study.

4 CONCLUSIONS

Purified compound from the leaves extract of *Clidemia hirta* was identified as phenolic derivative or phenolic acid. Antioxidant activity of EtOAc was determined using DPPH assay showed as IC_{50} = 49.9 µg/mL.

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