Antioxidant activity of butanolic extract from Madang Gatal (Schima wallichii Choisy) Leaves

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Abstract. Antioxidant activity of the butanolic extract from Madang Gatal (Schima wallichii Choisy) leaves was studied in the research. Antioxidant activity was analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical oxidation method. Furthermore, the extract was purified using column chromatography to yield 4 partially purified fractions (B1-B4). The extract and B2 fraction possess strong antioxidant action with IC50 value 12.55 and 24.50 μg/ml, respectively. The B1 and B3 fractions have moderate antioxidant activity with IC50 value 70.55 and 90.26 μg/ml, while B4 was not active as antioxidant with IC50 value of 228.28. The target compound of the fraction most active as an antioxidant (B2) was separated by preparative Thin Layer Chromatography (TLC). The isolate was then identified using Fourier Transform Infra-Red (FTIR) and Liquid Chromatography Mass Spectroscopy (LCMS). The IR spectra displayed that the bioactive compound of B2 was predicted as flavonoid with MS 290.25 g/mol. The major compound contained in a putative compound known as catechin with an IC50 value was 17.97 μg/ml.

1. Introduction

The environment is highly polluted by free radicals which may contribute to the aging process of tissue and cause various chronic and degenerative diseases in humans such as cancer and cardiovascular problems. Excessive amount of free radicals are harmful to human because they can interact with DNA and cell membrane which are important components of cell leading to mitochondrial dysfunction or cell death. The risk can be reduced by utilizing of free radical scavenger compounds known as antioxidants [1-3]. Antioxidants act as antiradical by donating their hydrogen atoms to radical compounds. The function of antioxidants are as free radical neutralizer, thus the aging process can be inhibited [4,5].

In addition, antioxidant ability in preventing cellular damage and scavenging free radicals could be related to its potential to prevent malignancies. Thus, antioxidant activity analysis is considered as one of screening processes for investigating potential candidates for natural antioxidant and anticancer agent [2]. A various number of Indonesian plants have been used traditional medicine to prevent chronic and degenerative diseases due to their remarkable potential as antioxidant. Madang gatal (Schima wallichii Choisy), also locally known as puspa or cheloni, belongs to the genus of Schima and tea family (Theaceae) [6]. Madang gatal is widely found in Indonesia and several countries in Asia, however, its scientific information and utilization for folk medication is still limited. The stem of Madang gatal is...
commonly used to produce high-quality carpentry wood, while the tree’s leaves are employed as a natural diet of primates [6,7]. In Indonesia, the plant has been traditionally used as a treatment for insomnia and hypertension.

Previous studies reported that Madang gatal extract has anti-inflammatory, antipyretic, analgesic [8], antimicrobial [9] and anti-mutagenic [10] activities, Kaemferol-3-O-ramnosida isolated from this plant known to exert anti-malarial [11], anticancer [12,13], and anti-plasmodial properties [14], while rotenone was found to possess antimicrobial and antifungal. In addition, another reported compound, theanine, potentially shows psychoactive properties for overcoming anxiety, depression, stress, insomnia, sleep disorders, hypertension and some symptoms of schizophrenia [15].

In this current research, the antioxidant activity of the butanolic extract from Madang gatal leaves will be examined. The bioactive compounds contained in the extract were purified and isolated. Further, the most active fraction as an antioxidant was isolated using column chromatography and continued by preparative TLC. The isolate was then identified using TLC, spectroscopy FTIR, and LCMS. Antioxidant activity was performed on the basis of the radical scavenging effect of the stable DPPH radical formula [17].

2. Materials and Methods

2.1. Materials

Madang gatal leaves were collected from PUSPIPTEK provincial garden. This plant was identified by a botanist of the Biology Laboratory, Research Center for Biology LIPI, Cibinong West Java, Indonesia and voucher specimens have been stored at the Biology Laboratory. All technical solvents such as methanol, n-hexane, ethyl acetate and butanol were re-distilled according to standard procedure. The butanolic extract obtained by partitioned of the metanolic extract using n-hexane, ethyl acetate, and butanol, successively.

Some analytical grades obtained from E. Merck were used without further purification. Silica gel plates (Kiesel gel 60F254 0.25 mm) and H2SO4 for TLC analysis, methanol and acetic acid for mass spectrometry (MS) analysis, DPPH for antioxidant activity analysis, and silica gel 60 (230-400 mesh) for column chromatography to purify bioactive compound of the extract. The equipment used in this study were FTIR Shimadzu prestige 21 using KBr pellets to determine the functional groups and LCMS Mariner Biospectrometry Electrospray Ionization (ESI) System to determine the MS of the isolate.

2.2. Methods

The bioactive compounds from the Madang gatal extract was isolated by using chromatography column techniques with silica gel 60 as the stationary phase. N-hexane and a gradient of ethyl acetate to 100% were selected as liquid-liquid extraction solvents to obtain a number of fractions. Purifications of the bioactive compounds were conducted on fraction which had the highest antioxidant activity by preparative TLC. The functional group of the isolate was then identified on FTIR Shimadzu prestige 21 using KBr pellets, while MS was analyzed by LCMS spectroscopy.

Antioxidant activity was evaluated using the DPPH method. All samples are prepared at the concentration of 1000 μg/ml. After that, the series solutions of 10-200 μg/ml were prepared to squeeze successively from the stock solution. Quercetin (10-25 μg/ml) was performed as a standard antioxidant. Subsequently, the series solution was diluted with methanol so the volume of the solution became 2000 μL at each concentration. Five hundred μL of DPPH solution was added to sample test and incubated for 30 min in the dark. Further, the absorbance was measured by using UV-Vis Cary 60 spectrophotometer at a wavelength of 517 nm. The percentage of DPPH inhibition and IC50 value were calculated [16,17].
3. Results and Discussion
In our previous study, extraction of Madang gatal leaves produced % yield of methanol (22.18%), butanol (20%), aqueous (19.67%), ethyl acetate (2.92%) and n-hexane (0, 06%) extract, sequentially [19]. This current study examined the antioxidant activity of Madang gatal butanolic extract by scavenging the free radicals 1,1-diphenyl-2-picryl-hydrazyl (DPPH). DPPH is converted to 1,1-diphenyl-2-picryl-hydrazine in the present of antioxidant compound, indicated by the reduction of the DPPH solution color from purple into a yellowed-colored DPPH-H molecule product. The color change was proportional to the number of radicals captured and can be measured by visible light spectrophotometer at wavelength 515-517 nm [16-18]. The antioxidant activity test was compared to quercetin as a standard antioxidant which is known as a strong antioxidant with IC\textsubscript{50} less than 10 μg/ml. Hence, the tested sample solutions will be known to have the same, less, or higher activity than standard materials [17].

The result showed that Madang gatal butanol extract was active as an antioxidant indicated by changing of the DPPH solution color, from purple to yellow. The IC\textsubscript{50} value of butanol extract in scavenging DPPH was 12.55 μg/ml. The IC\textsubscript{50} value lower than 50 μg/ml indicates that the butanol extract possess a robust antioxidant activity [16-18], but markedly lower than standard quercetin with IC\textsubscript{50} value of 9.35 μg/ml. Accordingly, Madang gatal butanol extract was found to be an effective antioxidant agent.

The extract was purified next by silica gel column chromatography eluting with n-hexane, a gradient of ethyl acetate to 100%. The separation by column chromatography yielded 35 fractions (F1– F35), and then combined into 4 purified fractions, which were B1(F1-F13), B2 (F14-F15), B3 (F16-F22), and B4 (F23-F35). The B1-B3 fractions have antioxidant activity, while B4 was not active as antioxidant indicated its IC\textsubscript{50} value of 228.28 μg/ml. B1 and B3 fractions with IC\textsubscript{50} value 90.26 and 70.55 μg/mL were categorized as moderate antioxidants, while B2 was categorized as a strong antioxidant with IC\textsubscript{50} value of 24.50 μg/ml. The antioxidant activity of the fractions is shown in Figure 1.

![Figure 1](image)

**Figure 1.** The IC\textsubscript{50} value of antioxidant activity from butanolic extract fractions of Madang gatal leaves.

The column chromatography has not yet produced pure compounds, therefore the next purification needs to be done. B2 which most active as an antioxidant was subjected to purified further by preparative TLC [20]. Preparative TLC produced 15.5 mg of isolate and then analyzed by using FTIR and LCMC spectrophotometers. The isolate was thought to have functional groups that are similar to flavonoid compounds. The previous research conducted by Barliana et al. showed that the main compound contained in S. wallachii was a flavonoid compound [14]. The phytochemical analysis of the Madang gatal leaves extracts from the previous study indicated the presence of flavonoids [19]. One of the main flavonoid compounds in theaceae plants are theaean and catechins [21].

The IR spectrum of B2 has similarities to the catechin IR spectrum. The compound exhibited absorption bands at 3246.20-3506.59 cm\textsuperscript{-1} indicating the presence of hydroxyl (OH) groups.
vibration of the carbonyl (C=O) group of the compound was indicated at 1072.42 cm\(^{-1}\). A wave number of 2922.16 cm\(^{-1}\), 678.94-837.11 cm\(^{-1}\), and 1608.63-1699.29 cm\(^{-1}\) were indicating the presence of C-H sp2 stretched, C-H aromatic, C=C stretched groups, respectively. Whilst catechins exhibited absorption bands at 3353.17 cm\(^{-1}\) and 1611.10 cm\(^{-1}\) indicating the presence of hydroxyl (OH) and C=C stretched groups. The IR spectrum similarities of B2 with catechins were presented in Figure 2. The molecular structure of catechin that one of the main flavonoids in theaceae was showed in Figure 3.

![Figure 2](image1.png)

**Figure 2.** The similarities of IR spectra of the putative compound contained in B2 fraction (A) and catechin compound as reference material (B).

![Figure 3](image2.png)

**Figure 3.** Molecular struture of catechin.

MS of the isolate from B2 identified using LCMS was presented in Figure 4. The chromatogram illustrated 1 main peak at the retention time of 1.68 minutes and MS of 290.25 g/mol was recognized as catechin, according to MS of catechin based on literature is 290.30 g/mol. Catechin and theanine were major compounds commonly found in theaceae plants [21, 22]. Catechine has been known as a powerful antioxidant for chronic and degerenetive disease prevention and health promotion due to its antioxidant, antidiabetic and anti-carcinogenic effects [23].
The isolate has powerful antioxidant with \( \text{IC}_{50} \) value 17.97 μg/ml.

4. Conclusion
The current report demonstrated that butanol extract of Madang gatal leaves exhibited a strong antioxidant property with the \( \text{IC}_{50} \) value of 12.55 μg/ml. Under the optimum purification and isolation condition, the extract produced 1 isolate (15.5 mg), which was predicted as a flavonoid. IR and mass spectrometry (MS) analysis displayed that the major compound of the isolate from B2 fraction might be a catechin compound which has some functional group similarities with MS 290.25 g/mol. The isolate performed a strong antioxidant activity with \( \text{IC}_{50} \) value of 17.97 μg/ml. Taken together, Madang gatal leaves potentially be developed as a natural antioxidant for preventing human diseases. Further well-design study using \(^1\text{H}\) and \(^{13}\text{C}\)-NMR analysis should be carried out to identify the isolate based on observed spectral.

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