Phytochemical Screening and Antioxidant Activity of Ethanol Extract of Leilem (Clerodendrum minahassae Teijsm. & Binn) as an Antihyperlipidemic and Antiatherosclerotic Agent

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Abstract. Leilem, which is known with the botanical name Clerodendrum minahassae, is a plant species commonly used as a component of almost all meat- and fish-based cuisine. In addition to enhancing the flavor and taste suited to the local people, leaves of leilem is also believed to possess high level of natural antioxidant. The leaves of this plant has also been used as traditional medicine to treat stomachache and lung diseases. Our previous study revealed that ethanol extract of leilem has beneficial antihyperlipidemic and antiatherosclerotic effects on the aorta of Wistar rats fed with high lipid and cholesterol levels. The present study was aimed at investigating the phytochemical contents and antioxidant activity of ethanol extract of leilem leaves. Phytochemical screening was carried out using standard methods of precipitation and coloration reactions. In addition, the total phenolics and flavonoids were determined by using spectrophotometric methods. Finally, the leaf extract of leilem was assayed to evaluate its in vitro antioxidant properties using 1,1-Diphenyl picryl hydrazyl (DPPH) radical assay and ferric reducing antioxidant potential (FRAP) assay. The phytochemical screening of leilem leaves revealed the presence of alkaloids, saponins, flavonoids, steroids, and phenols, while terpenes and tannins were not detected. The extract of leilem leaves showed estimated phenolics and flavonoid content of 139.88 mg/g and 34.46 mg/g respectively. Concentration of leilem leaf extract required for 50% inhibition of DPPH radical scavenging effect (IC50) was recorded as 565.45 μg/mL. At 1 mg/mL concentration, the aqueous extract of leilem leaves showed ferric reducing power of 123.62 μmoles/mg in FRAP assay. These findings suggest that the ethanol extract of leilem has potential in vitro antioxidant activities. Overall, results obtained from this study support our previous finding that the extract of leilem leaves has beneficial antihyperlipidemic and antiatherosclerotic effects and can be used as an alternative to synthetic antioxidants.

Keywords: Clerodendrum minahassae, antioxidant, antihyperlipidemic, antiatherosclerotic

1. Introduction

Plants have been one of the important sources of medicines since the dawn of human civilization and still remain one of the major sources of drugs in modern as well as in traditional systems of medicine. Pan et al. [1] speculated that to date approximately 80% of antimicrobial, cardiovascular, immunosuppressive, and anticancer drugs are of plant origin. Furthermore, it was estimated that more than 50% of prescribed drugs are either directly derived from herbs/plants or active ingredients obtained from plant substances [2].

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants that work with nutrients and dietary fiber to protect against diseases. Depending upon their biosynthetic origin, phytochemicals can be divided into several categories such as alkaloids, phenolics, flavonoids, steroids, terpenes, saponins, etc. The last several decades have seen increased research attention of potential phytochemicals from plants for therapeutic uses. This is because many phytochemicals have been demonstrated to have antioxidant activities and reduce the risk of many diseases, especially diseases that are related to oxidative stress such as cardiovascular diseases and cancer.
Leilem (*Clerodendrum minahassae* Teijsm. & Binn.) is a member of the plant genus *Clerodendrum*. The center of origin of this genus are Indonesia and the Philippines. Members of *Clerodendrum* have been cultivated in many countries in tropical Asia, Hawaiian Islands, Florida, West Indies, and Europe [3]. Grows as a wild uncultivated plant, *Clerodendrum minahassae* plays an important role in the diet of indigenous people of Minahasa and Manado, North Sulawesi, Indonesia. The leaves of leilem have long been used as vegetable and culinary herb in many kinds of meat recipes. Besides giving a pleasant flavour, leilem leaves are believed to contain natural antioxidants capable of counteracting the bad effects of animal fats for health. Indeed, leaves of leilem have traditionally been used as medicine for stomachache, trichinosis, and lung diseases. Although it has been demonstrated that the leaves of leilem contain polyphenolic compounds with potential antioxidant activity [4], there has been limited research on the biological activity of leilem leaves.

Our previous study revealed that ethanol extract of leilem has beneficial antihyperlipidemic and antiatherosclerotic effects on the aorta of Wistar rats fed with high lipid and cholesterol levels. Extract of leilem leaves was able to decrease triglyceride levels as well as increase HDL levels. Moreover, it also improved the aorta by reducing the number of foam cells and the thickness of the aortic wall. The purpose of this study was to investigate the phytochemical contents and antioxidant activity of ethanol extract of leilem leaves.

2. Experimental Method

2.1. Preparation of plant extract

Fresh leaves of *Clerodendrum minahassae* were obtained from Minahasa, North Sulawesi, Indonesia. The botanical identification and authentication was confirmed at the Department of Biology, Faculty of Mathematics and Natural Sciences, Indonesia. The leaves were air-dried at room temperature and in an oven at 40°C and then powdered by a homogenizer. The crude powder was macerated in 96% ethanol for 3 days. The ethanol extract was concentrated and evaporated at 40°C with a rotary evaporator under reduced pressure for 90 minutes to produce a thick extract. The extract was stored in a refrigerator until used in the study.

2.2. Qualitative Phytochemical Screening

Ethanol extract of leilem leaves was used for qualitative assessment for the major classes of phytochemicals namely alkaloids, saponins, flavonoids, steroids, phenols, terpenes, and tannins. The tests were performed according to various standard methods as described by Harborne [5] and Evans [6].

**Alkaloids.** The presence of alkaloids was tested using three reagents, *viz.* Mayer’s reagent, Dragendorff’s reagent, and Wagner’s reagent. A few drops of solution were added to the extract solution (0.5 mL). A reddish-brown precipitate demonstrated the test as positive.

**Saponins.** To the extract solution (0.5 mL), few drops of Na₂HCO₃ were added and was shaken vigorously to froth and was then allowed to stand for 15 – 20 minutes. A height of persistent foam greater than 1 cm indicated the presence of saponins.

**Flavonoids.** The 0.5 g of the extract was boiled with distilled water. To the extract solution (0.5 mL), few drops of 10% of ferric chloride solution were added. A green-blue or violet coloration demonstrated the presence of flavonoids.

**Steroids and Triterpenoids.** To the extract solution (0.5 mL), chloroform was added followed by sulphuric acid added slowly through the sides of the test tube. A reddish-brown color at the interface and turning the upper layer to green indicated the presence of steroids, while formation of deep red color indicated the presence of triterpenoids.

**Tannins.** To the extract solution (0.5 mL), few drops of 5% ferric chloride were added. Black or blue-green coloration or precipitate indicated the presence of tannins.
2.3. Quantitative Analysis

**Total phenolics determination.** Total phenolic content was estimated by the Folin–Ciocalteu method [7]. 200 µl of different concentrations of sample were added to 1 mL of 1:10 diluted Folin–Ciocalteu reagent. After 4 minutes, 800 µl of saturated sodium carbonate (75 g/L) was added. After 2 hours of incubation at room temperature, the absorbance at 765 nm was measured. Gallic acid (0–200 mg/L) was used for the standard calibration curve. The results were expressed as mg of Gallic acid equivalent (GAE)/g leilem leaf extract and calculated as mean value of three replicates.

**Flavonoid determination.** Total flavonoid content in the ethanol extract of leilem leaves was determined using the method described by Sakakana et al. [8]. The flavonoid content was determined by aluminum chloride method using quercetin as standard. Extract and quercetin were prepared in ethanol (1 mg/mL). 0.1 mL of extract was mixed with 0.9 mL of distilled water in test tubes, followed by addition of 75 µL of a 5% sodium nitrite solution. After 6 min, 150 µL of a 10% aluminum chloride solution was added and the mixture was allowed to stand for further 10 min after which 0.5 mL of 1M NaOH was added to the reaction mixture. The absorbance was determined at 415 nm. Quercetin (0–60 µg/mL) prepared in methanol was used as a standard. Results were expressed as milligram of Quercetin equivalent (QuE)/g of leilem extract, and calculated as mean value of three replicates.

**DPPH (2, 2-diphenyl-1-picril-hydrazil) Assay.** The free radical scavenging activity of the plant extracts was measured by DPPH [9], with some modifications. Briefly, 0.2 mM solution of DPPH in ethanol was prepared and 0.5 mL of this solution was added to 2.5 mL of plant extract and was allowed to stand at room temperature for 30 min, and then absorbance was read at 517 nm against blank samples. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. IC50 value was determined from the plotted graph of scavenging activity against the different concentrations of leilem extracts, which was defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%.

**FRAP (Ferric Reducing Ability of Plasma) Assay.** The determination of antioxidant activity using FRAP was performed using the modified method of Benzie and Strain [10]. Briefly, 3 mL of fresh FRAP reagent (300 mM acetate buffer (100 mL), 10 mM TPTZ solution (10 mL) and 20 mM FeCl3.6H2O (10 mL) solution) was added to the 0.1 mL extract solution in a test tube (the aqueous extract taken for the assay was 1 mg/mL) and was incubated in the dark for 30 minutes. Reading of the colored solution (ferrous tripyridyltriazine complex) of standard and sample was taken at 593 nm. Total antioxidant content was expressed as equivalent of Fe3+ converted to Fe2+ in µmoles/mg of extract. Calibration curve was made using Fe2+ solution as a standard.

3. Results and Discussion

In this study, ethanolic extract was chosen because on polarity basis, it is the nearest to the preparation used traditionally. Alcohol solvents are more capable of increasing the permeability of cell walls and facilitating the extraction of a greater number of polar molecules of both medium and low polarity [11, 12]. After the successful conventional hot Soxhlet extraction of Clerodendrum minahassae leaves in investigation, the preliminary phytochemical study revealed that ethanol extract of leilem contains alkaloids, saponins, flavonoids, steroids, and phenols, while terpenes and tannins were not detected (Table 1). These phytochemical compounds are known to support bioactivity [9, 13-16], thus responsible for the antioxidant activities.

The content of phenolic compounds in leaf extract of Clerodendrum minahassae was determined from regression equation of calibration curve of Gallic acid (Figure 1) and expressed as milligrams equivalent of gallic acid per gram of dry extract (mg GAE/g). Similarly, total flavonoid content was determined from regression equation of calibration curve of quercetin (Figure 2). Flavonoids content was expressed as milligrams equivalent of quercetin per gram of dry extract (mg QE/g). Total phenolics and flavonoids calculated in this study are presented in Table 2. Phenolic compounds are known as powerful chain breaking antioxidants, which may contribute directly to antioxidative action [11, 17, 18]. These phenolic compounds contribute to antioxidant activity due to the arrangement of functional groups (hydroxyl) about its nuclear structure for hydrogen donation in order to stabilize radical molecules [19, 20].
Table 1. Qualitative screening of phytochemicals from ethanol extract of *Clerodendrum minahassae*

| Phytochemicals | Present/Absent |
|----------------|----------------|
| Alkaloids      | +              |
| Saponins       | +              |
| Flavonoids     | +              |
| Terpenes       | -              |
| Steroids       | +              |
| Phenolics      | +              |
| Tannins        | -              |

Note: ‘+’ present ; ‘-’ absent

**Figure 1.** Total phenolic content for standard gallic acid, values expressed in terms of gallic acid equivalent.

**Figure 2.** Total flavonoid content for standard quercetin, values expressed in terms of quercetin equivalent.
Table 2. Total phenolic content (mg GAE/g) and flavonoid content (mg QE/g) of ethanol extract of *Clerodendrum minahassae*

| Extract   | Total phenolic Content (mg GAE/g) | Total Flavonoid Content (mg QE/g) |
|-----------|-----------------------------------|-----------------------------------|
| Ethanol   | 139.88                            | 34.46                             |

In the DPPH assay, the ability of the investigated leilem leaf extract to act as donors of hydrogen atoms or electrons in transformation of DPPH radical into its reduced form DPPH-H was investigated. It was found that leilem leaf extract was able to reduce the stable, purple-colored radical, DPPH, into the yellow-colored DPPH-H reaching 50% of reduction. The IC50 value was determined from a previously constructed standard curve (Figure 3) and was found to be 565.45 µg/mL. This low value of IC50 of the present investigation suggests that leilem leaf extract has a strong hydrogen donating ability and is comparable with the IC50 values of well-known plants with high antioxidant activity, such as blueberry and raspberry [21], and medicinal plants such as *Coronopus didymus* [22]. A significant relationship was found between antioxidant potential and total phenolic content, suggesting that phenolic compounds are the major contributors to the antioxidant activity of *Clerodendrum minahassae*.

![Figure 3](image-url)  
**Figure 3.** The scavenging activity of ethanol extract of leilem leaf assayed by DPPH free radical scavenging method.

The reducing ability of ethanol extract of leilem leaves as assayed by FRAP method measured the direct electron donating ability of the extract. The results for FRAP were calculated from a calibration graph which were linear over the calibration range with $R^2$ value of 0.9785 (Figure 4) and was found to be 123.62 μmoles/mg. The reducing ability of extract of leilem leaves is comparable with that of previously reported for other plants that are used for medicinal purposes such as *Goniothalamus velutinus* [23] and *Alstonia scholaris* [24].
Overall, the findings obtained from this study support the results of our previous study which revealed that ethanol extract of leilem leaves has beneficial antihyperlipidemic and antiatherosclerotic effects on the aorta of Wistar rats fed with high lipid and cholesterol levels. This high antioxidant activity and noticeable amount of polyphenolic compounds lend *Clerodendrum minahassae* as a potential plant to be used as a prospective source of natural antioxidant to food and health industries.

4. Conclusions
The phytochemical screening of this investigation attested the presence of several secondary metabolites with known biological antioxidant activities in the leaf extract of *Clerodendrum minahassae*. The ethanol extract of the leaves of this plant species showed a strong antioxidant activity by scavenging DPPH and FRAP methods. Furthermore, the extract was found to contain relatively high levels of total phenolics and flavonoids, which play a major role in controlling oxidation generated by free radicals. The results of this study suggest that the ethanol extract of *Clerodendrum minahassae* can be used as a prospective source of natural antioxidant. However, the phytoconstituents responsible for the antioxidant activity of the extract are still elusive. Therefore, further studies are needed to determine the mechanism behind the antioxidant activity of this plant.

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