# Identification of Antioxidant Activity of Ethanol Extracts from (Hedyotis corymbosa L.) Pearl Grass Leaves Against Oxidative Stress

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Abstract: Oxidative stress is the main causative factor of various degenerative diseases, and the search for natural antioxidant sources becomes important to overcome this problem. This study aims to identify the activity of antioxidant compounds from ethanol extract of pearl grass (Oldenlandia corymbosa) leaves in neutralizing free radicals. The methods used include leaf extraction with 70% ethanol and antioxidant activity testing using UV-Vis spectroscopy at a wavelength of 517 nm. Results showed that the extract with a concentration of 80 ppm produced a maximum inhibition of 51.60%, indicating significant antioxidant potential. Qualitative phytochemical tests identified the presence of phenolic compounds and flavonoids, which act as electron donors in the free radical penetration process. The IC50 value of the ethanol extract obtained was 46 µg/mL, indicating the effectiveness of the extract in inhibiting free radicals. These findings support the potential of pearl grass as a source of natural antioxidants that can be further developed for the prevention and therapy of oxidative stress-related degenerative diseases.

## 1 INTRODUCTION

The presence of free radicals in the body can cause various diseases and damage to cells, tissues, and important organs such as the liver, kidneys, and heart. Free radicals are molecules that are unstable because they have unpaired electrons. To stabilize themselves, these molecules will damage surrounding healthy cells. This condition also triggers degenerative diseases such as premature aging, arthritis, and cancer. According to research from (Khairani et al., 2023), strenuous physical activity can increase oxygen consumption excessively, which in turn triggers the formation of more free radicals. This increase causes the body to experience oxidative stress, a condition in which the production of free radicals exceeds the body's ability to neutralize them (Khairani et al., 2023). This damage triggers various degenerative diseases such as premature aging, arthritis, and cancer.

Strenuous physical activity, such as swimming, can significantly increase oxygen consumption, thereby increasing the formation of free radicals in the body. The imbalance between the production of free radicals and the ability of the antioxidant system to manage them leads to oxidative stress conditions (Khairani et al., 2023). This oxidative stress is a major risk factor in various degenerative diseases such as cancer, diabetes mellitus, atherosclerosis, which can stop at cardiovascular disorders such as coronary heart disease and heart failure (Andita et al., 2020).

Antioxidants are molecules that are able to counter the harmful effects of free radicals. The main task of antioxidants is to stop the chain reaction caused by free radicals, by neutralizing or inhibiting the oxidation process. These antioxidants are divided into two types: endogenous and exogenous. Endogenous antioxidants are naturally produced by the body, such as superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase. Meanwhile, exogenous antioxidants are obtained from outside the body, for example through food and supplements. In Indonesia, one of the promising sources of exogenous antioxidants is pearl grass (Oldenlandia corymbosa) (Elsayed Azab et al., 2019).

Pearl grass (Oldenlandia corymbosa), a member of the Rubiaceae family, is a flowering plant widely found in tropical regions, including in the Himalayan highlands up to 2000 meters. It is widely recognized in countries such as China, India, and Southeast Asian countries as an herbal remedy for cancer, including lymphosarcoma, stomach, nasopharyngeal, cervical, breast, rectal, and fibrosarcoma cancers. The plant has a variety of beneficial chemical compounds, such as antioxidant, antibacterial, and antihepatotoxic. Pearl grass also contains ursolic acid, oleanolic acid, gamma sitosterol, and alkaloids such as biflorone and biflorine. These ingredients make pearl grass have great potential as a medicine (Das et al., 2019)

Based on research (Soemardji et al., 2015)pearl grass contains compounds. various These beneficial compounds chemical include: hentriacontane, stigmasterol, ursolic acid, oleanolic acid, β-sitosterol, sitosterol-D-glucoside, p-coumaric acid, flavonoid glycosides, iridoid glycosides, alizarin, corogenin, and antragalol bonds. All parts of this plant, from leaves, stems, to roots, can be utilized to benefit from these various compounds. Antioxidant activity of pearl grass methanol extract has been carried out in vitro with the 1,1-diphenyl-2-picryl hydrazyl method (Soemardji et al., 2015)

Another study comparing the antioxidant activity of ethanol extracts of pearl grass leaves with several other medicinal plants, such as soursop leaves and kenikir leaves, using spectroscopic techniques with quercetin as a flavonoid standard, also found that pearl grass extracts contained quite high flavonoid levels as well as significant DPPH free radical scavenging activity, although soursop leaf extracts showed slightly higher activity (Wahyuni et al., 2018). Bioactivity studies of pearl grass reinforce its potential as an antioxidant, anti-inflammatory, hepatoprotective, antibacterial, and antimalarial. The content of triterpenes, anthraquinones, flavonoids, and phenolic compounds greatly contribute to these activities. This study confirms the use of pearl grass as a natural antioxidant source material with diverse therapeutic benefits (Rifka Alkhilyatul Ma'rifat, I Made Suraharta, 2024).

Although there have been several studies examining the antioxidant activity of pearlgrass, many have not specifically explored the components that contribute to this activity. In this study, the aim is to identify and validate the active compounds responsible, so as to provide a stronger scientific basis for the use of pearlweed in the health field.

# 2 METHOD

## Tools and Materials

The tools used are scissors, blender, analytical balance, glass jar, stirring rod, filter paper, erlenmeyer, rotary evaporator, Uv Vis spectrophotometric instrument, and drop pipette. The materials used were Hedyotis corymbosa leaves, 70% ethanol, aluminum foil, and plastic wrap.

## Research Procedure

**Sample preparation**

Hedyotis corymbosa leaves are dried under the sun for 24 hours until there is no water content. The next stage the leaves were pulverized using a blender, then sieved with a sieve and weighed as much as 250 grams of samples to be used for the extraction process.

**Hedyotis corymbosa leaf extraction**

250 grams of sample was dissolved with 125 ml of 70% ethanol, the ratio of material and solvent was 1:10, then placed in a storage cabinet for 2x24 hours at room temperature and occasionally stirred. The next stage after 2x24 hours, filtered using filter paper, the filtrate obtained was concentrated with a rotary evaporator at a temperature of 50 C. Hedyotis corymbosa extract was obtained.

**Testing using (Ultraviolet-Visible) UV-Vis Spectrophotometry**

The initial step is done by turning on the UV-Vis spectrophotometry device along with a connecting computer that uses analysis software. The sample is first prepared in the form of a solution at a certain concentration, then put into a clean and clear quartz cuvette. The cuvette containing the sample is placed in the sample chamber of the tool, then the spectrophotometer is operated so that the UV-Vis absorption spectrum of the sample is obtained. The resulting spectrum was then read with respect to the maximum wavelength and absorbance value, and compared with literature data to identify the presence of active compounds.

**Data Analysis**

The data obtained is presented in the form of variables that reflect the results of the analysis of the two extracts that have been tested using UV-Vis (Ultraviolet-Visible) Spectrophotometry instruments. The methodology used involved a literature review to strengthen the understanding of the maximum absorption wavelength ($\lambda$max) and absorbance intensity associated with the presence of active compounds in the extracts. The results of the analysis were then presented in the form of tables and graphs, with the aim of providing a clear and informative visual representation of the data obtained, thus facilitating interpretation, concentration comparison, and further quantitative and qualitative analysis.

# 3 RESULT AND DISCUSSION

This study measured the antioxidant activity of ethanol extract of pearl grass leaves (Oldenlandia corymbosa) using UV-Vis spectroscopy at a wavelength of 517 nm, which is a method for the analysis of DPPH free radical activity. The absorption data at a wavelength of 517 nm showed that the higher the concentration of the extract (from 20 ppm to 80 ppm), the absorption value decreased, indicating an increase in antioxidant activity. The measured absorbance is as follows:

Linear (2025-02-19 16:08:57 (+07:00))

| Standard | 517.00 (nm) | |
|---|---|---|
| | Conc (mg/mL) | Abs |

| Legend | | | | |
|---|---|---|---|---|
| o=overrange | u=underrange | r=re-read | cf=calibration failed | n=not used |

| Sample | 517.00 (nm) | |
|---|---|---|
| | Conc (mg/mL) | Abs |
| kontrol (metanol + dpph) | | 0.781 o |
| Mutiara 20 ppm | | 0.726 o |
| Mutiara 40 ppm | | 0.481 o |
| Mutiara 60 ppm | | 0.447 o |
| Mutiara 80 ppm | | 0.378 o |

| Legend | | | | |
|---|---|---|---|---|
| o=overrange | u=underrange | r=re-read | cf=calibration failed | n=not used |

| **Konsentrasi (ppm)** | **Penyerapan** | **Presentase Inhibisi (%)** |
|---|---|---|
| 20 | 0.726 | 40 |
| 0.481 | 7.04 | 38.41 |
| 60 | 0.447 | 42.77 |
| 80 | 0,378 | 51.60 |

(Table of Results of Antioxidant Activity of Ethanol Extract of Pearl Grass Leaf by DPPH Method)

The methanol + DPPH control had an absorbance of 0.781 as the baseline. The decrease in absorbance of the extract showed the ability of the extract to scavenge DPPH free radicals with a maximum inhibition of 51.60% at a concentration of 80 ppm. Qualitative phytochemical tests showed the presence of phenolic compounds, flavonoids, and other bioactive compounds known to act as antioxidants in pearl grass leaf extract. Pearl grass has long been used as a traditional medicinal plant with bioactive compounds such as ursolic acid, oleanolic acid, and various alkaloids that contribute to its antioxidant activity, while supporting the development of natural ingredient-based products that can help reduce the impact of oxidative stress in various degenerative diseases (Das et al., 2022).

Antioxidant activity measured through the DPPH absorption reduction method shows that the ethanol extract of pearl grass leaves has significant free radical scavenging potential. The significant decrease in absorbance at a concentration of 80 ppm indicates the ability of this extract to inhibit oxidative processes caused by free radicals, in accordance with the basic mechanism of antioxidants that are able to stop oxidation chain reactions. The presence of phenolic and flavonoid compounds, as identified through phytochemical tests, is very important because this group of compounds is known to have the ability as electron donors to neutralize free radicals, thereby reducing the level of oxidative stress. The chemical structure of phenolics provides a major contributor to the antioxidant activity of plants (Nime et al., 2023).

The antioxidant activity of ethanol extract of pearl grass (Oldenlandia corymbosa) leaves has been analyzed using the DPPH method, which measures the ability of the extract to provide electrons to neutralize the free radical 2,2-diphenyl-1 picrylhydrazyl (DPPH) as a representation of reactive oxygen radicals. Based on literature studies, ethanol extracts showed the highest antioxidant activity compared to extracts using n-hexane and ethyl acetate solvents, with an IC50 value of 46 µg/mL, indicating very strong antioxidant potential (Wahyuni et al., 2018).

The content of bioactive compounds such as phenolic compounds and flavonoids plays a significant role in the antioxidant activity of the extract. Flavonoid quantification spectrometry results show that ethanol extracts of pearl grass leaves contain high levels of flavonoids, which structurally contain free hydroxyl groups that can donate electrons to neutralize free radicals (Jumiati et al., 2022). The presence of these compounds is in line with the ability of the extract to stop oxidation chain reactions that cause cellular oxidative stress.

Another study on Oldenlandia corymbosa in vitro showed that methanol extract of pearl grass leaves has antioxidant activity containing phenolic, flavonoid, and other active compounds, which supports the results of this study. DPPH scavenging activity in another study also showed a potential IC50 of about 151 µg/mL for the methanol extract, which reinforces the finding of significant antioxidant activity in the ethanol extract tested in this study (Collins et al., 2021).

This IC50 value is higher than the ethanol extract in another study which showed an IC50 value of around 46 µg/mL, but both strengthen the evidence that the active compounds in pearl grass extract have the ability to neutralize free radicals. Phenolic compounds and flavonoids as electron donors play an important role in the mechanism of strengthening free radical oxidation reactions that cause oxidative stress (Ronzon et al., 2025). In addition, the antioxidant activity of the methanol extract is supported by the presence of other phytochemical compounds such as ursolic acid, oleanolic acid, and β-sitosterol which also play a role in increasing the ability to capture free radicals (Weik, 2000). The combination of these compounds synergistically contributes to cell protection activity against oxidative damage, as the basis for using this extract as a source of natural antioxidants.

## 4 CONCLUSIONS

The ethanol extract of pearl grass leaves showed significant antioxidant activity in overcoming oxidative stress, which was characterized by the ability of the extract to neutralize DPPH free radicals in vitro. The increase in extract concentration is directly proportional to the increase in antioxidant activity, with a maximum inhibition of 51.60% at a concentration of 80 ppm. The presence of phenolic compounds, flavonoids, and other bioactive compounds such as ursolic acid, oleanolic acid, and β-sitosterol in the extract play a major role as electron donors that are effective in stopping the chain reaction of free radical oxidation that causes oxidative stress. The IC50 value of the ethanol extract showed strong antioxidant potential (around 46 µg/mL), although the IC50 value of the methanol extract was higher (around 151 µg/mL), both strengthening the evidence that the bioactive compounds in pearlgrass effectively inhibit free radicals. These findings support the potential of pearl grass as a source of natural antioxidants that can be further developed into health products for the prevention and therapy of degenerative diseases associated with oxidative stress.

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