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Test Antioxidant Content In Herb *Kyllinga nemoralis* Extract With DPPH Method

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ABSTRACT

Antioxidants are compounds capable of slowing or preventing oxidation, even in relatively low concentrations, thereby contributing to various physiological functions in the body. The antioxidant components found in plants act as free radical scavengers by transforming them into less reactive molecules. *Jukut Pendul* (Kyllinga nemoralis) contains several bioactive substances, including flavonoids, tannins, and alkaloids, which are recognized for their potential as natural antioxidants. Flavonoids, for instance, exhibit antioxidant activity by donating hydrogen atoms or through their metal-chelating properties. These compounds can be present as glycosides (with glucose moieties) or in the free form known as aglycones. This research aimed to evaluate the antioxidant capacity of Kyllinga nemoralis extract. The plant material was sourced from Banyuwangi, East Java. Extraction was performed using the maceration method with 70% ethanol as the solvent. The free radical scavenging activity was assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl radical) method. Ascorbic acid was used as positive control. Measurement of absorbance was carried out through UV-Vis spectrophotometry. The extract was tested at concentrations of 20, 40, 60, 80, and 100 µg/mL. The findings revealed that the IC_{50} value of *Kyllinga nemoralis* extract was 11.351 µg/mL, whereas ascorbic acid exhibited an IC_{50} of 3.43 µg/mL. The antioxidant activity of *Kyllinga nemoralis* ethanol extract falls into the very strong category, ranging <50-100 µg/mL. These results suggest that although the antioxidant strength of Kyllinga nemoralis is lower than that of ascorbic acid, the extract still possesses detectable radical-scavenging activity.

Keywords: *Kyllinga nemoralis* Extract; DPPH Method; antioxidant; ascorbic acid;

Introduction

Research evidence highlights that antioxidants play a significant role in promoting health and in both the prevention and treatment of various diseases through the reduction of oxidative stress. Measuring their activity or capacity in food products and biological materials is necessary for ensuring the quality of functional foods and for understanding the therapeutic benefits of dietary antioxidants against oxidative stress–related conditions (Munteanu & Apetrei, 2021). Cellular damage triggers the release of free radicals, which play a significant role in cellular bioactive processes. Antioxidants are among the critical components responsible for preserving cellular function and integrity. They play a pivotal role in neutralize reactive oxygen species (ROS) and sustaining normal cellular activity. Antioxidants prevent the harmful effects of free radical configuration that lead to tissue damage either by inhibiting their formation or by promoting the breakdown of radical species (Kattappagari et al., 2015).

Indonesia is rich in natural resources that can be utilized as sources of natural antioxidants. One such example is *Kyllinga nemoralis*, which has long been used as traditional medicine. Beyond its traditional use, the medicinal potential of *Kyllinga nemoralis* has been scientifically investigated for its antidiarrhea, expectorant, anthelmintic, diuretic, antibacterial, and anti-acne properties (Niluwih et al., 2023) (Fadhliani, 2020) (Mulyani et al, 2022). This plant contains secondary metabolites, including essential oils (α-cyperone, α-humulene, and β-selinene), saponins, terpenoids, phenolic compounds, and flavonoids, which exhibit potential as natural antioxidants (Datta S, 2020). Further studies have reported that *Kyllinga nemoralis* contains flavonoids, phenolics, saponins, steroids, and terpenoids with antioxidant potential (Biosci et al., 2019)(Wahab & Rahman, 2022). Antioxidant activity of *Kyllinga nemoralis* was evaluated in South Kalimantan, Indonesia, using the DPPH assay with a UV-Vis spectrophotometer at 517 nm. The IC_{50} values were determined to be 79.842 ± 14.275 µg/mL for the ethanol extract, 95.632 ± 8.152 µg/mL for the n-hexane fraction, 42.616 ± 1.972 µg/mL for the ethyl acetate fraction, and 67.384 ± 11.678 µg/mL for the aqueous fraction. Among these, the ethyl acetate fraction exhibited the strongest antioxidant activity (Nabila et al., 2025). Antioxidant studies on *Kyllinga nemoralis* have also been carried out in the Philippines using the DPPH method, which yielded an IC_{50} value of 15.02 µg/mL, classified as very strong antioxidant activity, while the ethyl acetate extract showed an IC_{50} value of 62.9 µg/mL, classified as strong antioxidant activity (Ang & Uyangurin, 2022). Secondary metabolite content is influenced by geographic factors and growing environment. In light of the preceding results, this study was designed to evaluate the antioxidant potential of *Kyllinga nemoralis* originating from East Java, particularly Banyuwangi.

Methodology

An experimental design is a traditional approach to conducting quantitative research. It generated numerical data that describe the antioxidant activity of

Kyllinga nemoralis extract in inhibiting free radicals.

Instruments and Materials

The instruments used in this study included a UV-Vis spectrophotometer (GD-725N), rotary evaporator (SHZ-III), maceration vessel, a set of test tubes (Pyrex), analytical balance (Fujitsu), dropper pipettes, beakers (Herma), and volumetric flasks (Pyrex).

The materials employed in the study consisted of *Kyllinga nemoralis* simplicia powder, 70% ethanol, DPPH reagent, ascorbic acid, and distilled water.

Preparation of *Kyllinga nemoralis* Leaf Extract

Extraction of *Kyllinga nemoralis* herb was carried out using the maceration method with 70% ethanol as the solvent. A total of 400 grams of *Kyllinga nemoralis* simplicia powder was soaked in ethanol at a ratio of 1:10 in a macerator. The extraction process was performed for 3 × 24 hours with intermittent stirring. The liquid extract obtained was concentrated using a rotary evaporator at 50°C. The process was stopped once the solvent ceased dripping and the extract had thickened.

Determination of Antioxidant Activity

The antioxidant activity was tested in vitro using the DPPH method. A volume of 2.5 mL of *Kyllinga nemoralis* leaf extract at concentrations of 20, 40, 60, 80, and 100 µg/mL was placed into separate dark tubes and mixed with 2.5 mL of DPPH solution. The mixture was shaken and incubated for 30 minutes, after which the absorbance was measured at a wavelength of 517 nm. The percentage of radical scavenging activity was calculated using the following equation (Manongko et al., 2020):

$$\text{Activity} = \left(\frac{A\ blank - A\ sample}{A\ blank}\right) X100\%$$

Description:

A blank : Absorbance of DPPH

A sample : Absorbance of Sample + DPPH

Result and Discussion

Kyllinga nemoralis simplicia weighing 600 grams was extracted using with 70% ethanol yielded 54 grams of concentrated extract, corresponding to an extract yield of 9%. The choice of solvent greatly determines the effectiveness of extraction of

active compounds and metabolite extraction of more active compounds and metabolites. The selection of solvent is determined by several factors, including boiling point, toxicity, extraction efficiency, and cost. The maceration method offers advantages over other extraction techniques in terms of production cost, as well as the simplicity and practicality of the extraction process. The choice of ethanol concentration as a solvent is based on its polarity level: 90% ethanol is less polar, 70% ethanol is moderately polar, and 50% ethanol is highly polar (Fauziyah et al., 2022). The extraction was carried out for 2 × 24 hours. Seventy percent ethanol was selected as the solvent because it is considered optimal for dissolving bioactive compounds. The 30% water content allows for the effective extraction of both polar and semi-polar compounds (Dian Kartikasari, Ristia Rahman & Ridha, 2022).

The antioxidant activity of a plant extract is commonly assessed by determining its half-maximal inhibitory concentration (IC_{50}). This parameter reflects the concentration of the extract required to inhibit 50% of free radical activity. The IC_{50} value is obtained through linear regression analysis by substituting 50 for the *y* value in the equation $y = a + bx$. The antioxidant activity of the positive control, ascorbic acid, is presented in Table 1 and Figure 1, while the results for the antioxidant activity of the *Kyllinga nemoralis* extract are shown in Table 2 and Figure 2.

Table 1. Antioxidant Activity Test of Ascorbic Acid Standard Solution

No	Sample Concentration (µg/mL)	Average Absorbance	Antioxidant Activity (%)	IC_{50} (µg/mL)
1	20	0,252	55,367	3,43
2	40	0,253	55,744	
3	60	0,183	65,537	
4	80	0,235	52,354	
5	100	0,237	52,605	

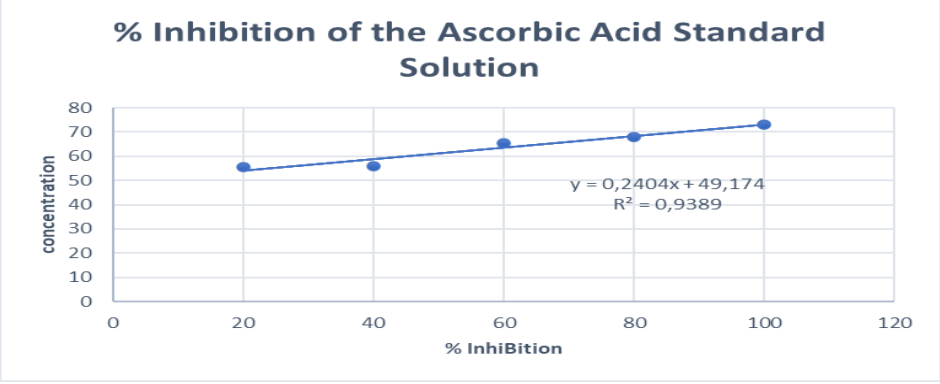

Figure 1. Precentage Inhibition The Ascorbic Acid Standard Solution

Table 2. Antioxidant Activity Test of *Kyllinga nemoralis* Extract

No	Sample Concentration (µg/mL)	Average Absorbance	Antioxidant Activity (%)	IC_{50} (µg/mL)
1	20	0,01	95	11,351
2	40	0,017	91	

3	60	0,021	89
4	80	0,030	85
5	100	0,040	79

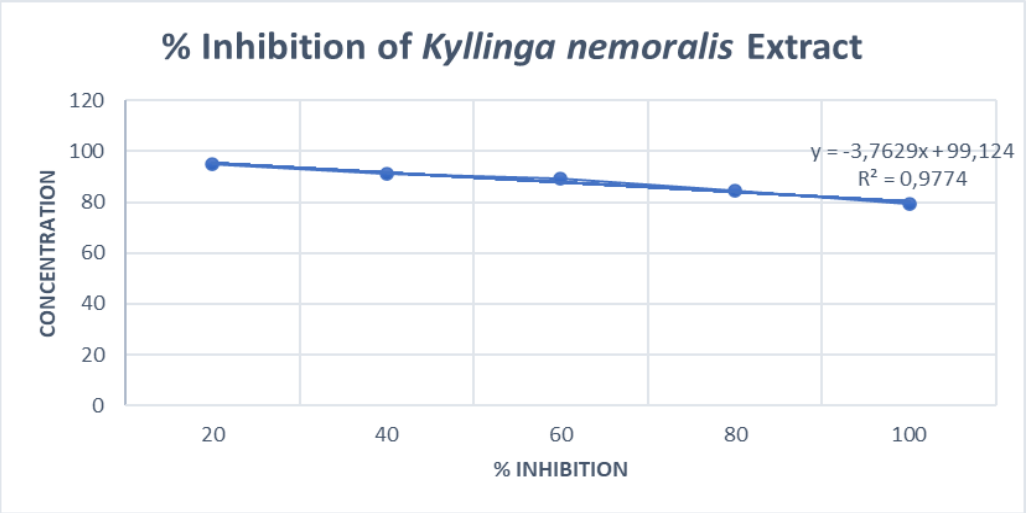

Figure 2. Precentage Inhibition of Kyllinga nemoralis Extract

Based on the figure 2, Based on the results obtained from the DPPH assay, the percent inhibition of *Kyllinga nemoralis* extract showed a decrease in antioxidant activity, starting from the lowest concentration of 20 µg/mL with 95% inhibition, down to 79% at the highest concentration of 100 µg/mL. This pattern in *Kyllinga nemoralis* extract indicates that higher extract concentrations correspond to lower inhibition percentages. This finding contrasts with the study conducted by (Damanis et al., 2020) which reported that the percentage of inhibition of free radical activity increases proportionally with concentration (Damanis et al., 2020). In samples containing antioxidant compounds, higher concentrations generally mean a greater number of molecules donating electrons or hydrogen atoms to DPPH free radicals, which leads to the fading of the DPPH color. As a result, lower absorbance values correspond to stronger antioxidant activity (Ika Fatmawati, Haeruddin, 2023).

The standard curve obtained was plotted between concentration and absorbance, resulting in a linear regression equation of y = -3,7629x + 99.124 with an R^2 value of 0.9774 standard and linear regression equation of ascorbic acid standard solution y=0,2404x + 49,174 R^2 = 0,9389. The R^2 value of *Kyllinga nemoralis* 0,9774 obtained in the curve of the relationship between concentration (µg/mL) and % inhibition in the test sample of *Kyllinga nemoralis* extract is above 0.900, indicating that the curve is linear. The IC_{50} value is obtained from linear regression by replacing the y value with 50 from the equation y = a + bx. The lower the IC_{50} value, the higher the antioxidant activity of a substance. The IC_{50} value of *Kyllinga nemoralis* extract was 11.351 µg/mL, whereas ascorbic acid exhibited an IC_{50} of 3.43 µg/mL. The antioxidant potential of a compound is classified by its IC_{50} value, ff the IC_{50} value is below 50 mg/L, it is categorized as very potent (Koes et al., 2024).

In this study, the IC_{50} value was obtained. This result differs from the percentage of inhibition reported by Nabila et al. (2025), in which the ethanol extract of *Kyllinga nemoralis* from South Kalimantan yielded an IC_{50} value of 79.842 ± 14.275 μg/mL with category very strong antioxidant (Nabila et al., 2025). These differences in antioxidant value can occur due to differences in environmental conditions, for example, at higher altitudes, which encourage the formation of higher secondary metabolites through light intensity, which can affect photosynthesis in plants. The presence of antioxidant compounds in plant organs is a byproduct of plant metabolism (Agus Aminurita, Galih Samodra, 2024). Such variations are often linked to distinct environmental characteristics that affect the organism's metabolic profile. External and internal factors significantly impact the antioxidant levels of the sample, making it possible to obtain different results in different regions. Factors influencing the life of the plant include nutrient availability, temperature, pH, topography, depth, water currents, and the presence of pollutants, all of which may affect the biosynthesis of active compounds (Marzukim I, 2018).

The methanolic and aqueous extracts of the plant leaves tested positive for terpenoids, saponins, and phenolic compounds. More recently, the ethanolic extract of the rhizomes was found to contain flavonoids, triterpenoids, and glycosides, while the petroleum ether extract contained triterpenoids and glycosides (Raju et al., 2011). Compounds such as flavonoids, polyphenols, and alkaloids can play a role in scavenging DPPH radicals (Rudiana et al., 2019). Flavonoids, a class of polyphenolic compounds with antioxidant properties, act by donating their hydrogen atoms or electrons to free radicals in order to stabilize these radical molecules (Dewi et al., 2018).

Conclusion

The extract was tested at concentrations of 20, 40, 60, 80, and 100 μg/mL. The IC_{50} value obtained for *Kyllinga nemoralis* extract was 11.351 μg/mL, compared with 3.43 μg/mL for ascorbic acid. These results suggest that the antioxidant capacity of *K. nemoralis* is weaker than that of ascorbic acid, although the extract still demonstrated noticeable radical scavenging activity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All aspects of the research, writing, and publication were conducted independently without any external influence.

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