






# ANTIOXIDANT ACTIVITY OF HYDROALCOHOLIC EXTRACT OF GREEN GEDI LEAVES (*Abelmoschus manihot* L.) USING CUPRAC (CUPRIC ION REDUCING ANTIOXIDANT CAPACITY) METHOD

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## ABSTRACT

Free radicals induce oxidative stress, which plays a pivotal role in the pathogenesis of various degenerative diseases. *Abelmoschus manihot* L., locally known as green gedi, is recognized for its rich secondary metabolite content with potential antioxidant properties. This study aimed to evaluate the antioxidant activity of the hydroalcoholic extract of green gedi leaves using the Cupric Ion Reducing Antioxidant Capacity (CUPRAC) method. Extraction was conducted via maceration using a 90% hydroalcoholic solvent, followed by comprehensive phytochemical screening and simplicia characterization. The antioxidant capacity was quantified by determining the IC<sub>50</sub> value through linear regression analysis of inhibition percentage versus concentration. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids/steroids. The hydroalcoholic extract exhibited "very strong" antioxidant activity, yielding an IC<sub>50</sub> value of 41.65 ± 0.30 µg/mL. Although this potency was slightly lower than the pure quercetin standard, it demonstrates a significant capacity for free radical neutralization. These findings underscore the potential of *Abelmoschus manihot* L. as a robust natural antioxidant source, supporting its further development into phytopharmaceuticals or functional health supplements for the prevention of oxidative stress-related conditions.

**Keywords:** *Abelmoschus manihot* L., antioxidant activity, CUPRAC, hydroalcoholic extract, IC<sub>50</sub>.

## ABSTRAK

Radikal bebas memicu stres oksidatif yang memainkan peran krusial dalam patogenesis berbagai penyakit degeneratif. *Abelmoschus manihot* L., yang secara lokal dikenal sebagai daun gedi hijau, diakui memiliki kandungan metabolit sekunder yang kaya dengan potensi sifat antioksidan. Penelitian ini bertujuan untuk mengevaluasi aktivitas antioksidan dari ekstrak hidroalkohol daun gedi hijau menggunakan metode Cupric Ion Reducing Antioxidant Capacity (CUPRAC). Ekstraksi dilakukan melalui maserasi menggunakan pelarut hidroalkohol 90%, diikuti dengan skrining fitokimia dan karakterisasi simplisia secara menyeluruh. Kapasitas antioksidan dikuantifikasi dengan menentukan nilai IC<sub>50</sub> melalui analisis regresi linear antara persentase inhibisi terhadap konsentrasi. Analisis fitokimia mengonfirmasi keberadaan alkaloid, flavonoid, glikosida, saponin, tanin, dan triterpenoid/steroid. Ekstrak hidroalkohol menunjukkan aktivitas antioksidan



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yang "sangat kuat", dengan nilai IC<sub>50</sub> sebesar  $41,65 \pm 0,30$  µg/mL. Meskipun potensi ini sedikit lebih rendah dibandingkan dengan standar kuersetin murni, hasil ini menunjukkan kapasitas yang signifikan dalam netralisasi radikal bebas. Temuan ini menegaskan potensi *Abelmoschus manihot* L. sebagai sumber antioksidan alami yang potensial, serta mendukung pengembangan lebih lanjut menjadi fitofarmaka atau suplemen kesehatan fungsional untuk pencegahan kondisi yang berkaitan dengan stres oksidatif.

Kata Kunci: *Abelmoschus manihot* L., aktivitas antioksidan, CUPRAC, ekstrak hidroalkohol, IC<sub>50</sub>.

## 1. Introduction

Free radicals are defined as molecules or atoms possessing one or more unpaired electrons, which induce oxidative damage to critical cellular components, including lipids, proteins, and DNA [1]. These reactive species pose a significant threat to human health due to their role in the pathogenesis of various degenerative diseases, such as diabetes mellitus, cancer, atherosclerosis, and hypertension [2]. Degenerative diseases are non-communicable conditions characterized by the chronic decline of organ function, often exacerbated by the aging process and chronic inflammation. Within the last five years, Indonesia has witnessed a substantial increase in the prevalence of non-communicable diseases (NCDs), specifically stroke, heart disease, and diabetes [3]. This rising trend underscores the urgent need for more effective interventions in NCD prevention and control. Strategies such as promoting healthy lifestyles, facilitating early detection, and enhancing access to healthcare services are pivotal in mitigating the future burden of these diseases [4]. Reactive Oxygen Species (ROS), which are byproducts of normal aerobic metabolism, are widely implicated in the etiology of premature aging, coronary heart disease, Alzheimer's disease, neurodegenerative disorders, and cataracts, often manifested through markers of oxidative stress. ROS include superoxide anion radicals, singlet oxygen, hydrogen peroxide, and highly reactive hydroxyl radicals [5].

Antioxidants are molecules capable of inhibiting or delaying the oxidation of other substrates by neutralizing free radicals through electron donation or capture, without becoming radicals themselves. These molecules play a crucial role in preventing and inhibiting the progression of degenerative diseases by maintaining cellular structural integrity and supporting both immune and metabolic functions. Consequently, the exploration and utilization of natural antioxidant compounds have become highly relevant and are currently focal points in research aimed at degenerative disease prevention [5], [6].

*Abelmoschus manihot* L., commonly known in Indonesia as green gedi leaves, is a tropical plant with a long history in traditional medicine across Asia. A member of the Malvaceae family, this plant is also a dietary staple in the Manadonese community, where it is used in the traditional porridge, Tinutuan. Previous phytochemical screening by Warongan (2017) identified the presence of secondary metabolites, including flavonoids, tannins, alkaloids, and steroids [7]. Furthermore, Suoth (2019) quantified the total flavonoid content in various fractions of *Abelmoschus manihot* L., reporting values of  $3.837 \pm 0.033$  mg QE/g for the ethanol extract,  $4.214 \pm 0.01$  mg QE/g for the n-hexane fraction,  $5.026 \pm 0.025$  mg QE/g for the ethyl acetate fraction, and  $2.435 \pm 0.007$  mg QE/g for the ethanol fraction. Flavonoid compounds are recognized for their diverse health benefits, most notably their potent antioxidant activity [8].

The CUPRAC (Cupric ion reducing antioxidant capacity) assay utilizes the Cu(II)-neocuproine (Cu(II)-Nc<sub>2</sub>) reagent as a chromogenic oxidizing agent to measure the reduction of Cu(II) ions. The CUPRAC method offers several advantages over other antioxidant assays; it is a selective reagent due to its lower redox potential and is capable of rapidly oxidizing thiol-type antioxidants. Additionally, the CUPRAC reagent exhibits greater stability compared to other chromogenic reagents such as ABTS and DPPH [9].

Based on this background, the present study aims to evaluate the antioxidant activity of the hydroalcoholic extract of *Abelmoschus manihot* L. leaves using the CUPRAC method. The use of a hydroalcoholic solvent system offers distinct advantages over single-solvent extraction, as it enables the simultaneous dissolution of both polar and semi-polar compounds, thereby capturing a broader range of bioactive constituents and potentially enhancing biological activity. It is anticipated that the findings of this research will provide robust scientific evidence regarding the potential of gedi leaves as a source of natural antioxidants and support the development of phytopharmaceuticals or natural-based supplements.

## 2. Methods

### 2.1 Tools and Materials

The tools used in this study include: aluminum foil, stirring rod, Beaker Glass (Iwaki), porcelain cup, chamber, 250 mL separating funnel (pyrex), desiccator, Erlenmeyer flask (pyrex), measuring cup (pyrex), plastic cuvette, micropipette (Eppendorf), tweezers, dropper pipette, rotary evaporator (Heidolph), sonicator, spatula, ultraviolet visible spectrophotometer (Shimadzu), analytical balance (Mettler toledo), vial, vortex and

maceration container. The materials used in this study were distilled ethanol, ammonium acetate buffer, Neocuproin (Sigma),  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , and Quercetin.

## 2.2 Preparation Extract

The extraction was performed using the maceration method. Initially, 40 g of *Abelmoschus manihot* L. leaf powder was weighed and transferred into a round-bottom flask. A hydroalcoholic solvent was prepared by mixing 99.9% absolute ethanol with distilled water to achieve a concentration of 90%. Maceration was conducted by adding 400 mL of the solvent to the sample. This process was maintained for a duration of 5 + 2 days with daily stirring. The resulting mixture was filtered using a Buchner funnel. The collected filtrate was then concentrated using a rotary evaporator at 50°C to remove the remaining solvent [9], [10].

## 2.3 Characteristic Examination and Phytochemical Screening of Hydroalcohol Extracts

The characterization of the sample was conducted through several determinations, including water content, water-soluble extractive content, ethanol-soluble extractive content, total ash content, and acid-insoluble ash content. Additionally, phytochemical screening was performed on the hydroalcoholic extract of green gedi leaves to identify the presence of bioactive secondary metabolites, specifically alkaloids, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids.

## 2.4 Preparation of CUPRAC Reagent Solution

A 0.01M solution of  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  was prepared by dissolving 0.4262 g of the salt in 250 mL of distilled water. The ammonium acetate buffer ( $\text{CH}_3\text{COONH}_4$ ) at pH 7.0 was prepared by dissolving 19.27 g of ammonium acetate in 250 mL of distilled water. Additionally, a 0.0075 M neocuproine (Nc) solution was prepared by dissolving 0.039 g of neocuproine in 25 mL of ethanol [11], [12].

## 2.5 CUPRAC Maximum Wavelength Determination ( $\lambda_{\text{Max}}$ )

The CUPRAC reagent mixture was prepared by combining 1 mL of 0.01 M  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , 1 mL of 0.0075 M neocuproine, 1 mL of ammonium acetate buffer, and 1 mL of distilled water in a vial. The absorbance of the resulting solution was measured across a wavelength range of 400–800 nm using a UV-Vis spectrophotometer. Based on the spectral scanning results, the maximum absorption wavelength ( $\lambda_{\text{max}}$ ) was identified at 452 nm [12].

## 2.6 Preparation of Quercetin Standard Stock Solution

A standard stock solution I (SSO I) was prepared by weighing 10 mg of quercetin and dissolving it in methanol within a 10 mL volumetric flask. The volume was adjusted to the mark with methanol to achieve a final concentration of 1000  $\mu\text{g/mL}$ . Subsequently, a standard stock solution II (SSO II) was prepared by pipetting 1 mL of SSO I into another 10 mL volumetric flask. The solution was diluted with methanol to the mark and shaken until homogeneous, resulting in a final concentration of 100  $\mu\text{g/mL}$  [12].

## 2.7 Preparation of Standard Stock Solution (SSR) of Hydroalcoholic Extract Sample of Green Gedi Leaves

A sample stock solution I (SSO I) was prepared by weighing 10 mg of the test sample and dissolving it in methanol within a 10 mL volumetric flask. The volume was adjusted to the mark with methanol to obtain a final concentration of 1000  $\mu\text{g/mL}$ . Subsequently, 5 mL of SSO I was pipetted into a 10 mL volumetric flask and diluted with methanol to the mark. The mixture was shaken until homogeneous to produce sample stock solution II (SSO II) with a final concentration of 500  $\mu\text{g/mL}$  [12], [13], [14].

## 2.8 Antioxidant Testing With CUPRAC Quercetin Free Radical Trap

Varying volumes of Standard Stock Solution II (SSO II)—specifically 0.0312 mL, 0.0625 mL, 0.125 mL, and 0.250 mL—were pipetted into 5 mL volumetric flasks to obtain a series of test solution concentrations (0.625  $\mu\text{g/mL}$ , 1.25  $\mu\text{g/mL}$ , 2.5  $\mu\text{g/mL}$ , and 5  $\mu\text{g/mL}$ ). To each flask, 1 mL of each CUPRAC reagent component (0.01 M  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.0075 M ethanolic neocuproine, and ammonium acetate buffer) and 1 mL of distilled water were added. The mixtures were then incubated for 30 minutes at room temperature. Subsequently, the absorbance of each solution was measured using a UV-Vis spectrophotometer at the maximum wavelength of 452 nm [12], [13].

## 2.9 Antioxidant Testing With CUPRAC Free Radical Trap Hydroalcoholic Extract of Green Gedi Leaves

Specific volumes of Standard Stock Solution II (SSO II)—namely 0.0625 mL, 0.125 mL, 0.250 mL, and 0.5 mL—were pipetted into 5 mL volumetric flasks to achieve a series of final concentrations (6.25  $\mu\text{g/mL}$ ,

12.5 µg/mL, 25 µg/mL, and 50 µg/mL, respectively). To each flask, 1 mL of each CUPRAC reagent component (0.01 M CuCl<sub>2</sub>·2H<sub>2</sub>O, 0.0075 M ethanolic neocuproine, and ammonium acetate buffer) and 1 mL of distilled water were added. The resulting mixtures were incubated for 30 minutes. Following incubation, the absorbance of each solution was measured using a UV-Vis spectrophotometer at a wavelength of 452 nm [8].

### 2.10 *IC<sub>50</sub> Value Analysis*

The free radical scavenging activity was determined by calculating the IC<sub>50</sub> (Inhibitory Concentration) value, which represents the concentration of the test compound required to neutralize 50% of free radicals. The IC<sub>50</sub> was derived from a linear regression equation, where the sample concentration (µg/mL) was plotted as the abscissa (x-axis) and the percentage of inhibition as the ordinate (y-axis) (Mardawati, 2008). According to the antioxidant potency classification, a compound is categorized as a very strong antioxidant if the IC<sub>50</sub> value is less than 50 µg/mL. Furthermore, it is classified as strong (50–100 µg/mL), moderate (100–150 µg/mL), or weak (151–200 µg/mL) [15].

## 3. Results

### 3.1. *Extraction Yield of Green Gedi Leaf*

The extraction of green gedi leaf powder (50 g) was conducted using a maceration method with a hydroalcoholic solvent (99.9% absolute ethanol and water in a 90:10 ratio). The process yielded 11.23 g of a concentrated thick extract, corresponding to a yield percentage of 22.46%.

### 3.2. *Simplicia Characterization and Phytochemical Screening*

The results of the characterization of green gedi leaf simplicia are presented in Table 1.

**Table 1.** Characterization Results of Green Gedi Leaf Simplicia

Parameter	Result (%)
Water content	6.5
Water soluble content	9.7
Ethanol soluble content	3.3
Total ash content	6.9
Acid insoluble ash content	2.8

The phytochemical screening of the hydroalcoholic extract revealed the presence of several secondary metabolites. As shown in Table 2, the extract tested positive for alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids/steroids.

**Table 2.** Phytochemical Screening of Hydroalcoholic Extract of Green Gedi Leaves

Parameter	Result (%)
Alkaloids	+
Flavonoids	+
Glycoside	+
Saponnin	+
Tannin	+
Triterpenoid/ steroid	+

### 3.3. *CUPRAC Antioxidant Activity*

The antioxidant capacity was evaluated based on the percentage of CUPRAC reduction. The data for the hydroalcoholic extract across various concentrations are summarized in Table 3.

**Table 3.** CUPRAC Antioxidant Activity of Green Gedi Leaf Extract

Concentration of test solution (µg/mL)	% Damping			Average
	I	II	III	
0	0.000	0.00	0.00	0.00
6.25	16.76	16.36	15.15	16.09
12.5	25.66	25.80	25.92	25.79
25	37.66	36.98	36.65	37.09
50	55.01	55.04	54.54	54.86

The antioxidant strength was further quantified using the IC<sub>50</sub> value, derived from the linear regression equations of three replicates. The average IC<sub>50</sub> value for the hydroalcoholic extract was  $41.65 \pm 0.30$  µg/mL (Table 4).

**Table 4.** IC<sub>50</sub> Values of Hydroalcoholic Extract of Green Gedi Leaves

Sample	Repetition	CUPRAC		
		Regression equation	IC <sub>50</sub> (µg/mL)	Average
Hydroalcoholic ethanol extract of green gedi leaves	I	$y = 1.0142x + 8.0078$ R <sup>2</sup> = 0.9267	41.40	41.65 ± 0.30
	II	$y = 1.0148x + 7.8138$ R <sup>2</sup> = 0.9301	41.57	
	III	$y = 1.0126x + 7.4687$ R <sup>2</sup> = 0.9309	42.00	

#### 4. Discussion

The characterization results indicate that the green gedi leaf *simplicia* meets the quality standards established by the *Materia Medika Indonesia* (MMI). Specifically, the water content of 6.5% is well below the maximum threshold of 10%, which is critical for preventing microbial and mold growth during storage [16], [17]. Low moisture levels are essential for maintaining the stability of bioactive compounds and preventing enzymatic degradation, ensuring the long-term quality of the herbal material. The total ash and acid-insoluble ash contents (6.9% and 2.8%, respectively) provide an overview of the mineral content and ensure low levels of metallic or external contamination, such as soil or sand, which often occur during the harvesting and drying process [7], [18].

Phytochemical screening confirmed that the hydroalcoholic extract contains a diverse range of bioactive compounds, including alkaloids, flavonoids, and tannins. These findings align with Hidayat (2022), suggesting that these metabolites contribute to the plant's therapeutic potential, particularly its antioxidant properties [7]. Recent studies on *Abelmoschus manihot* L. have highlighted that its secondary metabolites, particularly flavonoid glycosides like myricetin and quercetin derivatives, exhibit multi-target pharmacological activities, including anti-inflammatory and nephroprotective effects, which are closely linked to their antioxidant capacity.

In the CUPRAC assay, the extract demonstrated a dose-dependent antioxidant effect, where higher concentrations resulted in increased free radical scavenging activity [19]. The mechanism involves the reduction of the Cu(II)-neocuproine complex to the yellow-colored Cu(I)-neocuproine complex by the antioxidant compounds present in the sample [20]. Unlike other methods such as DPPH, the CUPRAC assay is considered more advantageous for plant extracts because it can detect both hydrophilic and lipophilic antioxidants and is carried out at a physiological pH (7.0), providing results that are more biologically relevant.

With an average IC<sub>50</sub> value of  $41.65 \pm 0.30$  µg/mL, the hydroalcoholic extract of green gedi leaves is classified as a "very strong" antioxidant (IC<sub>50</sub> < 50 µg/mL) [21], [22]. This high potency is primarily attributed to the presence of flavonoids, which neutralize radicals by donating hydrogen atoms or chelating metal ions [23]. Recent pharmacological reviews emphasize that the hydroalcoholic solvent (ethanol-water mixture) is highly effective in extracting polar and semi-polar phenolics, which often results in higher bioactivity compared to pure aqueous or pure organic extracts.

While the extract showed significant activity, it remained lower than the quercetin standard. This difference is expected as quercetin is a pure flavonoid compound, whereas the extract is a complex mixture of various phytochemicals where synergistic or antagonistic interactions may occur. However, the "very strong" classification of this extract underscores the potential of *Abelmoschus manihot* L. as a functional food ingredient or a source for developing natural antioxidant supplements. The use of a 90% hydroalcoholic solvent was instrumental in this study, as its polarity effectively extracts the active antioxidant constituents—specifically the flavonoid aglycones and glycosides—from the leaves, maximizing the total phenolic recovery [24].

#### 5. Conclusion

Based on the results of this study, it can be concluded that the 90% hydroalcoholic extract of green gedi leaves (*Abelmoschus manihot* L.) exhibits potent antioxidant activity when evaluated using the CUPRAC method. The extract achieved an IC<sub>50</sub> value of  $41.65 \pm 0.30$  µg/mL, which categorizes it as a "very strong" antioxidant (IC<sub>50</sub> < 50 µg/mL). These findings suggest that green gedi leaves serve as a significant natural source of antioxidant compounds, particularly flavonoids, with potential applications in the development of phytopharmaceuticals.

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## 7. Conflict of Interest

All authors declare that they have no conflict of interest related to this study and its publication.

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