



## Research Article

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# Specific and Non-Specific Safety Testing of Red Gedi Leaf Ethanol Extract (*Abelmoschus manihot* L. Medik)

Uji Keamanan Spesifik dan Non Spesifik Ekstrak Etanol Daun Gedi Merah (*Abelmoschus manihot* (L.) Medik)

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### ABSTRACT

Red gedi leaves (*Abelmoschus manihot* (L.) Medik) are traditionally used for their antidiabetic, antioxidant, and hepatoprotective properties. This study aimed to characterize the specific and non-specific quality parameters of red gedi leaf ethanol extract based on Herbal Pharmacopoeia standards. Extraction was performed by maceration using 96% ethanol, yielding 6.72%. Phytochemical screening confirmed the presence of flavonoids, saponins, tannins, quinones, and steroids, with a high total flavonoid content of  $56.97 \pm 3.75$  mg/g. Non-specific parameters, including water content, drying loss, ash values, microbial contamination, and heavy metal levels (Pb and Cd), complied with the required standards. Organoleptic evaluation showed a viscous extract with brownish color, slightly astringent taste, and characteristic odor. These results indicate that red gedi leaf ethanol extract meets Herbal Pharmacopoeia quality requirements and has potential for development as a standardized antioxidant herbal product.

### Kata kunci:

*Abelmoschus Manihot*

Ekstrak Etanol

Flavonoid

Gedi Merah

### ABSTRAK

Daun gedi merah (*Abelmoschus manihot* (L.) Medik) merupakan tanaman obat tradisional yang memiliki aktivitas antidiabetes, antioksidan, dan hepatoprotektif. Penelitian ini bertujuan untuk mengkarakterisasi parameter mutu spesifik dan non-spesifik ekstrak etanol daun gedi merah berdasarkan standar Farmakope Herbal Indonesia. Ekstraksi dilakukan dengan metode maserasi menggunakan etanol 96% dan menghasilkan rendemen sebesar 6,72%. Skrining fitokimia menunjukkan keberadaan flavonoid, saponin, tanin, kuinon, dan steroid, dengan kandungan flavonoid total sebesar  $56,97 \pm 3,75$  mg/g. Seluruh parameter non-spesifik, termasuk kadar air, kehilangan pengeringan, nilai abu, cemaran mikroba, dan logam berat Pb dan Cd, memenuhi standar yang ditetapkan. Secara organoleptik, ekstrak etanol daun gedi merah memiliki konsistensi kental, warna kecoklatan, rasa sedikit sepat, dan aroma khas. Hasil penelitian ini menunjukkan bahwa ekstrak etanol daun gedi merah memenuhi persyaratan mutu Farmakope Herbal dan berpotensi dikembangkan sebagai bahan baku obat herbal terstandarisasi.



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## 1. INTRODUCTION

Herbal plants are natural resources that have long been recognized to contain bioactive compounds capable of preventing and treating diseases based on empirical human experience. Traditional medicinal plants are often referred to as a “living pharmacy” because their use is generally associated with fewer adverse effects than synthetic drugs, which has encouraged widespread public acceptance (Kumontoy et al., 2023). One such medicinal plant is red gedi (*Abelmoschus manihot* (L.) Medik), which has been traditionally utilized by local communities, particularly in North Sulawesi and Eastern Indonesia. In Indonesia, red gedi is commonly consumed as a vegetable; in North Sulawesi, its leaves are processed into *tinutuan* (Manado porridge). Taxonomically, red gedi belongs to the family Malvaceae and possesses promising therapeutic potential for development as a standardized herbal medicine raw material.

Previous studies have demonstrated the considerable pharmacological potential of red gedi leaves, including antidiabetic, antioxidant, and hepatoprotective activities. Characterization of the ethanol extract has revealed the presence of flavonoid glycosides that significantly contribute to its antioxidant potential. In addition, red gedi leaves contain various bioactive compounds such as flavonoids, saponins, tannins, and terpenoids, which are responsible for their antibacterial, antifungal, and antioxidant activities (Handayani et al., 2024; Mutmainnah et al., 2018; Tandil et al., 2016; Tandil et al., 2017; Tandil et al., 2025).

The utilization of high-quality herbal raw materials requires comprehensive standardization testing to ensure consistent quality, safety, and efficacy. Standardization involves defined parameters, procedures, and analytical methods that guarantee product stability and compliance with regulatory requirements. Specific parameters include organoleptic properties, extract identity, levels of water- and ethanol-soluble compounds, and chromatographic profiles, while non-specific parameters encompass safety- and stability-related aspects such as water content, ash values, drying loss, microbial contamination, heavy metal contamination, and residual solvents (Imansyah & Alam, 2021; Kementerian Kesehatan Republik Indonesia [Kemenkes RI], 2022).

Despite extensive studies on the pharmacological activities of red gedi leaves, comprehensive characterization of both specific and non-specific quality parameters of red gedi leaf ethanol extract remains limited. This lack of data poses a major challenge in developing red gedi extract as a standardized herbal raw material in accordance with applicable regulatory requirements. Therefore, this study aimed to comprehensively characterize the specific and non-specific quality parameters of red gedi leaf ethanol extract based on Herbal Pharmacopoeia standards. The findings are expected to support the development of red gedi leaf extract as a standardized antioxidant herbal medicine and to strengthen

evidence-based utilization of endemic Indonesian medicinal plants.

## 2. METHODS

### 2.1. Materials

The samples used in this study were red gedi leaves obtained from three different locations in Palu City (South Palu, West Palu, and East Palu), Central Sulawesi, Indonesia. The equipment used included stirring rods, spray bottles, porcelain dishes (Haldenwanger), chambers, Buchner funnels (Haldenwanger), hotplate stirrers (Denville), UV lamps at 254 nm and 366 nm (Camag), ovens, dropping pipettes, complete glassware sets (Pyrex Iwaki), rotary evaporator systems, silica gel GF254 (Merck), a UV-Vis spectrophotometer (Shimadzu UV-1800), analytical balances, and maceration containers (glass jars).

The materials used were distilled water, aluminum chloride (Merck), amyl alcohol (Smart Lab), ammonia (Smart Lab), glacial acetic acid, iron(III) chloride (Smart Lab), red gedi leaf ethanol extract, ethanol, ether (Smart Lab), ethyl acetate, gelatin (Merck), hydrochloric acid (HCl; Smart Lab), chloroform (Smart Lab), quercetin (Merck), methanol, sodium hydroxide (NaOH; Smart Lab), sodium acetate (Merck), *n*-butanol, Dragendorff reagent, Mayer reagent, Liebermann–Burchard reagent, and magnesium powder.

### 2.2. Research Design

This study employed an experimental design using a quantitative descriptive approach. Each parameter was analyzed in triplicate ( $n = 3$ ) to ensure data reliability and validity. Sampling of red gedi leaves was conducted at three different locations in Palu City to represent geographical variability.

### 2.3. Preparation of Red Gedi Leaf Ethanol Extract

Red gedi leaf extract was prepared using the maceration method. Dried red gedi leaf powder was weighed and macerated using 96% ethanol. The mixture was agitated three times daily and protected from light. After maceration, the mixture was filtered to obtain the filtrate. The filtrate was concentrated using a rotary evaporator and further evaporated in a water bath at 60 °C until a thick extract was obtained, following the procedure described by Depkes RI (2000). The extract yield was calculated using the following equation:

$$\text{Extract yield (\%)} = \frac{\text{Weight of extract obtained}}{\text{Weight of initial sample}} \times 100\%$$

### 2.4. Standardization Test of Specific Extract Parameters

#### a. Organoleptic Evaluation

Organoleptic evaluation was performed by assessing the form, color, odor, and taste of the extract using the five senses.

#### b. Phytochemical Screening

**Alkaloid Test.** A total of 0.5 g of extract was mixed with 5 mL of 2 N hydrochloric acid and heated in a water bath for 2 min. Three

drops of Dragendorff reagent were then added. The presence of alkaloids was indicated by the formation of an orange coloration or precipitate (Tandi, 2018).

**Flavonoid Test.** A total of 0.5 g of extract was mixed with 10 mL of distilled water, heated in a water bath, and filtered. The filtrate was treated with concentrated hydrochloric acid, 1 mL of 96% ethanol, and magnesium powder. The appearance of a yellow coloration indicated the presence of flavonoids (Tandi, 2018).

**Saponin Test.** A total of 0.5 g of extract was mixed with 10 mL of hot distilled water in a test tube. After cooling, the solution was shaken vigorously. Stable foam formation after the addition of one drop of 2 N hydrochloric acid indicated the presence of saponins (Tandi, 2018).

**Tannin Test.** A total of 0.5 g of extract was mixed with 20 mL of hot water and three drops of 10% NaCl solution. Subsequently,

FeCl<sub>3</sub> solution was added. The formation of a dark blue coloration indicated the presence of tannins (Tandi, 2018).

**Steroid and Triterpenoid Test.** The extract was mixed with 5 mL of acetic acid and left to stand for 15 min. Subsequently, 2–3 drops of concentrated sulfuric acid were added. The appearance of orange, purple, or red colors indicated triterpenoids, whereas a blue coloration indicated steroids (Mangalu et al., 2022).

#### c. Determination of Water-Soluble Extract Content

A total of 1 g of extract was macerated with 20 mL of water–chloroform in a stoppered flask for 24 h, with intermittent shaking during the first 6 h. After standing for 18 h, the mixture was filtered. An aliquot of 4 mL of filtrate was evaporated in a pre-weighed shallow dish and heated at 105 °C until a constant weight was obtained. Water-soluble extract content was calculated as follows:

$$\text{Water soluble extract content (\%)} = \frac{\text{Weight of water soluble extract (g)}}{\text{Weight of extract (g)}} \times \frac{100}{20} \times 100$$

#### d. Determination of Ethanol-Soluble Extract Content

A total of 1 g of extract was dissolved in 20 mL of 96% ethanol in a stoppered flask for 24 h, with periodic stirring during the first 6 h. After 18 h, the solution was filtered. An aliquot of 4 mL of

filtrate was evaporated in a shallow dish, and the residue was heated at 105 °C until a constant weight was obtained. Ethanol-soluble extract content was calculated using the following equation:

$$\text{Ethanol – soluble extract content (\%)} = \frac{\text{Weight of ethanol – soluble extract (g)}}{\text{Weight of extract (g)}} \times \frac{100}{20} \times 100$$

## 2.5. Standardization of Non-Specific Extract Parameters

#### e. Determination of Drying Loss

A total of 1 g of extract was placed in a dish previously heated at 105 °C for 30 min and spread to form a layer 5–10 mm thick. The extract was dried at 105 °C until a constant weight was obtained. Drying loss was calculated using the following equation:

$$\text{Drying loss (\%)} = \frac{B_1 - B_2}{B_1} \times 100$$

where  $B_1$  is the initial sample weight and  $B_2$  is the final sample weight.

#### f. Determination of Water Content

A total of 1 g of extract was weighed in a tared dish and dried in an oven at 105 °C for approximately 3 h. After cooling in a desiccator, the constant weight was recorded. Water content was calculated as follows:

$$\text{Water content (\%)} = \frac{A - B}{A} \times 100$$

where  $A$  is the sample weight before heating and  $B$  is the sample weight after heating.

#### g. Determination of Total Ash Content

A previously incinerated and weighed silica dish was filled with 1 g of extract. The sample was gradually incinerated in a furnace until free from carbon. After cooling in a desiccator, the weight was recorded. Total ash content was calculated using the following equation:

$$\text{Total ash content (\%)} = \frac{W_2 - W_0}{W_1} \times 100$$

where  $W_0$  is the weight of the empty dish,  $W_1$  is the weight of the initial extract, and  $W_2$  is the weight of the dish plus ash.

#### h. Determination of Acid-Insoluble Ash Content

The total ash was boiled for 5 min with 25 mL of dilute sulfuric acid. The insoluble residue was collected by filtration using ash-free filter paper and washed with hot water. The residue and filter paper were incinerated in a furnace until a constant weight was obtained. Acid-insoluble ash content was calculated as follows:

$$\text{Acid-insoluble ash content (\%)} = \frac{A_1 - C - A_0}{B} \times 100$$

where  $A_1$  is the weight of dish plus residue after incineration,  $A_0$  is the weight of the empty dish,  $B$  is the initial sample weight, and  $C$  is the weight of the filter paper.

## 2.6. Microbiological Contamination Testing

#### a. Total Plate Count (TPC)

After sterilization of all equipment, 1 mL of each dilution was pipetted into sterile petri dishes using different pipettes for each dilution. Molten nutrient agar (NA) medium was added, mixed evenly, and incubated at 37 °C for 24 h. Colonies were counted on plates containing 30–300 colonies and calculated as follows:

$$\text{TPC} = \text{Number of colonies per plate} \times \frac{1}{\text{Dilution factor}}$$

#### b. Yeast and Mould Count (YMC)

One milliliter of each dilution was spread onto sterile petri dishes. Molten potato dextrose agar (PDA) medium at 45 °C was added and allowed to solidify. Plates were incubated at 25 °C for 3 days.

Colonies were counted on plates containing 10–100 colonies and calculated using the same formula as TPC.

### 2.7. Heavy Metal Contamination Analysis

Lead (Pb) and cadmium (Cd) contents were determined using Atomic Absorption Spectrophotometry (AAS) following wet digestion. A total of 1 g of extract was digested with 10 mL of concentrated nitric acid (HNO<sub>3</sub>) and heated until concentrated. After cooling, 5 mL of perchloric acid and 10 mL of distilled water were added, heated until concentrated, and filtered into a 50 mL volumetric flask. The volume was adjusted with distilled water, and the solution was analyzed using AAS.

### 2.8. Statistical Analysis

All test results were expressed as mean  $\pm$  standard deviation (SD) from three independent replications. Data normality was evaluated using the Shapiro–Wilk test. For normally distributed data, differences between sampling locations were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test when significant differences were observed ( $p < 0.05$ ). For non-normally distributed data, the Kruskal–Wallis test was applied. Pearson correlation analysis was used to assess relationships between specific and non-specific parameters. Statistical analyses were performed using SPSS version 25.0 with a 95% confidence level.

### 2.9. Data Analysis

Standardization results of red gedi leaf ethanol extract were evaluated based on the Indonesian Herbal Pharmacopoeia, 2nd Edition (2022). Microbiological and heavy metal parameters were assessed according to the standards issued by the Indonesian National Agency of Drug and Food Control (BPOM, 2019).

## 3. RESULTS AND DISCUSSION

### 3.1. Ethanol Extract Yield

**Table 1.** Yield of ethanol extract from red gedi leaves collected from different sampling locations

Sampling location	Simplicia powder weight (g)	Concentrated extract weight (g)	Extraction yield (%)
South Palu	626.55 $\pm$ 12.3	43.87 $\pm$ 2.1	7.00 $\pm$ 0.34
West Palu	626.56 $\pm$ 8.7	41.25 $\pm$ 1.8	6.58 $\pm$ 0.29
East Palu	626.55 $\pm$ 10.1	39.34 $\pm$ 2.3	6.28 $\pm$ 0.37
Mean	626.55 $\pm$ 10.4	41.49 $\pm$ 2.1	6.62 $\pm$ 0.36

Notes: Values are expressed as mean  $\pm$  standard deviation (SD) of three independent replicates ( $n = 3$ ). One-way ANOVA indicated no significant differences in extraction yield among sampling locations ( $F(2,6) = 2.14$ ,  $p > 0.05$ ;  $CV = 5.4\%$ ).

**Table 2.** Phytochemical screening results of the ethanol extract of red gedi leaves

No.	Phytochemical group	Result
1.	Flavonoid	+
2.	Saponin	+
3.	Tannin	+
4.	Quinone	+
5.	Alkaloid	-
	Mayer	-
	Dragendorff	-
6.	Steroid	+

Notes: (+) Presence of the tested phytochemical group; (-) absence of the tested phytochemical group.

Determination of ethanol extract yield represents the initial step in evaluating the specific quality parameters of red gedi leaf extract. Yield reflects extraction efficiency and provides an important indicator of economic feasibility and process effectiveness. In this study, yield was calculated as the ratio of concentrated extract weight to the initial weight of simplicia powder (Sobari et al., 2022). The ethanol extract yield obtained was relatively low, with an average value of 6.72% (Table 1). This result is likely attributable to the high fiber content of red gedi leaves, which may limit solvent penetration and reduce the diffusion of extractable compounds.

One-way analysis of variance (ANOVA) showed no significant differences in extract yield among the three sampling locations ( $F(2,6) = 2.14$ ,  $p > 0.05$ ). The low coefficient of variation ( $CV = 5.4\%$ ) indicates good consistency in the extraction process. These findings suggest that local geographical variation within Palu City did not significantly influence the yield of extractable compounds.

### 3.2. Phytochemical Composition

Qualitative phytochemical screening confirmed the presence of flavonoids, saponins, tannins, quinones, and steroids in the ethanol extract of red gedi leaves (Table 2). Alkaloids were not detected using either Mayer or Dragendorff reagents, distinguishing this extract from many other medicinal plants. This phytochemical profile is consistent with previous studies reporting the therapeutic potential of red gedi leaves, particularly in relation to antioxidant and antidiabetic activities (Handayani et al., 2024; Tandil et al., 2016). The presence of multiple bioactive compound classes indicates a broad pharmacological potential. Flavonoids are well known for their antioxidant and anti-inflammatory properties, saponins contribute to antimicrobial and immunomodulatory activities, tannins provide astringent and antimicrobial effects, while steroids and quinones contribute additional anti-inflammatory, antimicrobial, and antioxidant actions.

### 3.3. Specific Quality Parameters

Evaluation of specific quality parameters is essential to ensure that herbal extracts contain sufficient levels of bioactive compounds required to exert pharmacological effects (Wardani & Setianto, 2023). The specific parameters assessed in this study included extract identity, organoleptic characteristics, water- and ethanol-soluble extractive values, total flavonoid content (Table 3), and thin-layer chromatography (TLC) profiles (Figure 1 and Figure 2).

Organoleptic evaluation showed that the extract was viscous in form, brownish in color, slightly astringent in taste, and had a characteristic odor. These characteristics provide rapid and objective preliminary indicators for extract standardization and quality control. The ethanol-soluble extractive value ( $78.75 \pm 6.95\%$ ) was higher than the water–chloroform–soluble extractive value ( $44.16 \pm 4.01\%$ ), indicating the predominance of polar and semi-polar compounds in the extract. This polarity profile suggests that red gedi leaf extract is rich in hydrophilic bioactive compounds that contribute significantly to its pharmacological activity (Marpaung & Septiyani, 2020).

TLC analysis using two different solvent systems provided complementary information on the chemical composition of the extract. The ethyl acetate:methanol:glacial acetic acid solvent system produced a prominent fluorescent spot at an Rf value of 0.60, indicating the presence of major flavonoid compounds (Figure 1). Meanwhile, the n-butanol:glacial acetic acid:water solvent system produced two distinct yellow fluorescent spots at Rf values of 0.18 and 0.66 (Figure 2). The Rf value of the spot at 0.66 closely matched that of the quercetin standard (Rf = 0.68), confirming the presence of quercetin or structurally related flavonoid glycosides in the extract. These findings provide chromatographic evidence supporting the presence of structurally specific flavonoids with known nephroprotective, anti-inflammatory, and cardioprotective activities.

The total flavonoid content of the red gedi leaf ethanol extract was exceptionally high ( $56.97 \pm 3.75$  mg/g) (Table 3), positioning

this plant as a highly promising natural antioxidant source. Previous studies have demonstrated that flavonoids from red gedi exert antioxidant and hepatoprotective effects through activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway, which regulates cellular antioxidant defense mechanisms (Qiu et al., 2017). Activation of the Nrf2 pathway enhances the expression of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. In addition, flavonoids modulate cellular signaling pathways by inhibiting nuclear factor kappa B (NF- $\kappa$ B) and activating protein kinases involved in oxidative stress regulation, further contributing to their anti-inflammatory and cytoprotective effects (Diao et al., 2024).

### 3.4. Non-Specific Quality Parameters and Safety Evaluation

Assessment of non-specific quality parameters demonstrated that all measured values complied with the requirements of the Indonesian Herbal Pharmacopoeia (Table 4), confirming the safety of the extract for pharmaceutical applications. Water content ( $2.39 \pm 0.79\%$ ) and loss on drying ( $2.30 \pm 0.76\%$ ) were well below the maximum allowable limit of 10%, indicating effective moisture control and reduced risk of microbial growth.

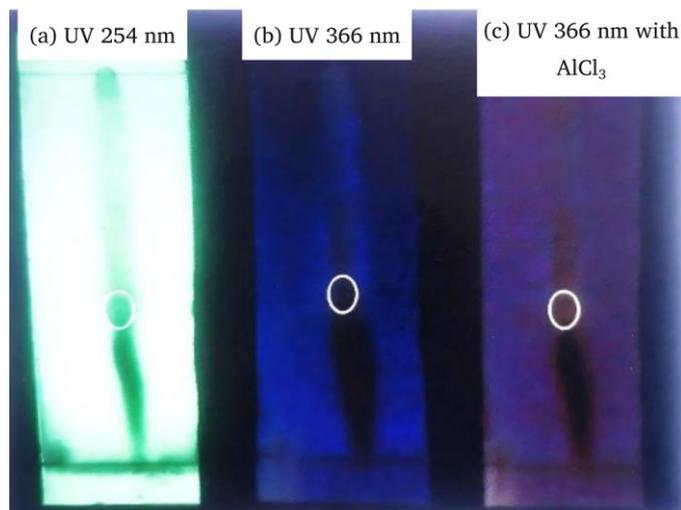
Minimal bacterial contamination was observed (0.23 colonies/g), and no fungal growth was detected, demonstrating good microbiological quality. This low microbial load is likely attributable to the antimicrobial effect of 96% ethanol used during extraction and the intrinsic antibacterial properties of flavonoids and triterpenoids present in the extract (Sun et al., 2024).

Heavy metal analysis revealed that lead and cadmium levels were below detection limits ( $<0.01$  mg/kg), far below the maximum permissible limits of 10 mg/kg for lead and 0.3 mg/kg for cadmium. In addition, the complete absence of ethanol solvent residue (0%) indicates effective solvent removal during the concentration process, eliminating potential toxicity concerns associated with residual solvents.

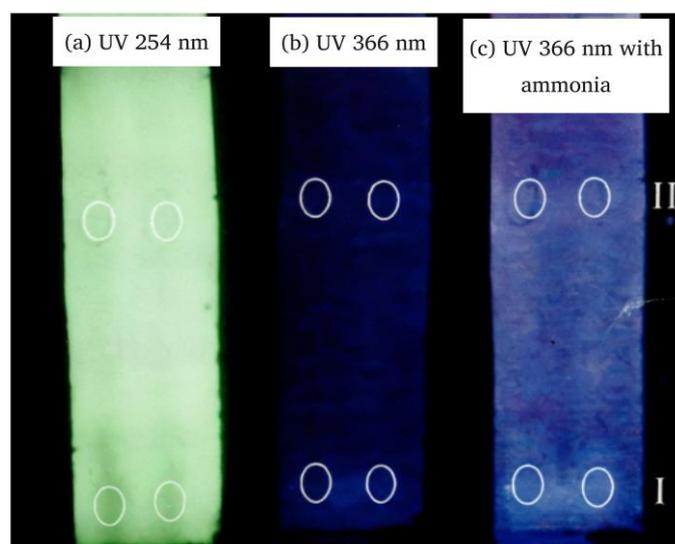
**Table 3.** Specific characterization of the ethanol extract of red gedi leaves

No.	Parameter	Result	CV (%)	p-value*
1	Extract identity			
a	Extract name	<i>Extractum Manihotae folium</i>	-	-
b	Botanical name	<i>Abelmoschus manihot</i> (L.) Medik	-	-
c	Plant part used	Leaves	-	-
d	Common name	Red gedi	-	-
2	Organoleptic properties			
a	Appearance	Thick/viscous	-	-
b	Color	Brownish	-	-
c	Taste	Slightly astringent	-	-
d	Odor	Characteristic	-	-
3	Compounds soluble in			
a	Water–chloroform (%)	$44.16 \pm 4.01$	9.08	0.032
b	Ethanol (%)	$78.75 \pm 6.95$	8.83	0.045
4	Total flavonoid content	$56.97 \pm 3.75$	6.58	0.028

\*p-values were obtained from one-way ANOVA comparing sampling locations. CV = coefficient of variation. Values are expressed as mean  $\pm$  standard deviation (SD) (n = 3). p < 0.05 indicates a statistically significant difference.



**Figure 1.** Thin-layer chromatography (TLC) profile of the ethanol extract of red gedi leaves. The TLC plate was developed using an ethyl acetate–methanol–glacial acetic acid mobile phase and visualized under UV 254 nm (a), UV 366 nm (b), and UV 366 nm after spraying with  $\text{AlCl}_3$  reagent (c). Fluorescent spots observed after  $\text{AlCl}_3$  derivatization indicate the presence of flavonoid compounds.



**Figure 2.** TLC profile of flavonoids in the ethanol extract of red gedi leaves. TLC was developed using *n*-butanol–glacial acetic acid–water (4:1:5, upper phase) with quercetin as the reference standard. Plates were visualized under UV 254 nm (a), UV 366 nm (b), and UV 366 nm after exposure to ammonia vapor (c). Yellow fluorescent spots at  $R_f$  0.18 and 0.66 were observed, with the spot at  $R_f$  0.66 corresponding to quercetin ( $R_f$  0.68).

**Table 4.** Non-specific quality parameters of red gedi leaf ethanol extract

Parameter	Sample	Result	CV (%)	Quality requirement	Status	<i>p</i> -value
Water content (%)	Simplicia powder	$3.62 \pm 0.53$	14.6	< 10%	Met	0.156
	Concentrated extract	$2.39 \pm 0.79$	33.1	< 10%	Met	0.089
Loss on drying (%)	Simplicia powder	$3.50 \pm 0.50$	14.3	< 10%	Met	0.234
	Concentrated extract	$2.30 \pm 0.76$	33.0	< 10%	Met	0.112
Total ash (%)	Simplicia powder	$8.33 \pm 0.76$	9.1	< 12.4%	Met	0.067
	Concentrated extract	$6.66 \pm 0.28$	4.2	< 12.4%	Met	0.445
Acid-insoluble ash (%)	Simplicia powder	$1.16 \pm 0.28$	24.1	< 1.2%	Met	0.178
	Concentrated extract	$0.83 \pm 0.28$	33.7	< 1.2%	Met	0.234
Total plate count (TPC)	—	0.23 colonies/g	—	< 10 colonies/g	Met	—
Yeast and mold count (YMC)	—	Not detected	—	< 10 colonies/g	Met	—
Pb contamination	—	< 0.01 mg/kg	—	< 10 mg/kg	Met	—
Cd contamination	—	< 0.01 mg/kg	—	< 0.3 mg/kg	Met	—
Residual ethanol (%)	—	0.0	—	< 1%	Met	—

**Notes:** Values are expressed as mean  $\pm$  standard deviation ( $n = 3$ ). *p*-values were obtained from one-way ANOVA for differences between sampling locations. CV = coefficient of variation. TPC = total plate count; YMC = yeast and mold count.  $p > 0.05$  indicates no significant difference.

#### 4. CONCLUSION

The comprehensive quality characterization of red gedi leaf ethanol extract demonstrated full compliance with Herbal Pharmacopoeia standards. The extract contained major bioactive compounds, including flavonoids, saponins, tannins, quinones, and steroids, with a high total flavonoid content of  $56.97 \pm 3.75$  mg/g. All evaluated specific and non-specific quality parameters met the established requirements, including water content ( $2.39 \pm 0.79\%$ ), drying loss ( $2.30 \pm 0.76\%$ ), total ash content ( $6.66 \pm 0.28\%$ ), and acid-insoluble ash content ( $0.83 \pm 0.28\%$ ), and the extract was confirmed to be free from microbial contamination, heavy metals, and residual ethanol. These findings indicate that red gedi leaf ethanol extract has strong potential for development as a standardized herbal medicine raw material, particularly as an antioxidant supplement, while future studies should focus on pesticide residue analysis and expanded quality assessment using samples from different regions of Central Sulawesi to support broader standardization and industrial application.

#### AUTHOR CONTRIBUTIONS

Conceptualization and supervision, JT and YS; methodology and investigation, JT, TWH, M, and MT; data analysis, M and MT; writing—original draft, JT and M; writing—review and editing, JT, YS, and TWH. All authors have read and agreed to the published version of the manuscript.

#### INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable.

#### INFORMED CONSENT STATEMENT

Not applicable.

#### DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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#### CONFLICTS OF INTEREST

The authors declare no conflict of interest.

#### DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist in improving clarity, structure, and readability. All

content was subsequently reviewed, edited, and verified by the authors, who take full responsibility for the integrity and originality of the work.

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