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ABSTRACT

The majority of traditional medicinal plants in Indonesia lack scientific validation. Scientific assessment, in conjunction with traditional knowledge, is crucial for acquiring effective pharmaceuticals for commercial use. Oldelandia corymbosa L., commonly known as rumput mutiara, belonging to the family Rubiaceae, is a member of the Rubiaceae family and has been utilized as a traditional medicinal plant for the treatment of various ailments. This study aims to evaluate the quality of both non-specific and specific parameters and to analyze the antioxidant activity of rumput mutiara. Antioxidant activity was evaluated with 1-diphenyl-2-picrylhydrazyl (DPPH). The findings for non-specific parameters indicated that the shrinkage drying of the extract and the water content were $18.00 \pm 0.000\%$ and $12.20 \pm 0.000\%$, respectively. Simultaneously, particular parameters indicate that the extracts possess a distinct odor, exhibit a blackish-brown hue, and display a viscous consistency. Microscopic parameters of rumput mutiara simplicia showed fragments such as anthers, leaf mesophyll, epidermis and stomata, transport bundles, stem parenchym, and sclerenchyma. Spesific parameters, such as the water-soluble content, and ethanolsoluble compounds were 72.00 ± 0.000 % and 35.00 ± 0.000 %, respectively. In addition, TLC profiles showed that secondary metabolites of extract were 6 alkaloids, 5 phenolics, 5 flavonoids, 5 tanins, 3 saponins, 5 steroid, and 5 glycosides. The extract has strong antioxidant activity with IC50 value of 14.11 $\pm 0.008 \,\mu\,\text{g/mL}$.

Keywords: rumput mutiara, non-specific parameters, specific parameters, antioxidant, DPPH.

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INTRODUCTION

Rumput mutiara (Oldenlanda corymbosa L.) is a plant in the Rubiaceae fam 10 hat has been utilized as a traditional medicinal plant. The plant is noted for its ability to eliminate heat and toxins, stimulate blood circulation, promote diuresis, and alleviate stranguria (urinary obstruction). It is also known to be effective against digestive tract malignancies, lymphosarcoma, and liver and laryngeal carcinoma. Appendicitis, hepatitis, pneumonia, cholecystesis, urinary infections, cellulites, and snake bites are also treated with it (Patel et al., 2014).

Medicinal plants require methods to establish identity, purity, and quality in order to sustain their properties. Standardization is an effort that can be performed to control the quality of medicinal plant raw materials. Standardization is the process of developing a set of distinctive standards in order to acquire assurances of quality, efficacy, and security. Standardization is a quality assurance technique that ensures that the parameters of medicinal plants remain constant (Muhtadi & Ningrum, 2019).

Seeing the many benefits and uses of rumput mutiara in society, a standard is needed to ensure the content of rumput mutiara. The goal of this research was to uncover specific and non-specific features for herbal medicinal compounds as indicated by the Indonesian Ministry of Health and the National Agency of Drug and Food Control Republic of Indonesia (Badan POM RI). Non-specific parameters such as shrinkage drying, and water content were measured. Extract identity and organoleptic evaluation, microscopic identification, determination of compound dissolved in certain solvents, phytochemical screening, and chromatogram profile are some of the specific characteristics. The antioxidant activity of the extract was also examined using the DPPH (2, 2-diphenyl-1-picrylhydrazy) assay in this study.

MATERIALS AND METHOD

Materials

Rumput mutiara herbs were collected from Kebun Percobaan Ganoko, Cikahuripan, Lembang, Kabupaten Bandung Barat, Jawa Barat. The plants were identified at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jatinangor, West Java, with number authentication 16/HB/06/2022, which claimed that the plant used was rumput mutiara (Oldelandia corymbosa L.). Ethanol and DPPH were analytical grade and bought from Sigma-Aldrich (St. Louis, USA).

Extraction

The herbs of rumput mutiara were macerated using 70% ethanol as the solvent for 3x24 h. The process was applied by changing the solvents every 24 hours. Then, the collected extracts were evaporated in a rotary vaporator at 40–50 °C until the extracts acquired their constant volume (Kusuma et al., 2017).

Shrinkage drying measurement

One gram of extract was put into a a pre-heated, closed weighing bottle, which was previously weighed when empty. The extract is placed in the oven at 105 °C with the lid ajar. The weighing bottle is positioned in the desiccator until it reaches room temperature. This procedure is performed iteratively until a constant weight is achieved, defined as a variation of no more than 0.0005g between successive weighings (Departemen Kesehatan, 2000).

Water content

Three grams of the extract are enclosed in aluminum foil and placed in a dry round-bottom flask. Fifty milliliters of toluene is introduced into the flask via a vertical condenser and heated

cautiously for one hour. The interior of the cooler is washed with toluene. Water and toluene droplets are observed until they are fully separated, at which point the water volume is measured (Departemen Kesehatan, 2000).

Extract identity and organoleptic evaluation of extracts

The extract identification encompasses a nomenclatural description, alternative designations of the plant, including its Indonesian name, and the specific plant parts utilized. The assessed organoleptic qualities encompass the extract's color, aroma, and flavor (Departemen Kesehatan, 2000).

Microscopic identification of simplicia from rumput mutiara

The powder of rumput mutiara herbs is positioned on a glass object, treated with a chloral hydrate solution, and sealed with a cover glass. The substance was further heated using a Bunsen burner with tube clamps, maintained below boiling point, until completely dry. The specimens were subsequently examined under a microscope at a magnification of 10x (Departemen Kesehatan, 2000).

Determination of compounds dissolved in certain solvents

The weight of the rumput mutiara herbs was 1.5 grams. Weighing was conducted twice, designating the samples as extract A and extract B. Extract A was macerated in a sealed flask for 24 hours with 50 mL of water-chloroform, whereas Extract B was macerated for 24 hours with 50 mL of 96% ethanol. The maceration products were filtered, and 10.0 mL of the filtrate was evaporated in a cup with a predetermined empty weight. The residue is subjected to heating at 105 °C until a constant weight is achieved (Departemen Kesehatan, 2000).

Phytochemical screening

The identification of chemical constituents in the ethanol extract of rumput mutiara plants is conducted via phytochemical screening, which includes the analysis of alkaloids, flavonoids, saponins, steroids, tannins, triterpenoids, and glycosides (Nur et al., 2022).

Chromatographic profile

The ethanol extract of rumput mutiara plants was manually spotted using a capillary tube on precoated silica gel GF254 plates of 15x5 cm with a thickness of 3 mm for the isolation of various phytochemical components. The spotted plates were placed in a solvent system to identify the appropriate mobile phase according to the procedure of Karthika et al. (2014), as detailed in Table 1. Following the separation of phytochemical ingredients, chemicals such as Dragendorff reagent and 5% ferric chloride were employed to identify the corresponding compounds. The color of the dots was recorded, and Rf values were computed using the subsequent formula:



Table 1. TLC system and spray reagent for determination secondary metabolite

Substances	Mobile Phases	Spraying Reagents	Color of the spot
Alkaloids	Chloroform: Methanol (3:2)	Dragendroff	Orange/ Brown
Polyphenols	Chloroform: Methanol (7: 3)	FeCl ₃	Blackish blue

Flavonoids	Ethylacetate: Methanol: Water: Glacial acetic acid	FeCl ₃	Grey
Tanins	(1,7: 0,5: 0,5: 0,5) Chloroform: Ethylacetate: Methanol	FeCl ₃	Blackish blue
Saponins	(5: 3: 3) Kloroform: Metanol (2,2: 0,5)	Vanillin H ₂ SO ₄ reagent	Violet Blue
Steroids	n-hexane: Ethylacetate (1,5: 0,5)	Vanillin H ₃ PO ₄ reagent	Blue
Glycosides	Ethylacetate-ethanol-water (8:2:1.2)	H_2SO_4	Pinkish Violet

Antioxidant activity assay

A 40-ppm DPPH solution was combined with 96% ethanol and left to incubate in the dark for 20 minutes. The absorbance was quantified within the range of 400 to 700 nm. Ascorbic acid and Rumput mutiara extract were solubilized in 96% ethanol and subsequently diluted to five distinct quantities. Each sample (1 mL) was combined with 2 mL of DPPH solution and subsequently incubated for 20 minutes. Absorbance was quantified at the peak wavelength, and inhibition was determined using formula 2. The IC50 value was calculated from a linear regression analysis of inhibition against concentration (Saptarini & Herawati, 2018).

$$\%inhibition = \frac{Abs\ DPPH - Abs\ sample}{Abs\ DPPH} \ x100\% \qquad \qquad(2)$$

RESULT AND DISCUSSION

The current research employed the maceration extraction method, with 70% ethanol as the solvent. During the maceration process, ethanol will permeate the simplicia cell wall and then penetrate the plant cell cavity, which houses the active constituents. The extraction solution will solubilize the secondary metabolites from the plant. Ethanol was chosen as the solvent due to its capacity to enhance cell wall permeability, facilitating the extraction of both polar and non-polar components. The concentrated fluid was expelled from the cell due to the disparity in active component concentration between the intracellular and extracellular environments (Kusuma et al., 2017). The extract yield from this research was 10.39%.

Non-specific parameters that have been studied in this research include shrinkage drying and moisture measurement from simplicia and extract. The drying shrinkage parameter aims to provide information on the value range of compounds lost in the drying process. Meanwhile, water content is related to the quality and storage power of simplicia and extract.

Table 2. The characterization of non-specific parameters from rumput mutiara

Parameters (%)	Specimens		
_	Simplicia	Extract	
Shrinkage drying	7.5	18.00 ± 0.000	
Water content	4.4	12.20 ± 0.000	
n=2			

Shrinkage drying and water content from simplicia do not surpass 10%, whereas the thick extract category has a water content range of 5-30%. According to **Table 2**, both simplicia and extract comply with the standards set by the Indonesian Herbal Pharmacopoeia.

The identification of extract and organoleptic characteristics is a specific criteria that seeks to furnish objective information regarding material identity through the straightforward introduction of materials. The parameter must be determined to provide an objective identify based on the compound's name and specific identity. The organoleptic features of the ethanolic extract of rumput mutiara were studied. **Table 3** presents the outcomes of assessments on form, color, aroma, and flavor.

Table 3. The identification and organoleptic characteristics of rumput mutiara

Parameters	Characteristics
Identity	
Scientific name for plants	Oldelandia corymbosa L.
Used parts	Whole parts
Indonesian name plants	Rumput mutiara
Organoleptic	
Form	Thick
Color	Blackish-brown
Smell	Specific
Taste	Bitter

The objective of utilizing a microscope to analyze rumput mutiara plant powder is to identify the characteristics of herb identification pieces. The chloral hydrate solution is designed to eliminate cellular components including protein and starch, hence enhancing the visibility of cellular identifying fragments in the herbs under a microscope (Fatmawati et al., 2021). **Table 4** illustrates the existence of anthers, leaf mesophyll, epidermis and stomata, vascular bundles, stem parenchyma, and sclerenchyma in the simplicia of rumput mutiara plant.

Table 4. Microscopic Tagments from rumput mutiara

Microscopic Observation Citation
(Herbal Pharmacopoeia of Indonesia, 2017)

Anthers

Mesophyll of leaves



The parameters of the levels of dissolved compounds aim to provide information of the type of chemical compounds in the extract.. Two categories of solvents are employed, specifically water and ethanol. . Water serves as a solvent for polar molecules, while ethanol is utilized for dissolving semi-polar and non-polar chemicals (Muhtadi & Ningrum, 2019).

Table 5. Water And Alcohol Soluble Compounds of Rumput Mutiara

Parameter	Characteristics
Water soluble compounds	72.00 ± 0.000 %
Ethanol soluble compounds	$35.00 \pm 0.000 \%$
n=2	

Table 5 indicates that rumput mutiara exhibits greater solubility in water compared to ethanol. The findings indicated that the compounds present in rumput mutiara extract are polar. The polar extract of rumput mutiara contains compounds including flavonoids, tannins, and anthraquinones (Selvan, 2015; Ezeabara, 2016).

Phytochemical screening reveals the constituents of plant extracts, identifying predominant components, and aids in the discovery of chemical compounds suitable for the development of effective pharmaceuticals. The color changes resulting from a reaction with a particular response are

observed qualitatively during phytochemical screening. The objective of this phytochemical screening is to determine the secondary metabolite content of the simplicia and extracts.

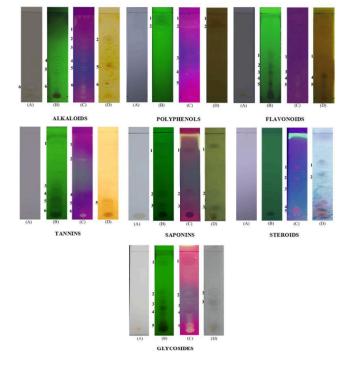
 $Table\ 6.\ Results\ of\ identification\ of\ secondary\ metabolites\ of\ rumput\ mutiara.$

Phytochemical Test	Simplicia	Extract	TLC
Alkaloids	+	+	+
Phenolics	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Glycosides	+	+	+

^{*(+) =} positive test (exist); (-) = negative test (not exist)

Table 6 presents the findings of the phytochemical screening. Phytochemical screening results indicate that rumput mutiara exhibits antioxidant activity owing to the presence of phenolic and flavonoid components.

Medicinal plants are rich in secondary metabolites, with alkaloids, flavonoids, glycosides, saponins, and terpenoids being particularly significant among the several bioactive compounds. The chromatographic method is the most often employed technique for the separation of plant constituents among the several accessible approaches.



 $Figure~1.~TLC~profile~on~observations~under~(A)~Visible;~B~(UV~254~nm);~C~(UV~366~nm);~D~(Dragendorff~for~alkaloids);~D~(FeCl_3~for~polyphenols,~flavonoids,~and~tannins);~D~(Vanillin~in~H_2SO_4~for~Saponin);~D~(Vanillin~in~H_3PO_4~for~steroids);~D~(H_2SO_4~for~glycosides)$

Table 7. Rf values of rumput mutiara extract on various secondary metabolites

Compounds	Spot	Visible	UV	UV	Dragendorff	Prediction
			254	366		Metabolites
			nm	nm		
Alkaloids	1	-	-	0.82	-	Alkaloids 1
	2	-	-	0.7	0.7	Alkaloids 2
	3	-	-	0.58	-	Alkaloids 3
	4	-	0.52	0.52	-	Alkaloids 4
	5	-	0.38	0.38	0.38	Alkaloids 4
	6	0.14	0.14	0.14	0.14	Alkaloids 5

	Spot	Visible	UV 254 nm	UV 366 nm	FeCl ₃	Prediction Metabolites
Polyphenols	1	-	0.9	0.9	0.9	Polyphenols 1
**	2	-	0.85	0.85	0.85	Polyphenols 2
	3	-	-	0.42	-	Polyphenols 3
	4	-	-	0.28	-	Polyphenols 4
	5	-	-	0.14	-	Polyphenols 5
Flavonoids	1	-	0.5	-	0.5	Flavonoids 1
	2	-	0.4	-	-	Flavonoids 2
	3	-	0.37	0.37	-	Flavonoids 3
	4	-	0.28	0.28	0.28	Flavonoids 4
	5	-	0.14	0.14	0.14	Fovonoids 5
Tannins	1	-	0.94	0.94	-	Tannins 1
	2	-	-	0.8	-	Tannins 2
	3	-	0.71	-	-	Tannins 3
	4	-	0.38	0.38	-	Tannins 4
	5	-	0.17	0.17	0.17	Tannins 5
	6	-	0.08	0.08	-	Tannins 6
	Spot	Visible	UV	UV	Vanillin in	Prediction
	•		254	366	H_2SO_4	Metabolites
			nm	nm		
Saponins	1	-	0.7	0.7	0.7	Saponin 1
	2	-	0.28	0.28	0.28	Saponin 2
	3	-	0.14	0.14	0.14	Saponin 3
	Spot	Visible	UV	UV	Vanillin in	Prediction
	•		254	366	H_3PO_4	Metabolites
			nm	nm		
Steroids	1	-	0.5	0.5	0.5	Steroids 1
	2	-	0.32	0.32	0.32	Steroids 2
	3	-	0.15	-	-	Steroids 3
	4	-	0.07	-	-	Steroids 4
	5	-	0.04	-	-	Steroids 5
	Spot	Visible	UV	UV	H ₂ SO ₄	Prediction
	-		254	366		Metabolites
			nm	nm		
Glycosides	1	-	0.92	0.92	-	Glycosides 1
	2	-	0.57	0.57	0.57	Glycosides 2
			0.4	0.4	0.4	Glycosides 3
	3	-	0.4			
	4	-	0.28	0.28	-	Glycosides 4

The ethanolic extract of rumput mutiara was analyzed using thin-layer chromatography (TLC), and various mobile phase compositions were investigated to isolate diverse secondary metabolites, including alkaloids, phenolics, flavonoids, tannins, saponins, steroids, and glycosides (Table 1, Figure 1). The samples were spotted on TLC plates that had been prepared in a suitable solvent system. The samples were applied to TLC plates prepared with an appropriate solvent solution. The color developed and was observed upon derivatization with the appropriate spraying reagent. Secondary metabolites were segregated according to color, and Rf values were assessed as presented in Table 7.

The TLC examination revealed the presence of alkaloids, phenolics, flavonoids, tannins, saponins, steroids, and glycosides in various solvent compositions, exhibiting diverse Rf values in the ethanolic extract of rumput mutiara (Table 7). In a study by Aprianto (2018), ursolic acid was detected in the ethanol extract of pearl grass following treatment with a 10% sulfuric acid reagent, resulting in the formation of purple spots. A comprehensive phytochemical analysis of rumput mutiara reveals the presence of proteins, polysaccharides, polyphenols, tannins, flavonoids, saponins, steroids, triterpenes, and glycosides (Das et al., 2019). Gosh et al (2018) investigated the total plant pigments in rumput mutiara, revealing variations in total pigments among different herbs, including chlorophyll-a, chlorophyll-b, total chlorophyll, and total carotenoids.

Alkaloids are predominantly biosynthesized from amino acids, yielding a verse array of chemical structures, most of which are derived from plants. Alkaloids function in both human medicine and an organism's innate defense mechanisms. Alkaloids comprise approximately 20% of the total identified secondary metabolites present in plants. Alkaloids are recognized for their medicinal applications as anesthetics, cardioprotective agents, and anti-inflammatory medications. Morphine, strychnine, quinine, ephedrine, and nicotine are prominent alkaloids employed in medical applications (Heinrich et al., 2021) This research identified six categories of alkaloids with diverse Rf values.

Polyphenols are a vast category of compounds generated from secondary metabolism, present throughout the plant kingdom. Polyphenolic acids, coumarins, flavonoids, stilbenes, and lignans exemplify polyphenols. Additional polymerized forms, including tannins and lignans, have been included. Some of them are responsible for the aroma, color, and antioxidant properties of the fruits, vegetables, seeds, and nuts we consume. Polyphenols are increasingly vital owing to their health-enhancing attributes. Moreover, their significance as natural antioxidants in the prevention and management of cancer, inflammatory, cardiovascular, and neurological diseases (Hano & Tungmunnithum, 2020). This stury identified five kinds of polyphenols with differing Rf values.

Flavonoids exemplify secondary metabolites found in various fruits, vegetables, herbs, stems, cereals, nuts, flowers, and seeds. Flavonoids possess biochemical and antioxidant properties that are advantageous in several diseases, including cardiovascular disorders, cancer, and neurological ailments. Flavonoids are scientifically associated with numerous health advantages and are vital in various nutraceutical, pharmacological, therapeutic, and cosmetic uses. This is primarily attributable to their anti-inflammatory, antioxidant, anti-carcinogenic, and anti-mutagenic properties, together with their capacity to regulate essential cellular enzyme activities (Chen et al., 2023). This research identified five types of flavonoids with distinct Rf values.

Tannins are astringent polyphenolic compounds derived from plants, commonly located in different parts of herbs. Tannin, a polyphenol, possesses numerous medicinal therapeutic properties in addition to its antioxidant capabilities, thereby demonstrating a range of pharmacological effects, including anti-toxic, anticancer, antiallergic, anti-inflammatory, anthelmintic, antimicrobial, antiviral, wound healing, and dysentery treatment, among others (Sharma et al., 2021). This research identifies five varieties of tannins with differing Rf values.

Saponins are a class of bioorganic molecules prevalent in the plant kingdom. They are naturally occurring glycosides that explicit soap-like foaming properties, resulting in foam formation when agitated in aqueous solutions. Saponins have biological functions and therapeutic properties, including hemolytic activity, anti-inflammatory, antibacterial, antifungal, antiviral, insecticidal, anticancer, cytotoxic, and molluscicidal effects, as documented in the literature. Moreover, saponins have demonstrated the ability to lower cholesterol levels in both animals and people (El Aziz et al., 2019). The ethanolic extract of rumput mutiara exhibited three distinct forms of saponins, each with varying Rf values.

Plant steroids are unique compounds present in the plant kingdom that have considerable physiological effects on plant growth, development, and reproduction. Plant steroids are a category of physiologically active secondary metabolites characterized by gonane carbon skeletons of 5 α and

 $5~\beta$ configurations. Plant steroids are categorized into groups according to their biological roles, structures, and synthesis mechanisms. The anti-cancer, immunomodulatory, anti-inflammatory, and anti-viral properties of all subtypes have been examined (Yerlikaya et al., 2023). This study has identified five types of steroids.

Plants synthesize a diverse array of secondary metabolites that may be glycosylated. The glycosylation of metabolites in plants serves multiple functions. Hydrophobic metabolites exhibit increased water solubility following glycosylation, enhancing their biodistribution and metabolic processes. The enhanced solubility and amphiphilicity of glycosylated metabolites may facilitate their transport across cellular membranes. Glycosylation can generate a non-toxic molecule that may subsequently be reactivated and employed as an aglycone for protection against parasites and herbivorous organisms (Kytidou et al., 2020). Among the results, five locations were identified as glycosides.

The DPPH method is a sensitive, rapid, and straightforward technique for assessing the antioxidant activity of plant extracts. The alteration of the DPPH solution demonstrated antioxidant action. The DPPH solution changes color from purple to yellow, with the intensity corresponding to the quantity of moles of the stabilized molecule. The literature indicates that the maximum wavelength of the DPPH solution is 515.0 nm (**Figure 2**). The transmitted color at this wavelength is green (500-520 nm). (N. Saptarini et al., 2019).

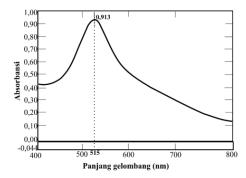


Figure 2. DPPH Wavelength in Spectrophotometer UV-Vis

Table 8. Results of the antioxidant activity of rumput mutiara extract

Sample	Concentrati on (ppm)	Absorbance ± Standard Deviation (SD)	Inhibition (%)	Linear Regression	IC ₅₀ (µg/mL)
Vitamin	1	0.462 ± 0.006	49.39		
C	2	0.408 ± 0.023	53.78		1.17 ± 0.016
(standar	3	0.359 ± 0.003	58.21	y=4.6025x+44.593	
d)	4	0.321 ± 0.034	62.63		
	5	0.275 ± 0.004	67.99		
Extract	10	0.468 ± 0.011	48.74		
	30	0.417 ± 0.005	54.33	y = 0.235x + 46.429	14.11 ±
	50	0.375 ± 0.004	58.93		0.008
	70	0.325 ± 0.011	64.40		

	90	0.283 ± 0.004	69.00	
(n=3)				

This study selected vitamin C as a control due to its efficacy as an antioxidant, which neutralizes oxidative stress through electron donation or transfer mechanisms. Vitamin C can diminish unstable radicals of 3 ygen, nitrogen, and sulfur, while also restoring other antioxidants inside the body. Moreover, human plasma assays have shown that vitamin C is effective in diminishing lipid peroxidation induced by peroxide radicals (Caritá et al., 2020).

The antioxidant activity of rumput mutiara extract was inferior to that of vitamin C, the positive control, due to the extracts not being pure components, as illustrated **Table 8**. Awe *et al.*, (2013) classify antioxidant activity as very strong, strong, moderate, or weak based on the IC50 value, as illustrated in **Table 9**. According to **Table 9**, the antioxidant activity of the extract from rumput mutiara is exceptionally robust, with an IC₅₀ of 14.11 μ g/mL, equivalent to that of vitamin C.

Table 9. Antioxidant potency determined by IC₅₀ value (Awe et 4., 2013)

IC ₅₀ (µg/mL)	Antioxidant activity
<50	Very strong
50-100	Strong
100-150	Medium
150-200	Weak

The extract's elevated antioxidant activity results from the presence of secondary metabolites, including phenolics and flavonoids (Sasikumar et al., 2009). According to prior research by Sasikumar et al. (2009), antioxidant assays utilizing DPPH exhibited a strong correlation with total phenolic and flavonoid concentration. This study's findings indicate that rumput mutiara possesses the potential to be developed into an antioxidant preparation.

CONCLUSION

The standardization of rumput mutiara extracts, conducted in accordance with established health department protocols, demonstrated that all metrics satisfied the requisite standards. The TLC examination of rumput mutiara extract revealed the presence of alkaloids, phenolics, flavonoids, tannins, saponins, steroids, and glycosides across several solvent compositions with differing Rf values. The antioxidant activity was demonstrated by IC50 values of $14.11 \pm 0.008 \, \mu g/mL$, indicating robust antioxidant efficacy.

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REFERENCES

Awe, F.B., Fagbemi, T.N., Ifesan, B.O.T. & Badejo, A.A. (2013). Antioxidant properties of cold and hot water extracts of cocoa, Hibiscus flower extract, and ginger beverage blends. Food Research International. 52(2): 490-495

Bronte, V., & Pittet, M. J. (2013). The Spleen in Local and Systemic Regulation of Immunity. *Immunity*, 39(5), 806–818. https://doi.org/10.1016/j.immuni.2013.10.010
Caritá, A. C., Fonseca-Santos, B., Shultz, J. D., Michniak-Kohn, B., Chorilli, M., & Leonardi, G. R. (2020).

Caritá, A. C., Fonseca-Santos, B., Shultz, J. D., Michniak-Kohn, B., Chorilli, M., & Leonardi, G. R. (2020). Vitamin C: One compound, several uses. Advances for delivery, efficiency and stability. Nanomedicine: Nanotechnology, Biology and Medicine, 24, 102117. https://doi.org/10.1016/j.nano.2019.102117

- Chen, S., Wang, X., Cheng, Y., Gao, H., & Chen, X. (2023). A Review of Classification, Biosynthesis, Biological Activities and Potential Applications of Flavonoids. *Molecules*, 28(13), 4982. https://doi.org/10.3390/molecules28134982
- Departemen Kesehatan. (2000). Parameter Standar Umum Ekstrak Tumbuhan Obat.
- El Aziz, M. M. A., Ashour, A. S., & Melad, A. G. (2019). A review on saponins from medicinal plants: chemistry, isolation, and determination. *Journal of Nanomedicine Research*, 8(1), 282-288.
- Fatmawati, A., Sucianingsih, D., Kurniawati, R., & Abdurrahman, M. (2021). Microscopic Identification and Determination of Total Flavonoid Content of Moringa Leaves Extract and Ethyl Acetate Fraction (Moringa oleifera L.). Indonesian Journal of Pharmaceutical Science and Technology, 1(1), 66–74.
- Ghosh P, Das P, Mukherjee R, Banik S, Karmakar S, Chatterjee S. Extraction and Quantification of Pigments from Indian Traditional Medicinal Plants: A Comparative Study between Tree, Shrub, and Herb, International Journal of Pharmaceutical Sciences and Research. 2018, 9(7):3052-3059, 0975-8232.
- Hano, C., & Tungmunnithum, D. (2020). Plant Polyphenols, More than Just Simple Natural Antioxidants: Oxidative Stress, Aging and Age-Related Diseases. *Medicines*, 7(5), 26. https://doi.org/10.3390/medicines7050026
- Heinrich, M., Mah, J., & Amirkia, V. (2021). Alkaloids Used as Medicines: Structural Phytochemistry Meets Biodiversity—An Update and Forward Look. *Molecules*, 26(7), 1836. https://doi.org/10.3390/molecules26071836
- Karthika K, Jamuna S, & Paulsamy S. (2014). TLC and HPTLC Fingerprint Profiles of Different Bioactive Components from the Tuber of Solena amplexicaulis. Journal of Pharmacognosy and Phytochemistry JPP, 3(31), 198–206. http://www.phytojournal.com/archives/2014/v013issue1/PartD/27.1-435.pdf Kusuma, S., Herawati, I., & Yuliasih, N. I. (2017). In vitro antifungal activity of the orange jasmine
- Kusuma, S., Herawati, I., & Yuliasih, N. I. (2017). In vitro antifungal activity of the orange jasmine (Murraya paniculata [L] Jack) leaves ethanol extract from Indonesia against Candida albicans. Int J Sci Eng Appl Sci, 273–278.
- Kytidou, K., Artola, M., Overkleeft, H. S., & Aerts, J. M. F. G. (2020). Plant Glycosides and Glycosidases: A Treasure-Trove for Therapeutics. Frontiers in Plant Science, 11. https://doi.org/10.3389/fpls.2020.00357
- Muhtadi, M., & Ningrum, U. (2019). Standardization of durian fruit peels (*Durio zibethinus* Murr.) extract and antioxidant activity using DPPH method. *Pharmaciana*, 9(2), 271. https://doi.org/10.12928/pharmaciana.v9i2.12652
- Nur, R. M., Dewi, R., & Kaliu, S. (2022). Antibacterial activity of methanol extract *Rhizophora mucronata* leaves toward *Salmonella typhi*: leading the typhoid fever. *Pharmaciana*, 12(3), 364. https://doi.org/10.12928/pharmaciana.v12i3.22475
- Nurkhasanah, Minangsari, D. N. I., & Yulianny, V. A. (2016). The combination of rosella (*Hibiscus sabdariffa* L) and Stevia (Stevia rebaudiana) extracts increase the antioxidant activity and stability. International Journal of Pharmacy and Pharmaceurical Sciences 8(5), 7–8.
- International Journal of Pharmacy and Pharmaceutical Sciences, 8(5), 7–8.

 Nurkhasanah, Nurani, L. H., & Hakim, Z. R. (2017). Effect of rosella (Hibiscus sabdariffa L.) extract on glutathione-S-transferase activity in rats. Tropical Journal of Pharmaceutical Research February, 16(10), 2411–2416.
- Patel, T., Jain, V., & Dodia, R. (2014). Oldenlandia corymbosa L.: A Phytopharmacological review. International Journal of Phytopharmacy, 4(3), 79–82.
- Saptarini, N. M., & Herawati, I. E. (2018). The Effect Acetic Acid On Total Anthocyanins Content and Antioxidant Activity of Tamarillo (Solanum betaceum Cav.). Journal of Pharmacy Research, 12, 398-401
- Saptarini, N., Rahayu, D., & Herawati, I. (2019). Antioxidant activity of crude bromelain of pineapple (Ananas comosus (L.) Merr) crown from Subang district, Indonesia. Journal of Pharmacy And Bioallied Sciences, 11(8), 551. https://doi.org/10.4103/jpbs.JPBS_200_19
- Sasikumar JM, Maheshu V, Aseervatham GS, Darsini DT. In vitro Antioxidant Activity of Hedyotis corymbosa (L.) Lam. Aerial Parts. Indian Journal of Biochemistry & Biophysics, 2010, 49-52.
- Sharma, K., Kumar, V., Kaur, J., Tanwar, B., Goyal, A., Sharma, R., Gat, Y., & Kumar, A. (2021). Health effects, sources, utilization and safety of tannins: a critical review. *Toxin Reviews*, 40(4), 432–444. https://doi.org/10.1080/15569543.2019.1662813
- Sulistyani, N., & Nurkhasanah. (2017). The cytotoxic effect of Elephantopus scaber Linn extract against breast cancer (T47D) cells The cytotoxic effect of Elephantopus scaber Linn extract against breast cancer (T47D) cells. IOP Conference Series: Materials Science and Engineering, 1–6. https://doi.org/10.1088/1757-899X/259/1/012006

- Ukoha, U., Mbagwu, S. I., Ndukwe, G. U., & Obiagboso, C. (2015). Histological And Biochemical Evaluation of The Kidney Following Chronic Consumption Of *Hibiscus sabdariffa*. Advances in Biology, 1–5.
- Evaluation of the Kidney Ponowing Chronic Consumption of *Hibiscus sabadryja*. Advances in Biology, 1–5.
 Yerlikaya, P. O., Arisan, E. D., Mehdizadehtapeh, L., Uysal-Onganer, P., & Gürkan, A. (2023). The Use of Plant Steroids in Viral Disease Treatments: Current Status and Future Perspectives. *European Journal of Biology*, https://doi.org/10.26650/EurJBiol.2023.1130357

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