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Analysis of antioxidant and standardization of ethanol extract of rumput mutiara (*Oldelandia corymbosa* L.)

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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. Rumput mutiara (*Oldelandia corymbosa* L) is a member of the Rubiaceae family and has been utilized as a traditional medicinal plant for the treatment of various ailments. The objective of this research was to assess the quality of both specific and non-specific parameters and to investigate the antioxidant potential of *rumput mutiara*. Antioxidant activity was evaluated with 2,2-diphenyl-1-picrylhydrazy (DPPH). The findings for non-specific parameters indicated that the shrinkage drying of the extract and the water content were 18.00 ± 0.000% and 12.20 ± 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 ethanol-soluble 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 IC_{50} value of 14.11 ±0.008 µg/mL.

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

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INTRODUCTION

Rumput mutiara (*Oldenlanda corymbosa* L.) is a member in the Rubiaceae that has been utilized as a traditional medicinal plant. The plant exhibits multiple therapeutic properties, such as detoxification, diuretic stimulation, blood circulation enhancement, and mitigation of urinary difficulties. It has also been applied in the treatment of gastrointestinal cancers, lymphatic tumors, hepatic and laryngeal carcinomas. Moreover, its traditional uses extend to ailments like appendicitis, hepatitis, pulmonary infections, gallbladder disorders, urinary tract infections, soft tissue inflammation, and snakebite management (Patel et al., 2014).

To maintain their qualities, medicinal plants need ways to prove their identification, purity, and quality. To maintain the quality of raw plant-derived materials used in medicine, standardization serves as a key regulatory approach. The process from standardisation 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. This study aimed to identify specific and non-specific characteristics of herbal medicinal substances in accordance with the standards set by the Indonesian Ministry of Health and the National Agency of Drug and Food Control (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.

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MATERIALS AND METHOD

Materials

Rumput mutiara herbs were collected from Kebun Percobaan Manoko, 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

A closed, preheated weighing container that had been previously weighed while empty was filled with one gramme of extract. With the container's lid removed, the extract was heated in an oven at a temperature of 105 °C. 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, 2020).

Water content

An amount of 3 g extract was wrapped in aluminum foil and placed into a dry round-bottom flask. A volume of 50 mL toluene was introduced via a vertical condenser, and the mixture was gently heated for one hour. After this, the condenser was rinsed with toluene, and after the water and toluene layers had fully separated, the volume of the aqueous phase was mensured (Departemen Kesehatan, 2020).

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, 2020).

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, 2020).

Identifying the substances that dissolve in particular solutions

The powdered *Rumput Mutiara* herb (3 g) was divided equally to obtain two samples named Extract A and Extract B. Extract A macerated in a closed container with 50 mL chloroform water for 24 hours, and Extract B in 96% ethanol under identical conditions. After filtration, 10 mL of each extract was evaporated in pre-weighed dishes at 105 ˚C until no further weight change was observed (Departemen Kesehatan, 2020).

Phytochemical screening

Using phytochemical screening, which involves examining alkaloids, flavonoids, saponins, steroids, tannins, triterpenoids, and glycosides, the chemical components in the ethanol extract of rumput mutiara plants are identified (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 & Paulsamy, 2015), 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 (1).~~:~~

$$Retention\ factor\ (Rf) = \frac{distance\ travelled\ by\ the\ solute}{distance\ travelled\ by\ the\ solvent} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

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Antioxidant activity assay

To determine antioxidant activity, a 40 ppm DPPH solution was prepared using 96% ethanol and left in the dark for 20 minutes. The absorbance was taken across 400–700 nm. *Rumput mutiara* extract and ascorbic acid were dissolved in 96% ethanol and diluted into five concentrations. Each sample (1 mL) was mixed with 2 mL of DPPH solution, then incubated for 20 minutes. Absorbance was measured at the absorbance maximum, and the inhibition percentage was calculated according to formula (2). A linear regression analysis was performed to calculate the IC_{50} value from the concentration–inhibition curve (Saptarini & Herawati, 2018).

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

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Table 1. TLC system and spray reagent for determination of 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 (1.7: 0.5: 0.5: 0.5)	$FeCl_3$	Grey
Tanins	Chloroform: Ethylacetate: Methanol (5: 3: 3)	$FeCl_3$	Blackish blue
Saponins	Kloroform: Metanol (2.2: 0.5)	Vanillin H_2SO_4 reagent	Violet Blue
Steroids	n-hexane: Ethylacetate (1.5: 0.5)	Vanillin H_3PO_4 reagent	Blue
Glycosides	Ethylacetate-ethanol-water (8:2:1.2)	H_2SO_4	Pinkish Violet

RESULT AND DISCUSSION

The current research employed maceration method, using 70% ethanol. During 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. Because of its potential to increase cell wall permeability and make it easier to extract both non-polar and polar components, ethanol was selected as the solvent. 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 is used to indicate the range of compound loss that occurs during the drying process. In the meanwhile, simplicia, extract quality, and storage capacity are correlated with water content.

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.

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Identifying extract and organoleptic properties is a particular criterion that aims to provide objective information about material identity by just introducing 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., 2022). 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.

Assessing dissolved compound levels helps in determining the nature of the chemical components within the extract. Water and ethanol are utilized as solvents, with water targeting polar substances and ethanol being effective for semi-polar and non-polar compounds (Muhtadi & Ningrum, 2019).

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 (Ezeabara & Egwuoba, 2016; Palanivelu et al., 2014) .

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 secondary metabolite of both simplicia and extracts.

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.

Secondary metabolites such as flavonoids, alkaloids, saponins, terpenoids and glycosides, are abundantly present in medicinal plants and are recognized for their significant bioactivity. The chromatographic method is the most often employed technique for the separation of plant constituents among the several accessible approaches.

Table 4. Microscopic fragments from rumput mutiara

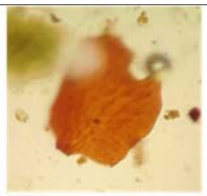
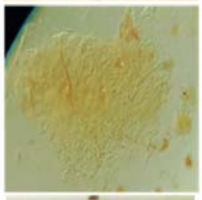
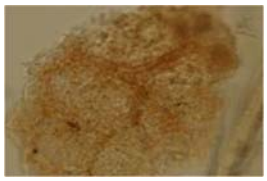
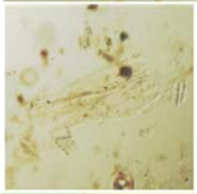
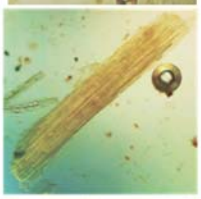
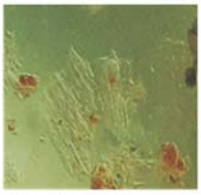
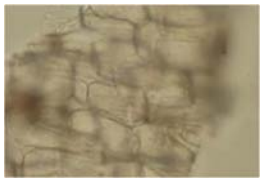
Microscopic Parameter	Observation	Citation (Herbal Pharmacopoeia of Indonesia, 2017)
Anthers		
Mesophyll of leaves		
Epidermis and Stomata		-
Transport bundles		
Stem parenchyma		
Sclerenchyma		-

Table 5. Water and alcohol soluble compounds of rumput mutiara

Parameter	Characteristics
Water soluble compounds	72.00 ± 0.000 %
Ethanol soluble compounds	35.00 ± 0.000 %

n=2

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)

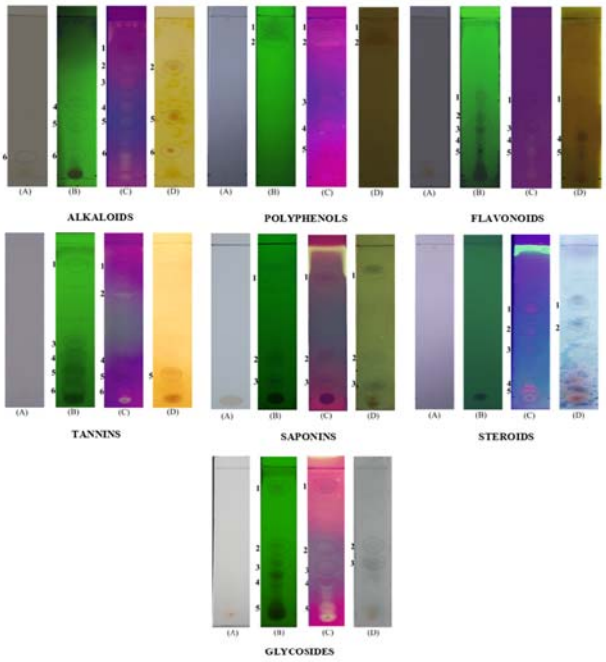


Figure 1. TLC profile on observations under (A) Visible; B (UV 254 nm); C (UV 366 nm); D (Dragendorff for alkaloids); D ($FeCl_3$ for polyphenols, flavonoids, and tannins); D (Vanillin in H_2SO_4 for Saponin); D (Vaniillin in H_3PO_4 for steroids); D (H_2SO_4 for glycosides)

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

Compounds	Spot	Visible	UV 254 nm	UV 366 nm	Dragendorff	Prediction Metabolites
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	Flavonoids 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 254 nm**	**UV 366 nm**	**Vanillin in H_2SO_4**	**Prediction Metabolites**
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 254 nm**	**UV 366 nm**	**Vanillin in H_3PO_4**	**Prediction Metabolites**
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

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Table 7. Rf values of rumput mutiara extract on various secondary metabolites (continue…)

Compounds	Spot	Visible	UV 254 nm	UV 366 nm	Dragendorff	Prediction Metabolites
	Spot	**Visible**	**UV 254 nm**	**UV 366 nm**	**H_2SO_4**	**Prediction Metabolites**
Glycosides	1	-	0.92	0.92	-	Glycosides 1
	2	-	0.57	0.57	0.57	Glycosides 2
	3	-	0.4	0.4	0.4	Glycosides 3
	4	-	0.28	0.28	-	Glycosides 4
	5	-	0.1	0.1	-	Glycosides 5

TLC analysis of the ethanolic *Rumput Mutiara* extract was conducted with various mobile phases to optimize the resolution of secondary metabolites such as phenolics, tannins, flavonoids, alkaloids, steroids, saponins, and glycosides detailed in Table 1 and illustrated in 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 phenolics, flavonoids, tannins, alkaloids, steroids, saponins and glycosides in various solvent compositions, exhibiting diverse Rf values in the ethanolic extract of rumput mutiara (Table 7). In a study by (Aprianto et al., 2017), 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. Phytochemical analyses of *Rumput Mutiara* have revealed the presence of several bioactive constituents, including polysaccharides, proteins, polyphenols, flavonoids, tannins, glycosides, saponins, steroids, and triterpenes (Ghosh et al., 2018). According to (Ghosh et al., 2018), a study on *Rumput Mutiara* revealed variations in total plant pigments, including chlorophyll and carotenoids across different herbal samples.

Alkaloids are mainly produced through the biosynthetic pathways of amino acids. Alkaloids, which constitute nearly 20% of the identified plant secondary metabolites, are essential to both biological defense systems and human pharmacotherapy. Their well-documented therapeutic effects include anesthetic, cardioprotective, and anti-inflammatory properties. Clinically relevant alkaloids include nicotine, morphine, quinine, strychnine, and ephedrine (Heinrich et al., 2021). This research identified six categories of alkaloids with diverse Rf values. Polyphenols comprise a broad group of secondary metabolites distributed widely in the plant. This group includes polyphenolic acids, flavonoids, coumarins, stilbenes, and lignans, along with polymeric forms like tannins. These compounds contribute to the sensory attributes—such as color and aroma—of plant-derived foods, including fruits, vegetables, seeds, and nuts. Their biological significance is increasingly recognized due to their antioxidant activity and potential roles in the prevention of cancer, inflammation, cardiovascular disorders, and neurodegenerative diseases (Hano & Tungmunnithum, 2020). This study identified five kinds of polyphenols with differing Rf values.

Flavonoids exemplify secondary metabolites found in various herbs, vegetables, fruits, flowers, stems, 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 antioxidant, anti-inflammatory, 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. As a polyphenol, tannin has a variety of therapeutic and medical uses, in addition to its antioxidant capabilities, thereby demonstrating a range of pharmacological effects, including anti-toxic, anticancer, anti-inflammatory, antiallergic, antimicrobial, anthelmintic, antiviral, wound healing, and dysentery treatment, among others (Sharma et al., 2021). This research identifies five varieties of tannins with differing Rf values.

Saponins, a class of glycosides found extensively in plants, are recognized for their foaming behavior in water, reminiscent of soap. These compounds play various biological roles and possess of many pharmacological effects, including hemolysis, anti-inflammatory, antimicrobial, cytotoxic, anticancer, and lipid-lowering activities. Evidence also supports their ability to reduce serum cholesterol (Ashour et al., 2019). The ethanolic extract of rumput mutiara exhibited three distinct forms of saponins, each with varying Rf values.

In plants, steroids represent a class of unique molecules that significantly influence physiological functions such as growth, development, and reproduction. Steroids found in plants are a class of bioactive secondary metabolites defined by their 5α and 5β gonane carbon backbones. They are categorized according to their structural features, biological functions, and biosynthetic pathways. All subtypes' antiviral, anti-inflammatory, immunomodulatory, and anti-cancer characteristics have been investigated (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 fulfils many tasks. Hydrophobic metabolites exhibit increased water solubility following glycosylation, enhancing their biodistribution and metabolic processes. Improved solubility and amphiphilic characteristics resulting from glycosylation may assist glycosylated compounds in crossing cell membranes more efficiently. 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 assay offers a sensitive, rapid, and simple approach for evaluating the antioxidant capacity of plant extracts. Antioxidant activity is indicated by the transformation of the DPPH solution, which changes color from purple to yellow, reflecting the amount of stabilized free radicals. The literature indicates that maximum wavelength of the DPPH solution is 515.0 nm (Figure 2). Transmitted color at this wavelength is green (500-520 nm) (Saptarini & Herawati, 2018).

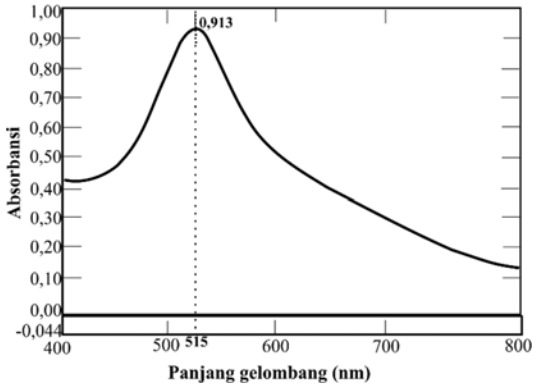

Figure 2. DPPH Wavelength in Spectrophotometer UV-Vis

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

Sample	Concentration (ppm)	Absorbance ± Standard Deviation (SD)	Inhibition (%)	Linear Regression	IC_{50} (µg/mL)
Vitamin C (standard)	1	0.462 ± 0.006	49.39	y=4.6025x+44.593	1.17 ± 0.016
	2	0.408 ± 0.023	53.78		
	3	0.359 ± 0.003	58.21		
	4	0.321 ± 0.034	62.63		
	5	0.275 ± 0.004	67.99		
Extract	10	0.468 ± 0.011	48.74	y = 0.235x + 46.429	14.11 ± 0.008
	30	0.417 ± 0.005	54.33		
	50	0.375 ± 0.004	58.93		
	70	0.325 ± 0.011	64.40		
	90	0.283 ± 0.004	69.00		

(n=3)

Vitamin C was chosen as the control in this study because of its proven antioxidant capabilities, primarily through electron donation and radical scavenging mechanisms. It effectively neutralizes reactive species such as oxygen, nitrogen, and sulfur radicals, and plays a role in regenerating other endogenous antioxidants. Additionally, studies in human plasma have shown that vitamin C significantly reduces lipid peroxidation triggered 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 based on the IC_{50} with value <50 µg/mL (very strong), 50-100 µg/mL (strong), 100-150 µg/mL (medium), and >200 µg/mL (weak). Antioxidant activity rumput mutiara extract is exceptionally robust, with an IC_{50} of 14.11 µg/mL, equivalent to vitamin C.

Extract's elevated antioxidant activity results from the presence of secondary metabolites, including phenolics and flavonoids (Sasikumar et al., 2010). 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 IC_{50} values of 14.11 ± 0.008 µg/mL, indicating robust antioxidant efficacy.

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