

Standardization of the ethanol extract from rumput mutiara (*Oldelandia corymbosa* L.) extract and its antioxidant activity using DPPH method

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ABSTRACT

Most of the traditional medicinal plants in Indonesia are not scientifically validated. Scientific evaluation, along with traditional knowledge, is essential to obtaining effective drugs for commercial purposes. Rumput mutiara (*Oldelandia corymbosa* L.), belonging to the family Rubiaceae, is one of the plants that have been used as traditional medicinal plants and is being prescribed to cure various diseases. The present study is to establish the quality of non-specific and specific parameters and analyze the antioxidant activity of rumput mutiara. Antioxidant activity was evaluated with 1-diphenyl-2-picrylhydrazyl (DPPH). The results for non-specific parameters show the moisture content of the extract (12.2%) and the water content (12.2%). Meanwhile, specific parameters show that extracts have a specific odor, are blackish-brown in color, and have a thick physical appearance. Microscopic parameters of rumput mutiara simplicia showed fragments such as anthers, leaf mesophyll, epidermis and stomata, transport bundles, stem parenchym, and sclerenchyma. the value of the water-soluble compounds (72%), and the value of the ethanol-soluble compounds (35%). The profiles of various individual secondary metabolites were made and developed for authentication. The ethanolic rumput mutiara extract contains derivatives 6 alkaloids, 2 phenolics, 5 flavonoids, 5 tanins, 3 saponins, 2 steroids, and 2 glycosides. And the result of the antioxidant activity of the extract is indicated by the IC₅₀ value (14.11 ppm), which indicates strong antioxidant activity.

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

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INTRODUCTION

Rumput mutiara (*Oldenlandia corymbosa* L.) is a plant in the Rubiaceae family that 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, cholecystitis, 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 purpose of this research was to discover specific and non-specific characteristics for herbal medicinal substances as recommended by the Indonesian Ministry of Health and the Food and Drug Administration of Indonesia. Non-specific characteristics such as shrinkage drying, and water content were measured. Extract identity and organoleptic evaluation, microscopic identification, determination of chemicals dissolved in certain solvents, phytochemical screening, and chromatogram profile are some of the specific characteristics. Antioxidants are a class of chemicals that neutralize free radicals and reactive oxygen species (ROS) in cells. Antioxidants protect against damage caused by free radicals and play significant roles in the development of many chronic diseases, including cardiovascular disorders, aging, heart disease, anaemia, cancer, and inflammation (Zehiroglu & Sarikaya, 2019). The antioxidant activity of the extract was also tested using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) technique in this study. DPPH is a stable free radical with an absorption band of 515 nm. It loses its absorption when it is decreased by an antioxidant or a free radical species. The DPPH technique is commonly used to measure the antioxidant activity of natural plant extracts (Shalaby & Shanab, 2013).

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MATERIALS AND METHOD

Materials

Rumput mutiara herbs were collected from Kebun Percobaan Manoko, Cikahuripan, Lembang, Kabupaten Bandung Barat, Jawa Barat on May 2022. 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 stated that the plant used was rumput mutiara (*Oldenlandia corymbosa* L.). Ethanol and DPPH were analytical grade and purchased from Sigma-Aldrich (St. Louis, USA).

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Extraction

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

Shrinkage drying measurement

One gram of extract was put into a closed-weighing bottle that had undergone heating conditioning and was weighed empty. The extract is put into the oven at 105 °C with the lid open. The weighing bottle is inserted into the excicator so that the temperature drops to room temperature.

This work is carried out repeatedly until a fixed weight is obtained (the difference between the weighing weight and weighing before is no more than 0,0005g) (Departemen Kesehatan, 2000).

Water content

The extract (3 g) is wrapped in aluminum foil and put in a dry round bottom flask. Fifty mL of toluene is added to the flask through a cooler (vertical condenser) and heated carefully for 1 hour. The inside of the cooler is rinsed with toluene. Water and toluene droplets are awaited until they are completely separated, and the water volume is read (Departemen Kesehatan, 2000).

Extract identity and organoleptic evaluation of extracts

Extract identity includes nomenclature description, plant's other names, including the Indonesian name, as well as the plants parts used. The organoleptic properties evaluated include the extract's color, smell, and taste (Departemen Kesehatan, 2000).

Microscopic identification of rumput mutiara herbs simplicia

Rumput mutiara herbs powder is placed on a glass object, given a solution of chloral hydrate, and covered with a cover glass. Then it was heated on a Bunsen fire with tube clamps, kept from boiling, and heated to dry. The preparations were then placed under a microscope and observed at a magnification of 10 times (Departemen Kesehatan, 2000).

Determination of compounds dissolved in certain solvents

Rumput mutiara herbs were weighed at 1.5 g. Weighing was done twice, labeling extract A and extract B. Extract A was macerated in a clogged flask for 1 day with 50 mL of water-chloroform, and B extract was macerated for 1 day with 50 mL of ethanol (96%). The results of the maceration were filtered, and the 10.0 mL of filtrate was evaporated in a cup with an empty weight. The residue is heated at a temperature of 105 °C to a fixed weight (Departemen Kesehatan, 2000).

Phytochemical screening

Identification of chemical compounds in the ethanol extract of rumput mutiara herbs is carried out through phytochemical screening, including examining alkaloid compounds, flavonoids, saponins, steroids, tannins, triterpenoids, and glycosides (Nur et al., 2022).

Chromatogram profile

For separation phytochemical compounds of rumput mutiara extract, sample was spotted manually using a capillary tube on silica gel GF₂₅₄ plates (15x5 cm with 3 mm thickness). The spotted plates were put into a solvent system to detect the suitable mobile phase as per the method of Karthika et al., (2014), as stated in Table 1. After the separation of phytochemical constituents, spraying reagents such as Dragendorff reagent and 5% ferric chloride were used to identify the respective compounds. The color of the spots was noted, and Rf values were calculated by using the following formula:

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

Table 1. Determination of phytochemicals with suitable mobile phases through TLC

Compounds	Mobile Phases	Spraying Reagents
Alkaloids	Chloroform:Methanol (3:2)	Dragendorff
Polyphenols	Chloroform:Methanol (7:3)	FeCl ₃

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Flavonoids	Ehtylacetate:Methanol:Water: Glacial acetic acid (1.7:0.5:0.5:0.5)	FeCl ₃
Tannins	Chloroform:Ehtylacetate:Methanol (5:3:3)	FeCl ₃
Saponins	Chloroform:Methanol (2.2:0.5)	Vanilin H ₂ SO ₄
Steroids	n-hexane:Ehtylacetate (1.5:0.5)	Vanilin H ₂ SO ₄
Glycosides	Ehtylacetate:Ethanol:Water (8:2:1.2)	Anisaldehyde sulphuric acid reagent

Antioxidant activity assay

A 40-ppm DPPH solution was added with 96% ethanol, and then it was allowed to stand for 20 minutes in a dark place. The absorbance was measured at 400–700 nm. Ascorbic acid as a standard and Rumpu mutiara extract were dissolved in 96% ethanol and diluted into five concentrations. Each sample (1 mL) was added to 2 mL of DPPH solution, then incubated for 20 min. Absorbance was measured at the maximum absorption wavelength, and inhibition was calculated using formula 2. The IC₅₀ value was calculated from a linear regression between inhibition versus concentration (N. M. Saptarini & Herawati, 2018).

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$$\%inhibition = \frac{Abs\ DPPH - Abs\ sample}{Abs\ DPPH} \times 100\% \quad \dots (2)$$

RESULT AND DISCUSSION

The maceration extraction method was used in the current research, using 70% ethanol as the solvent. Throughout the maceration process, ethanol will pass through the simplicia cell wall then enter the cavity of the cell plant, which contains the active components. The extraction solution will dissolve the active components. Ethanol was selected as the solvent because it increases cell wall permeability, providing both polar and non-polar components to be easily extracted. The concentrated fluid pushed out of the cell because to the different in active component concentration between the outside and the inside cells (Kusuma et al., 2017). 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 and specific parameters from rumpu mutiara

The characterization of non-specific parameters from rumpu mutiara		
Parameters	Results (%)	
	Simplicia	Extract
Shrinkage drying	7.5	18
Water content	4.4	12.2
The characterization of specific parameters from rumpu mutiara		
Parameters	Characteristics	
Identity		
Scientific name for plants	<i>Oldelandia corymbosa</i> L.	
Used parts	Whole parts	
Indonesian name plants	Rumpu mutiara	

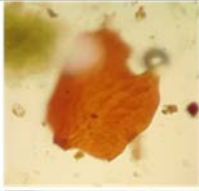
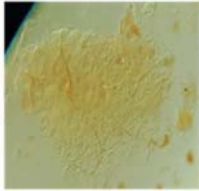
Organoleptic	
Form	Thick
Color	Blackish-brown
Smell	Specific
Taste	Bitter
Water soluble compounds	72%
Soluble ethanol compounds	35%
Parameters levels of dissolved compounds in specific solvents	
Parameter	Characteristics
Water soluble compounds	72%
Soluble ethanol compounds	35%

Shrinkage drying and water content from simplicia do not exceed 10%, while the thick extract category has a water content range of 5–30%. So, according to **Table 2**, both simplicia and extract meet the requirement from Farmakope Herbal Indonesia.

Identification of extract and organoleptic identity is one specific criterias that attempts to provide objective information about material identity through the simple introduction of materials. The determination of the parameter must be carried out in order to offer objective identity from the compound's name and specific identity. Rumput mutiara ethanolic extract organoleptic characteristics were analyzed. **Table 2** illustrates the results of form, color, smell, and taste tests.

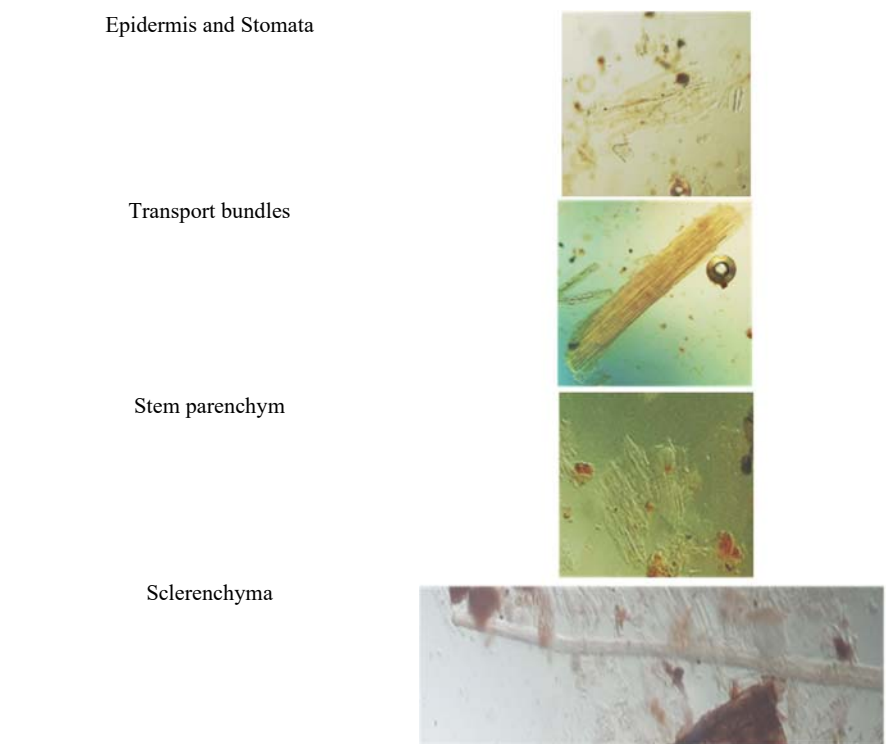
The purpose of using a microscope to examine rumput mutiara plant powder is to discover the properties of herb identification fragments. The chloral hydrate solution is meant to remove cell components such as protein and starch, allowing cell identification fragments on the herbs to be clearly visible under a microscope (Fatmawati et al., 2021). **Table 3** shows the presence of anthers, leaf mesophyll, epidermis and stomata, transport bundles, stem parenchym, and sclerenchyma in rumput mutiara herb simplicia.

Table 3. Microscopic from rumput mutiara powder fragments

Microscopic Parameter	Observation
Anthers	
Leaves mesophyll	

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The parameters of the levels of dissolved compounds aim to inform approximate number of compounds dissolved in certain solvents. Two types of solvents are used, namely water and ethanol. Water solvent to dissolve polar compounds, meanwhile ethanol solvent to dissolve semi polar and non-polar compounds (Muhtadi & Ningrum, 2019).

Table 5. Parameters levels of dissolved compounds in specific solvents

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According to **Table 2**, rumpit mutiara is more soluble in water than in ethanol. These findings suggested that the chemicals found in rumpit mutiara extract are polar.

Phytochemical screening not only exposes the components of plant extracts and what ones predominate among others, but it helps in the search for chemical compounds that can be used in the production of potent medications. The color or changes produced following a reaction with a specific response are seen qualitatively during phytochemical screening. The goal of this phytochemical screening is to figure out the secondary metabolite content of the simplicia and extracts.

Table 4. Results of identification of bioactive compounds of rumpit mutiara

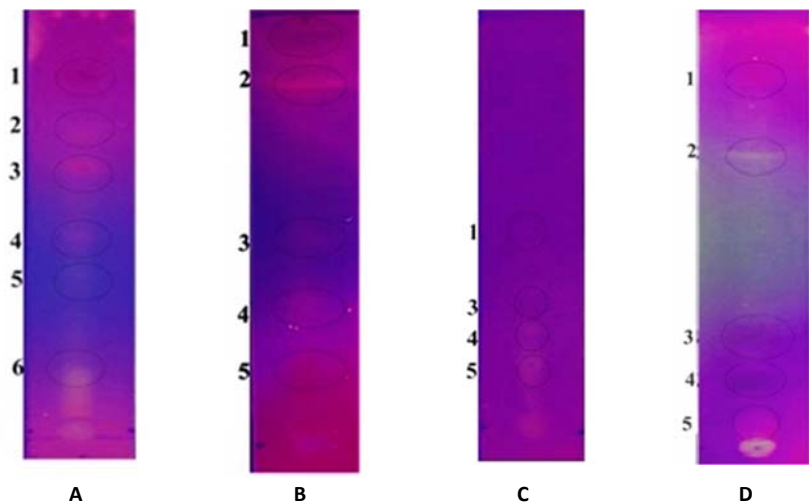
Phytochemical Test	Simplicia	Extract
Alkaloids	+	+
Phenolics	+	+

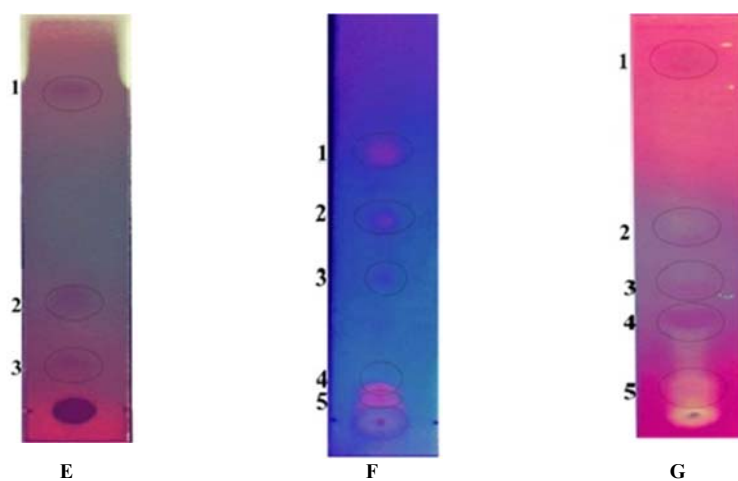
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Steroids	+	+
Glycosides	+	+

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

Table 4 presents the findings of the phytochemical screening. According to phytochemical screening results on simplicia and extracts, rumpu mutiara possesses antioxidant potential due to the presence of secondary phenolic metabolites and flavonoids.

Secondary metabolites are abundant in medicinal plants, and among the numerous bioactive substances, alkaloids, flavonoids, glycosides, saponins, and terpenoids are of particular importance. The chromatographic approach is the most often utilized technique for separating plant elements among the various methods available.





Picture 1. TLC profile on observations under 366 nm UV rays of ethanol of rumput mutiara extract for various secondary metabolites (A) alkaloids; (B) polyphenols; (C) flavonoids; (D) tannins; (E) saponins; (F) steroids; and (G) glycosides.

Table 5. Rf values of rumput mutiara extract on various secondary metabolites

Compounds	Spot	Rf	Keterangan
Alkaloids	1	0.61	Alkaloids 1
	2	0.58	Alkaoids 2
	3	0.48	Alkaloids 3
	4	0.42	Alkaloids 4
Polyphenols	1	0.92	Unknown
	2	0.75	Polyphenols 1
	3	0.65	Polyphenols 2
	4	0.52	Polyphenols 3
Flavonoids	1	0.61	Flavonoids 1
	2	0.48	Flavonoids 2
	3	0.40	Flavonoids 3
	4	0.32	Flavonoids 4
Tannins	1	0.91	Unknown
	2	0.85	Tannins 1
	3	0.24	Tannins 2
	4	0.11	Tannins 3
Saponins	1	0.32	Saponins 1
	2	0.22	Saponins 2
	3	0.14	Saponins 3
	4	0.07	Saponins 4
Steroids	1	0.32	Steroids 1
	2	0.24	Unknown
	3	0.14	Unknown
	4	0.07	Unknown
Glycosides	1	0.92	Unknown

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2	0.57	Glycosides 1
3	0.40	Glycosides 2
4	0.28	Unknown
5	0.10	Unknown

The ethanolic rumput mutiara extract was tested using TLC, and different mobile phase compositions were explored in order to separate the various 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 color evolved and was noted after derivatization with the suitable spraying reagent. Secondary metabolites were separated based on color, and Rf values were estimated as shown in Table 5.

The analysis using TLC identified the presence of alkaloids, phenolics, flavonoids, tannins, saponins, steroids, and glycosides in different solvent compositions with varying Rf levels in the ethanolic rumput mutiara extract (Table 5).

Alkaloids are primarily biosynthetically produced from amino acids, resulting in a wide range of chemical structures, the majority of which are obtained from plants. Alkaloids serve a purpose in both human medicine and an organism's natural defense. Alkaloids constitute roughly 20% of the total known secondary metabolites found in plants. Alkaloids are well known therapeutically as anesthetics, cardioprotective agents, and anti-inflammatory drugs. Morphine, strychnine, quinine, ephedrine, and nicotine are examples of well-known alkaloids utilized in clinical contexts (Heinrich et al., 2021). This study found four types of alkaloids with varied Rf levels, which occurs in orange/brown color.

Polyphenols are an extensive group of secondary metabolism-derived chemicals found throughout the plant environment. Polyphenolic acids, coumarins, flavonoids, stilbenes, and lignans are all examples of polyphenols. Other polymerized forms have been added, such as tannins and lignins. Some of them are in responsibility of the aroma, color, and antioxidant characteristics of the fruits, vegetables, seeds, and nuts we take. Polyphenols are becoming increasingly essential, due to their health-promoting properties. Furthermore, their importance as natural antioxidants in the prevention and treatment of cancer, inflammatory, cardiovascular, and neurodegenerative illnesses continued to increase (Hano & Tungmunthum, 2020). Three types of polyphenols with varied Rf levels were found in this study, in blackish blue spot.

Flavonoids are an example of secondary metabolite that occur in many kinds of fruits, vegetables, herbs, stems, cereals, nuts, flowers, and seeds. Flavonoids have biochemical and antioxidant actions that are beneficial in a variety of illnesses include cardiovascular disease, cancer, and neurological conditions. Flavonoids have been scientifically linked to a wide range of benefits for health and are an essential component in a wide range of nutraceutical, pharmacological, therapeutic, and cosmetic applications. This is due mostly to their anti-inflammatory, antioxidant, anti-carcinogenic, and anti-mutagenic capabilities, as well as their ability to modulate important cellular enzyme processes (Chen et al., 2023). There are four spots in grey color, which indicated hydrocarbon compounds. Color was occurred because there is flavonoid oxidation reaction in hydrocarbon compounds (Syarifah et al., 2019). Four kinds of flavonoids with varied Rf values were found in this research.

Tannins are astringent plant-based polyphenols that are often found in various portions of herbs. Tannin, a polyphenol, possesses several kinds of medicinal therapeutic properties besides to acting as an antioxidant, therefore it exhibits a variety of pharmacological properties such as anti-toxic, anticancer, antiallergic, anti-inflammatory, anthelmintic, antimicrobial, antiviral, wound healing, dysentery cure, and others (Sharma et al., 2021). In this research, three types of tannins with blackish blue spot and varying Rf levels have been identified.

Saponins are a type of bioorganic molecule that is abundant in the plant kingdom. They are naturally occurring glycosides with soap-like foaming, and as a result, they form foam when agitated

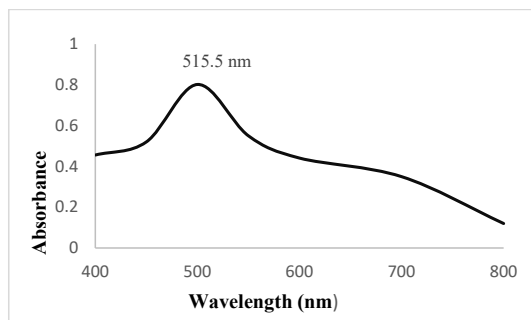
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in aqueous solutions. Saponins possess a biological role and medical capabilities such as hemolytic factor, anti-inflammatory, antibacterial, antifungal, antiviral, insecticidal, anticancer, cytotoxic, and molluscicidal effect, according to the literature. Furthermore, saponins have been shown to reduce cholesterol levels in both animals and humans (El Aziz et al., 2019). The ethanolic rumpup mutiara extract revealed four different types of saponins with different Rf levels in violet blue spot color by TLC.

Plant steroids are distinct chemicals found throughout the plant kingdom that have significant physiological implications for plant growth, development, and reproduction. Plant steroids are a type of physiologically active secondary metabolites having gonane carbon skeletons of 5 α and 5 β . Plant steroids are divided into groups based on their biological functions and structures, as well as their production mechanism. Anti-cancer, immunomodulatory, anti-inflammatory, and anti-viral activities of all subtypes have been studied (Yerlikaya et al., 2023). One type of steroid in blue spot color has been found in this study.

Plants produce a wide range of secondary metabolites that can be sugar-decorated, or glycosylated. Glycosylation of metabolites in plants has several functions. Hydrophobic metabolites become more water-soluble after glycosylation, which improves their biodistribution and metabolism. Glycosylated metabolites' increased solubility and amphiphilicity could help in their transport across cell membranes. Glycosylation may produce a non-toxic compound, which can then be reactivated and utilized as an aglycone in defense against parasites and plant-eating creatures like herbivores (Kytidou et al., 2020). From the results among the 5 spots detected only 2 were observed as glycosides which seen in pinkish violet spot.

The DPPH method is a sensitive, quick, and simple method to evaluate plant extract antioxidant activity. The modification of the DPPH solution indicated antioxidant activity. The color of the DPPH solution switches from purple to yellow, with the intensity equivalent to the number of moles of the stabilized molecule. According to the literature, the maximum wavelength of the DPPH solution was 515.5 nm (Picture 2). As transmitted color, this wavelength is green (500-520 nm). (N. Saptarini et al., 2019).



Picture Figure 2. DPPH Wavelength

Table 6. Results of the antioxidant activity of rumpup mutiara extract

Sample	Concentration (ppm)	Absorbance $\pm$ Standard Deviation (SD)	Inhibition (%)	Linear Regression	IC ₅₀ (ppm)
Vitamin C (standard)	1	0.462 $\pm$ 0.006	49.39	$y = -4.6025x + 44.593$	1.17
	2	0.408 $\pm$ 0.023	53.78		
	3	0.359 $\pm$ 0.003	58.21		

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	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		
	50	0.375 ± 0.004	58.93	$y = 0.235x + 46.429$	14.11
	70	0.325 ± 0.011	64.40		
	90	0.283 ± 0.004	69.00		
(n=3)					

In this study, vitamin C was chosen as a control because it is an effective antioxidant capable of neutralizing oxidative stress via an electron donation or transfer mechanism. Vitamin C has the ability to decrease unstable oxygen, nitrogen, and sulfur radicals, as well as regenerate other antioxidants in the body. Furthermore, human plasma tests have demonstrated that vitamin C is beneficial in reducing lipid peroxidation caused by peroxide radicals (Caritá et al., 2020).

Considering the extracts were not pure components, the antioxidant activity of rumput mutiara extract was lower than that of vitamin C as a positive control, as demonstrated in **Table 6**. The antioxidant activity category (Saptarini & Herawati, 2018) was very strong (<50 ppm) for the extract rumput mutiara, the same as vitamin C. The extract's high antioxidant activity is due to the presence of secondary metabolites such as phenolics and flavonoids. According to the findings of this study, rumput mutiara has the potential to be turned into an antioxidant preparation.

CONCLUSION

Standardization of rumput mutiara extracts according to standard procedures from the health department showed that all parameters met the criteria requirements. TLC analysis from rumput mutiara extract identified the presence of alkaloids, phenolics, flavonoids, tannins, saponins, steroids, and glycosides in different solvent compositions with varying R_f levels. The antioxidant activity was indicated by IC₅₀ values that were 14.11 ppm, which indicated **very strong** antioxidant activity.

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