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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 showed the presence of 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 IC_{50} 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 (*Oldenlanda 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, 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 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. The antioxidant activity of the extract was also tested using the DPPH (2, 2-diphenyl-1-picrylhydrazy) technique in this study.

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 stated that the plant used was rumput mutiara (*Oldelandia corymbosa* L.). Ethanol and DPPH were analytical grade and purchased 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 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 exicator 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 the separation of different phytochemical compounds in the ethanol extract of rumput mutiara herbs, the extract was spotted manually using a capillary tube on the precoated silica gel GF_{254} 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:

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

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

Compounds	Mobile Phases	Spraying Reagents	Colour 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	$FeCl_3$	Blackish blue

Saponins	(5: 3: 3) Kloroform: Metanol (2,2: 0,5)	Vanillin H_2SO_4 reagent	Violet Blue
Steroids	n-hexane: Ethylacetate (1,5: 0,5)	Vanillin H_2SO_4 reagent	Blue
Glycosides	Ethylacetate-ethanol-water (8:2:1.2)	Anisaldehyde sulphuric acid reagent	Pinkish Violet

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 Rumput 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 wavelength, and inhibition was calculated using formula 2. The IC_{50} value was calculated from a linear regression between inhibition versus concentration (N. M. Saptarini & Herawati, 2018).

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

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 parameters from rumput mutiara

Parameters	Results (%)	
	Simplicia	Extract
Shrinkage drying	7.5	18
Water content	4.4	12.2

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 criteria that attempts to provide objective information about material identity through the simple introduction of materials.

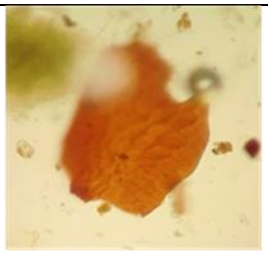
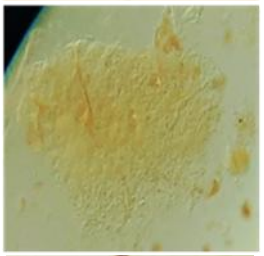
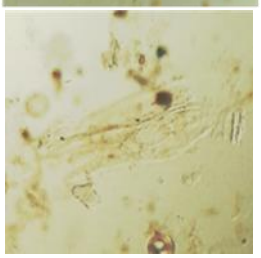
The determination of the parameter must be carried out in order to offer objective identity from the compound's name and specific identity. Rumput mutiara ethanolic extract organoleptic characteristics were analyzed. **Table 3** illustrates the results of form, color, smell, and taste tests.

Table 3. The characterization of specific parameters from rumput mutiara extract

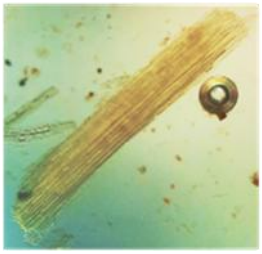
Parameters	Characteristics
Identity	
Scientific name for plants	*Oldelandia corymbosa* L.
Used parts	Whole parts
Indonesian name plants	Rumput mutiara
Organoleptic	
Form	Thick
Color	Blackish-brown
Smell	Specific
Taste	Bitter

The 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 4** shows the presence of anthers, leaf mesophyll, epidermis and stomata, transport bundles, stem parenchym, and sclerenchyma in rumput mutiara herb simplicia.

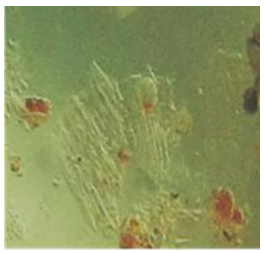
Table 4. Microscopic from rumput mutiara powder fragments

Microscopic Parameter	Observation
Anthers	
Leaves mesophyll	
Epidermis and Stomata	

Transport bundles



Stem parenchym



Sclerenchyma

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

Parameter	Characteristics
Water soluble compounds	72%
Soluble ethanol compounds	35%

According to **Table 5**, rumput mutiara is more soluble in water than in ethanol. These findings suggested that the chemicals found in rumput 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.

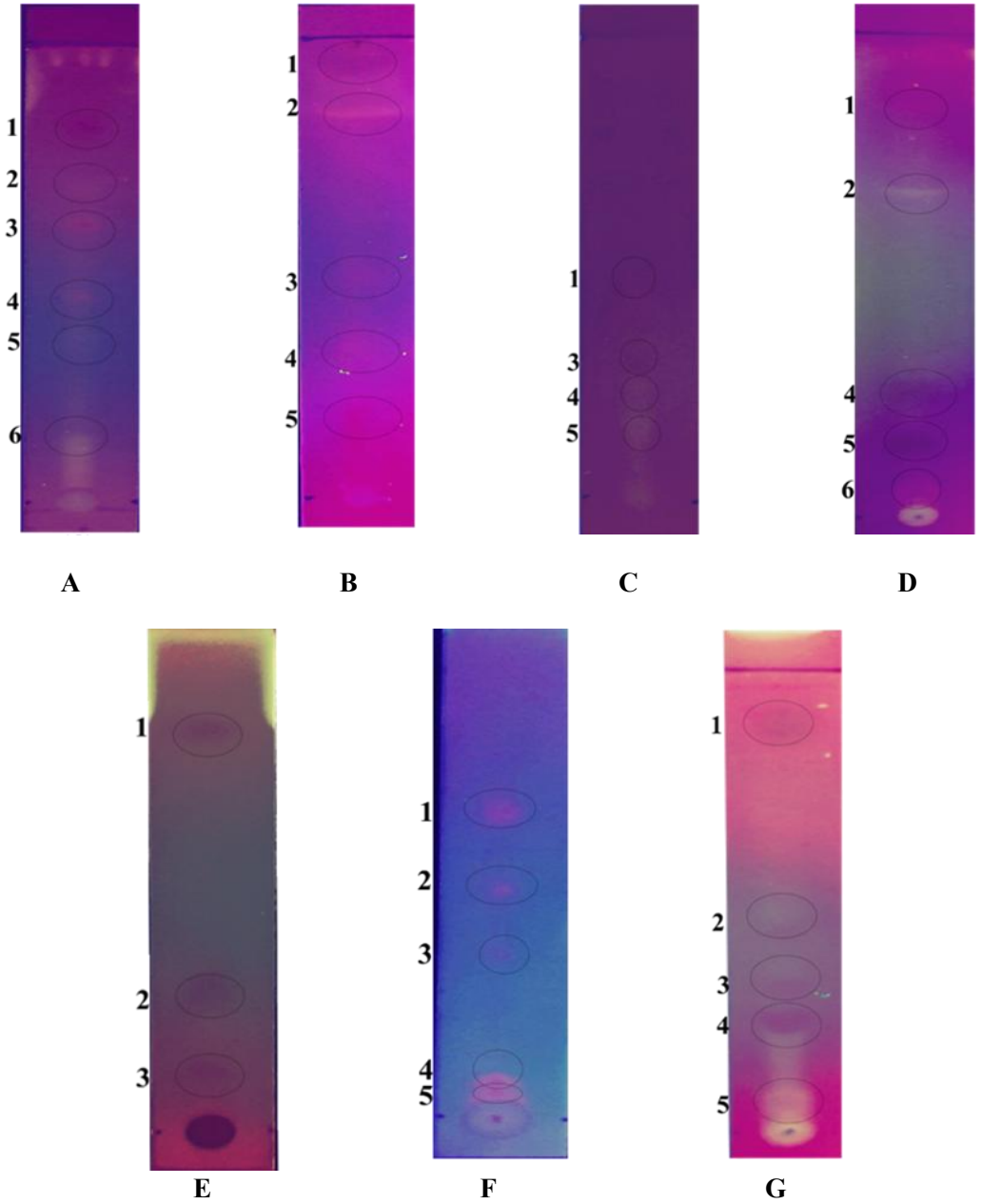
Table 6. Results of identification of bioactive compounds of *Oldelandia corymbosa* L.

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

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

Table 6 presents the findings of the phytochemical screening. According to phytochemical screening results on simplicia and extracts, rumput 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 7. 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
	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 derivarization with the suitable spraying reagent. Secondary metabolites were separated based on color, and Rf values were estimated as shown in **Table 7**.

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 7**).

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.

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 continues to increase (Hano & Tungmunnithum, 2020). Three types of polyphenols with varied Rf levels were found in this study.

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). 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 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 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 rumput mutiara extract revealed four different types of saponins with different Rf levels.

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 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.

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 (**Figure 2**). As transmitted color, this wavelength is green (500-520 nm). (N. Saptarini et al., 2019).

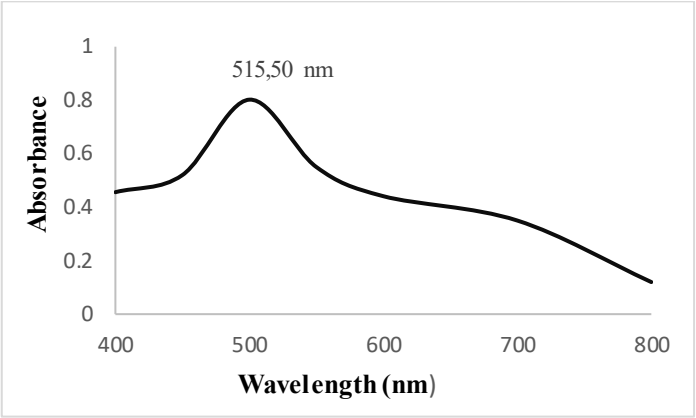


Figure 2. DPPH Wavelength

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

Sample	Concentration (ppm)	Absorbance ± Standard Deviation (SD)	Inhibition (%)	Linear Regression	IC_{50} (ppm)
Vitamin C (standard)	1	0.462 ± 0.006	49.39		
	2	0.408 ± 0.023	53.78		
	3	0.359 ± 0.003	58.21	y=4,6025x+44,593	1.17
	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 8**. 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 Rf levels. The antioxidant activity was indicated by IC_{50} values that were 14.11 ppm, which indicated strong antioxidant activity.

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2. Bukti Review dan Perbaikan Manuskrip (10 Mei 2024)

Pharmaciana

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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 IC_{50} value (14.11 ppm), which indicates strong antioxidant activity.

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

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INTRODUCTION

Rumput mutiara (*Oldenlanda 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, 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 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-picrylhydrazy) 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 (*Oldelandia 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 excator 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 GF_{254} 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:

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

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:Ethylacetate:Methanol (5:3:3)	$FeCl_3$
Saponins	Chloroform:Methanol (2.2:0.5)	Vanilin H_2SO_4
Steroids	n-hexane:Ethylacetate (1.5:0.5)	Vanilin H_2SO_4
Glycosides	Ethylacetate:Ethanol:Water (8:2:1.2)	Anisaldehyde suphuric 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 Rumput 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 IC_{50} value was calculated from a linear regression between inhibition versus concentration (N. M. Saptarini & Herawati, 2018).

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

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 rumput mutiara

The characterization of non-specific parameters from rumput mutiara		
Parameters	**Results (%)**	
	Simplicia	**Extract**
Shrinkage drying	7.5	18
Water content	4.4	12.2
The characterization of specific parameters from 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
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.

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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

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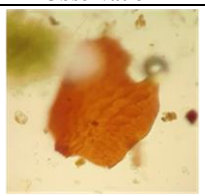
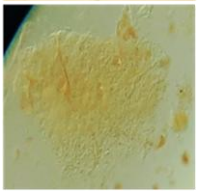
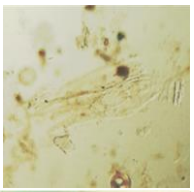
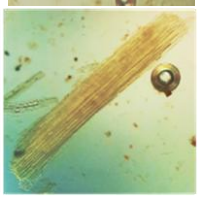
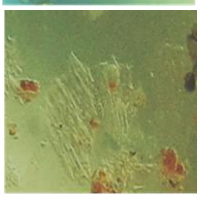
Microscopic Parameter	Observation
Anthers	
Leaves mesophyll	

Epidermis and Stomata	
Transport bundles	
Stem parenchym	
Sclerenchyma	

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**, rumput mutiara is more soluble in water than in ethanol. These findings suggested that the chemicals found in rumput 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 rumput mutiara

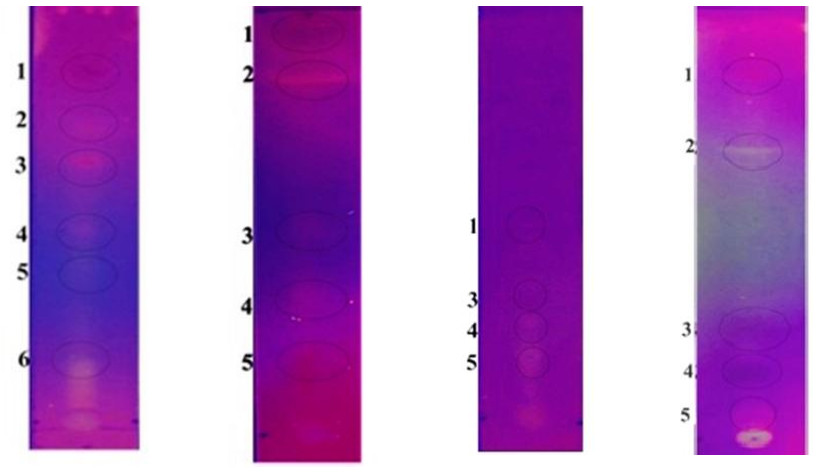
Phytochemical Test	Simplicia	Extract
Alkaloids	+	+
Phenolics	+	+

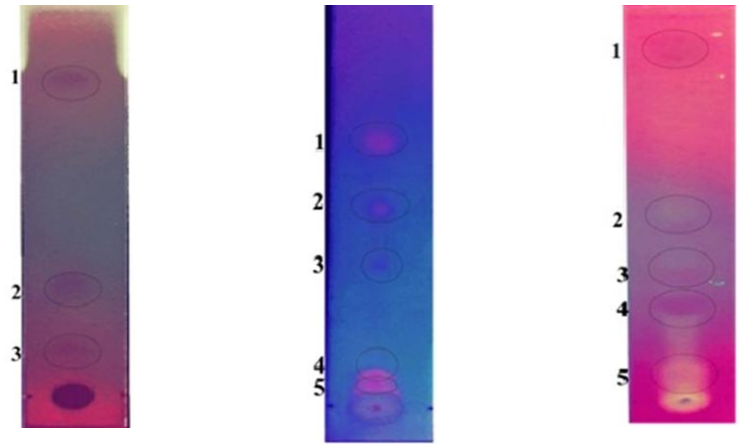
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Steroids	+	+
Glycosides	+	+

*(+) = 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, rumput 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.





E F G

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.

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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

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 derivarization with the suitable spraying reagent. Secondary metabolites were separated based on color, and Rf values were estimated as shown in **Table 5**.

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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 & Tungmunnithum, 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.

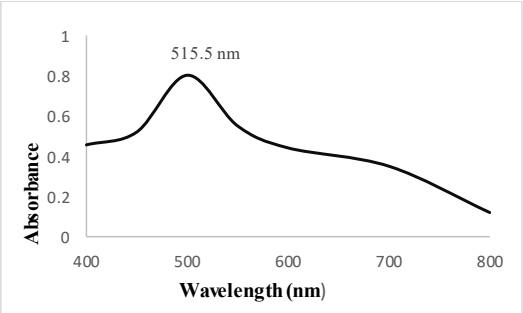
Saponins are a type of bioorganic molecule that is abundant in the plant kingdom. They are naturally occurring glycosides with soap-like foaming, and as a result, they form foam when agitated

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 rumput 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

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

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Sample	Concentration (ppm)	Absorbance ± Standard Deviation (SD)	Inhibition (%)	Linear Regression	IC_{50} (ppm)
Vitamin C (standard)	1	0.462 ± 0.006	49.39	y=4.6025x+44.593	1.17
	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		
	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 Rf levels. The antioxidant activity was indicated by IC_{50} values that were 14.11 ppm, which indicated very strong antioxidant activity.

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ACKNOWLEDGEMENT

The authors would like to thank Ita Inayah and Ira Rahmawati for technical assistance in this research.

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Editor Decision

Participants Edit

Dr.apt. Nina Salamah, M.Sc (ninasalamah)

Dr. apt. Laela Hayu Nurani, MSi. (laelafarmasi)

Dr Irma Erika Herawati (irmandap)

Messages

Note	From
Dear Editor, Sy sdh mengirimkan hasil revisi manuskrip saya dari REviewer A pada tgl 28 januari 2024. Apakah sdh ada hasil review terbaru? Terima kasih _____ Pharmaciana http://www.journal.uad.ac.id/index.php/Pharmaciana	irmandap 2024-03-03 02:04 PM
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Standardization of the ethanol extract from rumput mutiara (*Oldelandia corymbosa* L.) extract and its antioxidant activity using DPPH method

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Submitted :..... Reviewed :..... Accepted:.....

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~~ used 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 showed the S~~m~~hrinkage drying ~~oisture content~~ of the extract ~~(12.2%)~~ and the water content were 18~~2~~.0~~2~~0 -± 0.000 % and 12.20 ~~%~~± 0.000 %, respectively~~(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. Spesific parameters, such as ~~the value of~~ the water-soluble ~~compounds~~ content~~(72%)~~, and ~~the value of the~~ ethanol-soluble compounds were 72.00 ± 0.000 % and ~~(~~35.00~~%~~± 0.000 % , respectively~~)~~. ~~The profiles of various individual secondary metabolites were made and developed for authentication.~~ In addition, TLC profiles showed that secondary metabolites of extract were ~~The ethanolic rumput mutiara extract showed the presence of~~ 6 alkaloids, ~~2~~ 5 phenolics, ~~5~~ 5 flavonoids, ~~5~~ 5 tanins, 3 saponins, ~~2~~ 5 steroid~~s~~, and ~~2~~ 5 glycosides. ~~And the result of the~~ The ~~antioxidant activity of the~~ extract has strong antioxidant activity with ~~is indicated by the~~ IC_{50} value of ~~(~~14.11 ±0.00~~0~~8 µg/mL~~ppm~~)~~,~~ ~~which indicates strong antioxidant activity.~~

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

Corresponding author:

Name: Irma Erika Herawati
Affiliation of author: Sekolah Tinggi Farmasi Indonesia

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INTRODUCTION

Rumput mutiara (*Oldenlanda 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, 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 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 ~~F~~National Agency of Drug and Food Control Republic of Indonesia (Badan POM RI)~~ood 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. The antioxidant activity of the extract was also tested using the DPPH (2, 2-diphenyl-1-picrylhydrazy) technique in this study.

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 stated that the plant used was rumput mutiara (*Oldelandia corymbosa* L.). Ethanol and DPPH were analytical grade and purchased 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 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 volume~~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 ~~in~~placed at exicator~~serted into~~ ~~the exicator~~ 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).

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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 the separation of different phytochemical compounds in the ethanol extract of rumput mutiara herbs, the extract was spotted manually using a capillary tube on the precoated silica gel GF_{254} 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:

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

Table 1. ~~D~~TLC system and spray reagent for determination secondary metabolite ~~etermination of phytochemicals with suitable mobile phases through TLC~~

Commented [AS9]: TLC system and spray reagent for determination secondary metabolite

Compounds	Mobile Phases	Spraying Reagents	Colour 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_2SO_4~~ H_3PO_4 reagent	Blue
Glycosides	Ethylacetate-ethanol-water (8:2:1.2)	H_2SO_4~~Anisaldehyde sulphuric acid reagent~~	Pinkish Violet

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 Rumput 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 wavelength, and inhibition was calculated using formula 2. The IC_{50} value was calculated from a linear regression between inhibition versus concentration (~~N. M.~~ Saptarini & Herawati, 2018).

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

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 secondary metabolites from the plant~~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~~34~~utiara~~34~~al~~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~~34~~utiara~~34~~al~~simplicia and extract.

Table 2. The characterization of non-specific parameters from rumput ~~mutiara~~34mutiara

Parameters	~~Results~~ Samples (%)	
	Simplicia	Extract
Shrinkage drying (%)	7.5	18.00 ± 0.000
Water content (%)	4.4	12.20 ± 0.000

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Commented [AS10]: Extract not only contain active compounds but also non active compounds. We could state that compounds as secondary metabolites also.

Commented [AS11]: Shrinkage and water content of rumput Mutiara (n=?)
x ± SD

Commented [AS12]: Samples

n=2

Shrinkage drying and water content from ~~simplicia~~35utiara~~35al~~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~~35utiara~~35al~~simplicia and extract meet the requirement ~~f~~based on ~~rom~~ Farmakope Herbal Indonesia.

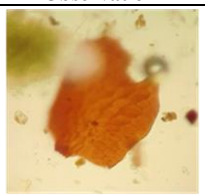
Identification of extract and organoleptic identity is one specific ~~e~~parameters~~riteria~~ that attempts to provide objective information about material identity through the simple introduction of materials. The determination of the parameter must be carried out in order to offer objective identity from the compound~~'~~s name and specific identity. Rumput ~~mutiara~~35utiara ethanolic extract organoleptic characteristics were analyzed. **Table 3** illustrates the results of form, color, smell, and taste tests.

Table 3. ~~The~~ ~~t~~The identity and organoleptic properties of rumput ~~Mutiara~~~~haracterization of specific parameters from~~ ~~rumput~~ ~~mutiara~~~~35utiara extract~~mutiara

Parameters	Characteristics
Identity	
Scientific name for plants	*Oldelandia ~~corymbosa~~ corymbosa*, ~~*35utiara35a*~~ L.
Used parts	Whole parts
Indonesian name plants	Rumput mutiara
Organoleptic	
Form	Thick
Color	Blackish-brown
Smell	Specific
Taste	Bitter

The purpose of using a microscope to examine rumput ~~mutiara~~~~35utiara~~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 4** shows the presence of anthers, leaf mesophyll, epidermis and stomata, transport bundles, stem parenchyma, and sclerenchyma in rumput ~~mutiara~~~~35utiara~~mutiara herb ~~simplicia~~~~35utiara35al~~simplicia.

Table 4. Microscopic from rumput ~~mutiara~~~~35utiara~~mutiara powder fragments

Microscopic Parameter	Observation
Anthers	

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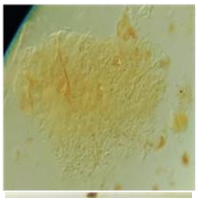
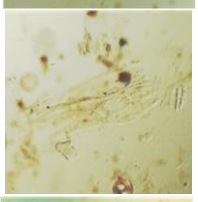
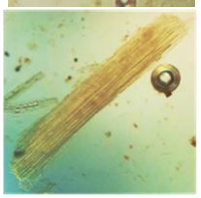
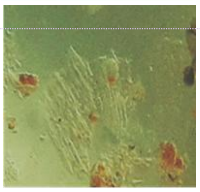
Leaves mesophyll 

Epidermis and Stomata 

Transport bundles 

Stem parenchyma 

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The parameters of the levels of dissolved compounds aim ~~to~~ to provide information of the type of chemical compounds in the extract. ~~nform 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. ~~P~~**Water And Alcohol Soluble Compounds ~~O~~o****f Rumput Mutiara** ~~**arameters levels of dissolved compounds in specific solvents**~~

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

n=2

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Commented [AS18]: Table 5. Water and alcohol soluble compounds of rumput Mutiara extract (n=?)
Value stated as x ± SD

According to **Table 5**, rumput ~~mutiara~~37~~utiara~~mutiara is more soluble in water than in ethanol. These findings suggested that the chemicals found in rumput ~~mutiara~~37~~utiara~~mutiara extract are polar. Compounds such as flavonoids, tannins, and anthraquinones are contained in the polar extract of rumput mutiara (Selvan, 2015; Ezeabara, 2016).

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 6. Results of identification of ~~bioactive compounds~~secondary metabolites of rumput mutiara *~~Oldelandia corymbosa~~* L.

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. According to phytochemical screening results on simplicia and extracts, rumput mutiara possesses antioxidant potential due to the presence of ~~secondary~~ phenolic ~~metabolites~~ and flavonoids compounds.

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.

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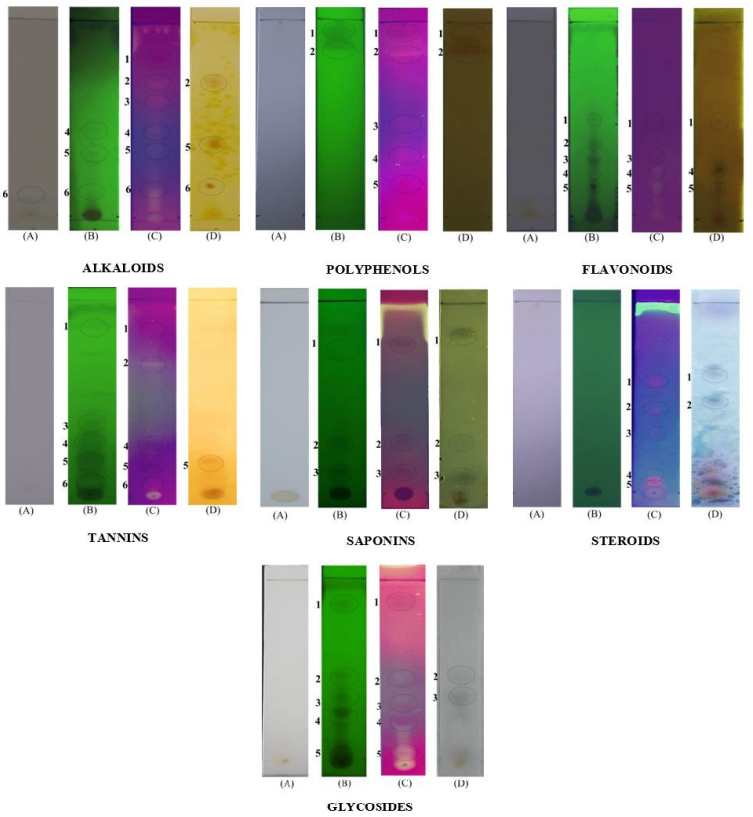

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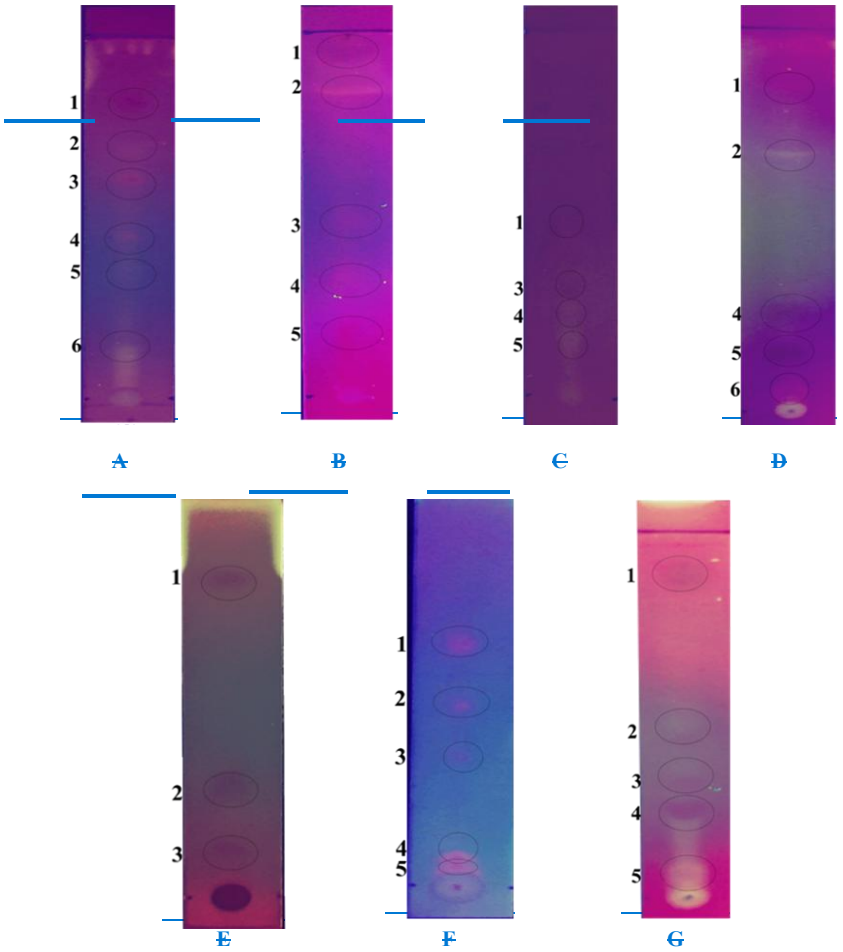

Picture 1. TLC profile on observations under (A) Visible; B (UV 245 nm); C (UV 366 nm); D (Dragendorff for alkaloids); D ($FeCl_3$ for polyphenols, flavonoids, and tannins); D (Vanillin in H_2SO_4 for Saponin); D (Vaniillin in H_3PO_4 for steroids); D (H_2SO_4 for glycosides)
~~366 nm UV rays of ethanol of rumput mutiara extract for various secondary metabolites (A) alkaloids; (B) polyphenols; (C) flavonoids; (D) tannins; (E) saponins; (F) steroids; and (G) glycosides.~~

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

Compounds	Spot	Visible ~~Rf~~	UV 254 nm	UV 366 nm	Dragendorff	Prediction Metabolites ~~KCompoun deterangan~~

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Alkaloids	1	0.61	-	0.82	-	Alkaloids 1
	2		-	0.7	0.7	Alkaloids 2
	3	0.58	-	0.58	-	Alkaloids 3
	4		0.52	0.52	-	Alkaloids 4
	5	0.48	0.38	0.38	0.38	Alkaloids 4
	6		0.14	0.14	0.14	Alkaloids 5
		0.42				
		-				
		0.14				
	Spot	**Visible**	**UV 254 nm**	**UV 366 nm**	**$FeCl_3$**	**Prediction Metabolites**
Polyphenols	1	0.92	0.9	0.9	0.9	UnknownPolyphenols 1
	2	0.75	0.85	0.85	0.85	
	3	0.65	-	0.42	-	Polyphenols 12
	4	0.52	-	0.28	-	
	5	-	-	0.14	-	Polyphenols 23
		-				
		-				Polyphenols 34
		-				
						Polyphenols 5
Flavonoids	1	0.61	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
		0.48				
		0.40				
		0.32				
Tannins	1	0.91	0.94	0.94	-	UnknownTannins 1
	2	-	-	0.8	-	
	3	-	0.71	-	-	Tannins 12
	4	-	0.38	0.38	-	Tannins 23
	5	-	0.17	0.17	0.17	Tannins 34
	6	-	0.08	0.08	-	Tannins 5
		0.85				Tannins 6
		0.24				
		0.11				
	Spot	**Visible**	**UV 254 nm**	**UV 366 nm**	**Vanillin in H_2SO_4**	**Prediction Metabolites**
Saponins	1	0.32	0.7	0.7	0.7	Saponin 1Saponins 1
	2		0.28	0.28	0.28	
	3	-	0.14	0.14	0.14	Saponin 2 Saponin 3Saponins 2 Saponins 3 Saponins 4
	4	0.22				
		0.14				
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	Spot	Visible	UV 254 nm	UV 366 nm	Vanillin in H_3PO_4	Prediction Metabolites
Steroids	1	0.32	0.5	0.5	0.5	Steroids 1
	2	0.24	0.32	0.32	0.32	Unknown
	3	0.14	0.15	-	-	Unknown
	4	0.07	0.07	-	-	UnknownSteroids 2
	5	-	0.04	-	-	Steroids 3
		-				Steroids 4
		-				Steroids 5
		-				
	Spot	**Visible**	**UV 254 nm**	**UV 366 nm**	**H_2SO_4**	**Prediction Metabolites**
Glycosides	1	0.92	0.92	0.92	-	Glycosides 1
	2	0.57	0.57	0.57	0.57	Unknown
	3	0.40	0.4	0.4	0.4	Glycosides 12
	4	0.28	0.28	0.28	-	Glycosides 23
	5	0.10	0.1	0.1	-	Glycosides 4
		-				Unknown
		-				Glycosides 5Unknown
		-				
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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 derivarization with the suitable spraying reagent. Secondary metabolites were separated based on color, and Rf values were estimated as shown in **Table 7**.

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 mutiara41mutiaramutiara extract (**Table 7**). In a study conducted by Aprianto (2018), ursolic acid was identified in the ethanol extract of pearl grass after spraying with 10% sulfuric acid reagent, which produced purple spots. A various phytochemical investigation of rumput mutiara shows the presence of proteins, polysaccharides, polyphenols, tannins, flavonoids, saponins, steroids, triterpene, and glycosides (Das et al., 2019). Gosh et al (2018) researched the total plant pigments in rumput mutiara, which showed different herbs total pigment such as chlorophyll-a, chlorophyll-bm total chlorophyll and total carotenoids.

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 six types of alkaloids with varied Rf levels.

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 lignains. Some of them are in responsibility of the aroma, color, and antioxidant characteristics of

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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 ~~continues to increase~~ (Hano & Tungmunnithum, 2020). ~~Three~~ Five types of polyphenols with varied Rf levels were found in this study.

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). ~~Four~~ Five kinds of flavonoids with varied Rf values were found in this research.

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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~~ five types of tannins with varying Rf levels have been identified.

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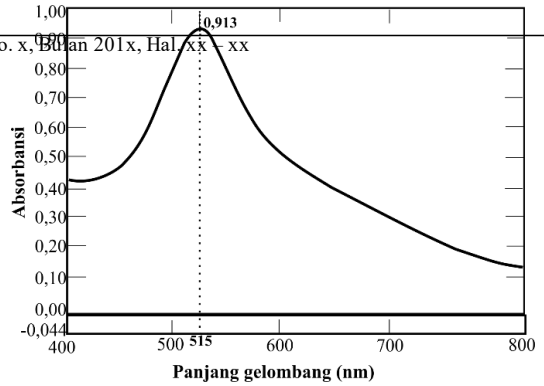
Saponins are a type of bioorganic molecule that is abundant in the plant kingdom. They are naturally occurring glycosides with soap-like foaming, and as a result, they form foam when agitated in aqueous solutions. Saponins possess a biological role and medical capabilities such as hemolytic factor, anti-inflammatory, 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 rumput ~~mutiara~~42~~utiara~~mutiara extract revealed ~~four~~ three different types of saponins with different Rf levels.

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~~ Five type of steroid 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 there are 5 spots detected ~~only 2 were observed~~ as glycosides.

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 515.0~~5~~ nm (**Figure 2**). As transmitted color, this wavelength is green (500-520 nm). (N. Saptarini et al., 2019).



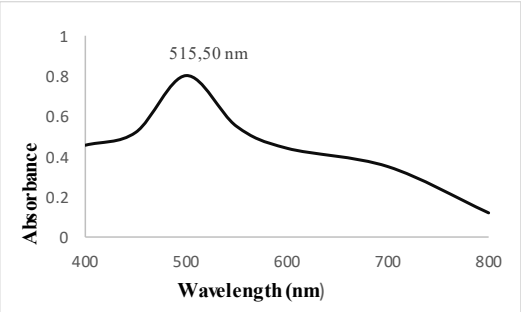


Figure 2. DPPH Wavelength at Spectrophotometer UV-Vis

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

Sample	Concentration (ppm)	Absorbance ± Standard Deviation (SD)	Inhibition (%)	Linear Regression	IC_{50} (ug/mL(~~ppm~~))
Vitamin C (standard)	1	0.462 ± 0.006	49.39	y=4,6025x+44,593	1.17 ± 0.~~000~~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 ± 0.0~~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)

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

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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 8**. According to Awe *et al*., (2013) the antioxidant activity is categorized as very strong, strong, moderate, and weak depending on the IC_{50} value as shown in Table 9. Based on Table 9, the antioxidant activity for the extract rumput mutiara is very strong, with IC_{50} 14.11 µg/mL, the same as vitamin C.

Table 9. Antioxidant power level based on IC_{50} value (Awe *et al*., 2013)

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

~~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 (Sasikumar *et al*., 2009). Based on previous research from Sasikumar et al 2009, antioxidant assays using DPPH, were well correlated with total phenol and total flavonoids content. 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 Rf levels. The antioxidant activity was indicated by IC_{50} values that were 14.11 ± 0.0098 µg/mL~~ppm~~, which indicated strong antioxidant activity.

ACKNOWLEDGEMENT

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Dr Irma Erika Herawati: 2024-05-10 08:00 AM

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Our decision is to: Revision Required

Dr.apt. Nina Salamah, M.Sc
Fakultas Farmasi, Universitas Ahmad Dahlan
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Reviewer A:

Does the paper contain an original contribution to the field?:
Is the paper technically sound?:
Does the title of the paper accurately reflect the major focus contribution
of this paper?:
Please suggest change of the title as appropriate (if any):
Is the abstract a clear description of the paper?:
Is the paper well written (clear, concise, and well organized)?:
Are the equations, figures and tables in this journal style, clear, relevant, and are the captions adequate?:

Please score the paper on a scale of 0 - 10 as per the directions below:
:
Please write down your suggestion to improve this manuscript:

Reviewer C:

Does the paper contain an original contribution to the field?:
Yes

Is the paper technically sound?:
Yes

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

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Submitted :..... Reviewed :..... Accepted:.....

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.), belonging to the family Rubiaceae, is one of the plants that have been used as traditional medicinal plants and is being used 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 showed the Shrinkage drying of the extract and the water contentwere 18.00 ± 0.000 % and 12.20 ± 0.000 %, respectively. 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. 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, non-specific parameters, specific parameters, antioxidant, DPPH.

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INTRODUCTION

Rumput mutiara (*Oldenlanda 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, 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 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 National Agency of Drug and Food Control Republic of Indonesia (Badan POM RI). 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. The antioxidant activity of the extract was also tested using the DPPH (2, 2-diphenyl-1-picrylhydrazy) technique 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 stated that the plant used was rumput mutiara (*Oldelandia corymbosa* L.). Ethanol and DPPH were analytical grade and purchased 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 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 volume (Kusuma et al., 2017).

Commented [AS7]: Constant volume

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 placed at exicator 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).

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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 the separation of different phytochemical compounds in the ethanol extract of rumput mutiara herbs, the extract was spotted manually using a capillary tube on the precoated silica gel GF_{254} 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:

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

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

Commented [AS9]: TLC system and spray reagent for determination secondary metabolite

Compounds	Mobile Phases	Spraying Reagents	Colour 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	$FeCl_3$	Blackish blue

Saponins	(5: 3: 3) 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

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 Rumput 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 wavelength, and inhibition was calculated using formula 2. The IC_{50} value was calculated from a linear regression between inhibition versus concentration (Saptarini & Herawati, 2018).

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

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 secondary metabolites from the plant. 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 parameters from rumput mutiara

Parameters (%)	Samples	
	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 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 based on Farmakope Herbal Indonesia.

Commented [AS10]: Shrinkage and water content of rumput Mutiara (n=?)
x ± SD

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Identification of extract and organoleptic identity is one specific parameters 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 35utiara ethanolic extract organoleptic characteristics were analyzed. **Table 3** illustrates the results of form, color, smell, and taste tests.

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Table 3. The identity and organoleptic properties of rumput mutiara

Commented [AS15]: The identity and organoleptic properties of rumput Mutiara extract

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 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 4** shows the presence of anthers, leaf mesophyll, epidermis and stomata, transport bundles, stem parenchyma, and sclerenchyma in rumput mutiara herb simplicia.

Table 4. Microscopic from rumput mutiara powder fragments

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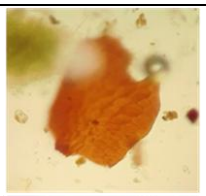
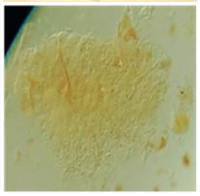
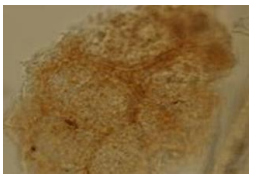
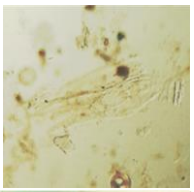
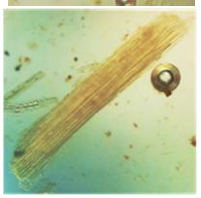
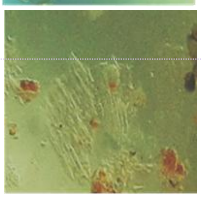
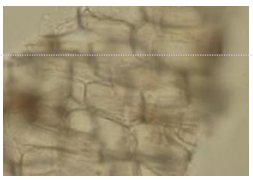
Microscopic Parameter	Observation	Reference (Herbal Pharmacopea Indonesia, 2017)
Anthers		
Leaves mesophyll		

Epidermis and Stomata		-
Transport bundles		
Stem parenchyma		
Sclerenchyma		-

The parameters of the levels of dissolved compounds aim to provide information of the type of chemical compounds in the extract. 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. 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

According to **Table 5**, rumput mutiara is more soluble in water than in ethanol. These findings suggested that the chemicals found in rumput mutiara extract are polar. Compounds such as flavonoids, tannins, and anthraquinones are contained in the polar extract of rumput mutiara (Selvan, 2015; Ezeabara, 2016).

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

Commented [AS17]: parenchyma

Commented [AS18]: to provide information of the type of chemical compounds in the extract.

Commented [AS19]: Table 5. Water and alcohol soluble compounds of rumput Mutiara extract (n=?)
Value stated as x ± SD

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 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)

Commented [AS20]: Secondary profiles of rumput Mutiara based on TLC-spray reagent

Table 6 presents the findings of the phytochemical screening. According to phytochemical screening results on simplicia and extracts, rumput mutiara possesses antioxidant potential due to the presence of phenolic and flavonoids compounds.

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.

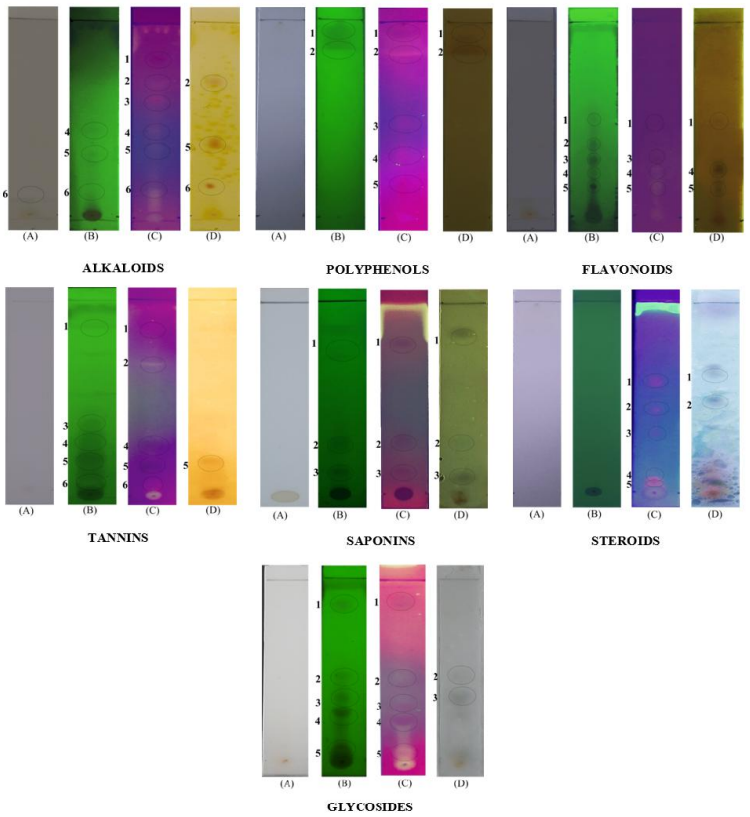


Figure 1. TLC profile on observations under (A) Visible; B (UV 254 nm); C (UV 366 nm); D (Dragendorff for alkaloids); D ($FeCl_3$ for polyphenols, flavonoids, and tannins); D (Vanillin in H_2SO_4 for Saponin); D (Vaniillin in H_3PO_4 for steroids); D (H_2SO_4 for glycosides)

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

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	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
	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

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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 derivarization with the suitable spraying reagent. Secondary metabolites were separated based on color, and Rf values were estimated as shown in **Table 7**.

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 7**). In a study conducted by Aprianto (2018), ursolic acid was identified in the ethanol extract of pearl grass after spraying with 10% sulfuric acid reagent, which produced purple spots. A various phytochemical investigation of rumput mutiara shows the presence of proteins, polysaccharides, polyphenols, tannins, flavonoids, saponins, steroids, triterpene, and glycosides (Das et al., 2019). Gosh et al (2018) researched the total plant pigments in rumput mutiara, which showed different herbs total pigment such as chlorophyll-a, chlorophyll-bm total chlorophyll and total carotenoids.

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 six types of alkaloids with varied Rf levels.

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 lignans. 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 (Hano & Tungmunnithum, 2020). Five types of polyphenols with varied Rf levels were found in this study.

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). Five 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, five types of tannins with 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 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 rumput mutiara extract revealed three different types of saponins with different Rf levels.

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-

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inflammatory, and anti-viral activities of all subtypes have been studied (Yerlikaya et al., 2023). Five type of steroid 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 there are 5 spots detected as glycosides.

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 515.0 nm (**Figure 2**). As transmitted color, this wavelength is green (500-520 nm). (N. Saptarini et al., 2019).

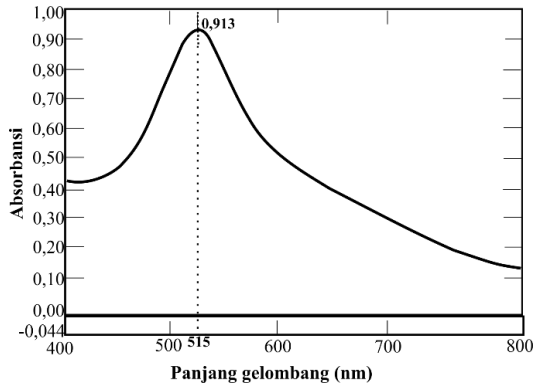

Figure 2. DPPH Wavelength at Spectrophotometer UV-Vis

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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)

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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 8**. According to Awe *et al*., (2013) the antioxidant activity is categorized as very strong, strong, moderate, and weak depending on the IC_{50} value as shown in Table 9. Based on Table 9, the antioxidant activity for the extract rumput mutiara is very strong, with IC_{50} 14.11 µg/mL, the same as vitamin C.

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Table 9. Antioxidant power level based on IC_{50} value (Awe *et al*., 2013)

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

The extract's high antioxidant activity is due to the presence of secondary metabolites such as phenolics and flavonoids (Sasikumar *et al*., 2009). Based on previous research from Sasikumar et al 2009, antioxidant assays using DPPH, were well correlated with total phenol and total flavonoids content. 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 Rf levels. The antioxidant activity was indicated by IC_{50} values that were 14.11 ± 0.008 µg/mL, which indicated strong antioxidant activity.

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Analysis of antioxidant and standardisation 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 3 alkaloids, 2 phenolics, 3 flavonoids, 1 tannin, 3 saponins, 2 steroid, and 2 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. To the best for our knowledge, this study is to investigate standardisation 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 herb were 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 powder of rumput mutiara herb (3 g) were 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 GF_{254} 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 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 no more than 14.00%. According to Table 2, both simplicia and extract comply with the standards set by the Indonesian Herbal Pharmacopoeia (Kemenkes, 2017).

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

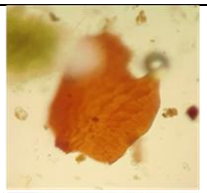
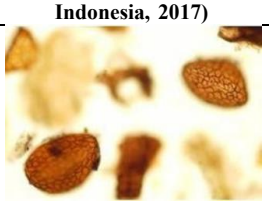
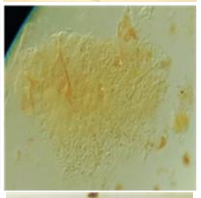
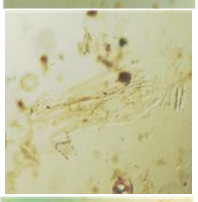
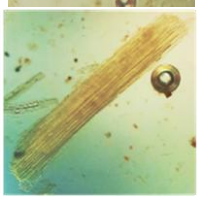
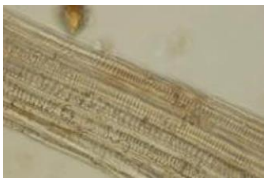
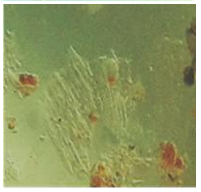
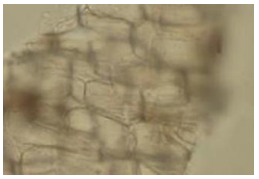
Microscopic Parameter	Observation	Citation (Herbal Pharmacopoeia of Indonesia, 2017)
Anthers		
Mesophyll of leaves		
Epidermis and Stomata		-
Transport bundles		
Stem parenchyma		
Sclerenchyma		-

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)

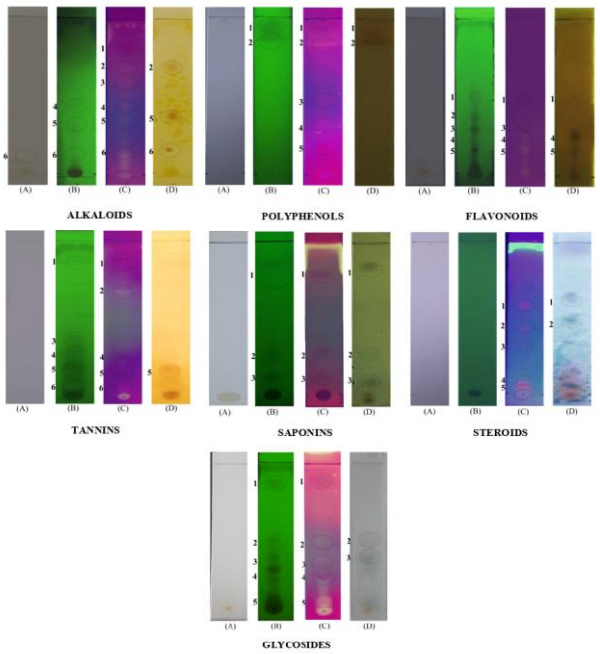


Figure 1. TLC profile on observations under (A) Visible; B (UV 254 nm); C (UV 366 nm); D (Dragendorff for alkaloids); D ($FeCl_3$ for polyphenols, flavonoids, and tannins); D (Vanillin in H_2SO_4 for Saponin); D (Vaniillin in H_3PO_4 for steroids); D (H_2SO_4 for glycosides)

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

Compounds	Spot	Visible	UV 254 nm	UV 366 nm	Dragendorff	Prediction Metabolites	Color of the spot
Alkaloids	1	-	-	0.82	-	NI	Orange/ Brown
	2	-	-	0.7	0.7	Alkaloids 1	
	3	-	-	0.58	-	NI	
	4	-	0.52	0.52	-	NI	
	5	-	0.38	0.38	0.38	Alkaloids 2	
	6	0.14	0.14	0.14	0.14	Alkaloids 3	
	Spot	**Visible**	**UV 254 nm**	**UV 366 nm**	**$FeCl_3$**	**Prediction Metabolites**	**Color of the spot**
Polyphenols	1	-	0.9	0.9	0.9	Polyphenols 1	Blackish blue
	2	-	0.85	0.85	0.85	Polyphenols 2	
	3	-	-	0.42	-	NI	
	4	-	-	0.28	-	NI	
	5	-	-	0.14	-	NI	
Flavonoids	1	-	0.5	-	0.5	Flavonoids 1	Grey
	2	-	0.4	-	-	NI	
	3	-	0.37	0.37	-	NI	
	4	-	0.28	0.28	0.28	Flavonoids 2	
	5	-	0.14	0.14	0.14	Flavonoids 3	
Tannins	1	-	0.94	0.94	-	NI	Blackish blue
	2	-	-	0.8	-	NI	
	3	-	0.71	-	-	NI	
	4	-	0.38	0.38	-	NI	
	5	-	0.17	0.17	0.17	Tannins 1	
	6	-	0.08	0.08	-	NI	
	Spot	**Visible**	**UV 254 nm**	**UV 366 nm**	**Vanillin in H_2SO_4**	**Prediction Metabolites**	**Color of the spot**
Saponins	1	-	0.7	0.7	0.7	Saponin 1	Violet Blue
	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**	**Color of the spot**
Steroids	1	-	0.5	0.5	0.5	Steroids 1	Blue
	2	-	0.32	0.32	0.32	Steroids 2	
	3	-	0.15	-	-	NI	
	4	-	0.07	-	-	NI	
	5	-	0.04	-	-	NI	

*NI = Not Identified

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Commented [H3]: Ini mestinya analisisnya tidak spt ini. Apakah semua spot yg muncul jadi alkaloid, mestinya hanya yg warna orange/coklat dg dragendorf yg alkaloid, sesuai di tabel 1. Tambahkan satu kolom lagi utk warna (setelah pereaksi semprot).

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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	H_2SO_4	Prediction Metabolites	Color of the spot
Glycosides	1	-	0.92	0.92	-	NI	Pinkish Violet
	2	-	0.57	0.57	0.57	Glycosides 1	
	3	-	0.4	0.4	0.4	Glycosides 2	
	4	-	0.28	0.28	-	NI	
	5	-	0.1	0.1	-	NI	

*NI = Not Identified

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 (Badri et al., 2019; Heinrich et al., 2021). This research identified six spots with diverse Rf values. Three spots showed an orange color change after being sprayed with Dragendorff reagent indicated the presence of alkaloid compounds. 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 spots with differing Rf values. Two spots showed a blackish blue color change after being sprayed with $FeCl_3$ reagent indicated the presence of polyphenols compounds.

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; Ekalu & Habila, 2020). This research identified

five spots with distinct Rf values. Three spots showed a gray color change after being sprayed with $FeCl_3$ reagent indicated the presence of flavonoids compounds.

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; Timilsena et al., 2023). This research identifies six varieties of tannins with differing Rf values. One spot showed a blackish blue color change after being sprayed with $FeCl_3$ reagent indicated the presence of tannin compounds.

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 and showed a violet blue color change after being sprayed with vanillin in H_2SO_4 reagent.

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 spots with varying Rf values. Two spots showed a blue color change after being sprayed with vanillin in H_3PO_4 reagent indicated the presence steroids compounds.

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 spots with varying Rf values. Two spots showed a pinkish violet color change after being sprayed with H_2SO_4 reagent indicated the presence glycosides compounds.

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).

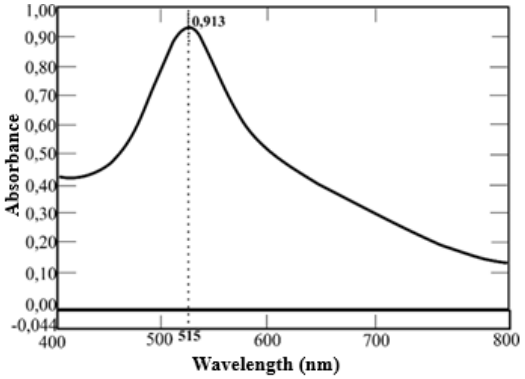

Figure 2. DPPH Wavelength in Spectrophotometer UV-Vis

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 standardisation 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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3. Bukti Manuskrip Diterima (26 Juli 2025)

Dr.apt. Nina Salamah, M.Sc <ninasalamah1996@gmail.com> Sat, Jul 26, 11:35 AM (7 days ago)
to Irma, me

Irma Erika Herawati, Syumillah Saepudin:

We have reached a decision regarding your submission to Pharmaciana, "Analysis of antioxidant and standardization of ethanol extract of rumput mutiara (Oldelandia corymbosa L.) ".

Our decision is to: Accepted

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[Pharmaciana] Editor Decision

2025-07-26 04:35 AM

Irma Erika Herawati, Syumillah Saepudin:

We have reached a decision regarding your submission to Pharmaciana, "Analysis of antioxidant and standardization of ethanol extract of rumput mutiara (Oldelandia corymbosa L.) ".

Our decision is to: Accepted

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Analysis of antioxidant and standardisation 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. 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 (*Oldenlandia 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 (Al Bashera et al., 2021; Archana et al., 2021; 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).

Despite the widespread traditional use and recognized benefits of rumput mutiara in the community, scientific research regarding its quality parameters remains limited. Therefore, standardization efforts are essential to ensure the consistency, efficacy, and safety of rumput mutiara as a medicinal plant. 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. 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 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 herb were 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 powder of rumput mutiara herb (3 g) were 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).

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 (1). A linear regression analysis was performed to calculate the IC_{50} value from the concentration–inhibition curve (Saptarini & Herawati, 2018).

$$\%inhibition = \frac{Abs\ DPPH - Abs\ sample}{Abs\ DPPH}\ x100\% \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

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 1. 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 no more than 14.00%. According to Table 1, both simplicia and extract comply with the standards set by the Indonesian Herbal Pharmacopoeia (Kemenkes, 2017).

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 2 presents the outcomes of assessments on form, color, aroma, and flavor.

Table 2. 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 3 illustrates the existence of anthers, leaf mesophyll, epidermis and stomata, vascular bundles, stem parenchyma, and sclerenchyma in the simplicia of rumput mutiara plant.

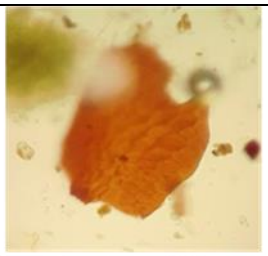
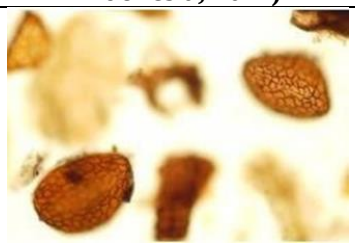
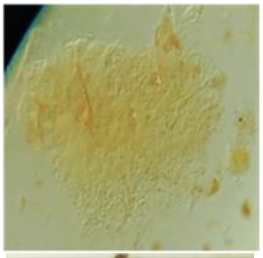
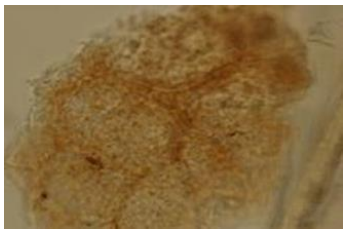
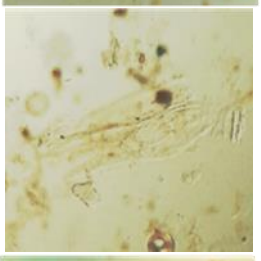
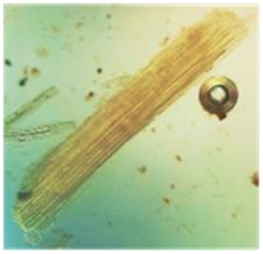
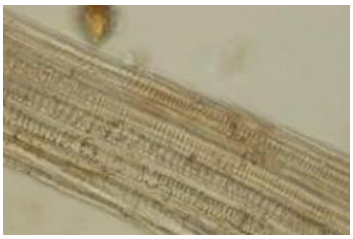
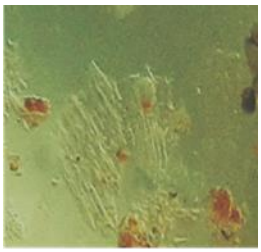
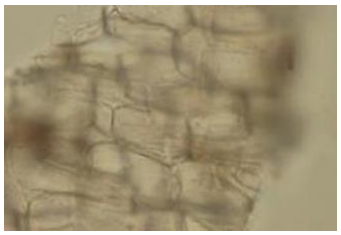
Assessing dissolved compound levels helps in determining the nature of the chemical components within the extract. Water and ethanol are utilized as solvents, with water targeting polar substances and ethanol being effective for semi-polar and non-polar compounds (Muhtadi & Ningrum, 2019).

Table 4 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 5 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.

Table 3. 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		-

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. 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

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.

Table 5. Results of identification of secondary metabolites of rumput mutiara

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

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

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 (Badri et al., 2019; Heinrich et al., 2021).

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).

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; Ekalu & Habila, 2020).

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; Timilsena et al., 2023).

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).

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).

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).

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 1). Transmitted color at this wavelength is green (500-520 nm) (Saptarini & Herawati, 2018).

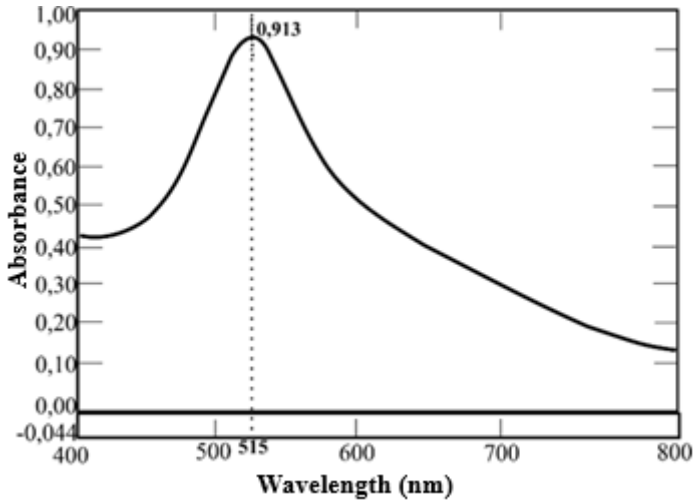


Figure 1. DPPH Wavelength in Spectrophotometer UV-Vis

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).

Table 6. 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)

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 6.
(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 standardisation 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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