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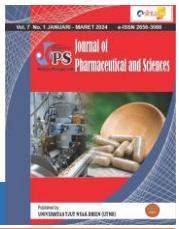

Standarisasi dan Pengujian Aktivitas Antioksidan Ekstrak Daun Karamunting (*Melastoma malabathricum* L.) Dengan Metode DPPH

Standardization and Antioxidant Activity Test of Karamunting Leaf Extract (*Melastoma malabathricum* L.) Using the DPPH Method

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Abstract

Introduction: Karamunting leaves (*Melastoma malabathricum* L.) are a typical plant of Kalimantan, empirically used by local communities as traditional medicine to treat diarrhea, dysentery, vaginal discharge, mouth ulcers, and to enhance immune function. The presence of phenolic compounds, flavonoids, and saponins in karamunting leaves indicates their potential as natural antioxidant agents. Standardization of simplicia and extracts is an essential prerequisite to ensure the quality, safety, and consistency of traditional medicinal raw materials in a scientific and measurable manner. **Objective:** This study aimed to standardize the 70% ethanol extract of karamunting leaves through specific and non-specific parameter testing and to evaluate its antioxidant activity using the DPPH method. **Methods:** Extraction was performed using the maceration method with 70% ethanol solvent for 24 hours. Standardization included specific parameter tests (organoleptic and phytochemical screening) and non-specific parameter tests (water content, drying loss, total ash content, acid-insoluble ash content, heavy metal contamination of Pb and Cd, as well as ethanol-soluble and water-soluble extractive values) in accordance with the Indonesian Herbal Pharmacopoeia (FHI) Edition II standards. Antioxidant activity was assessed using the DPPH radical scavenging method and expressed as the IC_{50} value. **Results:** The karamunting leaf extract exhibited a thick consistency, green color, characteristic odor, and bitter taste. Phytochemical screening showed positive results for phenolic compounds (blue-black coloration with $FeCl_3$) and flavonoids (orange coloration with Mg/HCl). Non-specific parameter testing revealed water content of 15.05±0.36%, drying loss of 6.54±0.51%, total ash content of 3.40±0.31%, acid-insoluble ash content of 0.79±0.02%, and ethanol-soluble and water-soluble extractive values of 98.52% and 96.60%, respectively. No Pb and Cd heavy metal contamination was detected. The antioxidant activity assay yielded an IC_{50} value of 54.63±1.97 ppm, which falls into the strong antioxidant category, although it remained lower than vitamin C (IC_{50}=8.80±1.27 ppm). **Conclusion:** The karamunting leaf extract has met the quality parameter requirements according to FHI standards and demonstrates significant potential as a source of natural antioxidants. Further research is required for the identification of specific active compounds, quantitative heavy metal analysis using AAS, and *in vivo* activity testing to confirm its efficacy and safety.

***Keywords*: Antioksidan, DPPH, Ekstrak Etanol, *Melastoma malabathricum* L., Standarisasi.**

Abstrak

Pendahuluan: Daun karamunting (*Melastoma malabathricum* L.) merupakan tanaman khas Kalimantan yang secara empiris dimanfaatkan masyarakat lokal sebagai obat tradisional untuk mengatasi diare, disentri, keputihan, sariawan, dan meningkatkan imunitas tubuh. Kandungan senyawa fenolik, flavonoid, dan saponin dalam daun karamunting berpotensi sebagai agen antioksidan alami. Standarisasi simplisia dan ekstrak menjadi prasyarat penting untuk menjamin mutu, keamanan, dan konsistensi bahan baku obat tradisional secara ilmiah dan terukur. **Tujuan:** Penelitian ini bertujuan untuk melakukan standarisasi ekstrak etanol 70% daun karamunting melalui uji parameter spesifik dan non-spesifik serta mengevaluasi aktivitas antioksidannya menggunakan metode DPPH. **Metode:** Ekstraksi dilakukan dengan metode maserasi menggunakan pelarut etanol 70% selama 24 jam. Standarisasi meliputi uji parameter spesifik (organoleptik dan fitokimia) serta parameter non-spesifik (kadar air, susut pengeringan, kadar abu total, kadar abu tidak larut asam, kontaminasi logam berat Pb dan Cd, serta kadar sari larut etanol dan air) sesuai dengan standar Farmakope Herbal Indonesia (FHI) Edisi II. Aktivitas antioksidan diuji dengan metode peredaman radikal DPPH dan dinyatakan sebagai nilai IC_{50}. **Hasil:** Ekstrak daun karamunting memiliki konsistensi kental, berwarna hijau, berbau khas, dan berasa pahit. Uji fitokimia menunjukkan hasil positif mengandung senyawa fenolik (warna biru kehitaman dengan $FeCl_3$) dan flavonoid (warna jingga dengan Mg/HCl). Parameter non-spesifik menunjukkan kadar air 15,05±0,36%, susut pengeringan 6,54±0,51%, kadar abu total 3,40±0,31%, kadar abu tidak larut asam 0,79±0,02%, serta kadar sari larut etanol dan air masing-masing 98,52% dan 96,60%. Tidak terdeteksi kontaminasi logam berat Pb dan Cd. Uji aktivitas antioksidan menghasilkan nilai IC_{50} sebesar 54,63±1,97 ppm, yang termasuk dalam kategori antioksidan kuat, meskipun masih lebih rendah dibandingkan vitamin C (IC_{50}=8,80±1,27 ppm). **Kesimpulan:** Ekstrak daun karamunting telah memenuhi persyaratan parameter mutu sesuai standar FHI dan menunjukkan potensi yang signifikan sebagai sumber antioksidan alami. Penelitian lanjutan diperlukan untuk identifikasi senyawa aktif spesifik, uji kuantitatif logam berat dengan AAS, serta uji aktivitas *in vivo* untuk mengonfirmasi efektivitas dan keamanannya.

***Kata Kunci: Antioxidant, DPPH, Ethanol Extract, Melastoma malabathricum* L., Standardization.**

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Introduction

The karamunting plant (*Melastoma malabathricum* L.) is a typical flora of the island of Borneo; its leaves are believed to contain hexadecanoic acid methyl ester and n-hexadecanoic acid [1]. Empirically, karamunting leaves are made into a brew or infusion and used as an anti-inflammatory, analgesic, and antioxidant [2]. However, this traditional processing is considered less effective because the compounds extracted during the brewing process are suboptimal due to the large number of unwanted residual compounds consumed, so the therapeutic effect takes a long time to be achieved. Therefore, it is necessary to process keramunting leaves into an extract to maximize the levels of target compounds.

Compounds in karamunting leaf extract have potential antioxidant activity. In previous studies, karamunting leaf extract had a phenolic content of 17.467 ± 0.035 mg/GAE/mg sample and a total flavonoid content of 4.403 ± 0.011 mg QE/mg sample [3]. As a good antioxidant agent, it is necessary to standardize natural ingredients to ensure they meet established criteria and are safe for the body [4]. Other supporting compounds such as alkaloids and tannins also play an active role in reducing free radicals. Free radicals need to be reduced to prevent serious diseases, such as degenerative diseases (diabetes mellitus, hypertension, cancer, and so on) [5]. A herbal raw material used as a therapeutic agent must be standardized before further processing.

Karamunting leaves, a natural ingredient, need to be standardized due to differences in quality and quantity, which are influenced by harvest age, growing location, and growth nutrition. Standardization of natural ingredients is crucial to ensure the safety, efficacy, and consistency of traditional medicinal products [6]. This way, the benefits of karamunting leaves can be maximized. This study aimed to standardize karamunting leaf simplicia and its activity as a natural antiaging agent.

Experimental Section

Materials and Apparatus

The materials used in this study include karamunting leaves (Banjarmasin, South Kalimantan), 70% ethanol (Medika, Indonesia), Ethanol pro analis (p.a.), chloroform p.a., distilled water p.a., 2,2-difenil-1-pikrilhidrazil (DPPH), Ascorbic acid p.a. from Merck, Germany. Reagents for tube tests, such as $FeCl_3$, sulfuric acid, NaOH, HCl, and Na_2S, were obtained from the chemistry laboratory at Universitas Muhammadiyah Surakarta.

Karamunting leaf extraction

The harvested karamunting leaves were then dried and powdered to a fineness of 40 mesh. The resulting powder was extracted with 70% ethanol solvent at a powder:solvent ratio of 1:10 w/v for 24 hours and remacerated with half the initial solvent [7].The resulting filtrate was evaporated with an IKA RV 10 rotary evaporator at 40ºC until a thick extract was obtained.

Specific Parameter Test

Organoleptic test. The resulting extract was observed visually using the five senses. Observations were based on odor, taste, color, and texture.

Phytochemical test. The extract was tested for phenolic and flavonoid compounds using the tube test and TLC methods. In the tube test, 0.5 g of the extract sample was dissolved in 10 mL of distilled water. The solution was filtered, and 2 mL of the filtrate was collected; 1-2 drops of iron(III) chloride were added to the

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sample. A color change to blue or blackish green indicates a positive phenolic content [8–10]. A 0.5 g extract sample was dissolved in 10 mL of 70% ethanol in a tube, then 0.5 g of magnesium (s) and 5-6 drops of HCl were added. If an orange color develops, the sample is considered to contain flavonoids [10,11].

Non-specific parameter testing

Moisture content test. Moisture content is determined using a calibrated moisture analyzer (by weighing 1-2 grams of thick extract into the instrument and waiting for the sample's water content to become constant [12].

Drying loss test. Accurately weigh 1g of the extract and transfer it to a shallow, stoppered weighing bottle that has been preheated to 105°C for 30 minutes and then tared. Before weighing, the extract is evenly distributed in the weighing bottle using a stirrer. The sample is placed in the drying chamber, the cap is removed, and it is dried at 105°C until a constant weight is reached. Before each drying period, allow the closed bottle to cool to room temperature in a desiccator. If the extract is difficult to dry and melts upon heating, add 1g of accurately weighed silica desiccant after drying and store in a desiccator at room temperature. Mix the silica thoroughly with the hot extract, then dry again at the specified temperature until a constant weight is reached [13].

Total ash content test. Approximately 2-3 g of the crushed, accurately weighed extract is placed in a tared, heated silicate crucible and mixed evenly. Ignite slowly until the carbon is gone, cool, and weigh. If the carbon cannot be removed this way, add hot water and filter through ash-free filter paper. Ignite the remaining paper and filter paper in the same crucible. Transfer the filtrate to the crucible, evaporate, ignite to a constant weight, and weigh. Calculate the ash content against the air-dried material [13].

Test for acid-insoluble ash. Boil the ash obtained in the ash content determination with 25 ml of dilute sulfuric acid for 5 minutes. Collect the acid-insoluble portion, filter through a sintered-glass crucible or ash-free filter paper, wash with hot water, ignite to constant weight, and weigh. Calculate the acid-insoluble ash against the air-dried material [13].

Test for heavy metal contamination. Lead metal contamination test: 5 mL of each sample was placed in a test tube and labeled. The sample was then dripped with HCl solution, and the results were observed. If no precipitate had formed, the sample was heated. The formation of a white precipitate indicated a positive test. The treatment was repeated by replacing the reagents with H_2SO_4 and NaOH [14]. The Cd metal contamination test: 5 mL of each sample was placed in a test tube, then 5 drops of 2 M NaOH were added. Samples indicating the presence of Cd were those that produced a white precipitate. Another test can be performed with Sodium Sulfide (Na_2S) by adding 5 mL of the sample solution to the test tube, then adding 5 drops of 1 M Na2S. The sample was declared positive if a yellow precipitate formed [15].

Ethanol-soluble extract content. Extract (W1) was weighed (2.5 g), macerated with 50 mL of 95% ethanol for 24 hours in a stoppered flask. Shake occasionally for the first 6 hours, let stand for 18 hours, and filter quickly to prevent ethanol evaporation. The filtrate obtained was evaporated to dryness in a shallow, flat-bottomed dish that had been tared (W0) by leaving it until the solvent evaporated and the residue remained. The residue was heated at 105ºC until the weight remained constant (W2), and the ethanol-soluble extract content was calculated as %w/w [7].

Water-soluble extract content test. Extract (W1) was weighed at 2.5 g, then distilled for 24 hours with 50 mL of chloroform pa in a measuring flask. Shake occasionally for the first 6 hours, let stand for 18 hours, and filter. The filtrate obtained was evaporated to dryness in a shallow, flat-bottomed dish that had been tared (W0) by letting it stand until the solvent evaporated and the residue remained. The residue was heated at 105ºC until the weight remained constant (W2). Calculate the content of water-soluble extract in %w/w [7].

Antioxidant assay using the DPPH method

DPPH solution was prepared by weighing 15.6 mg of DPPH powder and dissolving it in ethanol p.a. in a 100 mL volumetric flask. Blank used ethanol p.a. without DPPH. The test was continued with maximum wavelength screening using a Shimadzu 1280 UV-Vis spectrophotometer, Japan. The DPPH solution was incubated for 30 minutes and scanned at 500-600 nm to determine the maximum absorbance. Vitamin C stock solution was used as a positive control at 1000 ppm and diluted with ethanol p.a. to obtain solutions of 2.5, 5, 7.5, 10, and 12.5 ppm. The test sample solution in the form of an extract was diluted up to 1000 ppm using ethanol p.a. to 1, 25, 50, 75, and 100 ppm. The sample was tested with a ratio of 1 ml sample added with 1 ml DPPH added with ethanol up to 5 ml [16].

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Result Analysis Test

The antioxidant activity of Karamunting leaves was assessed using the DPPH method, and ANOVA was used to determine differences in sample activity relative to the reference standard, namely vitamin C.

Results and Discussion

Standardization of herbal samples is crucial to maintain the quality of medicinal raw materials, due to differences in plant composition [17]. Standardization tests are divided into two types: specific and non-specific. The specific standardization tests used in this study were organoleptic and phytochemical tests of karamunting leaves.

Organoleptic testing is the initial step in standardizing plant extracts to ensure the identity and consistency of herbal ingredient quality through sensory observation. Based on observations of leaf extracts from the karamunting plant (Table 1), the results were similar to those previously reported by Sutomo [18]. Texturally, the resulting extract had a thick consistency, indicating that the solvent evaporation process had reached its optimal point and yielded an extract rich in active compounds. The presence of resin, gum, or natural mucus may increase viscosity. In terms of color, the green color of the leaves reflects the distribution of natural pigments and secondary metabolites. The dominant green color in the leaves comes from chlorophyll. However, the most striking aspect is the similarity in the bitter taste profile and distinctive odor of karamunting leaves. The strong bitter taste is a specific indicator of the high tannin and flavonoid content in the karamunting plant. Phytochemically, tannin compounds can bind and precipitate proteins, which is sensorially translated as a tart or bitter taste on the tongue. The presence of these compounds in the leaves also confirms the pharmacological potential of karamunting as an antibacterial and anti-inflammatory agent, because this astringent property is traditionally used to treat diarrhea and accelerate the healing of open wounds by shrinking tissue. The distinctive odor detected also indicates the presence of essential oils or compounds.

The results of the phytochemical screening provided scientific confirmation of previous organoleptic observations, particularly on phenolic parameters. Testing with specific reagents, such as $FeCl_3$, showed a positive result, with a blue-black color change in the leaf extract. This demonstrates the presence of phenolic hydroxyl groups that react with Fe^{3+} ions to form complexes. This finding is in line with the research by Roni & Astary Tahun in 2018, which found that all parts of the karamunting plant contain high levels of phenolic compounds that function as natural antioxidant agents [3]. The presence of these phenolic compounds significantly contributes to the bitter taste experienced during organoleptic testing, as they bind proteins in the tongue mucosa. In addition to phenolics, the presence of flavonoid compounds was also consistently identified throughout all parts of the plant organs. The chemical reaction that produced an orange color in all samples indicated a strong positive result for the presence of flavonoid-derived compounds. This is supported by the literature of Roni & Astary Tahun in 2018, which emphasized that an orange color in the screening test is a typical indicator of flavonoid presence. Flavonoids are widely known in the pharmaceutical world for their biological activities as anti-inflammatory and antimicrobial agents. Thus, the alignment between the organoleptic data, which indicates a bitter taste and distinctive odor, and the phytochemical data, which positively contain phenolics and flavonoids, confirms that karamunting leaves have equal potential to be developed as a raw material for multifunctional herbal medicines.

Table 1. Standardization test of karamunting leaf extract with specific parameters

Parameters	Results
Organoleptic	
Odor	Distinctive
Taste	Bitter
Color	Green
Texture	Thick
Phytochemical Content	
Phenolics	Blue-black
Flavonoids	Orange

The non-specific standardization tests conducted included water content, drying loss, total ash content, acid-insoluble ash content, heavy metal contamination with Pb and Cd, ethanol-soluble extract content, and

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water-soluble extract content. The test results are shown in Table 2. The water content test was conducted to establish a minimum limit for the material's water content, with a low water content ensuring the stability of the extract against microbial growth and chemical hydrolysis. Based on three replications, the average water content of the leaf extract was 15.05 ± 0.36%. The water content in leaves extract is relatively higher, enabling them to absorb more water vapor from the environment than other, denser tissues. The higher water's content of the extract, it can increase the risk of contamination during storage. The water content required for thick extracts or dry simplicia is ideally 5-30% [19]. Previous research stated that the water content of karamunting leaf extract is 10% [3]. Although there are differences in the results, they can be attributed to the length of the evaporation process and differences in solvent use. In this study, 70% ethanol was used, whereas in the previous study, 96% ethanol was used, resulting in higher water content. The lower the ethanol concentration, the higher the boiling point, resulting in a higher water content in the extract.

The drying loss parameter is one of the non-specific standardization criteria that aims to determine the range of compound losses during the drying process. In contrast to moisture content, which measures only specific water content, drying loss measures all volatile materials, including water and residual organic solvents, at a specific heating temperature (generally 105 °C). Based on the testing of Karamunting leaf extract, an average drying loss of 6.54±0.51% w/w was obtained. This value indicates that the extract is stable. Compared with the Indonesian Herbal Pharmacopoeia (FHI) standard, the drying loss for thick extracts of medicinal plants is generally required to be <10% w/w [13]. Thus, the Karamunting leaf extract sample in this test has met the recommended standards. This low drying loss is highly beneficial from a pharmaceutical perspective, as it minimizes the risk of microbial growth (fungi and bacteria) and prevents hydrolysis reactions that can damage active compounds such as flavonoids and phenolics during storage. When compared with previous research, these results align with the findings of Nurhayati et al. (2022), who reported that the physicochemical parameters of karamunting leaves exhibited a stable profile within the 5-9% range [20]. The consistency of the data obtained indicates that the evaporation process of the filtrate solvent during extract preparation was carried out optimally. Furthermore, the small standard deviation indicates a high level of precision in the testing procedure, ensuring reliable data as a quality parameter for the preparation and a longer shelf life and consistent quality.

Determination of total ash content aims to provide an overview of the mineral content, both internal minerals originating from the plant tissue itself and external mineral contamination, such as sand or soil, that may be carried during harvesting and processing. Based on the test results, the average total ash content of karamunting leaf extract was 3.40±0.31% w/w. This value indicates the amount of inorganic residue remaining after a thorough ashing at high temperatures, reflecting the purity and inorganic content of the extract. Compared with the quality standards for natural ingredients, these results meet the safety and quality criteria. Based on FHI Edition II of 2017, in general, the total ash content for thick extracts of medicinal plants is required not to exceed <8.4% [7], which indicates that the extract has a good level of cleanliness and minimal contamination of unwanted external inorganic materials. These results also show consistency when compared with previous studies. For comparison, research by Zetiara et al. in 2025 on karamunting leaves reported a similar ash content range of 5.53% [21]. The ash content of a plant extract depends on the location where it grows and the mineral composition of the soil. The values produced in this study support the finding that karamunting leaves naturally have a fairly stable mineral content (such as calcium, magnesium, and potassium). The low standard deviation also indicates that the ashing method used has good precision, so this data is valid for use as a parameter for standardizing the quality of karamunting leaf extract as a raw material for pharmaceutical preparations.

Determination of acid-insoluble ash content is a more specific purity parameter than total ash content. This test aims to determine the level of silica contamination, such as sand, soil, or certain metal elements, remaining after ashing and boiling in dilute hydrochloric acid. Based on the test results on the thick Karamunting leaf extract, an average value of 0.79 ± 0.02% w/w was obtained. This result is not much different from previous research, which was 0.42% [21]. This very low value indicates that the cleanliness of the sample during harvesting, wet sorting, and processing into extracts is well maintained. Chemically, most natural minerals in plants are soluble in acid, while external minerals from the environment (silicates from the soil) are resistant. Therefore, the low acid-insoluble ash value indicates that this Karamunting leaf extract is almost completely free from external inorganic impurities that are toxic or abrasive. In terms of standardization, this result meets the requirements set out in FHI Edition II 2017, which is <0.8% [7]. The simplex washing technique affects these results before extraction; the cleaner the washing, the lower the possibility of acid-insoluble ash

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content, so to improve the quality of the extract, it is better to carry out a clean washing so that the total ash content value is smaller.

In addition to testing the active compound content, the safety aspect of the extract against environmental pollution is a mandatory parameter in the standardization of natural materials. Based on the results of qualitative testing for lead (Pb) using specific reagents such as HCl, H_2SO_4, and NaOH, no white deposits or color changes were observed, indicating the presence of lead ions in the extracts of the leaves of karamunting. Likewise, in the cadmium (Cd) test using the Na_2S reagent, no yellow deposits were found, typical of Cadmium Sulfide (CdS), in all parts of the plant organs. These negative results indicate that the karamunting samples used grew in an environment not contaminated by industrial waste or human activities containing dangerous heavy metals. The absence of Pb and Cd contamination in leaves. According to the Indonesian Food and Drug Authority (BPOM RI) in 2014, regarding the Quality Requirements for Traditional Medicines, the maximum limit of heavy metal contamination is highly considered because of its nature, which is difficult to excrete and can accumulate in the body, which has the potential to cause damage to the kidneys and nervous system [22]. This result is in line with the principle of herbal safety, which states that quality raw materials must be free of heavy metal contaminants. In this study, no Pb or Cd contamination was found in the leaf extracts of the karamunting plant, and the extracts are declared safe for use as raw materials for pharmaceutical preparations or traditional medicines, as they meet the quality and safety standards for simple drugs and medicinal plant extracts. This test can be further investigated qualitatively with an atomic absorption spectrometer instrument.

Determination of the ethanol-soluble and water-soluble extract contents is one of the specific parameters of extract standardization, aiming to provide an initial picture of the amount of chemical compounds that can be extracted in a particular solvent. Based on the test results, the ethanol-soluble extract content of karamunting leaves was 98.52% w/w, while the water-soluble extract content was 96.60% w/w. This slight difference in values indicates that the karamunting leaf simplicia has an excellent solubility profile at various levels of solvent polarity. The dominance of the ethanol-soluble extract content (98.52%) indicates that most secondary metabolites in karamunting leaves, such as flavonoids, terpenoids, and phenolics, are more optimally attracted by the universal ethanol solvent. On the other hand, the high value of the water-soluble extract content reflects the presence of abundant polar compounds, such as tannins, saponins, and glycosides, which are known to be the main components of the karamunting plant. The high percentage of both extract levels indicates that the resulting extract is highly pure, with very little inorganic material or insoluble impurities. The standardization test carried out, and the meeting of the established requirements, are expected to maintain the quality of karamunting leaves as an antioxidant agent.

Tebel 2. Standardization test of karamunting leaf extract with non-specific parameters

Non-specific parameters	Results	Literature
Moisture content (%w/w)	15,05±0,36	5-30%w/w [19]
Drying loss (%w/w)	6,54±0,51	<10% [13]
Total ash content	3,40±0,31%w/w	<8,4% [13]
Acid-insoluble ash content	0,79±0,02%w/w	<0,8% [13]
Pb contamination	Not detected	
Cd contamination	Not detected	
Ethanol-soluble extract content	98,52% w/w	
Water-soluble extract content	96,60% w/w	

Based on the results of the antioxidant activity test, the IC_{50} value of the 70% ethanol extract of Karamunting leaves (*Melastoma malabathricum* L.) was 54.63±1.97 ppm. This result is obtained from the linear regression equation y = 23.7777+0.4807x (R = 0.9996). Referring to the antioxidant strength classification, this plant part is categorized as strong because it has an IC_{50} value below 100 ppm. However, when compared to the positive control of Vitamin C with an IC_{50} value of 8.80±1.27 ppm, the antioxidant strength of this natural extract is still below that of pure ascorbic acid. This is understandable because vitamin C is a pure compound, while the 70% ethanol extract still contains a variety of secondary metabolites. This result is obtained from the linear regression equation y = 5.5313+5.0246x (R = 0.9942).

These results were supported by the results of the one-way ANOVA statistical test, which showed that the test extract sample was quite sensitive to free radical scavenging. Several studies report that the ethanol extract of *M. malabathricum* fruit has an IC_{50} of around 16.82 ppm, which is classified as strong antioxidant

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activity [23]. These differences in IC_{50} values indicate that antioxidant activity is strongly influenced by the plant part, total flavonoid content, and extraction method used. Extracts with higher flavonoid content generally exhibit stronger antioxidant activity.

Table 3. Antioxidant activity test of vitamin C and karamunting leaves

Sample	IC_{50} (ppm)	Antioxidant Category	Significance
Ascorbic acid	8,80±1,27	Very strong	<0,05 (significant)
Karamunting leaf extract	54,63±1,97	Strong	

These results corroborate previous findings that karamunting leaf extract contains high flavonoid compounds, which directly correlate with free radical scavenging activity with a value of 44.78±0.18 mgQE/g [24]. This activity is likely influenced by the distribution of phytochemical profiles such as anthocyanins, tannins, and quercetin, which are more concentrated in the leaf mesophyll tissue as a plant defense mechanism against UV exposure. The use of 70% ethanol solvent in this study proved effective in extracting the polar and semipolar compounds responsible for this antioxidant activity. Based on these results, a liquid chromatography-mass spectrometry test can be carried out at a further test stage to find out in more detail about the various compounds contained in the caramunting leaf extract sample.

The *Melastoma malabathricum* L. plant is known to contain various flavonoid compounds that play an important role as antioxidants. Some of the main flavonoids commonly found include quercetin, kaempferol, myricetin, and the anthocyanin group [25]. Flavonoid derivatives such as quercetrin and other phenolic compounds have also been reported in the leaves of this plant. This flavonoid diversity underlies the strong antioxidant activity of this plant.

The use of 70% ethanol as a solvent in the extraction also has a strong scientific basis. Ethanol 70% is a solvent with medium polarity that is able to extract polar and semipolar compounds optimally. The water content in ethanol increases the solvent's ability to dissolve polar compounds such as flavonoid glycosides and phenolics, while ethanol is able to dissolve semipolar compounds such as flavonoid aglycones (e.g., quercetin and kaempferol). Therefore, this solvent is often used to obtain optimal yields and bioactive compound content. Studies also show that variations in ethanol concentration can affect the resulting phenolic and flavonoid content [26].

The antioxidant activity of flavonoids in the DPPH method is strongly influenced by their chemical structure, particularly the presence of hydroxyl groups (-OH) in the aromatic ring. Hydroxyl groups in the ortho (o-) or para (p-) positions play a crucial role in donating hydrogen atoms (H•) to reduce DPPH free radicals to a more stable form. After hydrogen donation, the formed flavonoid radical is stabilized by electron delocalization in the aromatic ring system. This mechanism explains why flavonoids like quercetin (which have many -OH groups) exhibit very strong antioxidant activity compared to other flavonoids with fewer hydroxyl groups [27].

Conclusions

This study concluded that the karamunting leaf extract had met the specific parameter standards (organoleptic and containing phenolic also flavonoid compounds) and non-specific (moisture content, drying loss, total ash content, acid-insoluble ash content, did not contain Pb and Cd metal contamination, and solubility test with ethanol and water solvents). The karamunting leaf extract had a potential antioxidant activity of 54.63±1.97 ppm, which was categorized as strong. This study only used one antioxidant test method (DPPH), so it needs to be confirmed with other methods such as ABTS or FRAP; heavy metal contamination tests are still qualitative, so further quantitative tests can be carried out with the AAS instrument; identification and isolation of specific active compounds responsible for antioxidant activity have not been carried out; activity tests are only in vitro, so further in vivo tests are needed.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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