East Asian Journal of Multidisciplinary Research (EAJMR)
Vol. 4, No. 8, 2025: 4119 - 4134

Antioxidant Activity of Ethanol Extract, Fractions and Subfractions from Malay Apple Seed (*Syzygium Malaccense*)

Tien Ellita Endah Puspitasari[1*], Tri Wijayanti[2], Nuraini Harmastuti[3]
Setia Budi University, Surakarta, Indonesia
Corresponding Author: Tien Ellita Endah Puspitasari litawork22@gmail.com

A R T I C L E I N F O

Keywords: Malay Apple Seed, Fraction, Subfraction, Antioxidant, DPPH

Received : 15, July
Revised : 15, August
Accepted: 26, August

A B S T R A C T

Malay apple *(Syzygium malaccense*) plants are known to have various benefits. Parts of the fruit, seeds, leaves and stem bark are known to have antioxidant activity. Previous studies showed that ethanol extracts and methanol extracts of malay apple (*Syzygium malaccense)* seeds have antioxidant activity using the DPPH method. The extract was fractionated tested for antioxidant activity. The most active fraction was separated using VLC and then separated using preparative TLC. The subfractions tested for antioxidant activity using DPPH method. The results showed that ethanol extract sample had antioxidant activity, while the fraction showed that ethyl acetate fraction showed the best antioxidant activity. The subfraction separation results that the subfraction F2 showed the best antioxidant activity with very strong intensity.

DOI prefix: https://doi.org/10.55927/eajmr.v4i8.318
ISSN-E: 2828 – 1519 (Online)
https://mtiformosapublisher.org/index.php/eajmr

INTRODUCTION

Antioxidant is a compound that can absorb or neutralize free radicals so as to prevent degenerative diseases that can cause various diseases such as cardiovascular, carcinogenesis, and other diseases that can be caused by free radicals. Antioxidant compounds are substance compounds that are indispensable by the body to prevent damage caused by free radicals to normal cells, proteins, and weak cells. Antioxidants that are widely found in nature are examples of natural antioxidants. Natural antioxidants can be obtained from plant parts such as wood, bark, roots, leaves, fruits, flowers, seeds, and others (Parwata, 2016).

One of the plants that have good antioxidant activity is plants from the *Myrtaceae* family, one of which is the malay apple (*Syzygium malaccense*). Plants in the *Myrtaceae* family are very commonly used as medicinal plants to treat various diseases such as bronchitis, asthma, diabetes mellitus, and as anti-inflammatory. *Syzygium malaccense* or better known as guava bol belongs to the myrtaceae family and has many opportunities as a medicinal plant. There is a natural antioxidant content contained in the plant that has the ability to prevent degenerative diseases. Malay apple is one of the good sources of antioxidants so it has the potential to maintain human health (Fauziah et al., 2019). From the research that has been done, the stem bark extracted using ethanol has good antioxidant activity (Fauziah et al., 2019). Then the fruit also has good antioxidant activity in ethanol extracts (Nurhasnawati et al., 2017), methanol extract (Gibbert et al., 2021; Nunes et al., 2016). In addition, the ethanol extract (Devitria et al., 2023) and methanol extract (Devitria et al., 2022) of malay apple has antioxidant activity.

Flavonoids are phenolic compounds that are widely isolated from plants for their benefits as antioxidants. As antioxidants, flavonoids can capture free radicals that can damage body cells. Medicinal plants that contain flavonoid chemical compounds are an alternative therapeutic option to prevent the formation of free radicals and reduce tissue damage due to inflammation. Apart from being empirically proven, treatment with medicinal plants also has the advantage of being cost-effective and easy to find and cultivate. Flavonoids can almost be found in all parts of the plant, namely roots, leaves, fruits, stems, skin, flowers, and seeds (Husna et al., 2022).

Until now, studies on the content of chemical compounds in guava plants (*Syzygium malaccense*) are still limited. There are various studies on the fruit, stem, bark and seeds of the guava plant that have good antioxidant activity. In addition to the normal chemical content found in all plant tissues, seeds have a number of additional chemical contents stored as food reserves to accommodate germination. These food reserves are stored mainly as carbohydrates, fats, and proteins. Seeds also contain other chemicals, some of which play minor storage roles, but most of which function as growth and metabolic control agents. Many reports on the potential antioxidant activity of *Syzygium malaccense* plants. leaves, fruits, and seeds show the content of phenolic compounds, flavonoids, and carotenoids which are one of the sources of antioxidant activity (Batista et al., 2017). *Syzygium malaccense* has many properties, one of which is widely used for

inflammation of the mucous membranes in the community. This study aims to determine the antioxidant activity in the content of extracts and fractions in *Syzygium malaccense* seeds. In addition, to determine the class of compounds contained in the sample which may be compounds that have antioxidant activity in the *Syzygium malaccense* sample.

THEORETICAL REVIEW

A frequently used method for traditional drug discovery is the extraction method. The choice of extraction method depends on the nature of the material and the compound to be isolated. Extraction can be done by maceration, percolation, digestion, reflux. In addition, the maceration method can be modified using an extractor equipped with a heating mantle. Maceration is commonly used in the preparation of homemade tonics for a long time and is a popular and inexpensive way to obtain bioactive compounds (Senapati & Behera, 2023). Maceration is an old technique used for drug preparation. Maceration is a solid-liquid extraction method in which the bioactive compounds (solutes) in the plant material are extracted by soaking the plant material in a specific solvent for a certain period of time. Maceration is a simple method using uncomplicated equipment and for this reason is a popular choice for researchers. Unfortunately, the duration of the extraction time is long and sometimes time-consuming (Njila et al., 2017).

When large quantities of extractions are performed, it is not possible to extract every plant sample with a customized solvent system. A common procedure should be developed and validated, taking into account the detection rates of active extracts ("hits") obtained using multiple extraction methods, and followed by an analysis of false positive response rates. This approach has been used to develop common extraction protocols to be used in extracting plant constituents used to perform in vivo or in vitro biological screening (Sabotič et al., 2024). Fractionation is the process of separating a plant extract into various fractions. It further separates the fractions into parts consisting of a number of compounds. The process continues until the pure compound is isolated. When multiple solvents are required for fractionation, they should be added according to the order of increasing polarity (Abubakar & Haque, 2020).

Plant secondary metabolites play an important role in determining the biological activity of medicinal plants used in traditional medicine. Therefore, identification and isolation of secondary metabolites are important for standardization and quality improvement of plant extracts (Sholikhah, 2016). Flavonoids are secondary metabolites of polyphenols found widely in plants as well as foods and have various bioactive effects including one as an antioxidant. Flavonoid compounds are polyphenolic compounds that have 15 carbon atoms arranged in the C6-C3-C6 configuration, meaning that the carbon skeleton consists of two C6 groups (substituted benzene rings) connected by a three-carbon aliphatic chain. Flavonoids are significantly found in several parts of plants such as fruits and vegetables which play a role as neurotrophins in mammals, reduce angiogenesis, antioxidant substances, resistance to aging morphological changes (Arifin et al., 2018).

Chromatography is a separation method based on the difference in the movement of compound components between two phases, namely the stationary phase (which can be a liquid or solid substance) and the mobile phase (which can be a gas or liquid substance). The concept of chromatography involves the use of a mobile phase, which is an extraction solvent and a stationary phase such as silica gel and sephadex which is then mixed with calcium sulfate as a binder. The KLT process involves the use of adsorption mechanisms to separate compounds from the mixture. The separation that occurs is based on the interaction between the compounds in the mixture and the stationary phase (Abubakar & Haque, 2020). Vacuum Liquid Chromatography is considered as preparative thin layer chromatography as the separation is carried out on thin layer chromatography grade silica gel or aluminum oxide and the column is dried after each fraction as in preparative thin layer chromatography plates are dried and re-run to enhance the separation. A packed Vacuum Liquid Chromatography column can be reused for the same or similar separation by thoroughly washing the column with methanol and removing any decomposed polar material or bands from the top of the adsorbent column. Gradient elution is very effective and can be used for the separation of small as well as large quantities of mixtures (Ferrante et al., 2018).

Antioxidants are now an important part of our lives today because they neutralize or destroy "Reactive Oxygen Species" (ROS) or so-called radicals before they can damage cells. ROS-induced oxidation can result in cell membrane disintegration, membrane protein damage, and can cause DNA mutations that can lead to premature aging and can further initiate or propagate the development of many diseases such as arteriosclerosis, cancer, diabetes mellitus, liver injury, inflammation, skin damage, coronary heart disease, and joint inflammation (Dontha, 2016). The antioxidant effectiveness of a compound depends on its chemical characteristics and physical location in the food (proximity to the phospholipid membrane, emulsion interface, or aqueous phase). Antioxidants such as flavonoids, phenolic acids, tannins, Vitamin C, and Vitamin E (Pruteanu et al., 2023). The methods and instruments used to measure antioxidant activity have evolved over the past decade. So far, various chemical assays coupled with highly sensitive and automated detection technologies have been used to evaluate antioxidant activity by specialized methods, such as scavenging activity against different types of free radicals or ROS, reducing power and metal chelation (Munteanu & Apetrei, 2021).

METHODOLOGY

Tools

UV-Vis spectrophotometer (Shimadzu®), analytical balance (Acis®), hot plate (IKA®), micropipette, pipette, filter paper, beaker glass (Pyrex®), measuring cup (Pyrex®), beaker (Pyrex®), rotary evaporator (Buchi®) and blender (Philips®).

Materials

The materials used in this study include malay apple (*Syzygium malaccense*) seeds samples, ethanol 96% (Brataco®), methanol p.a (Brataco®), ethanol p.a (Brataco®), DPPH (Sigma Aldrich®), aquadest.

Experimental Procedure

Sample Preparation

Malay apple (*Syzygium malaccense*) seeds samples were sorted, washed, and dried. The seeds is traditionally cleaned, dried under the sun, and collected at night. The procedure is repeated for seven cycles each day, totaling seven days. The dried samples were pulverized using a blender and then stored in a dry, tightly closed place.

Preparation of Ethanol Extract and Fractions of Malay Apple (Syzygium malaccense) Seeds

A 500 g sample was macerated using 96% ethanol for 3x24 hours with occasional stirring and then filtered. The filters were obtained and thus concentrated using a rotary evaporator to get a thick extract. The extract obtained was computed as a percentage yield using the equation:

$$\% \text{ Rendement} = \frac{weight\ extract}{Ssimplicia\ weight} \times 100\%$$

The viscous extract was subjected to a liquid-liquid fractionation process, which entailed the separation of the extract and its subsequent partitioning using water, n-hexane, and ethyl acetate. The extract was dissolved in a 1:1 mixture of n-hexane and distilled water. After approximately 10 to 15 minutes, the aqueous and n-hexane layers will separate in a separatory funnel. Three additional fractionations of the resulting two layers are required to obtain the secondary compounds. Subsequently, the residual water in the n-hexane fraction was combined with ethyl acetate in a 1:1 ratio (ethyl acetate:distilled water), and the separatory funnel was shaken thoroughly until separation occurred. The water fraction was extracted from the residual layer of distilled water separated from the ethyl acetate fraction in the third fractionation. The solvent-soluble components were then evaporated using a rotary evaporator, yielding the condensed fractions of water, n-hexane, and ethyl acetate.

Preparation of Ethanol Extract and Fractions of Malay Apple (Syzygium malaccense) Seeds

A fraction separation procedure is conducted by weighing 5 grams of selected fractions based on the results of antioxidant testing of the fractions in question. Subsequently, the aforementioned fractions are added to a 5-gram silica gel column stationary phase, which has been pulverized into a powdered state. Subsequently, fractionation was conducted once more through vacuum column chromatography, utilizing a silica gel column as the stationary phase and a combination of solvents as the mobile phase. This process permitted the

examination of fractions displaying the most effective separation, as observed through thin-layer chromatography, with the addition of 50 mL of solvent in the initial stage. The elution solvent, which ranges in polarity from non-polar (n-hexane) to polar (ethyl acetate) to polar (ethanol), exhibits an increase in polarity[31]. To ensure optimal density, the sample was deposited at the apex of the column and distributed in a uniform manner, in accordance with the established protocol. Subsequently, the filter paper was positioned on top of the column, and the apparatus was activated. Subsequently, the column was completely emptied following each collection. The liquid vacuum chromatography eluate was then collected in a glass container and subjected to further analysis by thin-layer chromatography using silica gel GF_{254} as the stationary phase and an optimal n-hexan:ethyl acetate:methanol mobile phase. In the event of a similar stain pattern appearing in UV light, the fractions can be collected and combined, thereby facilitating the production of simpler subfractions.

Antioxidant Activity Assay with DPPH Method
Preparation of 50 ppm DPPH Stock Solution

Antioxidant testing with the DPPH method as done in previous studies 5 mg of DPPH was weighed and then dissolved with 100 mL of methanol p.a in a glass volumetric flask.

Blank Absorbance Measurement

3 mL of DPPH stock solution was taken, then wavelength scanning was carried out with a UV-VIS spectrophotometer at a wavelength of 400-800 nm. The absorbance was measured using a UV-VIS spectrophotometer at the maximum wavelength obtained.

Analysis of DPPH Binding Activity with Malay Apple (Syzygium malaccense) Seeds

Subsequently, the fraction samples must be diluted using methanol p.a. as the solvent. This process should be conducted in a manner that allows for the creation of a series of concentration variations within each sample. Subsequently, a 50 ppm DPPH stock solution should be prepared by dissolving 5 mg of DPPH in 100 ml of methanol p.a. A comparison solution, which serves as a control, should then be prepared by combining 2 ml of methanol p.a. with 1 ml of the aforementioned 50 ppm DPPH solution. For the test sample, 2 ml of sample solution and 2 ml of DPPH solution were prepared separately. The samples were then incubated for 30 minutes at 27°C until a color change indicative of DPPH activity occurred. All samples were prepared in triplicate. Absorbance values for all samples, namely those that had been incubated, were determined using a UV-vis spectrophotometer at a wavelength of 517 nm.

Data Analysis

Antioxidant activity can be known by obtaining % inhibition data and $IC_{50,}$ which states the concentration that can reduce free radicals by 50%. The use of the calculation below can determine the percent inhibition value:

$$\% \text{ inhibition} = \frac{Blanko\ Absorbance - Sample\ Absorbance}{Blanko\ Absorbance} \times 100\%$$

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening of simplisia, extracts and fractions aims to determine the presence of secondary metabolite compounds present in the guava bol seed simplisia used and can also be used as a qualitative description of the simplisia content. The chemical content test aims to provide an initial description of the composition of the chemical content. Positive results of metabolite compound content can be seen in the reaction caused after giving the reagent according to the compound group. Qualitative testing carried out in this study was carried out on simplisia, extracts, ethyl acetate fractions, water fractions, and n-hexane fractions, which included identification of alkaloids, flavonoids, tannins, saponins, and steroids / triterpenoids using reagents according to the class of compounds to be tested. And from the tests carried out, it can be seen the class of compounds contained in the research samples used. From the qualitative testing carried out can be seen in table 1.

Table 1. Phytochemical Screening

Compound	Reagents	Sample					Description
		S	E	FE	FH	FA	
Flavonoid	+Mg+HCl+amil alkohol	+	+	+	+	+	Orange
Alkaloid	H_2SO_4 + P.Dragendorf	+	+	+	+	+	Brick Sediment
	+ P.Mayer	+	+	+	+	+	White sediment
Tannin	+NaCl 10% + Gelatin 0,5%	+	+	+	+	+	White sediment
Saponin	shaking + HCl 2N	+	+	+	+	+	Stabilized foam
Steroid/Triterpenoid	Eter+Asam Asetat Anhidrat+H_2SO_4	+	+	+	+	+	Blue green color

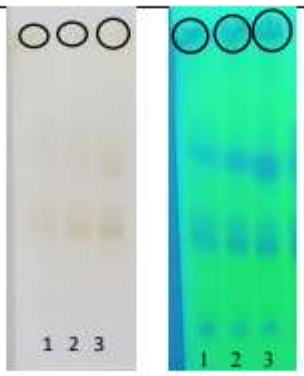
Keterangan : S = Simplisia (+) = Detected
E = Extract (-) = not Detected
FE = Ethyl Fraction
FH = N-hexan Fraction
FA = Water Fraction

From the phytochemical screening carried out on simplisia, water fraction extracts, n-hexane fractions, and ethyl acetate fractions, it was found that the simplisia, water fraction extracts, n-hexane fractions, and ethyl acetate in guava bol seeds positively contained flavonoid compounds, alkaloids, tannins,

saponins, and steroids/triterpenoids seen from the reactions caused after giving reagents to the test samples.

In the fraction sample testing, TLC testing was also carried out for flavonoid compounds because it was suspected that flavonoid compounds had a role as antioxidants from guava seeds. In the flavonoid compound testing using TLC, the stationary phase was silica gel GF254 and the mobile phase was Butanol: Glacial Acetic Acid: Water (4:1:5) which produced spots as shown in the following table 2.

Table 2. Chromatography Profile of Qualitative Test

Senyawa	Plat KLT	Ket
Flavonoid		(1) Fraksi Air (2) Fraksi n-Heksan (3) Fraksi Etil Asetat Positif = warna kuning setelah diberi uap amonia

From the test results obtained the results of the n-hexane fraction, the water fraction and the ethyl fraction showed a yellow stain after being given ammonia vapor which indicated that the positive fraction contained flavonoid compounds so that the antioxidant activity test was continued. The compounds contained in the ethyl acetate fraction extract are flavonoids, This shows that the compound is semipolar because it dissolves in semipolar solvents. While for the compounds contained in the water fraction extract are flavonoids. This shows that all these compounds are polar because they dissolve in polar solvents. The n-hexane fraction also positively contains flavonoid compounds. N-hexane is a non-polar compound, there are several types of flavonoids that can dissolve in non-polar solvents such as polymethoxy aglycones or isoflavones whose sugar groups or glycoside forms have been released so that they can only dissolve in non-polar solvents.

Extraction and Fractination

Extraction was carried out using the maceration method. The extraction process uses a cold extraction method, namely maceration, because the guava bol seed sample is thought to have active substances that have the potential to act as antioxidants which are generally non-volatile components. Using this extraction method is expected not to cause degradation of metabolites that are not resistant to heating. The yield of guava seed extract obtained can be seen in Table 3.

Table 3. Yield of Extract

Sample	Weight of Dry Simplified Powder (g)	Weight of Ethanol Extract (g)	Rendement (%)
Extract	500	66,67	13,34

Bioactive compounds are more effectively bound by solvents that have high polarity. Polar bioactive compounds will be optimal if extracted using polar solvents compared to non-polar solvents. The choice of solvent used in this study is based on the polarity index and the polarity of the intended compound. Based on the polarity index, it is known that the polarity index in ethanol is 5.2 (Abarca-Vargas et al., 2016), so that ethanol solvent can be used as a solvent for extraction which is expected to attract compounds that can provide antioxidant activity in malay apple seed samples used in this study..

Table 4. Yield of Fractions

Ethanol Extract (g)	Solvent	Weight of Fraction	Rendement (%)
35,25	Water	14,55	41,28
	N-Heksan	10,68	30,3
	Ethyl Acetat	7,14	20,26

Fractionation is carried out with the aim of separating compounds into simpler groups of compounds. In this study, fractionation was carried out using the liquid-liquid extraction method. The thick extract of malay apple seeds was fractionated using liquid-liquid fractionation to separate the compound content contained in the extract according to the level of polarity. The partition separation method uses two immiscible solvents whose polarity increases. The principle of partition separation is like dissolve like which means polar organic compounds will mostly exist in the polar phase, while non-polar organic compounds will mostly exist in the non-polar phase. The three solvents used in this study are n-hexane which has a polarity index of 0 which means it is non-polar, ethyl acetate which has a polarity index of 4.4 which means ethyl acetate is able to dissolve semi-polar compounds, while water has a polarity of 9.0 which means it is very polar so that it can attract polar compounds.

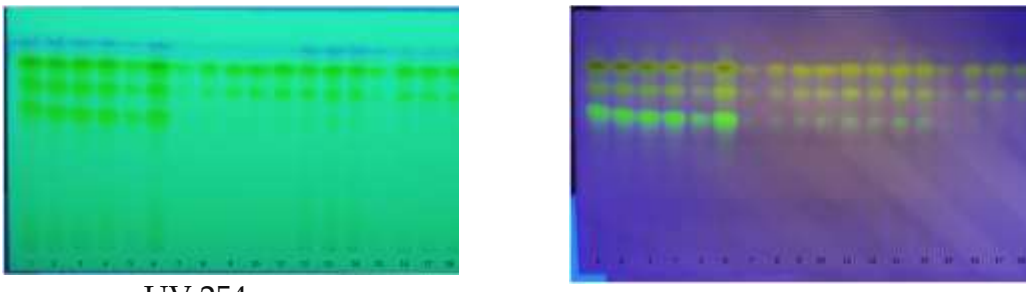
Liquid Vacuum Chromatography

In the selected ethyl acetate fraction results then continued the separation process using the VLC method to separate the active compounds contained in the guava bol seed sample which has the best antioxidant activity seen from the IC50 value obtained previously.

The analysis was performed using TLC using silica gel GF_{254} stationary phase and the most optimal mobile phase of n-hexan:ethyl acetate and n-hexan:ethyl acetate:methanol. From the various phases used, the second mobile phase, namely n-hexan:ethyl acetate (2:8) is the optimal mobile phase for TLC of VLC results. Then the selected ethyl acetate fraction was separated using the VLC method using silica gel GF_{254} stationary phase and the mobile phase

obtained from the optimization results using n hexanes: ethyl acetate (2:8). The selection of the VLC method is because the sample is expected to migrate quickly in the stationary phase and mobile phase during vacuum conditions so that it can produce compounds better (Zhang et al., 2018). From the separation using VLC, 19 eluate results were obtained. The eluate produced through VLC is collected into a glass bottle, the separation of a number of fractions can be seen from the appearance of color differences in the compounds that come out with the mobile phase where the darkest color to near clear resembles the mobile phase is considered not to contain active compounds.

The eluate obtained was then eluted using TLC using the optimal mobile phase, namely hexanes: ethyl acetate (2:8) and obtained the same chromatogram pattern visible in UV light which was then combined so that the subfraction results were obtained. The chromatogram results obtained can be seen in Figure 1.



UV 254 nm UV 366 nm

Figure 1. Chromatogram Result

On the observation of UV 366 nm TLC plate clearly visible single stain separation. Grouping on TLC separation that has been done based on the color and height of the stain produced after separation. In the eluate stains 1-6 have a fluorescent color when viewed at UV 366 nm so grouped into 1 subfraction. The stains of eluate 1-6 have the same stain so that they are combined into subfraction F1, as well as for stains 7-14 have the same color and stain when viewed using UV 366 nm so that they are combined into subfraction F2. Stains of eluate 15-19 have colors and stains that are getting fainter after elution when viewed using UV 366 nm so that they can be combined into subfraction F3. The TLC results that have been carried out also produce a yellow color in visible light which can also be concluded that the possibility of compounds suspected of having antioxidant activity is a flavonoid group compound.

Antioxidant Activity

1. Antioxidant Activity from Extract and Fractions

Antioxidant activity testing in this study was carried out with samples of water, ethyl acetate, and n-hexane fractions with a comparison used vitamin C using the DPPH method with a wavelength of 514 nm obtained from the results of scanning the maximum wavelength to measure the absorbance of samples and vitamin C which was then used to determine the IC_{50} value with an absorbance of 0.712 (range 0.2-0.8) which can be seen in the appendix. IC_{50} value is a value to measure antiradical activity that can reduce 50% of free radical activity by

antioxidant compounds. Then the next operating time obtained from the Vitamin C control sample is at the 30th minute. From the results of antioxidant activity testing that has been done, the results are obtained as in the following table:

Table 5. Antioxidant Activity (IC_{50}) from Extract and Fractions

Sample	IC_{50} (ppm)	AAI	Category
Vitamin C	29,814±0,532	1,174±0,021	Strong with Strong Intensity
Ekstrak	110,167±7,038	0,319±0,02	Medium with Weak Intensity
Fraksi Air	190,803±3,985	0,183±0,004	Low with Weak Intensity
Fraksi N-Heksan	161,954±3,196	0,216±0,004	Low with Weak Intensity
Fraksi Etil	84,094±2,291	0,416±0,012	Strong with Weak Intensity

In this study, the antioxidant activity of the sample was tested using the DPPH method with Vitamin C as a positive control. Vitamin C is one of the compounds that is widely recognized as an antioxidant. Vitamin C donates electrons so as to prevent other compounds from being reduced by radical compounds so that it can be used as one of the positive controls for antioxidant testing (Reskita Cahyani et al., n.d.).

The use of the DPPH test method itself is one of the most commonly used methods because it is one of the most stable radical compounds compared to other examples of radicals, so that when used as a reagent it is simply dissolved and does not need to be reacted with other reagents. When DPPH reacts with hydrogen donors, reduced DPPH is formed as seen from the loss of purple color to yellow as antioxidants are added (Reskita Cahyani et al., n.d.). Antioxidant activity is indicated by the IC_{50} value which is the concentration of antioxidant to reduce 50% of antioxidant activity. The IC_{50} value is inversely proportional to the antioxidant ability of the compound. The smaller the IC_{50}, the greater the ability of the compound to reduce free radicals. IC_{50} value < 50 ppm indicates very strong antioxidant power, IC_{50} value 51-100 ppm indicates strong antioxidant power, IC_{50} value 101-250 ppm indicates moderate antioxidant power, IC_{50} value 250-500 ppm indicates weak antioxidant power, and IC_{50} value > 500 ppm indicates inactive antioxidant power. Furthermore, observations were made through the Antioxidant Activity Index (AAI) parameter. AAI is calculated by considering the mass of DPPH and the mass of the test compound in the reaction, resulting in a constant for each compound.

In the study of antioxidant activity using DPPH with positive control comparator vitamin C and obtained data as in table 5. In the table, it is known that there is antioxidant activity in the ethanol extract of jambu bol seeds used which has a level of antioxidant power that is included in the medium seen from the IC_{50} value of 110.167 ppm (100-150 ppm) and has low antioxidant activity when viewed from the AAI value of 0.319 (<0.5). This may be due to the fact that there are still many compounds contained in the extract that allow relatively moderate antioxidant activity compared to the vitamin C control used, which has a very strong antioxidant strength level (<50 ppm) seen from the IC_{50} value of 29.814 ppm and strong antioxidant capacity (1-2) seen from the resulting AAI value of 1.174. Furthermore, the ethyl acetate, water, and n-hexane fractions were

also tested and the results showed that the level of antioxidant power tested in the ethyl fraction had a better level of antioxidant activity compared to the water and n-hexane fractions. The IC_{50} value of the ethyl fraction has a value of 84.094 ppm which has a relatively moderate level of antioxidant power (50-100 ppm) and has a weak antioxidant capacity of 0.416 (<0.5). While from table 5. shows the results that the water fraction and n-hexane fraction samples have a relatively weak level of antioxidant activity (150-200 ppm) seen from the IC_{50} value of 190.803 ppm in the water fraction and the IC_{50} value of 161.954 ppm and a relatively low antioxidant capacity (<0.5) seen in the AAI value of 0.183 in the water fraction and 0.216 in the n-hexane fraction. The ethyl fraction has the highest antioxidant activity compared to the n-hexane and water fractions, possibly because the compounds contained act as antioxidants, namely tannins and flavonoids, which are attracted to the semipolar ethyl acetate solvent. The homogeneity test using SPSS also showed that the data used were homogeneous ($p>0.05$). A normality test was then performed, indicating that the data were normally distributed ($p>0.05$). A further ANOVA test showed that the data used showed significant differences ($p<0.05$). The ethyl fraction had the highest antioxidant activity compared to the n-hexane and water fractions, possibly due to the presence of compounds that act as antioxidants, namely tannins and flavonoids, which are attracted to the semipolar ethyl acetate solvent.

Ethyl acetate solvent can attract semipolar secondary metabolite compounds such as flavonoids and tannins. Ethyl acetate solvent is a solvent used to extract compounds with intermediate polarity such as flavonoids and tannins. Free flavonoids such as flavones, flavones, isoflavones, and flavanols are semi-polar so they dissolve in semi-polar solvents (Rahayu Santi dan Made Sukadana, n.d.). This allows the antioxidant activity in the ethyl fraction to be stronger. From the observation through the IC_{50} value that has been done on the ethanol extract, water fraction, n-hexane fraction, and ethyl acetate fraction samples, it is known that these samples have the strongest antioxidant activity in the ethyl acetate fraction sample so that further separation can be continued using the VLC method.

2. Antioxidant Activity from Subfractions

Furthermore, the combined subfractions were tested for antioxidant activity quantitatively using the DPPH method with the same testing procedure as that of the fractions. From the results of antioxidant activity testing that has been done obtained the results as in the following table:

Table 6. Antioxidant Activity (IC_{50}) from Subfractions

Sample	**IC_{50} (ppm)**	**AAI**	**Category**
F1	31,009±0,495	1,129±0,018	Very Strong with Strong Intensity
F2	19,618±0,187	1,784±0,017	Very Strong with Strong Intensity
F3	82,021±2,130	0,427±0,011	Strong with Weak Intensity

Description: F1 eluate 1-6**, F2** eluate 7-14**, F3** eluate 15-19

The analysis of the test results presented in Table 6 reveals that the F2 subfraction exhibits notably potent antioxidant properties, evident from the IC_{50}

value of 19.615 ppm (<50 ppm) and an AAI value of 1.784, signifying a robust antioxidant capacity falling within the range of 1.0-2.0. Similarly, Table 6 illustrates that the F1 subfraction also demonstrates significant antioxidant activity, with an IC_{50} value of 31.009 ppm (<50 ppm) and an AAI value of 1.129, indicating a strong intensity antioxidant capacity within the AAI value range of 1.0-2.0. Furthermore, the F3 subfraction displays antioxidant activity with an IC_{50} value of 82.021 ppm (50-100 ppm) and an AAI value of 0.427, suggesting a weak intensity antioxidant capacity level (AAI <0.51). The comprehensive antioxidant assessments conducted confirm that the F2 subfraction exhibits remarkably potent antioxidant activity, reflected in the IC_{50} value of 19.615 ppm (<50 ppm) and an AAI value of 2.549, denoting an exceptionally strong antioxidant capacity (>2). The homogeneity test using SPSS also showed that the data used were homogeneous ($p>0.05$). A normality test was then performed, indicating that the data were normally distributed ($p>0.05$). ANOVA test was then conducted, which showed significant differences between the data used ($p<0.05$), as seen in the appendix. Based on the antioxidant testing, the results showed that subfraction F2 had relatively strong antioxidant activity, as seen from the IC50 value of 19.615 ppm (<50 ppm), and the capacity level was seen from the AAI value of 2.549, indicating very strong antioxidant capacity (>2).

Antioxidants are stable molecules or compounds capable of donating electrons or hydrogen to free radicals, thereby neutralizing them and reducing their capacity to initiate chain reactions. These antioxidants effectively interact with free radicals, halting chain reactions and safeguarding essential molecules from damage. Within the human body, flavonoids serve as potent antioxidants, particularly beneficial for cancer prevention. Their advantages encompass shielding cell structures, enhancing vitamin C efficacy, anti-inflammatory properties, osteoporosis prevention, and antibiotic effects. Flavonoids can directly act as antibiotics by interfering with the function of microorganisms like bacteria or viruses (Sembiring et al., 2016). Flavonoids, a class of polyphenols, are categorized according to their chemical composition and synthesis. The classification of flavonoids is based on structural distinctions, particularly in carbon substitution within the central aromatic ring, leading to various pharmacological effects. Flavonoids encompass flavones, flavanones, flavonols, catechins, flavanols, chalcones, and anthocyanins. Due to their capacity to neutralize free radicals, flavonoids are closely associated with antioxidants. The process by which flavonoids combat free radicals can be segmented into three main actions: inhibiting the generation of Reactive Oxygen Species (ROS), neutralizing ROS, and regulating/protecting against oxidative stress with antioxidants. Additionally, flavonoids promote the activity of internal antioxidant enzymes, inhibit enzymes involved in free radical production, and chelate metals. The hydroxyl group located in ring B is recognized for its pivotal role in neutralizing ROS. This hydroxyl group is deemed crucial in the neutralization of free radicals as it facilitates the donation of hydrogen. The hydroxyl group within flavonoids exhibits significant reactivity as a hydrogen donor, thereby stabilizing free radicals. By donating hydrogen and electrons to

hydroxyl, peroxyl, and peroxynitrate radicals, the hydroxyl group on the aromatic ring B effectively renders them harmless (Alfaridz et al., n.d.).

CONCLUSIONS AND RECOMMENDATIONS

From the research concluded that there is antioxidant activity with relatively moderate in the extract seen from the IC50 value of 110.167 ppm with relatively weak AAI antioxidant intensity, there is antioxidant activity in the fraction seen from the IC_{50} value of 161.954 ppm in the N-hexan fraction; IC50 value of 190.803 ppm in the water fraction; and IC50 value of 84.094 ppm in the Ethyl fraction with weak antioxidant intensity seen in the AAI parameters of the three fractions and there is antioxidant activity in subfractions seen from IC50 value of 31.009 ppm with relatively strong AAI antioxidant intensity in subfraction F1, IC50 value of 19.618 ppm with relatively strong AAI antioxidant intensity in subfraction F2 and IC50 value of 82.021 ppm with relatively weak AAI antioxidant intensity in subfraction F3.

FURTHER STUDY

From the research, researchers hope to further expand research on the benefits of the malay apple plant and research hopes that there will be more extensive research on antioxidants in various plants.

ACKNOWLEDGMENT

The researcher would like to thank all those who helped in completing this research.

REFERENCES

Abarca-Vargas, R., Peña Malacara, C. F., & Petricevich, V. L. (2016). Characterization of chemical compounds with antioxidant and cytotoxic activities in bougainvillea x buttiana holttum and standl, (Var. rose) extracts. *Antioxidants*, *5*(4). https://doi.org/10.3390/antiox5040045

Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. In *Journal of Pharmacy and Bioallied Sciences*. https://doi.org/10.4103/jpbs.JPBS_175_19

Alfaridz, F., Amalia, R., Kunci, K., Flavonoid, :, & Klasifikasi, A.-O. (n.d.). *REVIEW JURNAL : KLASIFIKASI DAN AKTIVITAS FARMAKOLOGI DARI SENYAWA AKTIF FLAVONOID*.

Arifin, B., Ibrahim, S., Kimia, J., Matematika, F., Ilmu, D., & Alam, P. (2018). STRUKTUR, BIOAKTIVITAS DAN ANTIOKSIDAN FLAVONOID STRUCTURE, BIOACTIVITY AND ANTIOXIDAN OF FLAVONOID. *Jurnal Zarah*, *6*(1), 21–29.

Batista, Â. G., da Silva, J. K., Betim Cazarin, C. B., Biasoto, A. C. T., Sawaya, A. C. H. F., Prado, M. A., & Maróstica Júnior, M. R. (2017). Red-jambo (Syzygium malaccense): Bioactive compounds in fruits and leaves. *LWT - Food Science and Technology*, *76*, 284–291. https://doi.org/10.1016/j.lwt.2016.05.013

Devitria, R., Juariah, S., putri, L., Analis Kesehatan Klinikal, J., Putri, L., Studi DIII Analis Kesehatan, P., Farmasi dan Ilmu Kesehatan, F., Juni, D., & Jambu Bol,

B. (2022). UJI AKTIVITAS ANTIOKSIDAN EKSTRAK METANOL BIJI JAMBU BOL (Syzygium malaccense L) DENGAN METODE DPPH (2,2-Diphenyl-1-picrylhidrazil). In *Klinikal Sains* (Vol. 10, Issue 1). http://jurnal.univrab.ac.id/index.php/klinikal

Devitria, R., Wulandari, R., & Elfia, M. (2023). WATER SOLUBLE ASH CONTENT AND ACID INSOLUBLE ASH CONTENT TEST ON GUAVA SEED SIMPLICIA (Sygyzium malaccense) UJI KADAR ABU LARUT AIR DAN KADAR ABU TIDAK LARUT ASAM PADA SIMPLISIA BIJI JAMBU BOL (Sygyzium malaccense). *Jurnal Ilmu Kesehatan Abdurrab)*, *1*(2), 12–16.

Dontha, S. (2016). A review on antioxidant methods. *Asian Journal of Pharmaceutical and Clinical Research*, *9*(2), 14–32. https://doi.org/10.22159/ajpcr.2016.v9s2.13092

Fauziah, N., Noviyanti, N., & Musthapa, I. (2019). PEMANFAATAN KAYU BATANG JAMBU BOL (Syzygium malaccense (L). Merr. & Perry) SEBAGAI SUMBER ANTIOKSIDAN BARU. *Jurnal Ilmiah Farmako Bahari*, *10*(1), 33. https://doi.org/10.52434/jfb.v10i1.522

Ferrante, R., Boccuni, F., Tombolini, F., & Iavicoli, S. (2018). Measurement techniques of exposure to nanomaterials in workplaces. *Nanotechnology in Eco-Efficient Construction: Materials, Processes and Applications*, 785–813. https://doi.org/10.1016/B978-0-08-102641-0.00030-X

Gibbert, L., Sereno, A. B., Andrade, M. T. P. de, Silva, M. A. B. da, Miguel, M. D., Montrucchio, D. P., Messias-Reason, I. J. de, Dantas, A. M., Borges, G. da S. C., Miguel, O. G., Kruger, C. C. H., & Dias, J. de F. G. (2021). Nutritional composition, antioxidant activity and anticancer potential of Syzygium cumini (L.) and Syzygium malaccense (L.) fruits. *Research, Society and Development*, *10*(4), e5210413743. https://doi.org/10.33448/rsd-v10i4.13743

Husna, P. A. U., Kairupan, C. F., & Lintong, P. M. (2022). Tinjauan Mengenai Manfaat Flavonoid pada Tumbuhan Obat Sebagai Antioksidan dan Antiinflamasi. *EBiomedik*, *10*(1), 76–83. https://doi.org/10.35790/ebm.v10.i1.38173

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences*, *22*(7). https://doi.org/10.3390/ijms22073380

Njila, M. I. N., Mahdi, E., Lembe, D. M., Nde, Z., & Nyonseu, D. (2017). Review on Extraction and Isolation of Plant Secondary Metabolites. *7th Int'l Conference on Agricultural, Chemical, Biological and Environmental Sciences*. https://doi.org/10.15242/iie.c0517024

Nunes, P. C., De Souza Aquino, J., Rockenbach, I. I., & Stamford, T. L. M. (2016). Physico-chemical characterization, bioactive compounds and antioxidant activity of Malay apple [Syzygium malaccense (L.) Merr. & L.M. Perry]. *PLoS ONE*, *11*(6), 1–11. https://doi.org/10.1371/journal.pone.0158134

Nurhasnawati, H., Handayani, F., & Sukarmi. (2017). Sokletasi Terhadap Aktivitas Antioksidan Ekstrak Etanol Daun Jambu Bol (Syzygium malaccense L .). *Jurnal Ilmiah Manuntung*, *3*(1), 91–95.

Pruteanu, L. L., Bailey, D. S., Grădinaru, A. C., & Jäntschi, L. (2023). The Biochemistry and Effectiveness of Antioxidants in Food, Fruits, and Marine

Algae. In *Antioxidants* (Vol. 12, Issue 4). MDPI. https://doi.org/10.3390/antiox12040860

Rahayu Santi dan Made Sukadana, S. I. (n.d.). AKTIVITAS ANTIOKSIDAN TOTAL FLAVONOID DAN FENOL KULIT BATANG GAYAM (Inocarpus fagiferus Fosb). In *JURNAL KIMIA* (Vol. 9, Issue 2).

Reskita Cahyani, D., Fitri Faradilla, R., & Ilmu dan Teknologi Pangan Fakultas Pertanian Univeristas Halu Oleo Kendari, J. (n.d.). Evaluasi Metode In Vitro Pada Analisis Aktivitas Antioksidan Beberapa Buah Tropis: Studi Kepustakaan [Evaluation In Vitro Method Analysis In Antioxidant Activities of Some Tropical Fruits:A Review]. *J. Sains Dan Teknologi Pangan*, *5*(6).

Sabotič, J., Bayram, E., Ezra, D., Gaudêncio, S. P., Haznedaroğlu, B. Z., Janež, N., Ktari, L., Luganini, A., Mandalakis, M., Safarik, I., Simes, D., Strode, E., Toruńska-Sitarz, A., Varamogianni-Mamatsi, D., Varese, G. C., & Vasquez, M. I. (2024). A guide to the use of bioassays in exploration of natural resources. *Biotechnology Advances*, *71*, 108307. https://doi.org/10.1016/J.BIOTECHADV.2024.108307

Sembiring, E., Sangi, M. S., & Suryanto, E. (2016). AKTIVITAS ANTIOKSIDAN EKSTRAK DAN FRAKSI DARI BIJI JAGUNG (Zea mays L.). *Chem. Prog*, *9*(1). https://doi.org/10.35799/cp.9.1.2016.13908

Senapati, M. R., & Behera, P. C. (2023). Novel extraction conditions for phytochemicals. *Recent Frontiers of Phytochemicals: Applications in Food, Pharmacy, Cosmetics, and Biotechnology*, 27–61. https://doi.org/10.1016/B978-0-443-19143-5.00019-0

Sholikhah, E. N. (2016). Indonesian medicinal plants as sources of secondary metabolites for pharmaceutical industry. *Journal of Thee Medical Sciences (Berkala Ilmu Kedokteran)*, *48*(04), 226–239. https://doi.org/10.19106/jmedsci004804201606

Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. In *Chinese Medicine (United Kingdom)* (Vol. 13, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s13020-018-0177-x