Research Article

DOI: 10.22146/ifnp.76137 https://journal.ugm.ac.id/ifnp
ISSN 2597-9388

Journal of Indonesian Food and Nutrition Progress



# Chemical Characteristics of Waru Leaf (*Hibiscus tiliaceus*) As Food Packaging Material

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Submitted: July 9th, 2022; Revised: October 10th, 2023,
Accepted: October 13th, 2023; Published: November 27th, 2023

**ABSTRACT**: Waru leaf (*Hibiscus tilaceus*) is commonly used as a traditional food packaging material in Indonesia. Waru leaf is known to contain bioactive components with antioxidant and antimicrobial activity. This research aimed to determine the chemical properties of waru leaf of various maturity levels and provide information on the potency of leaf extract that can be used as an active packaging additive. The chemical properties include fatty acids, volatile compounds, antioxidant activity, and total phenolic content. It was discovered that both the young and mature waru leaf contained 4 types of fatty acids: predominantly linoleic acid and linolenic acid. In the young waru leaf, the primary volatile compounds were acids, with cis-vaccenic acid (21%) and glycidyl palmitate comprising (16.8%). As for the mature leaf, the main volatile compounds were acids and esters, with glycidyl oleate at (18.52%), glycidyl palmitate (12.28%), and Decocanoic acid, 3-hydroxy- (7.85%). The crude methanol extract was fractionated with hexane, ethyl acetate, and butanol to analyze bioactive compounds. The highest antioxidant activity was found in ethyl acetate fraction with an average of %Radical Scavenging Activity (%RSA) of 36.25% on the young waru leaf and 29.92% on the mature waru leaf. The ethyl acetate fraction had the highest total phenolic content of 164.66 mg GAE/g and 146.50 mg GAE/g for young and mature waru leaves, respectively. The result shows that waru leaf extract is a potential ingredient for developing active packaging.

**Keywords:** antioxidant, bioactive, food packaging material, total phenolic, waru leaf

## INTRODUCTION

Packaging plays an important role in preserving and maintaining the quality of agricultural products. A wrapper can protect the food inside it from exposure to moisture, oxygen, and other gases and from physical disturbances (Risch, 2009). There are two types of packaging materials: natural and artificial packaging. Natural packaging materials are biodegradable and environmentally friendly (Casey, 2015). People in some regions of Indonesia use packaging made from natural materials to package traditional foods, especially from leaves (Opara, 2013). Waru (*Hibiscus tilaceus*) leaf has been commonly used as food packaging material since long ago. The modern lifestyle has shifted from traditional packaging materials to more contemporary ones, such as paper cups and plastics (Noviadji, 2014). However, these materials are not environmentally friendly, and hence it is crucial to find alternative materials that are more eco-friendly. Common materials used for food packaging include paper, glass, and plastic (Pergiovanni & Limbo, 2016). The packaging industry is the largest contributor to plastic production globally, as per De Kock (2020), which poses a significant threat to the environment due to the resulting plastic waste.

Waru leaves are used as the packaging of tempe in Yogyakarta and Central Java. The reason for choosing waru leaf for packaging materials on traditional foods has not been supported by scientific studies. It is expected that waru leaf contains bioactive components with antioxidant and antimicrobial activity. Previous studies reported that young leaf of waru had higher free radical scavenging activity (76%), total phenolic (26.23 mg GAE/g), and flavonoid (17 mg Quercetin/g) content than the mature one (71%, 25 mg GAE/g, 12 Quercetin/g), respectively (Nivas *et al.*, 2010). In addition, it has been found that waru leaf exhibited antioxidant activity with an $IC_{50}$ of 86.5 µg/ml, as Ramproshad *et al.* (2012) reported. However, the leaf itself as food packaging material, especially in the most optimum age of maturation of waru leaf for use in food packaging, has not yet been studied.

In 2017, a study conducted by Sari found that tempeh packaged with waru leaf has the highest antioxidant activity of 53.43%, compared to that packed with banana leaf, teak leaf, and plastic. The presence of antioxidants in waru leaf indicates that it contains bioactive compounds that play a significant role in natural product packaging. A different study has confirmed that certain leaves used for natural packaging contain phenolic content. Guava leaves with 100 ppm ethyl acetate fraction, for instance, had phenolic content of 643.7 GAE/g (Amalina, 2017)

and steamed banana leaves with a fraction of 100 ppm ethyl acetate fraction contained a total phenolic content of 923.0 GAE/g (Putra, 2017). Food packaging made from leaves has the potential to be active packaging due to its bioactive compounds. Active packaging refers to packaging that is specifically designed to preserve or extend the shelf-life of food. A technique called active packaging involves adding specific elements to food packaging that can either release or absorb compounds from the food or its surroundings. This helps to preserve the quality of the food and prevent damage (Abreu *et al*., 2012).

Based on the research from Nandangopalan *et al*. (2015), waru leaf contains some volatile compounds, such as 2-nitro-tertiary butanol, 2-propanone, 1-(1-methyl ethoxy), 2-cyclopentene-1-one, 2-hydroxy, n-dimethylglycine, 4h-piran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, nonane nitrile, 2-asam furancarboxylic, 73etrahydro-3-metil-5-oxo, methyl ester, 2-metoksi-4-vinilfenol, dimethoxyphenol, benzenamin, fitol, octadecanoic acid, methyl ester, eicosatrienoic acid, dan asam hexadecanoate. According to Lazo *et al*. (2009), when unsaturated fatty acids undergo oxidation, they form volatile compounds such as aldehydes, ketones, and alcohol. Oleic acid oxidation, in particular, produces aromatic compounds like hexanal, heptanal, octanal, and nonanal which have an impact on flavor.

In this research, we studied the chemical properties of waru leaves and provided information on their potential use as additives in food packaging materials. Chemical properties included fatty acids profile analysis, volatile compounds analysis, antioxidant activity, and total phenolic compounds of waru leaf were evaluated.

## MATERIALS AND METHOD

### *Samples*

The leaves utilized were young waru leaves, specifically shoots one and two, and mature waru leaves, specifically shoots three, four, and five. The leaves were taken from Gunungkidul, Yogyakarta Special Region, Indonesia.

### *Chemicals and reagents*

Chemicals used were methanol 80%, methanol P.A., ethyl acetate, n-hexane, n-butanol, gallic acid, Folin Ciocalteu (Merck), 2,2-Diphenyl-l-picrylhydrazyl (DPPH) (Sigma Aldrich), Butylated hydroxytoluene (BHT) 9Sigma Aldrich), anhydrous $Na_2SO_4$, pentane, dichlorometane, liquid nitrogen and gas, sodium carbonate, Petroleum Ether (PE), sodium methanolic, boron trifluoride methanoate.

### *Sample Preparation*

The leaves were plucked, sorted, and trimmed into good-quality pieces for the leaf preparation. After sample preparation, analysis of chemical properties including fatty acid profile, volatile compounds, total phenolic, and antioxidant activity were performed.

### *Extraction*

Leaf powder was used for extraction, antioxidant, and total phenolic content analysis. First, waru leaves were dried in a cabinet dryer at 50 $^oC$ for 17 hours to decrease water content and to facilitate the grinding to produce leaf powder. After the drying process ended, the dried leaf was grinded using a blender (Philips) until it became powder and sieved using a 40 mesh sieve. The extraction was done by dissolving 10 g of waru leaf powder in 800 ml of 80% methanol (1:8) and macerating using a shaker for 48 hours. Next, the leaves residue was separated from the extract using Whatman filter paper (no. 42) to get the crude extract. After the filtration, the extract was evaporated with a rotary evaporator at 50 $^oC$ and 335 atm pressure to get the concentrated extract. The concentrated extract was dissolved in aquades:methanol (9:1) mixture with extract and solvent ratio of 1:2. The mixture was partitioned using a separatory funnel with ethyl acetate (1:5), butanol (1:3), and hexane (1:5). Each solvent used had a different dielectric constant; methanol was 32.70 (at 25 °C), ethyl acetate was 6.02 (at 25 °C), butanol was 9.9 (at 25°C), and hexane was 1.88 (at 25 °C). Each fraction was evaporated using rotary evaporation and exhaled with nitrogen gas to get the dry extract. The next step was to determine the total phenolic and antioxidant activity from each fraction of dry extract (Firdausi *et al.*, 2015).

### *Fatty acid profile*

The fatty acid profile was performed using an Agilent Gas Chromatograph (GC) equipped with a Flame Ionized Detector (FID). The analysis was performed using a Gas DB-23 column, a length of 30 m, an inner diameter of 0.25mm, a particle layer diameter 0.25 µm, and nitrogen as the carrier gas. The volume of sample injected was 1 µL, the injector temperature was held at 100 °C for 5 minutes, the oven temperature rose to 275 °C at a rate of 4 °C/min and was held for 10 minutes. The heating temperature system was isothermal at an initial temperature of 290 °C then held for 15 minutes (Ji *et al.*, 2017). The fat of the obtained leaf samples was methylated, 1.5 ml of sodium solution was added to 0.5 ml of fat sample, and the mixture was heated at 80 °C for 20 minutes while shaking. The sample was cooled after the heating was completed. Next, 2 ml of methanoate boron trifluoride was added to the sample, heated again at 80 °C for 20 minutes, and cooled. Then, 1 ml of hexane and 1 ml of saturated NaCl are added and extracted. The top of layer formed was removed, and placed in an Eppendorf tube, the methylated sample was injected into the GC and detected with a FID detector (detector temperature of 285 °C). This test used a Supelco branded Methyl Ester (FAME) the standard mixture (Socaci *et. al.*, 2009). This was a standard containing a mixture of several types of fatty acids. The method of identifying the leaf fatty acid profile was to compare the peak retention time

of the fatty acid chromatogram sample with the retention time of the chromatogram peak at an external standard of known composition and concentration and to compare the peak area of each sample of fatty acid to the standard so

that the concentration is known (Socaci *et. al.*, 2009).

***Analysis of volatile compounds***

Waru leaves were reduced to ± 2cmx2cm and weighed 30 g. Nitrogen (liquid) was added to the leaf and ground to obtain powder. Subsequently, pentane:dichloromethane (2:1, 200mL:100mL) was added to this powder and mixed with a shaker (190 rpm). The mixture was then macerated at 4°C overnight. The next step was to separate the leaf extract from the leaf residue and treat it with anhydrous $Na_2SO_4$ to remove the water. After filtration with Whatman filter paper (no. 42), the residual solvent was carefully concentrated to a small amount (about 5 mL) using a rotary evaporator. The concentrated extract (1-2 µL) in pentane:dichloromethane was analyzed by GC–MS to detect chemical components. Testing of volatile compounds was carried out using Agilent GC-MS, 30m DB-WAX column, 0.25 mm diameter and 0.25 µm diameter stationary particle layer diameter, helium carrier gas at a flow rate of 0.8 ml/min, 0.5 µL sample with a 100: 1 split ratio and 250 °C injector temperature. The temperature system used was isothermal at an oven temperature of 45 °C. Then, it increased to 250 °C at a rate of 4 °C/min and held for 5 minutes. The properties of the volatile compound were determined by comparing the mass spectra of the database pre-programmed with the sample analyte on GC-MS (Kuo, *et. al.*, 2006).

***Total phenolic content***

The total phenolic content was determined by putting 1 ml of dry extract in a test tube, then adding 5 ml of 2% $Na_2CO_3$ into the tube and incubating the mixture for 10 minutes. The next step was the addition of 0.5 ml of Follin Ciocalteu into the mixture, homogenization using a vortex, and incubation for 30 minutes. The absorbance was measured using a spectrophotometer at 760 nm wavelength. The absorbance result was compared to the gallic acid calibration curve to get the Gallic Acid Equivalent result (Perez *et al*., 2015). The total phenolic was presented in mg GAE/g.

***Antioxidant activity***

Antioxidant activity was determined using DPPH (2,2-Diphenyl-1-picrylhydrazyl) method. The first step, the dry extract was diluted in 95% ethanol, and then 0,1 ml was put in a test tube. 2.9 ml DPPH 0.2 M was added into the test tube and mixed using a vortex and incubated for 30 minutes in a dark room. Absorbance was measured in a spectrophotometer UV Vis (Thermoscientific Spectronic 200) at 517 nm wavelength (Aree *et al.*, 2016).

***Statistical analysis***

The data from the analysis of antioxidant activity and total phenolic were analyzed using multivariate analysis (MANOVA) with a 95% confidence level and Duncan Multiple Range Test to determine the significance of the difference if there were significantly different data results ($p < 0.05$).

**Table 1.** Fatty Acid Profile of Waru Leaf

| Fatty Acid | Relative area (%) | |
|---|---|---|
| | Waru Leaf | |
| | Young | Mature |
| Linoleic Acid | 34.67 | 26.82 |
| Cis-9-Oleic Acid | 0.001 | 9.07 |
| Linolenic Acid | 44.63 | 36.52 |
| Palmitoleic Acid | 20.68 | 23.30 |

## RESULT AND DISCUSSION

***Fatty Acid Profile***

In young and mature waru leaf, four types of fatty acids were detected, dominated by linolenic and linoleic acid, palmitoleic acid and cis-9-oleic acid. The result of fatty acid analysis is shown in Table 1.

The four types of fatty acids are classified as unsaturated fatty acids. The high content of unsaturated fatty acid in waru leaf was due to the photosynthetic process. Free fatty acid, which can interfere with electron transfer for energy production, is undesirable in photosynthesis. Free fatty acids may be present when the amount of undeveloped saturated fat leaf is high (Zhang *et. al.*, 2010). The different stages of plant development can affect the amount, type, and quantity of the plant metabolite (Nayeem & Karvekar, 2010). Some fatty acids increase during the maturity process, while some decrease. Linoleic and linolenic acids are dominant fatty acids from both levels of maturity. The two polyunsaturated fatty acids are dietary essential fatty acids because the human body is incapable of their biosynthesis (He *et al*, 2020). The result showed that both linoleic and linolenic acid decreased as long as the leaf matured. The changes that occurred were thought to be due to the activity of the desaturase enzyme, an enzyme that removes two hydrogen atoms from a single-bond fatty acid to form a double-bond carbon (Arregui *et. al*., 2012).

***Volatile Compounds***

Nowadays, the most ideal method for analysis of volatile bioactive compounds is using GC-MS (Grover & Patni, 2013). Table 2. shows the results of GC–MS analysis of volatile compounds in waru leaf. The waru leaf extracts consisted of a complex mixture of different types of

**Table 2.** Volatile Compounds of Waru Leaf Extract

| Classification | Volatile Compounds | Area (%) | |
|---|---|---|---|
| | | Young Waru Leaf | Mature Waru Leaf |
| Acid | cis-Vaccenic acid | **21.97** | 0.09 |
| Acid | Glycidyl palmitate | **16.80** | **12.28** |
| Ester | 9-Octadecenoic acid (Z)-, hexyl ester | 5.21 | - |
| Acid | trans-13-Octadecenoic acid | 3.87 | 1.37 |
| Ester | Glycidyl oleate | 2.86 | **18.52** |
| Acid | Eicosanoic acid | 2.43 | 7.85 |
| Ester | 11-Octadecenoic acid, methyl ester | 1.17 | - |
| Acid | cis-13-Eicosenoic acid | 0.78 | - |
| Acid | 9-Hexadecenoic acid | 0.68 | 4.77 |
| Alcohol | 2-Methyl-Z,Z-3,13-octadecadienol | 0.32 | - |
| Ether | Ethyl iso-allocholate | 0.27 | 2.91 |
| Acid | Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester | 0.17 | 2.92 |
| Acid | trans-13-Octadecenoic acid | 0.15 | - |
| Alcohol | 1-Heptatriacotanol | - | 2.66 |
| Acid | 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester | - | 2.43 |
| Ester | Oleic acid, 3-(octadecyloxy)propyl ester | - | 2.07 |
| Ester | trans-9-Octadecenoic acid, pentyl ester | - | 1.45 |
| Acid | cis-13-Octadecenoic acid | - | 1.27 |
| Ester | Cyclopropanebutanoic acid, methyl ester | - | 0.90 |
| Acid | 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- | - | 0.81 |
| Alcohol | Octadecanal, 2-bromo- | - | 0.33 |
| Acid | Oleic Acid | - | 0.12 |
| Acid | Dodecanoic acid, 3-hydroxy- | - | 0.04 |
| Acid | Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate | - | 0.03 |

organic compounds. The identified compound contained esters, acids, ethers, and their derivatives.

Based on the analysis result of volatile compounds in Table 2., it was revealed that young and mature waru leaf contained 24 volatile compounds in total, 13 compounds found in young waru leaf and 19 compounds found in mature waru leaf. 4 major compounds of young waru leaf extract were cis-vaccenic acid (21.97%), glycidyl palmitate (16.80%), 9-Octadecenoic acid (Z)-, hexyl ester (5.21%) and trans-13-Octadecenoic acid (3.87%). In comparison, mature waru leaf contained glycidyl oleate (18.52%), glycidyl palmitate (12.28%), Eicosanoic acid (7.85%), and 9-Hexadecenoic acid (4.77%). Cis-vaccenic acid, which is the largest amount of volatile compounds in young waru leaf is found in teak leaf (Dewi, 2020), and in some fruits such as apples, durians, and lemons (Shibahara *et al.*, 1987), also in mango pulp (Shibahara *et al.*, 1986). On the other hand, glycidyl oleate, found in mature waru leaf, can also be found in teak leaf (Dewi, 2020) and vegetable oils (Haines *et al.*, 2011).

The result of the analysis of the volatile compounds showed that most of the compounds detected were in the majority of the acid group. Volatile compounds from the aldehydes, ketones, and alcohol groups are known to derive from various reactions involving fatty acids (Lazo *et al.*, 2017). According to Primary *et al.* (2018), aldehydes and ketones are formed from activities related to chemical reactions, including enzymatic reactions and autooxidation of fats. Therefore, the study confirmed that the number of volatile compounds increases with maturity.

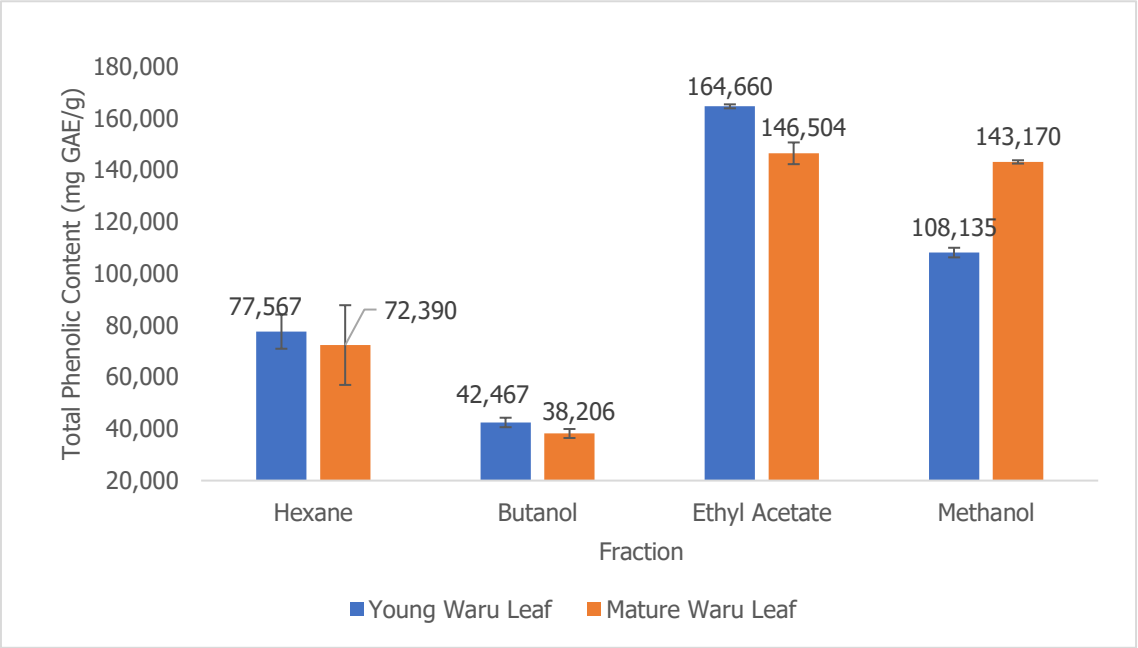


**Figure 1.** Total phenolic of Waru Leaves

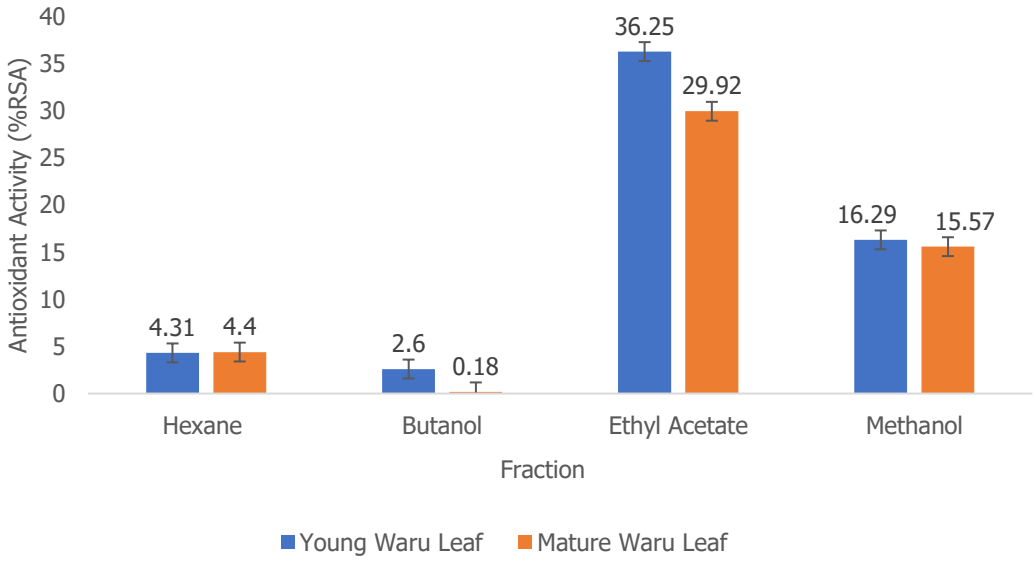


**Figure 2**. Antioxidant Activity of Waru Leaves

Thus, young waru leaves showed a much lower diversity of the compounds than mature waru leaves (Taveira *et al.*, 2009).

***Total Phenolic Content***

The total phenolic from waru leaf extract was analyzed at a concentration of 500 ppm. From the result, the value of young waru extract in the hexane fraction was 77,567 mg GAE/g, the butanol fraction was 42,674 mg GAE/g, the ethyl acetate fraction was 164,660 mg GAE/g, and the methanol fraction was 108,135 mg GAE/g. On the other hand, the result of the total phenolic mature waru leaf extract hexane fraction was 72,390 mg GAE/g, the butanol fraction was 38,206 mg GAE/g, the ethyl acetate fraction was 146,504 mg GAE/g, and the methanol fraction was 143,170 mg GAE/g. The analysis result shows that the fraction with the highest total phenolic yield was the ethyl acetate, followed by the methanol fraction, hexane fraction, and the butanol fraction with the lowest total phenolic content. Figure 1 shows the analysis result of the total phenolic from young and mature waru leaf extract.

The total phenolic content in waru leaf extract was influenced by the type of solvent used during the fractionation process, depending on the degree of polarity. Ethyl acetate is a semi-polar solvent. In other words, the total phenolic compounds involved in the

antioxidant activity of waru leaf extract are semi-polar. In a study by Meenashree *et al.* (2014), the total phenolic content of the petroleum ether extract of banana leaf was 12.19 mg/mL, which was higher than the other solvents such as ethanol and acetone. In a study by Kusumowati *et al.* (2012), the total phenolic ethanol extract of teak leaf was 95.46 mg/g.

***Antioxidant Activity***

The result of the antioxidant activity analysis of young and mature waru leaf extract was carried out with a concentration of 200 ppm. From the result, it was known that the average value of Radical Scavenging Activity (%RSA) of the young waru extract in hexane fraction was 4.307%, the butanol fraction was 2.602%, the ethyl acetate fraction was 36.249%, and the methanol fraction was 16.285%. Meanwhile, the mature waru leaf result for the hexane fraction was 4.397%, the ethyl acetate fraction was 29.924%, the butanol fraction was 0.179%, and the methanol fraction was 15.568%. The analysis shows that the ethyl acetate fraction had the highest antioxidant activity, followed by the methanol fraction, the hexane fraction, and the butanol fraction as the lowest. The result of the antioxidant activity analysis of young and mature waru leaves are shown in Figure 2.

Ethyl acetate is included in the semi-polar solvent that can extract semi-polar compounds. Wikanta *et al.* (2012) stated that chemistry applies the rule of like dissolves like. Semi-polar compounds extracted during the fractionation process are bioactive and have antioxidant potential. This occurred because some antioxidant compounds, whose solubility depends on the polarity of the solvent used during the extraction process, became selective due to the fraction of the crude extract and were concentrated in the ethyl acetate fraction. In the Tanduk banana leaf found that the EC50 value of Tanduk banana leaf extract was 104.36 (yg/mL) and the EC50 value of Cavendish banana leaf ethanol extract was 157.36 (yg/mL) (Nugraheni *et al.*, 2017). A study by Immawati and Marliani (2017) found that the ethanol-water fraction of the read teak leaf extract showed the most active activity compared to the n-hexane and ethyl acetate fractions. The compounds that are thought to be active are total phenolics and flavonoids.

## CONCLUSION

This study shows that the maturity of the leaf affected the chemical component in waru leaf extract, but not significantly. The ethyl acetate fraction showed the highest antioxidant activity and phenolic content, but the difference between mature and young was not significant. It was found that both the young and mature waru leaf contained four types of fatty acids, which were predominantly linolenic acid and linoleic acid. The main volatile compounds in young waru leaf were acids, while the main volatile compounds in mature waru leaf were acids and esters. In the analysis of bioactive compounds, the crude methanol extract was fractionated with hexane, ethyl acetate, and butanol, and the highest antioxidant activity and total phenolic was found in the ethyl acetate fraction. The results show that extract of waru leaf can potentially be used in active food packaging. Further study is needed to determine detailed phenolic content using LC-MS and more trials to measure the product's shelf life with active packaging containing waru leaf extract.

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