LIMPASU PERICARPIUM : AN ALTENATIVE SOURCE OF ANTIOXIDANT FROM BORNEO WITH SEQUENTIAL MACERATION METHOD

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ABSTRACT

Antioxidants are substances that can slow down the oxidation process of free radicals. Limpasu plant (*Baccaurea lanceolata* (Miq) Muell. Arg), an indigenous plant of Borneo, is a natural antioxidant source. The purpose of this study was to determine the antioxidant activity of the limpasu pericarpium extract. The extraction of the limpasu pericarpium was done by maceration method using solvents with increasing polarity ranging from n-hexane, ethyl acetate, and methanol. Antioxidant activities of the three extracts were measured by the DPPH and FRAP methods. The IC_{50} values of n-hexane, ethyl acetate, and methanol extracts, as well as quercetin using the DPPH method were 517,45 µg/mL, 530,64 µg/mL, 10,63 µg/mL and 6,83 µg/mL, respectively. Meanwhile, the IC_{50} values obtained from FRAP method were 198,96 µg/mL, 190,07 µg/mL, 661,36 µg/mL, and 7,09 µg/mL, respectively. The results revealed that the methanol extract is more potent than other extracts tested for antioxidant activity.

Keywords: *Baccaurea lanceolata*; Antioxidant; DPPH; FRAP

INTRODUCTION

A free radical is a molecular atom or compound with one or more unpaired electrons in its outer shell so that it is highly reactive. An imbalance between the number of free radicals and the number of endogenous antioxidants can cause cell damage. Reactive oxygen species can cause oxidative damage leading to chronic diseases such as cancer, neurodegenerative diseases, arthritis, and diabetes mellitus. In a biological system, the body can usually produce its own antioxidants in the form of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. However, the amount of these enzymes is often insufficient. Therefore it requires the intake of foods that contain lots of antioxidants.

Previous studies reported that high consumption of fresh fruits and vegetables may have a protective effect against various chronic diseases caused by oxidative stress in the body.[1] This is mainly due to the presence of antioxidants and bioactive compounds such as vitamin C, carotenoids, phenolics, flavonoids, tannins, and anthocyanidins which can scavenge free radicals and inhibit lipid peroxidation.[2,3]

Based on epidemiological studies with observations of comprehensive analysis in the literature, it is stated that there is unquestionable evidence that consuming higher fruit and vegetables also reduces the risk of cardiovascular disease and cancer.[4,5]

Many people believe that products with natural labels are safer and better for the body. The World Health Organization (WHO) estimates that 80% of the world's population uses herbal medicine to maintain their primary health.[6] Exploration of biological materials and their potential as herbal medicine is currently being carried out. Kalimantan or Borneo is an island rich in medicinal plants that have been used for a long time, especially people in the interior of the Kalimantan forest. One of the native plants of Kalimantan is limpasu (*Baccaurea*

lanceolata), which has been used from generation to generation as traditional medicine. It has been used for headaches, stomach aches, acne medication, and skin care by pounding it and applying it to the part of the skin exposed to the sun. Limpasu is often used for skincare by the Banjar tribe in Central and South Kalimantan which is used topically on the skin to protect the skin from the sun.[7]

Limpasu plant extracts have been studied for their antioxidant activity,[8–10] antibacterial,[11] and sunscreens.[12] Secondary metabolites are contained in the form of phenolic compounds, which generally act as antioxidants. The chemical components in limpasu are included in the phenol, flavonoid, anthocyanin, and carotenoid groups.[8]

In a previous study, Limpasu (*Baccauera lanceolata*) has been analyzed for antioxidants assay (DPPH, FRAP, and ABTS) on it's pericarp, pulp, and seed with a common maceration method and showed the highest antioxidant activity found in the pulp extract.[8] This study aims to determine the antioxidant activity of the limpasu pericarpium extracts with sequential maceration method. Sequential maceration was still not widely practiced.

Figure 1. Plants and fresh fruit of *Baccaurea lanceolata*

MATERIAL AND METHODS

Chemical Materials

This study has used analytical grade ethyl acetate (EtOAc), n-hexane, and methanol (MeOH), Quercetin (Sigma Aldrich, India), DPPH (1, 1-*diphenyl*-2-*picrylhydrazyl*) (Sigma Aldrich, Germany), TPTZ (2,4,6-*tripyridylstriazine*) (Sigma Aldrich, Switzerland), Iron (II) sulfate (pa, sigma-aldrich), Iron (III) sulfate (pa, sigma-aldrich), Tween 20 (pa, sigma-aldrich), FeCl3, HCl, sodium acetate, and acetic acid (glacial) (Merck, Germany).

Instruments

This study has used ultraviolet (UV) - 1600 spectrophotometer (Shimadzu) as spectrophotometric measurements.

Raw Material

A total of 15 kg of fresh limpasu fruit was taken from Batu Harang Village, Haruyan District, Hulu Sungai Tengah Regency, South Kalimantan, in August 2020. A taxonomist determined the sample at the Department of Mathematics and Natural Sciences Basic Laboratory, University of Lambung Mangkurat, South Kalimantan, Indonesia, Dr. Totok Wianto. The result of determination was the type of *Baccaurea lanceolata* from the Phyllantaceae family. A voucher specimen (BLc 2910) has been deposited in the Department of Mathematics and Natural Sciences Basic Laboratory, University of Lambung Mangkurat.

Limpasu Powder Extraction

The pericarpium of the limpasu fruit was then washed and cut pieces, dried by being placed in a room at a temperature of 18-19^{0}C for ten consecutive days, and the dried pericarpium was obtained. Dry pericarpium was crushed using a grinding machine to produce 1.5 kg.

A total of 1.5 kg of powder was macerated using solvents with increasing polarity starting from 15 L of n-hexane followed by 15 L of ethyl acetate and 15 L of methanol. The maceration results form n-hexane solvent was filtered, and the residue was aerated to remove the solvent so that n-hexane extract is obtained. Furthermore, the residue was macerated again with the same procedure and treatment to obtain ethyl acetate and methanol extracts. Solvent will be changed for each 5 days.

Calculation of Antioxidant Activity
DPPH assay

The antioxidant test method against DPPH free radicals used in this study was a modification of several previously reported procedures.[13] A total of 50 mg of the extract was dissolved with 100 mL of methanol p.a (500 ppm). Then a series of solutions were made in each test tube with a concentration of 200, 300, 400, and 500 ppm. About 1 ml sample was added to test tube, 1 mL DPPH were then added to the mixture followed by 2 mL methanol p.a, incubated at 37 °C for 30 minutes. The control blank used was methanol. Quercetin was used as a positive control. Furthermore, the absorption was measured using a UV-Vis spectrophotometer at a wavelength of 515 nm. The IC_{50} values are calculated respectively using the regression equation formula. Measurement of inhibition activity was determined from % inhibition and IC_{50} that the following formula can calculate:

% DPPH Scavenging = [1-(A sample-A blank)/(A control-A blank)] x 100%

FRAP assay

The FRAP antioxidant test method used in this study is a modification of the procedure reported by Panda.[14] A total of 50 mg of the extract were dissolved with 100 mL of methanol p.a (500 ppm). Then a series of solutions were made in each test tube with a concentration of 100, 200, 300, and 400 ppm. About 300 µL sample was added to the test tube, 3 mL FRAP, incubated at 37 °C for 30 minutes. The control blank used was 300 µL methanol pa and 3 mL FRAP. Quercetin was used as a positive control.

Furthermore, the absorption was measured using a UV-Vis spectrophotometer at a wavelength of 594 nm. The IC_{50} values are calculated respectively using the regression equation formula. Measurement of inhibition activity was determined from % inhibition and IC_{50} that the following formula can calculate.

% Antioxidant Activity = [A sample-A blank] x 100%

RESULTS

Extraction using the multilevel maceration method was used to prevent damage to compounds that are not resistant to heating, whereas n-hexane, ethyl acetate, and methanol are used to extract compounds based on different polarities. Thus, all the compounds contained in the pericardium *Baccaurea lanceolata* can be extracted based on the polarity of the solvent and produce thick, non-polar extracts (n-hexane extract) semi-polar (ethyl acetate extract), and polar compounds (methanol extract). The resulting thick n-hexane extract is 30,22 gram, ethyl acetate extract of 45,31 gram, and methanol extract of 75,45 gram. Methanol extract gave the highest yield. Thus it is concluded that the pericarpium *Baccaurea lanceolata* contains many polar compounds.

The free radical scavenging activity of three limpasu extracts was determined using the DPPH method over the range of concentration (200 to 500 ppm). The results (Figure 2) showed that the free radical scavenging activity was dose-dependent. The methanol extract (MeOH) showed the highest activity of 56,71% to 71,01%.

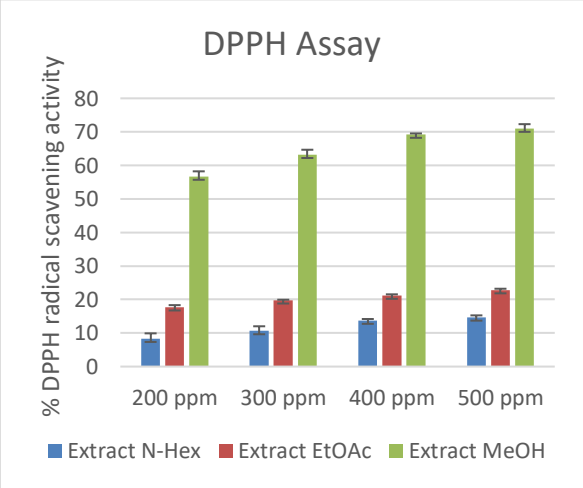

Figure 2. Scavenging activity of limpasu pericarpium extracts using the DPPH method. Values are mean ± SEM of three replicates

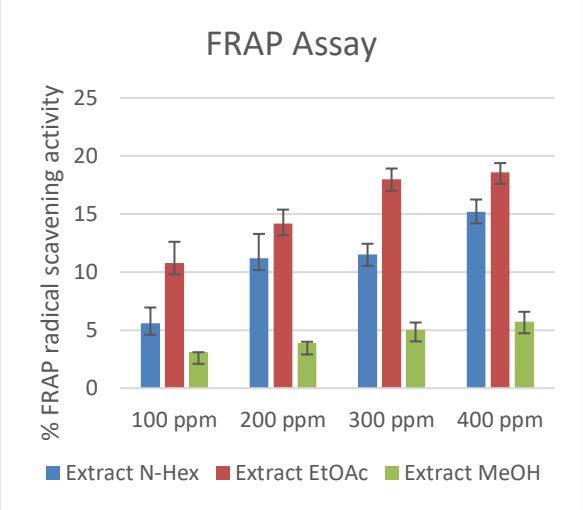

Figure 3. Scavenging activity of limpasu pericarpium extracts using the FRAP method. Values are mean ± SEM of three replicates

The free radical scavenging activity of three limpasu extracts were determined using FRAP method over the range of concentration (100 to 400 ppm), and the results (Figure 3) showed that the free radical scavenging activity was dose-dependent and that the ethyl acetate extract (EtOAc) showed the highest investigated free radical scavenging activity of 10,80% to 18,60%.

Table 1. Antioxidant activity of limpasu pericarpium extracts using the DPPH and FRAP method

Sampel-Standart	IC_{50}	
	DPPH	FRAP
Extract N-Hex	517,45 µg/mL	198,96 µg/mL
Extract EtOAc	530,64 µg/mL	190,07 µg/mL
Extract MeOH	10,63 µg/mL	661,36 µg/mL
Quercetin	6,83 µg/mL	7,09 µg/mL

In the DPPH method, the low IC_{50} value shows the high radical scavenging property. The free radical scavenging capacity of extracts from this plant is between 10,63-530,64 µg/mL. The strong DPPH inhibition was shown in methanol extract with an IC50 value of 10,63 µg/mL (antioxidant activity categories shown in Table 2).

In the FRAP method, the low IC_{50} value shows the high radical scavenging property. The free radical scavenging capacity of extracts from this plant is between 190,07-661,36 µg/mL. The results (Table 1) shown that the samples for FRAP inhibition were in the weak category (Table 2), with the weakest inhibition was shown by methanol extract with IC_{50} 661,36 µg/mL.

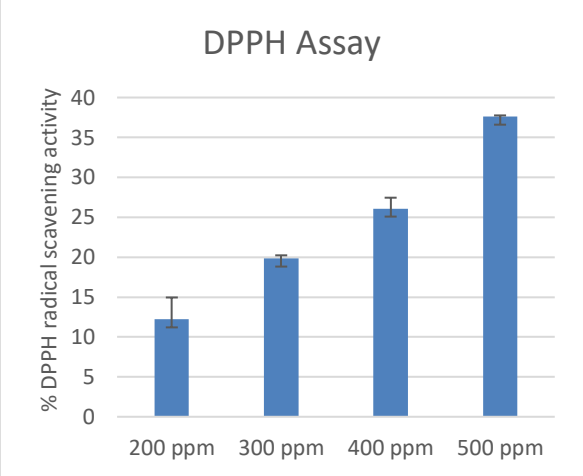

Figure 4. Scavenging activity of Quercetin using the DPPH method. Values are mean ± SEM of three replicates

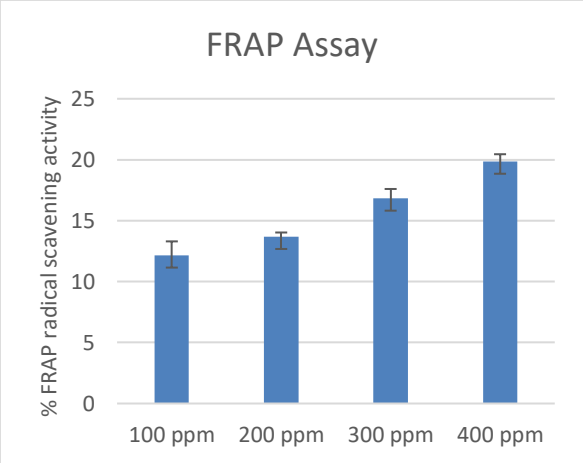

Figure 5. Scavenging activity of Quercetin using the FRAP method. Values are mean ± SEM of three replicates

The free radical scavenging activity of Quercetin was determined using DPPH and FRAP. The activity was determined over a range of concentrations (100 to 500 ppm), and the results (Figure 4 and Figure 5) showed that the free radical scavenging activity was dose-dependent. The IC_{50} values, the concentration giving 50% inhibition of DPPH, and FRAP

inhibition is very strong were 6,83 μg/mL and 7,09 μg/mL, respectively.

Table 2. Antioxidant activity categories[15]

IC_{50}	Antioxidant properties
50 ppm	Very Strong
50-100 ppm	Strong
100-150 ppm	Moderate
150-200 ppm	Weak

DISCUSSION

The antioxidant activity of extracts of *Baccaurea lanceolata* pericarpium was carried out using the DPPH and FRAP methods compared to Quercetin as a positive control. Quercetin (Figure 6) is a typical flavonoid compound that can be found in many fruits and vegetables. Quercetin has been widely used in medicine because it has strong antioxidant activity.[15] The mechanism of Quercetin as an antioxidant can be through Hydrogen Atom Transfer (HAT), Single-Electron Transfer-Proton Transfer (SET-PT), and Sequential Proton Loss Electron Transfer (SPLET).[16,17] The HAT mechanism in antioxidant activity is through the transfer of H atoms from breaking homolytic OH bonds and binding with free radicals to break the chain reaction. The stability of the hydroxyl group can be seen from the bonding dissociation enthalpy (BDE) value, the lower the BDE value, the lower the stability, so that the breaking of the OH bond can occur easily.

The HAT mechanism of Quercetin is influenced by the 4'-OH (ring B) and 3-OH (C ring) groups. This is because the BDE value of each of these groups is lower than the other hydroxyl groups. The SET-PT mechanism in antioxidant activity is through single electron transfer and deprotonation of the –OH group. The ionization potentials (IPs) strongly influence the single-electron transfer, while the –OH group deprotonation is strongly influenced by the proton dissociation enthalpy (PDE) value. The lower the IP value, the easier it will be to release electrons. Likewise with the PDE value, if the lower PDE value deprotonates the –OH group will be more accessible. The lower PDE value of the quercetin hydroxyl group was found in the 4'-OH (ring B) and 3-OH (C ring) groups compared to other –OH groups, so that the –OH groups on ring B and ring C contributed greatly to this mechanism. SPLET mechanism in antioxidant activity is through the formation of quercetin-O-anion and the formation of quercetin radicals. This mechanism is influenced by the value of the proton affinities (PAs) and the value of the electron transfer enthalpy (ETE). The lower the PAs value, the easier it is to form quercetin-O-anion, and the lower the ETE value, the easier it will be to form quercetin radicals. The PAs value in the 4'-OH group (ring B) of Quercetin is the lowest compared to other hydroxyl groups. In addition, the lowest ETE value is indicated by the formation of 3'-O ● in ring B compared to the formation of other groups, so that the –OH group on ring B contributes greatly to the SPLET mechanism. Some of these mechanisms indicate that the antioxidant activity of Quercetin is strongly influenced by the -OH groups on ring B and ring C compared to the –OH group in ring A.[16]

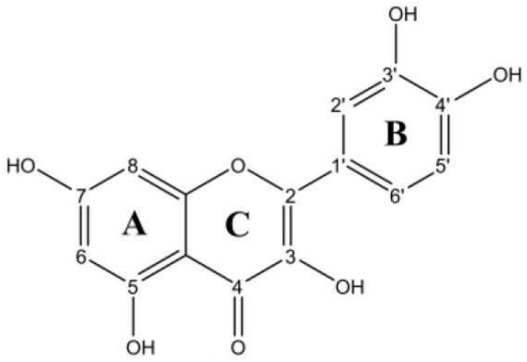

Figure 6. The structure of quercetin.[17]

The test results are expressed as a percent of inhibition (%) which describes the antioxidant activity pericarpium extracts from *Baccaurea lanceolata* in percent units. In the DPPH method, the higher percent shows the higher radical scavenging property**.** If the resulting higher percent value, the higher the antioxidant activity of the sample.[18] In addition, the test results are also stated with the $IC_{50,}$ which is used to

determine the concentration of antioxidant compounds which can inhibit 50% of free radicals. Antioxidant activity was called 'very strong' category on IC_{50} when the value is <50 µg/mL, 'strong' when the value is 50-100 µg/mL, 'moderate' when the value is 101-250 µg/mL, 'weak' when the value is 250-500 µg/mL and does not act as an antioxidant if the value is> 500 µg/mL (Table 2).[19]

The DPPH method is the most commonly used in testing antioxidant activity. DPPH solution will react with antioxidants to become *diphenyl-pycrylhydrazine*. The changes in DPPH compounds were detected by seeing the decrease in the absorbance of DPPH solution when added with antioxidant compounds.[20] The higher of absorbance show the lower of the antioxidant activity of the test solution. Based on Figure 2, it can be seen that the methanol extract showed the percent of inhibition that close to the standard. The IC_{50} value of in Table 1 shows that the methanol extract provided a stronger antioxidant activity of 10,63 µg/mL, than the n-hexane extract (517,45 µg/mL) and ethyl acetate extract (530,64 µg/mL). The methanol extract provides antioxidant activity in the same category as the standard, so that the methanol extract of the pericarpium of *Baccaurea lanceolata* acts as better antioxidant agent than the other extracts for this method. This is probably because the polar compounds that contained in the methanol extract have a mechanism principle according to the DPPH method. The DPPH method can be used for compounds that have an antioxidant mechanism by donating hydrogen atoms.[21] Thus, it is possible that the compounds contained in the methanol extract are compounds that donate hydrogen to their antioxidant activity.

The results obtained are in line with the research of Indradi[22,] which reported that the ethanol extract of Pluche indica provided the best antioxidant activity based on IC_{50} compared to ethyl acetate extract and n-hexane extract using the DPPH method. In addition, Abarca[23] also reported that the methanol extract of *Bougainvillea x buttiana* provided the best antioxidant activity based on IC_{50} compared to ethanol, distilled water, acetone, ethyl acetate, n-hexane and dichloromethane using the DPPH method.

The FRAP method is the most commonly used in testing antioxidant activity. The FRAP method involves the reduction of Fe3+ to Fe2+. The Fe3+ species is sourced from the FRAP reagent, a mixture of acetate buffer, TPTZ, and FeCl3. If the test solution has antioxidant activity (causing other compounds to experience reduction), a reduction reaction will occur from Fe3+ to Fe2+. This Fe reduction reaction causes the formation of the blue complex Fe (II) *tripyridyltriazine,* which can be detected spectrophotometrically at a wavelength of 594 nm.[24] The formation of an increasingly dark blue color indicates the formation of more Fe2+ ions. The higher of absorbance show, the higher the antioxidant activity of the test solution. Based on Figure 3, it can be seen that all of pericarpium extracts *Baccaurea lanceolata* have a small percentage of inhibition compared to the standard (Figure 5). The IC_{50} value of Table 1 shows that the ethyl acetate extract and n-hexane extract both provide moderate antioxidant activity of 190,07 µg/mL and 198,96 µg/mL, while the methanol extract (661,36 µg/mL) does not provide antioxidant activity based on the FRAP method. Thus, ethyl acetate extract and n-hexane extract pericarpium *Baccaurea lanceolata* act as antioxidant agents better than methanol extract. This is probably because the semi-polar or non-polar compounds contained in ethyl acetate extract or n-hexane extract have a mechanism principle according to the FRAP method. The FRAP method can be used for compounds that have an antioxidant mechanism by donating electrons.[25] So, it is possible that the compounds contained in ethyl acetate extract and n-hexane extract are compounds that donate electrons to their antioxidant activity. The results obtained are in line with the research of Eom[26,] which reported that the ethyl acetate fraction of *Rumex crispus* provided the best antioxidant

activity compared to dichloromethane fraction, ethanol extract, water fraction, n-butanol fraction, and n-hexane fraction based on the FRAP method. In addition, Salimikia[27] also reported that the ethyl acetate extract of *Salvia chloroleuca* provided the best antioxidant activity compared to methanol extract and n-hexane extract based on the FRAP method.

The difference in antioxidant activity of each extract can be caused by differences in the type or quality of secondary metabolites.[10] The antioxidant activity in extracts can be caused by the content of electron donor compounds, hydrogen donor compounds or the possibility of a synergistic effect between compounds that provide antioxidant activity.[21,25] Phytoconstituents included in the pericarpium *Baccaurea lanceolata*, such as flavonoids, can play a role in antioxidant activity.[10,28] Flavonoids have antioxidant properties due to the main structures such as catechol structures, double bonds, and hydroxyl groups. The ability of flavonoids to chelate free radicals by donating electron or hydrogen atoms shows their activity as strong antioxidants.[29] In addition, differences in the mechanism of free radical inhibition of the compounds contained in the pericarpium *Baccaurea lenceolata* may affect differences in antioxidant activity in different methods.

CONCLUSION

This study has determined the antioxidant activity of limpasu percarpium. It was tested by the antioxidant activity of the pericarpium extracts of *Baccaurea lanceolata* (Miq) Muell. Arg. using the DPPH method, the methanol extract provided has the very strong antioxidant activity category and uses the FRAP method, which is the ethyl acetate extract provided has the moderate category antioxidant activity. However, methanol extract has a good antioxidant activity agent for the DPPH method, so it is feasible to do further research to isolate compounds that act as antioxidants.

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