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Article

Antioxidant Activity of Bioactive Compounds in the Ethyl Acetate Extract of Putat Leaves (*Planchonia valida*)

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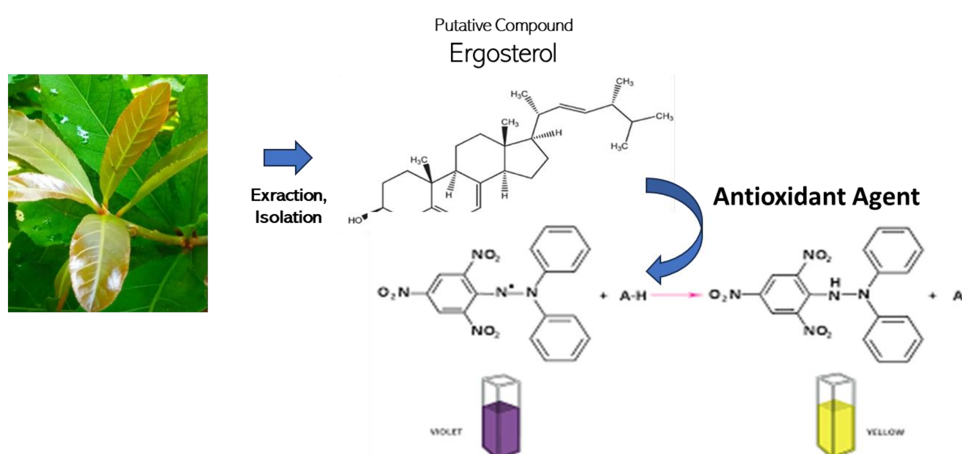
Abstract

The Putat leaf (*Planchonia valida*) is a plant traditionally used by the community in the Tanjung Lanjut area, Jambi, both as a salad ingredient and as a traditional medicine to treat skin diseases and promote health. This identifies that the putat plant contains chemical compounds that potentially have antioxidant activity. In this study, putat leaf samples (*Planchonia valida*) that potentially have antioxidant activity. In this study, *Planchonia valida (P. valida)* leaf samples using n-hexane and ethyl acetate solvents through a multistep maceration process. Subsequently, phytochemical screening and antioxidant activity tests were conducted to obtain information about fractions that have the potential to be further isolated. The extraction percentage with a yield value of 1,28% was obtained in the maceration process. Further isolation was carried out using isolate liquid chromatography and FT-IR instruments. The obtained isolate was in the form of a wet solid, and through characterization, it was classified as a steroid compound suspected to be ergosterol. Free radicals or oxidants have reactive effects on the human body and can trigger various types of diseases. Antioxidants are known to have the ability to inhibit or neutralize free radicals in the human body. This study tested antioxidant activity using the DPPH (1,1-diphenyl-1,1-picrylhydrazyl) method on isolating F1. Isolate F1 was found to have antioxidant activity with a value of 714.637 ppm, falling into the range of weak antioxidant activity

Keywords: *Antioxidant, Bioactive Compounds, Planchonia valida*

Graphical Abstract



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Introduction

Indonesia is a country that has a high wealth of natural resources. Various plant species thrive in this country, one of which is the putat plant. *Planchonia valida* or better known as putat can be seen in the Lake Tangkas area and is the most dominant plant compared to various types of plants. Based on Jamiat *et al* (2019), putat plants are woody plants with taproots, spherical stems and have branches. Putat plants are theoretically generally found in swampy areas that are inundated by water or sometimes inundated by water (Syukur, 2016).

Putat leaves can be used to treat skin diseases such as itching by pounding the leaves then attached to the itchy area. Putat leaves are also used as a mixture of postpartum bath water. In addition to being a medicine, generally people use putat leaves as a mixture of cold powder and used to protect the skin from sun exposure when in the field and can eliminate dark spots on the face. (Supriningrum et al., 2019). This identifies that putat plants contain chemical compounds that are bioactive.

Based on research conducted by Shaumi (2019), through phytochemical screening of putat leaf shoot powder (*Planchonia valida)* stated that it positively contains all chemical compounds, namely alkaloids, tannins, flavonoids, steroids, and saponins. This statement is also supported by research conducted by Supriningrum et al., (2019) which explains that alkaloids, flavonoids, tannins, saponins and steroids are among the chemical components contained in putat leaves.
Putat leaves have many useful ingredients for the body that can be used as antibacterials. Plants that contain antibacterial are usually used as medicinal plants. Most medicinal plants are plants that are often used for traditional medicine and have even been widely managed as modern medicine.
According to Syamsudin et al., (2019), research from the genus *Planchonia that has been reported includes phytochemical tests there are saponin compounds acylated with triterpenoids from* Planchonia careya species. 6 chemical compounds from *Planchonia careya* have been isolated that have antibacterial activity against (+)-galokatekin, galokatekin-(4α→8)-galokatekin, α-dimorphphenolic acid, hiptatatic acid, 3-β-Otrans-p coumaroyltormentic acid, 3-β-O-cis-p-kumaroyltormentic acid.

Antioxidants are defined as compounds that can delay, slow down and inhibit the process of lipid oxidation. This compound can reduce the negative influence of free radicals. Free radicals are highly reactive molecules, which can disrupt cell integrity, can react with cell structure components, can react with cell structure components such as enzin and DNA. In the body, free radicals are continuously formed. This leads to the formation of new, more reactive free radicals, causing cell damage and death. Therefore, antioxidants are needed in order to protect the body from free radicals and reduce their negative impacts (Handayani, 2020). In order to fulfill the issues, the search for natural antioxidant compounds is directed at natural resources (Ipandi et al., 2016).

Free radicals in the body are very dangerous materials. Free radical material is actually a compound or molecule containing one or more unpaired electrons in its outer orbital. The presence of unpaired electrons results in the compound being very reactive to find a partner. You do this by binding or attacking the electrons of molecules around it. Free radicals are generally bound to large molecules such as lipids, proteins, and DNA (carriers of traits). If this happens, it will result in cell damage or cell growth that cannot be controlled (Leksono, 2018).

Chemically, free radicals are compounds that have unpaired electrons, which often look for other molecules to be stable. Free radicals or oxidants can have an effect on cell damage in the human body due to oxidation reactions (Hasanah et al., 2017). According to Iglesias *et al* (2016), the DPPH test is considered more sensitive for determining the antioxidant activity of compounds that are less polar. According to Karadag (2009) in Wulansari (2018), the DPPH method has the advantage that the analysis method is simpler, faster, easier to observe and

more sensitive to samples with relatively small concentrations so that the results obtained are more accurate (Wahdaningsih et al., 2013).

The results of research conducted by Pratiwi *et al* (2019), obtained data showing that the ethyl acetate fraction has better antioxidant activity than n-hexane fraction extract. Antioxidant activity comes from compounds found in the fraction of red dragon fruit skin among which are known to be positive based on phytochemicals are alkaloids, terpenoids, phenolics and flavonoids. Tests were carried out on the n-hexane fraction, ethyl acetate fraction and methanol fraction, it was found that in the n-hexane fraction, ethyl acetate fraction and methanol fraction had less active antioxidant activity with an IC_{50} *value of* n-hexane fraction of 198.05μg/ml while in the ethyl acetate fraction of 199.527μg/ml and methanol fraction of 445.255μg/ml.

Material and Methods

Materials and Instrumentations

The materials used are Putat Leaves (*Planchonia valida)* obtained from Tanjung Lanjut Village, Muaro Jambi regency, Indonesia. Chemical were used in this research Ethyl Acetate, Aquadest (Sigma-Aldrich), N-Hexan, Silica Gel G60 (0.063-0.200 mm and 0.040-0.063 mm), H_{2SO4} 2N, HCl 2N, Mayer Reagent, Dragendrof Reagent, Bouchardat Reagent, HCl, Magnesium powder, DPPH powder, Standard solution, Vitamin C (ascorbic acid), Methanol p.a.. The instrumentation used are vacuum *rotary evaporator* (IKA RV 10), Vacuum Liquid Chromatography (KVC), Thin Layer Chromatography (KLT), UV-Vis Spectrophotometry (Thermo Fisher Scientific, Singapore), FT-IR Spectrophotometry (Thermo Fisher Scientific), as well as other equipment such as analytical balances.

Methods

Sample Preparation and Extraction. The sample to be studied is the leaf part of the putat plant. The sample is washed with water and dried by aeration. Next, the putat leaves are mashed with a blender and filtered using a 60 mesh sieve so that putat leaf simplisia is produced. A dry sample of 1.4 kg was macerated in stages. Multilevel maceration is performed to separate the compounds in the sample according to their degree of polarity. The initial solvent used for maceration is n-hexane as much as 1.5 L. Then maceration is continued with ethyl acetate solvent. Stirring is carried out for 3 hours then allowed to stand for 48 hours. The extract obtained from ethyl acetate solvent is then concentrated using a *rotary evaporator*. From ethyl acetate extract, dry extract was obtained as much as 16.8 grams with a yield of 1.28%.

Phytochemical screening. Phytochemical analysis was performed to identify various bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and steroids. A positive result was characterized by a distinct color change and/or the formation of a precipitate, signifying a chemical interaction between the reagent and the plant extract.

The detection of alkaloids involved the addition of 2N hydrochloric acid (HCl) to the sample, followed by treatment with Mayer's reagent, which resulted in the formation of a yellow precipitate. Furthermore, Bouchardat reagent induced a red coloration, whereas Dragendorff reagent generated a brown precipitate, confirming the presence of alkaloids (Ningrum et al., 2016). The presence of flavonoids was verified through the Flavonoid Test, where the ethyl acetate extract of putat leaves exhibited an orange coloration, indicative of flavonoid compounds (Vanessa et al., 2014). The Tannin Test yielded a blackish-green coloration upon the addition of 1% FeCl3 solution, signifying a positive result. Similarly, the presence of saponins was confirmed by the formation of stable, persistent foam, which remained intact even after the addition of 2N HCl (Wardana and Tukiran, 2016). The detection of steroids was established through the Steroid Test, in which treatment with Liebermann-Burchard reagents (anhydrous acetic acid and concentrated sulfuric acid) produced a bluish-green coloration, characteristic of steroid compounds. Additionally, the test differentiated terpenoids, which exhibited an orange or purple-red

coloration, from steroids that displayed a bluish-green hue (Sangi et al., 2013).

Isolation Active Compound. Liquid vacuum column chromatography (KVC) was performed using stationary phase silica gel with sample ratio: silica gel (1:20). The sample extract is impregging using silica gel, then added to the column that has contained the stationary phase. While the mobile phase used is n-hexane: ethyl acetate and ethyl acetate: methanol with variations in ratio (10:0; 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9; and 0:10). The fraction obtained is accommodated and then evaporated. The results of column chromatography were performed again KLT. Silhouettes that have identical smudge patterns are combined based on the Rf value on the chromatogram. Eluents that have one spot stain are then tested using 3 different eluents, where if KLT remains one spot stain then isolates are obtained.

Antioxidant activity. The preparation of the DPPH solution involves dissolving 1.97 mg of DPPH powder into 100 mL of methanol p.a. to achieve a 50 μM concentration, resulting in a dark purple solution (Furqan and Nurman, 2020). For the preparation of the test solution, variations in concentrations of 50 ppm, 30 ppm, and 10 ppm are achieved by first weighing 50 mg of putat extract and dissolving it in 10 mL of methanol p.a. to create a 1000 ppm standard solution. Dilution is then performed to obtain the desired concentrations. The positive control solution is prepared by dissolving 0.001 g of ascorbic acid in 5 mL of methanol p.a., forming a 100ppm standard solution, from which further dilutions are made to create solutions with concentrations of 50 ppm, 30 ppm, and 10 ppm. The negative control solution is composed of methanol p.a. with the addition of DPPH solution. For the antioxidant activity test, 0.2 mL of each test solution, as well as the negative and positive control solutions, are pipetted into separate vials and combined with 3.8 mL of 50 μM DPPH solution. After homogenizing the mixtures, they are incubated in the dark for 30 minutes. Absorbance is then measured at a wavelength of 517 nm using UV-Visible spectrophotometry. The percentage of inhibition, which reflects the DPPH scavenging activity, is calculated, along with the IC_{50} value, indicating the concentration at which 50% inhibition is achieved.

Data Analysis.

Determination of %Yield. Determination of the percentage of extract yield was calculated using equation 1.

$$\%\text{Yield} = \frac{\text{Mass of Extract (g)}}{\text{Mass of Simplicia (g)}} \times 100\% \quad (1$$

Determination of Antioxidant Performance. The determination of the % inhibition value is done using the equation 2.

$$\%\ \text{inhibition} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}}\ \text{x}\ 100\% \quad (2)$$

IC_{50} Value. The IC_{50} value can be obtained by plotting a graph of the relationship of sample concentration with % inhibition. The x-axis is the sample concentration while the y-axis is %inhibition. Then calculated the regression equation y = a + bx where to calculate the value of IC_{50} can be known from replacing the value of y in the regression equation with the value of 50.

Results and Discussions

The results of the data stated that in ethyl acetate extract of putat leaves there are flavonoid compounds, tannins and steroids. Method in conducting phytochemical tests by looking at color change reactions using color reagents. The results of phytochemical tests on ethyl acetate extract of putat leaves *(Planchonia valida)* can be seen in Table 1.

Table 1. Secondary metabolites of Ethyl Acetate Extract

Secondary Methabolite	Result
Alkaloids	-
Flavonoids	+
Tannins	+
Saponins	-
Steroids	+

Antioxidant of Crude Extract

The results of the antioxidant activity test of ethyl acetate extract of putat leaves can be seen in

Table 2. Antioxidant activity of ethyl acetate extract

Samples	Linear Regression	IC_{50} (ppm)	Activity
Extract Ethyl Acetate	y= 0.331x + 32.828 R2= 0.8421	51.87	Strong
Ascorbic acid	Y = 2.34x + 14.943 R2 = 0.9731	14.94	Very Strong

The IC_{50} value of ethyl acetate extract of putat leaves was obtained using the calculation of the linear regression equation of ethyl acetate extract of putat leaves in Figure 5 is y = 0.331x + 32.828 and R 2 = 0.8421. The y coefficient of this equation expresses, while the x coefficient expresses the amount of concentration required to reduce 50% of DPPH radical activity. The linear regression curve in Figure 1 illustrates that with increasing concentration of extract, the greater the % inhibition, which means the higher the antioxidant activity. The following is a calculation of the IC_{50} value of ethyl acetate extract of putat leaves.

In Table 2, it was found that the IC_{50} value of 51.87 ppm is still in the strong range at the level of antioxidant power according to Jun et al (2003), due to it has an IC_{50} value in the range (50-100 ppm), so that ethyl acetate extract has the potential to proceed to compound isolation at the next stage

Isolate of Planchonia valida

A total of 15 grams of extract was impregnated using 15 grams of silica gel. The stationary phase used is 40 grams of silica gel. The sample was eluted using ethyl acetate eluent: methanol in a gradient, the polarity increased by 10%. From the isolation process carried out, 22 vials were accommodated based on the color of the resulting tape. Furthermore, a Thin Layer Chromatography (TLC) test is carried out by observing the same stain pattern from each vial to identify the combined fraction. The following is a table of KVC result fraction grouping.

Table 3. Grouping of KVC yield fraction of ethyl acetate extract of putat leaves

Faction	Vial Bottle Order	Fraction Weight (g)
1	4	0.050
2	5-8	0.056
3	9-15	0.060
4	16-18	0.050

Furthermore, qualitative antioxidant activity tests were carried out on all fractions obtained to determine the intensity of the resulting color change. The qualitative test begins with the elution of the fraction obtained using ethyl methanol eluents in a ratio of 7 and 3 so that stains are obtained on the KLT plate. Furthermore, the KLT plate is sprayed using DPPH solution to see the resulting color change and presented through Figure 2.

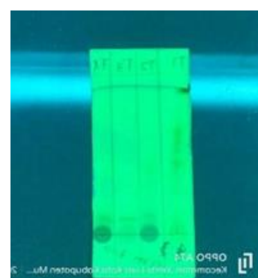


Figure 2. Qualitative activity test using DPPH sprayer against Ethyl Acetate Fraction

From Figure 2, it can be seen that the dominant color intensity is found in fraction 1. In addition to being based on the resulting stain pattern, grouping fraction 1 is based on a less tailless stain pattern, it can be possible that fraction 1 could potentially contain pure compounds. Therefore, recrystallization is carried out to remove the conyaminants contained in the sample. Purification is carried out by recrystallization using solvents from nonpolar (n-hexane), semipolar (ethyl acetate), polar (methanol) after which it is dried.

Isolates were monitored by TLC using appropriate eluents (Methanol : ethyl acetate = 3:7) and stains were obtained. A single stain was obtained on the KLT plate using Methanol eluent: Ethyl Acetate = 3: 7. This identifies that the isolates obtained are already pure. However, to ensure that the monitor is carried out again by KLT using a different eluent development (3 eluent system). The eluent used is chloroform ;

ethyl acetate (1:9); DCM: Chloroform (2:8) and DCM: n-hexane (1:9) and produced a dead stain. This identifies that the compounds contained in F1 isolates are pure (Figure 3).

Isolates were monitored by TLC using appropriate eluents (Methanol : ethyl acetate = 3:7) and stains were obtained. A single stain was obtained on the KLT plate using Methanol eluent: Ethyl Acetate = 3: 7. This identifies that the isolates obtained are already pure. However, to ensure that the monitor is carried out again by KLT using a different eluent development (3 eluent system). The eluent used is chloroform ; ethyl acetate (1:9); DCM: Chloroform (2:8) and DCM: n-hexane (1:9) and produced a dead stain. This identifies that the compounds contained in F1 isolates are pure (Figure 3).

Figure 3. F1 isolate KLT results using eluents (from left to right) DCM : ethyl acetate = 1:9, chloroform : ethyl acetate = (1:9) and DCM : Chloroform = (2:8).

Phytochemical screening

Based on the table above, it can be seen that ethyl acetate isolate gave positive results in testing steroid class compounds using Lieberman Burchard reagents which are characterized by the formation of green / blue color in the sample. After obtaining preliminary information related to the compounds contained in the isolate, it is necessary to characterize spectrophotometrically at the next stage.

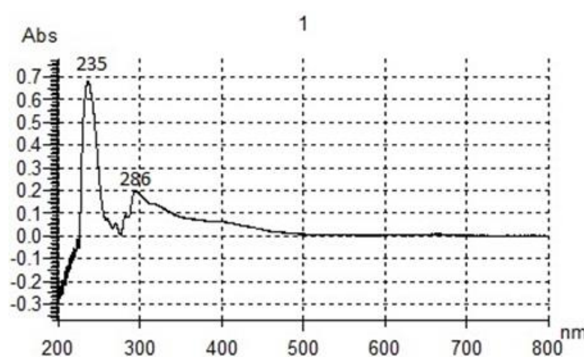
Table 4. Phytochemical screening results of ethyl acetate isolate

Secondary Metabolites	Result
Alkaloids	-
Tannins	-
Flavonoids	-
Saponins	-
Steroids	+

Chemical Structure Determination

Characterization using a UV-Vis spectrophotometer to determine the presence of chromophore groups contained in an organic compound. The working principle of the UV-Vis spectrophotometer is the interaction between radiation in the range of 200-800nm wavelengths passed to a compound. This interaction results in transitions between electronic energy in organic molecules. The following are the results of measuring the wavelength of isolates using a UV-Vis spectrophotometer (Figure 4).

Figure 4. UV-Vis Spectrum of F1 Isolates Ethyl



Acetate 1λ = 235nm and 2λ = 286nm.

The purpose of characterization by UV-Vis spectrophotometer aims to analyze compounds containing chromophore groups, which are part of molecules that absorb UV light and visible light. Figure 4 shows the maximum absorption at wavelength. UV-Vis spectra show that isolates provide two absorption peaks: in band 1 λ = 235nm and band 2 λ = 286nm. Absorption at 235 indicates the existence of a conjugated diema system in the structure. Meanwhile, absorption in 286 indicates the existence of an aromatic system with certain substituents.

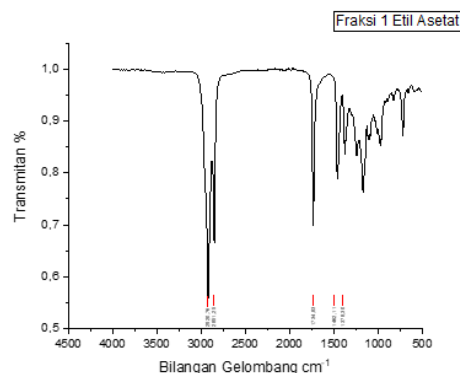


Figure 6. IR Spectrum of F1 Ethyl Acetate

Identification of isolates by IR spectrophotometry showed that absorption at wavenumbers 2920.76 cm-1 and 2851.25 cm-1 is suspected to be absorption of the C-H group. This conjecture is strengthened by the presence of bending vibrations in the wavenumber region 1462.83 cm-1 which indicates the presence of CH 2 groups. The absorption band in the wavenumber region 13,78,30cm-1 shows the bending of the C-H group of CH_3. The absorption band at wavenumber 1734.83 cm-1 is thought to be an absorption for the C=O group, as Hiroshi et al (1976) reported, there is an O-H bond (hydroxyl) and has a double bond C=C (ena) and a C=O bond (carbonyl). The presence of O-H (hydroxyl) bonds, having a C=C (ena) double bond and a C=O (carbonyl) bond identifies that isolates of Fraction 1 Ethyl Acetate are Ergosterol compounds.

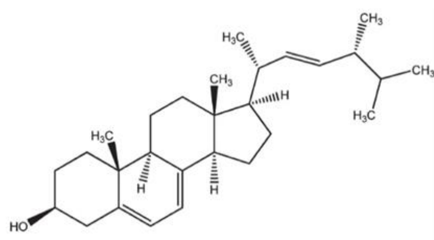


Figure 6. Chemical structure of Ergosterol

Table 5. Interpretation and Comparison of IR Spectrum

Interpretation	Wavenumbers (cm^{-1})	
	Isolate	Ergosterol
C-H Alkanes/aromatics	2920.76	2956.29
C-H Alkanes/aromatics	2851.25	2871.43
C=O	1734.83	-
C=N, C=C	1462.11	-
OH Bending	1378.30	1460.98
C-N aromatic	1244.29	1369.64
C-OH	1172.16	1158.32

From the results of antioxidant activity tests in Table 6 conducted on F1 isolates and ascorbate acid, it is known that F1 isolates have an IC_{50} value of 714.6377 ppm. This indicates that F1 isolates have weak antioxidant levels (Jun *et al*, 2003). This is reinforced by comparing the IC_{50} value of isolates with the IC_{50} value of ascorbate, it can be seen that the IC_{50} value of isolates is greater than the IC_{50} value of ascorbate acid, which is 14.9488 ppm < 714.6377 ppm. A higher IC_{50} value of a sample indicates weaker antioxidant activity. Therefore, it can be concluded that the compound demonstrates antioxidant activity that is classified as weak, yet it still holds potential as an antioxidant

Table 6. Antioxidant activity of Isolate

Samples	Linear Regression	IC_{50} (ppm)
Isolate (F1)	y= 0.0138x + 40.138 R2 = 1	714.63
Ascorbic acids	Y = 2.34x + 14.943 R2 = 0.9731	14.94

Conclusion

The ethyl acetate fraction of Putat Leaves (*Planchonia valida*) contains a class of steroid compounds. The antioxidant activity of extracts and isolates of ethyl acetate fraction of Putat Leaves (Planchonia valida) expressed in IC50 values is classified as strong and classified as very weak

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Author Contributions

Conceptualization, N and DVS.; Methodology, FF and RDP; Software, ILT and ML.; Validation: N and ML; Formal Analysis, ILT and ML.; Investigation, IIR and N.; Resources, N and FF.; Data Curation, DVS and RDP; Writing – Original Draft Preparation, DVS, N; Writing – Review & Editing, ILT and ML; Visualization: N and ML.; Supervision, N and ML; Project Administration, ML

Conflic of Interest

The authors declare no conflict of interest

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