

Cytotoxic activity of isolate compounds from derendan (*Lansium parasiticum* (Osbeck) K.C. Sahni & Bennet) fruit peel

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ABSTRACT: Derendan (*Lansium parasiticum* (Osbeck) K.C. Sahni & Bennet) is a plant from the Meliaceae family that grows in the tropics, especially in Sumatra (Indonesia). It was reported that this plant has antimalarial, antimutagenic, and anticancer properties. This study aims to determine the cytotoxic activity of compounds isolated from the methanol extract of *Lansium parasiticum* bark. Extraction using maceration method with methanol solvent. Vacuum Liquid Chromatography (KVC) with 21 different eluent ratios (n-hexane: ethyl acetate and ethyl acetate; methanol) was utilized for the fractionation. The result was 21 fractions. The washing technique was used to purify fraction 17. The obtained compound LPR17 with a melting point of 239-241°C. Based on the UV spectrum, the wavelengths of the LPR17 compound were 277.0 nm and 205.0 nm. The FT-IR spectrum shows aliphatic OH, CH, C=O, C-H, and C-C functional groups. This compound reacts positively with the Liebermann-Burchard reagent. The compound obtained is a triterpenoid group. Cytotoxic test using BSLT method. LPR17 compounds provide very strong cytotoxic activity (LC₅₀ = 67.15 g/mL).

KEYWORDS: BSLT; cytotoxic; *Lansium parasiticum*.

INTRODUCTION

The public has widely used the empirical use of medicinal plants for the needs of medicinal preparations [1]. One of the plant families that is widely used as a source of medicinal compounds is Meliaceae. Among the Meliaceae family plants, there are several varieties of duku, including duku (*Lansium domesticum* var. duku), kokosan (*L. Parasiticum* var. aquaeum), derendan (*L. parasiticum* (Osbeck) Sahni & Bennet). *Lansium* contains various bioactive compounds with pharmacological activities, such as anticancer, antimalarial, anti-melanogenic, antimicrobial, and antioxidant [2]. *Lansium parasiticum* was reported that this plant has antimalarial, antimutagenic, and anticancer properties [3].

Several studies on *L. parasiticum* fruit as cytotoxic The ethanolic extract of the stem bark of *L. parasiticum* has strong cytotoxic activity using BSLT method with a *Lethal Concentration 50* (LC₅₀) value of 93,48 ppm [4]. The ethanolic extract of Langsat is toxic (LC₅₀=32,713 µg/mL) [5].

Isolation of the polar fraction from the fruit skin of *L. parasiticum* obtained a new compound, namely Methyl lansioside C, lansioside C, and Lansioside B. Methyl lansioside C and Lansioside B compounds gave moderate free radical inhibitory activity with a value of Scavenging Concentration 50 (SC₅₀) 14.5 and 13.7 mM [6].

Based on the literature, it is known that the Derenden has many medicinal properties. However, the skin of the derendan fruit has not been studied in depth and is only considered waste. Based on this background, the researchers tested the antioxidant and cytotoxic activity of isolated compounds contained in the total methanol extract of derenden fruit peel (*L. parasiticum* K.C.Sahni & Bannet).

MATERIALS AND METHODS

Materials

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Derendan fruit peel. N-hexane (Brataco®, Indonesia), ethyl acetate (Brataco®, Indonesia) and methanol (Brataco®, Indonesia), Chloroform (Millipore®, German), Ammonia (Millipore®, German), FeCl₃ (Millipore®, German), H₂SO₄ (Millipore®, German), acetic acid anhydrides (Millipore®, German), Mayer reagent, Lieberman-Burchard reagent, TLC plate (Millipore®, German), Vitamin C (Brataco®, Indonesia), DMSO (Millipore®, German). Distillation kit (Gopal®), dark bottle, analytical balance, TLC plate (Merck®, Indonesia), VLC kit, chamber (Pyrex®, U.S), UV stain viewer lamp (Camag®, German), vial, capillary tube, Stuart Melting Point Apparatus (SMP11), UV-Vis Spectrophotometer (Shimadzu® UV-1800, Japan), IR Spectrophotometer (Shimadzu®IR Prestige-21, Japan), HPLC (Shimadzu®, Shim-Pack VP-ODS column (150x4.6) mm, injection volume 20µL, rate flow 1,0 mL/minute, UV detector), a magnifying glass, a dark container, aeration, a low light intensity lamp (15 Watt PLC LED), seawater, and commonly used glassware.

Isolation of secondary metabolic compounds

The rind of the derendan fruit was obtained from Lalang Village, Sungai Apit District, Siak Sri Indrapura Regency, Riau Province. Furthermore, the sample was identified at the Botanical Laboratory of the Biology Department, Faculty of Mathematics and Natural Sciences, Riau University (Identification Letter No. 98/UN19.5.1.1.3/Bio/Botani/2018).

A total of 7 kg of derendan fruit skin is separated from the flesh and then dried (750 grams). Then it was macerated for 3x5 days with methanol (192.7 grams extract). The phytochemical profiles were observed using various reagents to determine the class of secondary metabolites, including alkaloid, phenolic, terpenoid, steroid, and saponin [7].

Twenty five gram of methanol extract was fractionated using Vacuum Liquid Chromatography (VLC) with 1 x 200 mL eluent in the ratio: n-hexane 100%, n-hexane: ethyl acetate; (9:1), (8:2), (7:3), (6:4), (5:5), (4:6), (3:7), (2:8), (1:9), ethyl acetate 100%, ethyl acetate:methanol; (9:1), (8:2), (7:3), (6:4), (5:5), (4:6), (3:7), (2:8), (1:9) and 100% methanol. Twenty-one fractions are concentrated. Each fraction was observed separation pattern by TLC. Based on observations, crystals formed spontaneously in fraction 17, so the isolation process and cytotoxic test activity were continued.

Fraction 17 was separated by using the decantation method. The crystals were washed with n-hexane, ethyl acetate, and methanol to obtain colorless crystals (compound LPR17). LPR17 compounds were observed for purity by TLC and High-Performance Liquid Chromatography (HPLC) (Shimidzu®, ODS Shimpack®, UV detectors 205 and 277 nm). Examination of LPR17 compounds includes an examination of organoleptic, chemical properties, phytochemical tests, and melting point. Characterization of LPR17 using UV-Vis and FT-IR spectrophotometers.

Cytotoxic test

Artemia salina Leach shrimp cysts were hatched in a culture container filled with seawater and equipped with aeration and lamp (PLC LED® 15watt), used 48 hours after the larval hatched. The test vial was calibrated at 5 mL. The test was carried out with concentrations of 1000, 100, and 10 µg/mL (5 vials for each dose and 1 for control (DMSO/seawater)). Five replicates were prepared for each dose level. Each test vial was allowed to evaporate the solvent, redissolved the sample with 50 µL DMSO, and added seawater almost to the calibration limit. Ten shrimp larvae were added to each vial. Sea water was added a few drops to the calibration limit, and the mortality of shrimp larvae was observed after 24 hours. The data obtained is calculated LC₅₀ using a probit table. The negative control is 50 µL DMSO.

Dead larvae calculated by the total number of each concentration minus the number of live larvae. The percentage of mortality was calculated by comparing the number of dead larvae to the total number of larvae. Then seen in the table of probit values, from these values will be known the value of probit, then entered in the regression equation so that the value of LC₅₀ is obtained.

$$y = a + bx$$

Where y is the probit value, a is the intercept, b is the slope of the linear regression line, and x is the concentration log.

RESULTS AND DISCUSSION

In this study, isolation and testing of cytotoxic activity of the isolates from the methanol extract of the

rind of (*Lansium parasiticum* (Osbeck) K.C Sahni & Bennet) fruit were carried out. The purpose of drying is to get a sample that is not easily damaged so that it can be stored for a longer time [8]. Dry sorting is the final stage of sample preparation, and this stage aims to separate foreign objects still left in the dry sample, such as sand, small stones, and dust [8]. The sample was refined to increase the sample's surface area, and the contact between the solvent and the sample was wider to facilitate the penetration of the solvent into the plant cell membrane. Extracting the compounds contained in the sample was more optimal [9]. The results of the refining stage obtained as much as 750 g of dry powder from the fresh skin of the derendan fruit. Maceration was chosen because it is a simple extraction method and can extract compound substances from the sample, both resistant to heating and those not resistant to heating [10]. Meanwhile, the methanol solvent was chosen because methanol is a universal solvent consisting of non-polar molecules in the form of hydrocarbon compounds (R- or CH₃-) and polar molecules (hydroxyl groups or -OH), which can attract non-polar compounds to polar compounds [11].

The extract was concentrated using a rotary evaporator to separate the methanol solvent from the extract. The rotary evaporator is equipped with a vacuum system. A vacuum in the tool helps lower the pressure on the system, so the solvent will boil at a lower temperature than its boiling point and accelerate the solvent evaporation process. Evaporation aims to reduce damage to thermolabile compounds contained in the extract [12]. Evaporation of the methanol solvent produced a thick brown extract of 192.7 g with a yield of 25.7%. In testing the content of secondary metabolites for the methanol extract of derendan fruit peel, it was positive that it contained alkaloids, flavonoids, and terpenoids.

The VLC results obtained were 21 fractions. In the purification stage, the F17 fraction was chosen because the F17 fraction formed crystals (colorless cubic crystals) at the bottom of the vial. Purification of fraction 17 was continued by washing and recrystallization methods. Fraction weighing 0.7 g was recrystallized using methanol as solvent, and crystals formed spontaneously. The separation was carried out by the decantation method. The solvent that cannot dissolve the crystals is poured into another container. The decantation method results in a separation between the solvent and the crystals. The crystals obtained are purified again by a washing technique, namely by dissolving the compound with a solvent that does not dissolve (methanol). Washing aims to dissolve impurities in the sample so that cleaner crystals are obtained. The compound obtained was labeled LPR17.

Identification of the purity of the LPR17 compound using HPLC. The type of HPLC used is reverse phase HPLC, where the mobile phase used is polar (acetonitrile: water 9:1), the column size used by Shim-Pack VP-ODS (150x4.6 mm), and the method used is a gradient elution system, injection volume 20 μL, flow rate 1.0 mL/min, UV detector at wavelengths of 205 nm and 277 nm. The HPLC chromatogram showed a dominant peak with a retention time of 3,282 minutes (205 nm and 277 nm detectors). Asymmetric chromatogram due to fronting and tailings at the peak (Figure 1). Fronting and tailings can occur because the stationary phase used in HPLC is overloaded, so the column must hold the solute more strongly [13].

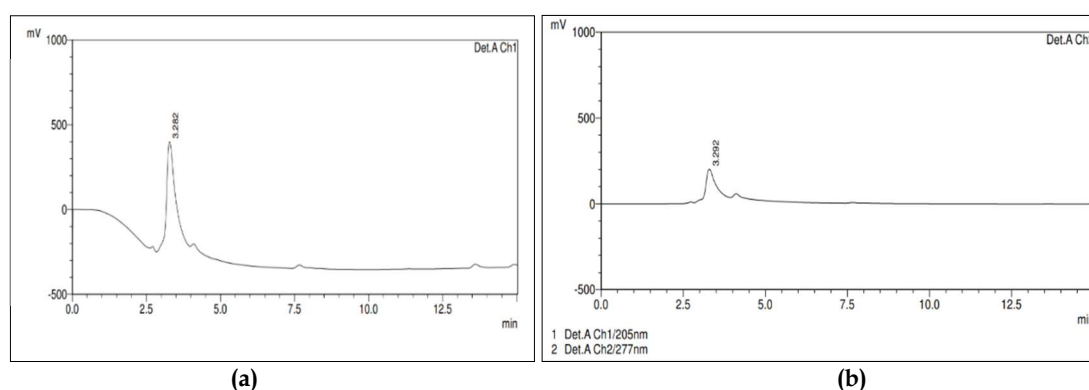


Figure 1. LPR17 Chromatogram ; (a) detector UV : 205 nm and (b) detector UV : 277 nm.

On organoleptic examination, the LPR17 compound was in the form of white crystals. Chemical examination of the compound LPR17 with Liebermann-Burchard reagent is pink, indicating that the compound belongs to the terpenoid group, characterized by the appearance of pink color [14]. This color is formed due

to the compound's conjugation extension, which is initiated by the acetylation process of anhydrous acetic acid [15].

The solubility and melting point measurements were carried out during the physical examination. The LPR17 compound is known to be slightly soluble in methanol and soluble in water. Solubility is primarily due to the polarity or dipole moment of the solvent [16]. In this case, the LPR17 compound, which is thought to be a terpenoid compound that dissolves in the water, is polar. This is caused by intermolecular forces, namely the dipole-dipole force, inducing hydrogen. A polar molecule with a permanent dipole will induce a non-polar molecule that does not have a dipole, so there is an electrostatic force between them or the so-called induced dipole-dipole force [17].

The compound LPR17 has a melting point of 239-241°C. In determining the melting point of a compound, if the melting point obtained has a difference in numbers less than 2°C, then the compound can be said to have better purity. However, if the difference is more significant than 2°C, the compound is not pure [7]. The LPR17 compound gave an absorbance of 0.118 at 277.0 nm and a maximum absorbance (0.765) at a wavelength of 205.0 nm (Figure 2). The wavelength indicates a chromophore group due to the electron transition from π - π^* , as in terpenoid compounds.

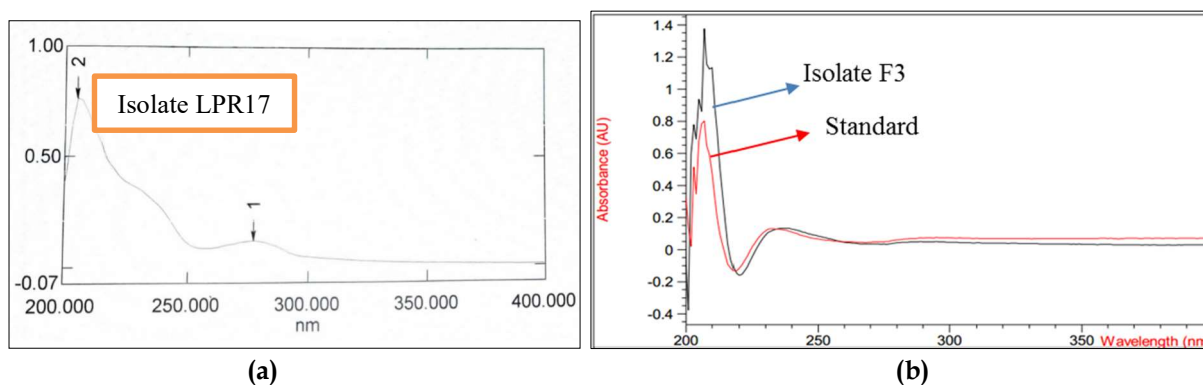


Figure 2. UV spectrum ; (a) LPR17 and (b) Triterpenoid standart (Cucurbitacin Standard)[18].

Identification of the LPR17 compound with FT-IR spectrophotometer aims to see the functional groups in the isolated compound. The results of the measurement of the FT-IR spectrum at a wave number of 326.5 cm^{-1} indicate a relatively broad and sharp absorption with a strong enough intensity indicating the presence of a hydroxy functional group (O-H). The wave number 2944.82 cm^{-1} area indicates the presence of the C-H aliphatic functional group. The wave number 2832.6 cm^{-1} area indicates the presence of the C-H aliphatic functional group.

The wave number area of 1651.1 cm^{-1} indicates the presence of the C=O functional group. The wave number area of 1449.54 cm^{-1} indicates the presence of the C-H functional group. The wave number area of 1020.68 cm^{-1} shows the presence of stretching vibrations -C-O -C-OH or C-O-C bonds (Figure 3).

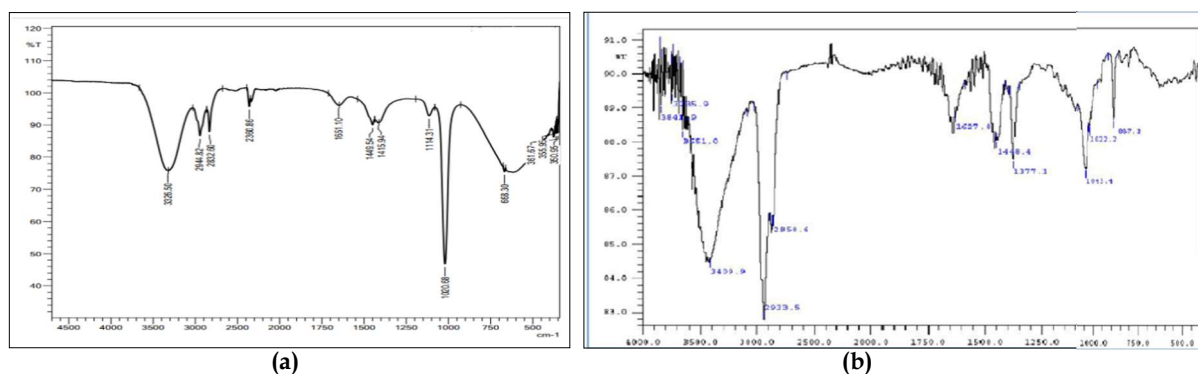


Figure 3. FT-IR spectrum; (a) Compound LPR17 and (b) Comparison of Triterpenoids [19].

The results of the alleged LPR17 compound are triterpenoid compounds, and this is also reinforced by the similarity of UV and FTIR spectra (Isolate F3 and Cucubirtacin standard) with previous research, which show the number of identical absorptions [18], [19].

From the observations, after 24 hours, the percentage of deaths at each concentration of 1000, 100, and 10 g/mL showed the average mortality was $73.3\% \pm 5.77$, $53.3\% \pm 3.78$ and $33.3\% \pm 5.77$, respectively. The control was not found to exert a killing effect on *Artemia salina*. After obtaining the percent mortality of *Artemia salina* larvae, it can be seen in the probit table of each percent mortality value obtained. The probit value is known using the regression equation, and the regression value is $y = 0.5268x + 4.075$. Then the concentration that caused 50% of experimental animal deaths obtained an LC_{50} value of 67.154 g/mL with a very toxic category (Table 1).

Table 1. BSLT test result of LPR17.

Compound	Concentration ($\mu\text{g/mL}$)	Concentration log (x)	% mortality	Probit value (y)	LC_{50} ($\mu\text{g/mL}$)	Result
LPR17	1000	3	73.3	5.6219	67.15	Toxic
	100	2	53.3	5.0828		
	10	1	33.3	4.5684		

Larval death was caused by the development of *Artemia salina* nauplii which were more sensitive to toxic compounds. The route of exposure to toxic compounds begins through the oral and dermal parts. In the mouth, these toxic compounds are absorbed into the digestive tract. At the same time, in the dermal part, there is an absorption process through cell membranes followed by the distribution of toxic compounds into the body of *Artemia salina*, and a metabolic reaction process occurs. After these toxic compounds enter orally and dermally, are absorbed into the body's tissues, and ultimately attack the cells, there is functional and metabolic damage to *Artemia salina* cells. The effects occur quickly within 24 hours, causing 50% of the deaths of *Artemia salina* [20].

CONCLUSION

Isolation of methanol extract of Derendan obtained LPR17 compound in the form of crystals (colorless cubic crystals), melting point 239-241°C. Based on the identification using the Liebermann-Burchard reagent, LPR17 is a terpenoid group. It is proven by the UV and FT-IR spectra data which have been compared with the literature. The LPR17 compound provided the cytotoxic activity was very strong $LC_{50} = 67.15 \mu\text{g/mL}$.

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