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Antioxidant Activity Test (DPPH) and Cytotoxicity of Jengkol Peel Ethanol Extract (*Pithecellobium jiringa*) on Shrimp Larvae (*Artemia salina Leach*)

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Abstract

This study aims to determine the antioxidant and cytotoxic activity of the ethanol extract of jengkol peel (*Pithecellobium jiringa*). Jengkol peel ethanol extract (*Pithecellobium jiringa*) was obtained by maceration using 70% ethanol as a solvent. In the phytochemical screening test it was found that chemical compounds contained in jengkol peel (*Pithecellobium jiringa*) including alkaloids, flavonoids, and saponins. The antioxidant activity of the ethanol extract was tested using the 35 ppm DPPH method. The test results on the ethanol extract showed moderate antioxidant activity with an IC_{50} value of 209.45 µg/mL. The cytotoxic activity of the ethanol extract was tested by the BSLT method using shrimp larvae (*Artemia salina* L.). The results showed that the ethanol extract of jengkol peel (*Pithecellobium jiringa*) has cytotoxic activity with LC_{50} value minimum of 17.875 mg/L and LC_{50} a maximum of 360.714 mg/L with an average of 180.136 mg/L.

Keywords: *Pithecellobium jiringa*; Antioxidant; cytotoxic.

INTRODUCTION

Jengkol (*Pithecellobium jiringa*) is one of the horticultural plants used as food for Indonesian people (Maxiselly *et al.*, 2016). Jengkol fruit is a smelly legume and is popular in Southeast Asia (Indonesia, Malaysia, Thailand and Cambodia) with a characteristic of an unpleasant aroma but is commonly consumed in various local dishes (Asikin *et al.*, 2017). Aside from being a food ingredient, jengkol seeds are shielded by black seed shells, during this time, the jengkol skin does not function properly and degrades into organic waste. According to previous studies, the skin of jengkol (*Pithecellobium jiringa*) contains chemical compounds including alkaloids, flavonoids, saponins, steroids/triterpenoids, and tannins, which are believed to have antioxidant and cytotoxic activity (Rahayu *et al.*, 2001). Jengkol can also be used to treat diabetes, high blood pressure, dysentery, gastric disorders, and bladder stones (Maxiselly *et al.*, 2016) (Shukri *et al.*, 2011).

This research focuses on the antioxidant and cytotoxic activity in jengkol peel. Antioxidants are molecules that limit the formation of radicals, complete their radical reactions, and neutralize the radicals formed (Erdogan *et al.*, 2020). Free radicals, in small amounts, initially serve to reduce inflammation and fight microorganisms in the body. These populations of free radicals are controlled by the body's antioxidants so that the amount is stable and non-destructive, but recently the number of free radicals rises as a result of changes in human lifestyle and environment, resulting in degenerative diseases such as cancer, heart disease, and premature aging caused by an imbalance of the amount of free radical produced in the body with the amount of endogenous antioxidant produced in the body, also known as oxidative stress (Khan *et al.*, 2013). As a result of the occurrence of oxidative stress, there is a decrease in the amount of oxygen and nutrients, which effects the microvascular and isochemic damage (referfusion injury) and causes cell and tissue damage due to the excessive production of free radicals as a result of the metabolism of fat and protein stored in the body. The body requires a large intake of antioxidants from outside or exogenous antioxidant sources to prevent this condition (Şeker *et al.*, 2021).

The DPPH method is a single procedure to figure out a material's antioxidant capacity. (2,2-diphenyl-1-picrylhydrazil or 1,1-diphenyl-2-picrylhydrazil). DPPH is a free radical molecule with a dark purple shade that is stable in methanol solution. The mechanism that occurs is the process of reducing DPPH compounds by

antioxidants which results in decomposition of the color intensity of the DPPH solution (Jabbar *et al.*, 2019).

Toxicity test is a preliminary test conducted to determine the toxic effect and threshold for the use of a plant as medicine. The initial method that can be used for cytotoxic testing is the Brine Shrimp Lethality Test (BSLT) method using shrimp larvae (Artemia salina Leach) (Fadhli *et al.*, 2019). The results of this test can be used to identify a wider range of plant bioactivity (Rahmi *et al.*, 2022). Therefore, this study aims to identify the antioxidant activity (DPPH) and cytotoxicity of the ethanol extract of jengkol peel (*Pithecellobium jiringa*) on shrimp larvae (*Artemia salina Leach*).

MATERIALS AND METHODS

Tools and Materials

Tools

This study used a Hitachi U-2000 spectrophotometer, technical balances, analytical balances, vacuum rotary evaporators, vessels for hatching shrimp eggs, lamps for BSLT, micro pipettes 10 – 1000 µL, a set of distillation apparatus, test tubes, whatman 42 filter paper, aluminum foil, and other glassware.

Chemicals and Samples

The chemicals used were 70% ethanol, methanol p.a, distilled water, 1,1-diphenyl-2-picrylhydrazyl (DPPH), dimethyl sulfoksida (DMSO), NH_4OH, chloroform, concentrated HCl, Mg metal.

Sample

Jengkol skin (*Pithecellobium jiringa*) was taken from the people's garden in Lubuk Alung, Padang Pariaman, West Sumatra.

Experimental Animals

The shrimp larvae (Artemia salina Leach) used by this study were obtained from the UNAND Biology Laboratory in Padang, West Sumatra.

Works procedure

Sample Preparation

A total of 2 kg of fresh jengkol peel samples (*Pithecellobium jiringa*) was taken and all materials are separated from dirt or other foreign materials then washed thoroughly. The sample was dried by air drying for 4-5 days until the moisture content is less than 10% so that the material obtained was not easily damaged. The dried samples were then chopped and mashed with *blender*. The simplicia obtained was wrapped in plastic and stored for further testing.

Extraction

As much as 1 kg of dry jengkol peel powder was extracted using 70% ethanol solvent by maceration method for 2x24 hours. The macerated filtrate is combined and the solvent was evaporated using a Rotary Evaporator at 60°C until a thick extract was obtained. The resulting extract was then used as a research sample.

Phytochemical Analysis

A total of 1 gram of extract was weighed, then basified with NH4OH, then 5 ml of chloroform:distilled water (1:1) was added, shaken in a separatory funnel, and left for a few moments until two layers formed. Water layer (flavonoids, phenolics, and saponins) for analysis, chloroform layer (terpenoids, steroids, and alkaloids).

Antioxidant Testing (DPPH)

Dissolving 10 mg of DPPH with methanol p.a in a 100 mL volumetric flask, pipetting 17.5 mL into a 50 mL volumetric flask to obtain a 35 ppm DPPH solution. A total of 3.8 mL of 35 ppm DPPH solution was dissolved with 0.2 mL of p.a methanol then left in the dark for 30 minutes. The solution's absorbance was measured through a UV-Vis spectrophotometer at 400-600 nm.

To reach a concentration of 1000 ppm, 25 mg of the extract was dissolved in a 25 mL volumetric flask with methanol p.a. The test solution was then pipetted with concentrations of 80, 120, 160, 200, and 240 ppm. As a comparison, vitamin C (ascorbic acid) levels in distilled water were 10, 20, 30, 40, and 50 g/mL, respectively.

Pipette 0.2 mL of each test solution into a test tube, then add 3.8 mL of 35 ppm DPPH solution and leave for 30 minutes in a dark spot. The absorbance of this solution is then measured at the maximum wavelength. In comparison, vitamin C was tested in the same manner, with each test being performed three times.

Toxicity Testing (BSLT)

The shrimp larvae were prepared by incubating 2 grams of Artemia salina L. eggs, soaking the eggs using 600 mL of seawater and lighting them with a 40-60 watt incandescent lamp, and aerating for 48 hours. The test solution was prepared by dissolving the sample in salt water with a concentration of 0, 25, 50, 100, 500, 1000 ppm, respectively. Control solution (0 ppm) was carried out without the addition of extracts. Furthermore, cytotoxic testing was carried out using the BSLT method, in which the test solution with concentrations of 0, 25, 50, 100, 500, 1000 ppm was pipetted as much as 6 mL and put into a test tube, then added 10 Artemia salina L. shrimp larvae into each test tube. Make observations for 24 hours, then count the number of shrimp larvae that died within 24 hours. For each test solution was repeated 3 times.The total number of Artemia salina L. larvae used was 180 individuals. The average mortality of larvae is obtained by dividing the total mortality of larvae at each concentration by the number of replications carried out, namely three times. Toxic activity is expressed in LC_{50} values which was calculated by probit analysis using Minitab.

RESULTS AND DISCUSSION

In this study, the results of the determination of jengkol plants (*Pithecellobium to the ring*) belongs to the family; *Mimosaceae* (legume), Genus; *Pithecellobium*, Species; *Pithecellobium lobatum* (Benth). The results of the phytochemical screening test on jengkol peel, found flavonoids, alkaloids, and saponins (table 1). In testing the antioxidant activity of the ethanol extract of jengkol peel, the IC_{50} value was obtained of 209.45 µg/mL (table 2; figure 1), as well as the LC_{50} value minimum 17,875 mg/L dan LC_{50} maximum of 360.714 mg/L with an average of 180.136 mg/L for the cytotoxic activity of the ethanol extract of jengkol peel, where the number of deaths *Artemia salina* L. is directly proportional to the increase in concentration (table 3; figure 2).

Table 1. Phytochemical Screening Test Results.

Compound	Identification
Alkaloid	+
Flavonoid	+
Saponin	+

Note: (+) contains the compound in question

Table 2. Test Results for Antioxidant Activity of Jengkol Peel Ethanol Extract against DPPH.

Concentration (ppm)	Absorbance Control	Extract Absorbance	Percent Inhibition (%)	Regression	IC_{50}
80	0,786	0,695	11,57		
120	0,786	0,601	23,53		
160	0,786	0,502	35,13	y = 0.294x – 11.58	209,45 µg/mL
200	0,786	0,405	48,47	R^2 = 0,997	
240	0,786	0,330	58,01		

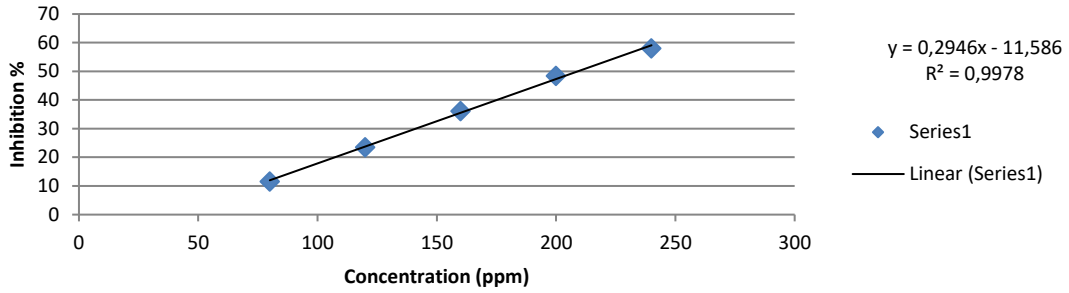

Figure 1. Curve for Determining Antioxidant Activity of Jengkol Skin.

Table 3. Test Results for the Cytotoxic Activity of Jengkol Peel Ethanol Extract (*Pithecellobium jiringa)* with the BSLT method.

Concentration (ppm)	Test			Total Deaths	Death Percent (%)
	P1	P2	P3		
0	0	0	0	0	0
25	1	2	2	5	16,67
50	3	2	3	8	26,67
100	5	5	4	14	46,67
500	6	8	7	21	70
1000	9	10	10	29	96,67

P1 (First try); P2 (Second try); P3 (Third try)

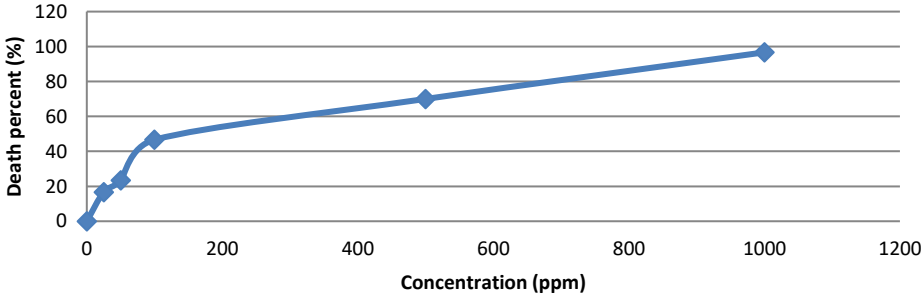

Figure 2. Relationship between Concentration and Death Percentage.

Discussion

This study used jengkol (*Pithecellobium jiringa*) taken from plantations in Lubuk Alung, Padang Pariaman, West Sumatra. 1 kg of dry jengkol peel powder was extracted using the maceration process with 70% ethanol solvent for 2x24 hours. The maceration method yielded 43.4695 grams of viscous extract with a yield of 14.29%. The DPPH technique was used to assess the antioxidant activity of an ethanol extract of jengkol peel. This method was chosen because it is a simple method, easy to measure antioxidants, sensitive and requires a small sample with a relatively short time. Compounds with antioxidant activity will react with DPPH, resulting in a purple to yellow color shift (Martinus *et al.*, 2014). The absorbance was then determined using a Uv-Vis spectrophotometer with a maximum wavelength of 515-520 nm. The IC50 number represents the quantity of antioxidant activity. The highest absorption wavelength of DPPH 35 ppm in methanol p.a recorded using a Uv-Vis spectrophotometer was 515 nm with an absorbance value of 0.869. Measurement of the antioxidant activity of the ethanol extract of jengkol peel (*Pithecellobium jiringa*) was prepared in several concentrations (table 2), namely 240, 200, 160, 120, 80 ppm, and vitamin C was used as a comparison. Examination of antioxidants in ethanol extract of jengkol peel (*Pithecellobium jiringa*) obtained the IC_{50} value of 209.45 ppm with moderate antioxidant activity because it has an IC value$_{50}$ between 101-250 ppm, when compared to vitamin C with IC_{50} value 34.11 ppm has a very strong antioxidant activity, because it has an IC_{50} value less than 50 ppm (Badarinath *et al.*, 2010).

Testing the cytotoxic activity of the ethanol extract of jengkol peel using *Artemia salina* L. The death test of Artemia salina L. represents a fast, cheap, and simple biological test to test the bioactivity of plant extracts which in many cases correlates quite well with cytotoxic and antitumor properties (Hossain *et al.*, 2012). The results obtained from the research using the Brine Shirmp Lethality Test (BSLT) method were the number of Artemia salina L. that died due to the administration of ethanol extract of jengkol peel (*Pithecellobium jiringa*) at a concentration of 1000 ppm, total death of 29 Artemia salina L., concentration of 500 ppm, total death of 21 Artemia salina L., concentration of 100 ppm, total death of 14 Artemia salina L., concentration of 50 ppm, total death of 7 Artemia salina L., concentration of 25 ppm, total death of 5 Artemia salina L., and concentration of 0 ppm no dead Artemia salina L. was found. This shows that the number of dead Artemia salina L. is directly proportional to the increase in concentration (Table 3; Figure 2). Furthermore, the cytotoxic activity of the ethanol extract of jengkol peel calculated by probit analysis using Minitab 15 Which expressed in LC_{50} values. To get the LC_{50} value first, mortality is calculated by means of the accumulation of dead divided by the accumulation of life and death (total) multiplied by 100% (Agustian *et al.*, 2013). The concentration that can result in 50% of the death of an animal population is obtained by drawing a line from 50% of the mortality probit (Arifuddin *et al.*, 2014) (Eroglu *et al.*, 2018). Probit analysis using Minitab 15 which was carried out on the ethanol extract of jengkol peel obtained cytotoxic activity with LC_{50} values minimum 17,875 mg/L and LC_{50} maximum 360.714 mg/L, with LC_{50} value average 180.136 mg/L.

CONCLUSIONS

Testing the antioxidant and cytotoxic activity of the ethanol extract of jengkol peel (*Pithecellobium jiringa*) showed that the ethanol extract of jengkol peel (*Pithecellobium jiringa*) has moderate antioxidant activity with an IC_{50} value of 209.45 µg/mL and cytotoxic activity with LC_{50} values minimum 17.875 mg/L dan LC_{50} a maximum of 360,714 mg/L, with an average value of 180,136 mg/L.

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Competing Interests: The authors declared no conflict of interest in the manuscript.

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