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BSLT and DPPH Methods to Determine the Potential Toxicity of Archidendron Pauciflorum Skin to Artemia Salina and the Antioxidant Potential as an Early Exploration of Anti-Cancer Drugs

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ABSTRACT

Archidendron pauciflorum skin is a dense waste that pollutes the environment and can be a problem if not properly cleaned. According to previous research, Archidendron pauciflorum skin has a very high potential because it contains flavonoid components and polyphenols with anti-cancer and antibacterial properties. To evaluate the toxicity activity with the BSLT Method and the antioxidant DPPH Method, the scalp is cut into small pieces, dried in the oven at 60 °C, and then macerated for 24 hours in a mixture of methanol and ethyl acetate solvents as well as hexane to produce polar, semipolar, and non-polar chemicals. The fibre from each solvent is collected, and the rotary evaporator is used to evaporate it until a thick extract is produced. According to the final findings of the study, the LC50 value (µg/mL) for each solvent is: the methanol value is 111 ppm, while ethyl acetate is 192 ppm. While for the test of antioxidant activity, significant results were obtained on the methanol solvent with an IC50 value of 51.13 µg/mL The conclusion of this study is that the 24-hour maceration period is the most efficient solvent for toxicity to Artemia salina and antioxidants to continue further research in the search for anti-cancer drugs.

1. Introduction

There will be a lot of cancer patients between 2010 and 2020, and the disease will still be the leading cause of death worldwide. Using pharmaceutical substances is one of many possibilities for treating cancer. The role of conventional medicine is actually strengthened rather than diminished by recent scientific and technological developments [1]. One of the many causes of death in Indonesia is cancer. Indonesia experienced 396,914 cancer cases in the year, according to the World Health Organisation's (WHO) Global Burden of Cancer Study (Globocan). Of these, 234,511 perished in 2020 as a result of the aforementioned illnesses. One area of the body is where cancer cells first begin to

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proliferate, but in the early phases of their growth, they exhibit no symptoms. When cancer cells travel to other tissues, symptoms start to appear. Consequently, this illness can be regarded as one that is difficult to identify and is therefore exceedingly hazardous. This process allows the cancer cells to move towards the blood vessels [2,3]. Cancer cells have the ability to modify their energy metabolism to accommodate the higher biogenetic requirements necessary for their accelerated and unchecked proliferation [4]. Surgery, radiation therapy, and chemotherapy are some of the therapies used to slow the growth of cancer cells. Natural chemicals are still employed as supplementary treatments [5].

A preliminary toxicity test using a 50% lethal concentration (LC_{50}) determination, one of the procedures in the Toxicity Test (BSLT) for Artemia salina Leach, can be used to identify a natural material that contains anti-cancer chemicals. Additionally, prior researchers claim that one of the test procedures is frequently utilised to discover new anticancer chemicals. The BSLT test method is also simple to use, quick, inexpensive, and fairly accurate. Artemia salina is used as the test animal for this procedure. It is well known that A. salina is relatively sensitive to toxins. This is due to the fact that A. salina larvae have thin skin and are therefore sensitive to their surroundings. As a result, foreign substances found in the larvae of A. Salina's living environment have the potential to diffuse into their bodies and have an immediate impact on their cells. A. salina larvae will perish if the chemical entering the cell has hazardous characteristics. [1,6].

Potential chemical compounds in the skin of Archidendron pauciflorum that are believed to have antioxidant properties can vary, but some classes of compounds commonly found in them include:

Phenolate: This component is commonly found in plants and is famous for its antioxidant activity. Phenolates can help fight free radicals that can damage body cells and cause oxidative stress.

Flavonoids: These are polyphenol compounds that are also found in a variety of plants. Flavonoids have been shown to have powerful antioxidant activity and can help protect body cells from oxidative damage [7].

Saponins are compounds that have a variety of health benefits, including antioxidant properties. They can help fight free radicals and reduce the risk of cell damage. Tanins: Tanins are polyphenol compounds found in many plants. They have the ability to bind and dampen proteins, as well as having strong antioxidant activity. Based on previous research according to Archidendron pauciflorum skin contained 9.56% polyphenols, 7.82% tannins, and 56.92% saponins, and Archidendron pauciflorum skin contains active compounds such as alkaloids, flavonoids, anthracinone glycosides, tannins, triterpenoids, and saponins [8]. The number of active compounds it has is considered by many Archidendron pauciflorum skins to be organic waste that is beneficial for health because it contains abundant flavonoids, thus allowing it to have strong antioxidant activity [9].

To obtain compounds that are potentially antioxidants, organic solvents such as ethyl acetate and methanol are needed, as they are good enough to dissolve various antioxidants, including phenolates, flavonoids, and tannins, which are commonly found in plants. This allows for the efficient extraction of such active ingredients from plant raw materials. Besides, it is easy to find, relatively inexpensive, and has chemical properties that facilitate the extraction process. can be used in a variety of extraction conditions, both at room temperature and at lower or higher temperatures, depending on the research needs. As well as being able to dissolve various kinds of compounds efficiently. This can increase the likelihood of detecting and measuring antioxidant activity in the sample.

2. Methods

2.1 Tools

The tools used in this study are: maserators, lumpangs, distillation equipment, analytical scales, a set of shrimp larva breeding tools, vials, aluminium voils, and glassware commonly used in laboratories. analytical scales, a set of microplate reader 96-well (Berthold) tools, UV-Vis 10S Genesis spectronics, vials, aluminium foil, drip plates, and glassware commonly used in laboratories.

2.2 Ingredients

The sample used in this study was Pithecellobium jiringa *Archidendron pauciflorum* skin obtained from the Rumbai market in Pekanbaru. The materials used are ethyl acetate (Pro Analis Merck 1.09623.2500), methanol (Methanol merck 1.06009), aluminium foil, aquades, Artemia salina shrimp eggs (Artemia Supreme Plus Pakan Burayak Cupang Guppy Repack Artemia Golden), and dimethyl sulfoxide (DMSO) 99,9%, Ultrapure, HPLC-grade methanol, DPPH Sigma Aldrich 50 mg, cotton, aluminum foil, and aquades.

2.3 Sample Extraction

Archidendron pauciflorum skin is dried in an oven at 40°C and then chopped into small pieces. A sample of 10 grammes was macerated for 24 hours using ethyl acetate and methanol solvents. The solvent fibre is collected, the solvent is evaporated until a viscous extract is obtained, and then the desired concentration is made [10].

2.4 Shrimp Hatchery

Artemia Salina L. *brine shrimp eggs* are hatched in containers or vessels filled with seawater. The vessel is stored in a room equipped with a bulb or lamp with good aeration for 48 hours at room temperature. After hatching, the larvae are separated from the eggs and collected on the bright side of the container (close to the light source) using a micropipette [11].

2.5 Toxicity Test

One cytotoxicity test that's employed from with minor modification Artemia salina Shrimp cysts are hatched in breeding containers filled with seawater and used 48 hours after the larvae hatch. Tests are carried out with concentrations of 1000, 100, and 10 ppm with three repetitions each. A total of 40 mg of the test extract was dissolved in 4 mL of solvent (10.000 ppm mother solution) [12]. Making a concentration of 1000 ppm by diluting the mother solution of 10.000 ppm as much as 2 mL with methanol up to 20 mL, we obtained a test extract concentration of 1000 ppm. A pipette of 2 mL with a concentration of 1000 was then added to solvent up to 20 mL so that a concentration of 100 was obtained. A concentration of 100 was taken in 2 mL, and then a solvent was added up to 20 mL so that a concentration of 10 ppm was obtained. Furthermore, 3 vials were prepared for each concentration, filled with 5 mL each, and then given a calibration limit. Each test vial is allowed to evaporate and dry its solvent. Re-dissolve the test extract with 50µL of DMSO using a micropipette, then add seawater to the calibration limit (5 mL). Put shrimp larvae in each vial—as many as 10 heads. Then observe the shrimp larvae after 24 hours. From the resulting data, LC_{50} is calculated by the probit table curve method [12].

2.6 Antioxidant Activity

An antioxidant activity test was performed using a microplate reader with two-fold dilution with the DPPH method [13-15]. At a wavelength of 520 nm. A sample of 2 mg is dissolved in 2 mL MeOH so that the sample concentration becomes 1000 µg/mL. Into the plate Microplate reader 96 wells in row A inserted a sample of 100 µL (*the plate* consists of rows A-H each totalling 12 wells). A total of 50 µL MeOH is inserted in each well on lines B-F. Line A is pipettes of 50 µL and inserted into row B, row B is pipetted 50 µL is inserted into row C and carried out until row F, row F is pipetted 50 µL and then discarded, so that concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, and 31.25 µg/mL are obtained. While the G-H row was filled with 50 µL MeOH, specifically in the H row filled only wells 1-6. Rows A-G added DPPH as much as 80 µL with a concentration of 80 µg / mL, then incubated for 30 minutes. Radical disclosure activity is measured as a decrease in DPPH absorbance with a *microplate reader* and data processing. The positive control used as a comparison was vitamin C with a concentration of 50 µg/mL Antioxidant activity is expressed as percent inhibition (% inhibition) with the following equation [16,17]

3. Results

The results of research conducted for toxicity tests with the BSLT method from Ethyl Acetate extract and Methanol of *Archidendron pauciflorum* fruit peel obtained LC_{50} values as in Table 1 [18]

Table 1
Toxicity activity of ethyl acetate extract and methanol of Jerngkol fruit peel

Extract Sample	Concentration (µg /mL)	Concentration Log (x)	Death (%)	Probit Value (y)	LC_{50} (µg /mL)
Ethyl Acetate	10	1	10	3.72	192
	100	2	35	4.61	
	1000	3	75	5.67	
Methanol	10	1	15	3.96	111
	100	2	48	4.95	
	1000	3	83	5.95	

The LC_{50} result of the ethyl acetate and methanol solvent is obtained from the linear regression equation by way of the concentration log versus the death probit of the larva, as seen in Figure 1 below.

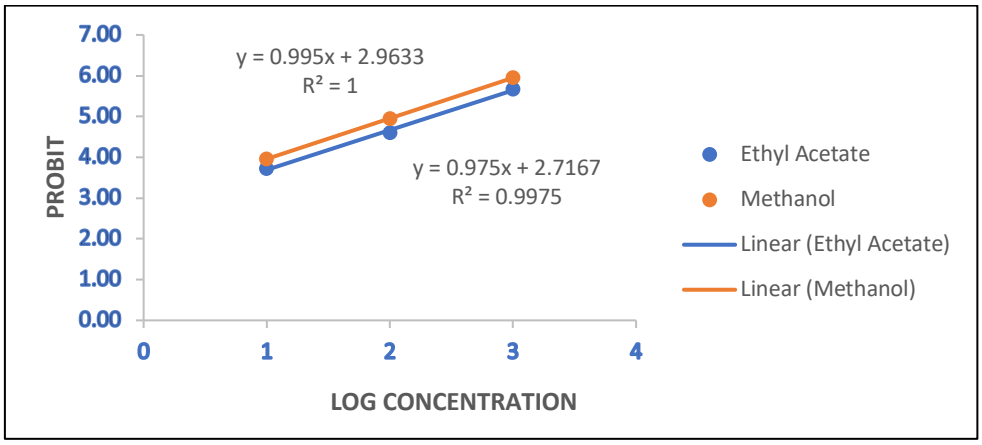

Fig. 1. Log Concentration vs. Probit larval death with ethyl acetate extract and methanol extract

An analysis of antioxidant activity using the DPPH method with a microplate reader 96 well (Berthold technologies) at a wavelength of 520 nm yielded an IC_{50} value as seen on with maceration time 24 hours in Table 2.

Table 2
LC_{50} calculation

LC_{50} methanol extract calculation	LC_{50} Ethyl Acetate extract calculation
y = 0.995x + 2.9633	y = 0.975x + 2.7167
5 = 0.995x + 2.9633	5 = 0.975x + 2.7167
0.995 x = 5 - 2.9633	0.975x = 5-2.7167
0.995 x = 2.0367	0.975x = 2.2833
x = 2.0469	x = 2.2833
LC_{50} = anti log 2.0469	LC_{50} = anti log 2.2833
= 111 µg/mL	= 192 µg/mL

Tabel 3
Measurement results: inhibition of maceration sample for 24 hours

Ln Konsentrasi	% Inhibition	
	Ethyl Acetate	methanol
6.9078	47.185	98.494
6.2146	39.235	88.377
5.5215	29.84	76.814
4.8283	22.885	66.697
4.1352	12.677	52.424
3.442	6.7148	41.223

Here is a linear regression equation (Figure 2) to obtain the IC_{50} value of an ethyl acetate solvent and methanol solvent with a plot between Ln Concentration versus % barrier to DPPH

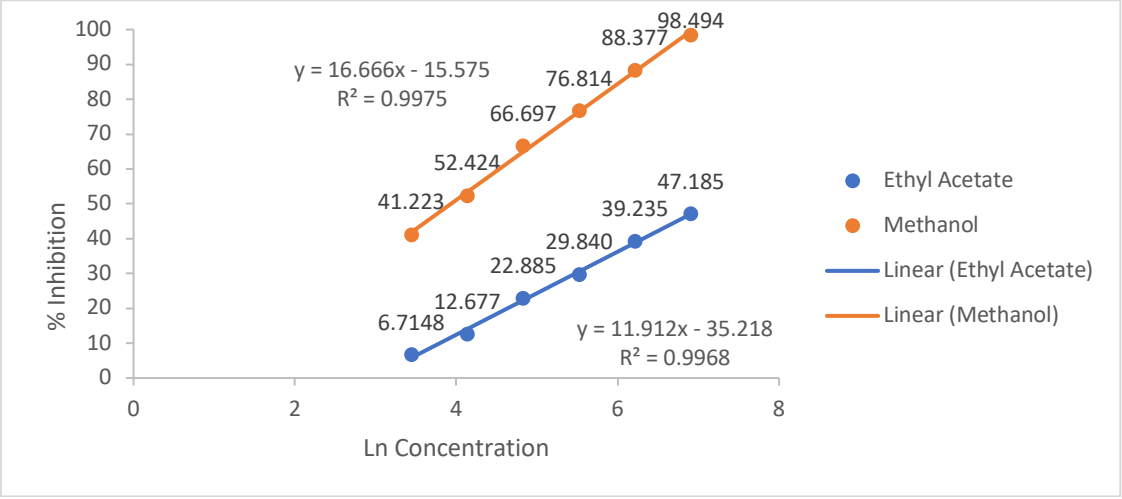

Fig. 2. Ln Concentration vs. % Inhibition Extract Type methanol and Ethyl Acetate

Table 4
IC_{50} calculation

IC_{50} methanol extract calculation	IC_{50} Ethyl Acetate extract calculation
y = 16.667x – 15.577	y = 11.912x – 35.218
y = 50	y = 50
50 = 16.667x – 15.577	50= 11.912x – 35.218
16.667x = 50 + 15.577	11.912x = 85.218
Lnx = 3,9345	Lnx = 7,1539
(IC_{50})= 51.13 µg/mL	(IC_{50}) = 1279,08 µg/mL

4. Discussion

Plant compounds are an important source of therapeutic compounds for cancer treatment. The BSLT method cytotoxicity test is a crucial preliminary test for anticancer characteristics. Human cancer cells can be used to continue the cytotoxicity test if the results demonstrate cytotoxicity [12]. The preliminary screening for cytotoxic activity was performed using the BSLT method [19].

Another opinion also says the toxicity test of the brine shrimp lethality test (BSLT) method is one method that is widely used as a first step in the search for new anticancer compounds [20]. The results of toxicity tests with this method have been shown to have a correlation with the cytotoxic power of anti-cancer compounds. The advantages of this method include being easy to do, fast, easy to reproduce, and showing a correlation with a specific anti-cancer It can be seen that the concentration of methanol extract of *Archidendron pauciflorum* skin in this experiment shows different effects on the death of Artemia salina larvae.

The number of larvae per test vial is 10 and each concentration is carried out 3 repetitions. The total number of *Artemia salina* Leach larvae used was 100 larvae. The larvae used are 48 hours old because at this age the larval limbs are complete compared to when the larvae hatched. In observing the growth and development of larvae to test the toxicity of the extract, an auxiliary tool is used to observe, namely a magnifying glass or loupe.

Mortality is obtained by adding up dead larvae on each vial of equal concentration. The results of probit analysis using a regression equation, showed LC_{50} methanol extract of *Archidendron pauciflorum* bark of 111 µg/mL. dan untuk ekstrak etil asetat 192 µg/mL that extracts with LC_{50} values are categorized as highly toxic to Artemia salina shrimp larvae, as shown in Table 1. The Lethal Concentration 50 (LC50) is the concentration of a substance that is needed to kill 50% of the organisms tested in a given period of time. The higher the LC50, the lower the toxicity of the substance. This is due to the polarity of both solvents, which can be explained as follows: Ethyl acetate is a less polar organic solvent compared to methanol. This means that ethyl aceticate is more likely to dissolve non-polar or slightly polar compounds. Methanol, on the other hand, is a more polar solvent than ethylacetate. This makes it more capable of dissolving polar compounds. So, when doing extraction using ethyl acetate, it is highly likely to extract more non-polar or a little polar composition, whereas methanol will be more efficient in extracting polar compounds, which is why the LC50 from the sample of ethyl aceticate extract is higher than that of methane because the ethyl acid extract contains less compounds that are toxic to the organism being tested.

The DPPH method (2,2-diphenyl-1-picryl-hydrazyl) is used to determine the antioxidant that can contribute one hydrogen atom. DPPH is a free radical that is widely used to test the corrosion and inhibition of free radicals from samples by measuring at concentrations ranging from 1000 µg/mL to 31.25 µg / mL. As for the measured deficit of DPPH radical absorption at 520 nm wavelengths [21]. The absorption decreases when the DPPH radicals are inhibited by antioxidants through a hydrogen donor to form a stable DPPH. The reaction causes a colour change from purple to yellow, as seen in

the picture. The antioxidant activity is indicated by a 50% barrier, which means that the sample can inhibit 50 % of the free radicals of DPPH, as shown in Table 4 [22, 23].

The results from the IC_{50} for methanol solvents and ethyl acetate were, respectively, 51.13 µg/mL and 1279.08 µg/mL. The IC_{50} values can be explained as methanol has a better ability to extract polar compounds, such as flavonoids, tannins, and phenolic acids, which often have antioxidant activity. As a result, methanol extracts often contain more antioxidants than ethylacetate extract, as well as because most of the antioxidants found in plants are more soluble in polar solvents such as methanol than in non-polar solvents such as ethyl acetate. Therefore, methanol is more effective in extracting this compound from the sample. Although ethyl acetate solvents do not necessarily show strong antioxidant activity in 24-hour mass extraction methods, this does not mean that the anti-oxidant compounds are not present in the extracts. Sometimes, certain antioxidant compounds may be less soluble in ethyl acetate, or their presence in ethanyl aceticate extract may not be detected in certain test methods. Therefore, it is important to consider the type of compound to be extracted and the method of analysis to be used when choosing solvents for extraction.

Following from previous research by [24], on the antioxidant quality of the plant Archidendron pauciflorum with the methods Radical NO and DPPH seen in Table 5.

Table 5
Phytochemicals and antioxidant properties of A. pouchiflorum fruit

Phytochemicals	Quantity
Total phenolics (mg GAE/g)	97.50 ± 2.45
Total flavonoid (mg QE/g)	6.67 ± 0.38
Total carotenoid (mg/100 g)	1.13 ± 0.12
Total β-carotene (µg/100 g)	12.51 ± 0.48
Ascorbic acid (mg/100 g)	39.61 ± 0.61
Total anthocyanin (mg/100 g)	94.12 ± 0.12
Antioxidant activity	Quantity
Total antioxidant capacity (µg AA/g) DPPH	194.32 ± 0.06
radical scavenging activity (%)	91.97 ± 0.39
Reducing power assay (µg AA/g)	2.54 ± 0.03
Ferric reducing antioxidant power (µM Fe2SO4/100g)	3288 ± 0.46
Metal chelating sapacity (%)	18.65 ± 0.27
NO Radical scavenging activity (%)	53.76 ± 0.06
IC_{50} (µg/g)	11.28 ± 0.37

Source : [24]

Interactions between the secondary metabolite compounds in the skin of Archidendron pauciflorum and DPPH (2,2-diphenyl-1-picrylhydrazil) in the context of antioxidant activity can occur through several mechanisms that contribute to the potential of the compound as a free radical inhibitor. Here are some possible interaction mechanisms:

i. Electron donation: The antioxidant compounds in the skin of Archidendron pauciflorum can donate their electrons to DPPH-free radicals. DPPH is an unstable molecule because it has one free electron on its nitrogen atom. When the antioxidants donate one or more of their electrons, DPPH becomes stable and no longer reactive as a free radical.
ii. Radical capture: Antioxidant compounds can physically react with free radicals, such as DPPH, by capturing them and forming a complex that is less reactive or not reactive at all. In the case of DPHP, this radical capture will result in a colour change from purple to yellow, which can be measured spectrophotometrically to evaluate antioxidant activity.

iii. Reduction: Some of the antioxidant compounds in the skin of Archidendron pauciflorum may have the ability to reduce free radicals by taking or receiving one or more electrons, thereby reducing the activity of such free radicals.
iv. Intermediate reaction: Some of the secondary metabolite compounds in the skin of Archidendron pauciflorum may inhibit the formation of free radicals or alter the reaction between free radicals and other molecules, thus preventing damage caused by free radicals.

This mechanism of interaction collectively contributes to the potential of the secondary metabolite compound in the skin of Archidendron pauciflorum as a free radical inhibitor. By showing powerful antioxidant activity through these interactions, these compounds can help protect the body's cells from damage caused by oxidative stress induced by free radicals. However, further research is needed to understand in more detail the mechanisms of these interactions and the potential antioxidant activity of these compounds.

5. Conclusion

From the research can we conclude :

i. From the toxicity test of ethyl acetate extract samples of *Archidendron pauciflorum* fruit peel, LC_{50} = 192 µg / mL was obtained
ii. From the toxicity test from methanol extract samples of *Archidendron pauciflorum* fruit peel, LC_{50} = 111 µg / mL was obtained
iii. Based on the table of LC_{50} probit values about toxite levels, a sample is categorized as a toxite for ethyl acetate solvent and methanol solvent
iv. From the LC50 results obtained, it can be explained that LC50 is the concentration of a substance needed to kill 50% of the organisms tested in a given period of time. The higher the LC50, the lower the toxicity of the substance to the organism tested
v. Among the three solvents that have good antioxidant activity are methanol solvents as big as 51.13 µg/mL , while ethyl acetate is as large as 1279.08 µg/mL

For Antioxidant Activity: IC50 is the concentration of a substance required to inhibit antioxidant activity by 50%. The lower the IC50 value, the stronger the antioxidants activity of the substance, because the lower concentration is required to the same effect. IC50 data provides information about the effectiveness of a substance in combating oxidative damage and protecting body cells from free radicals.

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