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Cytotoxic Test of Ethyl Acetate Extract of Breadfruit Leaves (*Artocarpus altilis* (Parkinson's ex F.A. Zorn) Fosberg) by Brine *Shrimp Lethality Test* Method

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A B S T R A C T

Breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A. Zorn) Fosberg) belonging to the Moraceae family are one of the plants that can be used as traditional medicine that is found near all regions in Indonesia. Breadfruit leaves (*Artocarpus altilis* (Parkinson's ex F.A. Zorn) Fosberg) have a high antioxidant activity that can be used as an alternative therapy in the treatment of cancer. The purpose of this study was to determine the cytotoxic activity of ethyl acetate extract of breadfruit leaves (*Artocarpus altilis* (Parkinson's ex F.A. Zorn) Fosberg). Observation of cytotoxic activity using the Brine Shrimp Lethality Test method with various test concentrations of 1000 µg / ml, 100 µg / ml, 10 µg / ml. The results of the study found that the percentage of death from each test concentration was different, namely 100%, 83.33% and 56.66%. Ethyl acetate extract from breadfruit leaves (*Artocarpus altilis* (Parkinson's ex F.A.Zorn) Fosberg) has cytotoxic activity with a value of ,LC-50. 11,180 µg/ml belongs to the category of highly toxic. Research can be concluded that breadfruit leaves (*Artocarpus altilis* (Parkinson's ex F.A. Zorn) Fosberg) have the potential to have cytotoxic activity.

Keywords: Breadfruit leaves; *Artocarpus altilis* (Parkinson's ex F.A. Zorn) Fosberg; Cytotoxic test

INTRODUCTION

One of the treatments of cancer is chemotherapy. Chemotherapy is the treatment with chemical compounds or drugs called cytostatics. Cytostatics is a class of drugs that can inhibit cancer growth or kill cancer cells[1]. These chemotherapy drugs work throughout the body as a result of which they can affect normal and healthy cells, so that there can be damage to healthy cells and cause side effects[2].

Currently, the treatment of cancer from chemicals is still at risk of side effects, so this makes people turn to traditional medicine derived from plants. One of the plants that can be used as a folk remedy is breadfruit (*Artocarpus altilis* (Parkinson's ex F.A. Zorn) Fosberg), belonging to the Moraceae family. The breadfruit plant (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) is easily found in almost all regions in Indonesia. The part of the breadfruit plant (*Artocarpus altilis* (Parkinson's ex F.A.Zorn) Fosberg) that has been used by the community is fruit, because it is rich in fiber. Breadfruit leaves are another part that is used for the treatment and prevention of diseases[3].

Breadfruit leaves (*Artocarpus altilis* (Parkinson's ex F.A.Zorn) Fosberg) contain alkaloids, steroids, terpenoids, flavonoids, phenolics and saponins[4]. Some plant parts of breadfruit are known to have antioxidant activity, including in the leaves. Based on research that has been carried out by Fakhrudin et al[5]., (2016) ethyl acetate extract from breadfruit leaves has a high antioxidant activity with the results obtained showing a value of IC_50 66.5 µg / ml.

In previous studies that have been carried out by Rosmawaty & Tehubijuluw (2013)[4] stated that methanol extract from breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) was tested by the Brine Shrimp Lethality Test method with a value of LC_50, [6]namely 392,826 µg / ml is toxic. Research that has been conducted by Ramadhani (2009)[6] states that ethanol extract of breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) was tested by the Brine Shrimp Lethality Test method with a value of LC_50 namely >1000 µg / ml which is non-toxic. In additio[7]n, the activity of the n-butanol fraction of breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) was tested by the Brine Shrimp Lethality Test method with a value of LC_50 the n¬-butanol fraction was 670.5 µg/ml which is toxic[7].

Based on the previous literature, there has not been reported a cytotoxic test of ethyl acetate extract of breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) using the Brine Shrimp Lethality Test method, so researchers are interested in conducting a cytotoxic test study of ethyl acetate extract of breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) using the Brine Shrimp Lethality Test method.

METHOD

TOOLS and Materials

The tools used in this study were *Rotary evaporator* (Ika Rv 10), micro pipette 1000 µg (Hamilton), micro pipette 50 µg (Hamilton), aerator (air bubble forming), UV lamp (Camag), aquarium / larval breeding container, desiccator (Duren), oven (Menmert), glass tools that support the study, KLT plate.

The ingredients used in this study were breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) , *eggs of Artemia salina* Leach, distilled water (PT. Novalindo), ethyl acetate (PT. Novalindo), seawater, dimethyl sulfoxide (DMSO) solution (PT. Brataco), mercury (II) chloride (PT. Brataco), potassium iodide (PT. Brataco), hydrochloric acid (PT. Brataco), iron (III) chloride (PT. Brataco), iodine (PT. Brataco).

PROCEDURE

Sampling

The sample used was fresh leaves of breadfruit (*Artocarpus altilis* (Parkinson's ex F.A. Zorn) Fosberg) as much as 1.5 kg taken from the Kalak Hantu area, Padang Sarai Village, Koto Tangah District, Padang City, West Sumatra.

Plant Identification

The identification was carried out at the ANDA Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang West Sumatra.

Creation of Simplisia

In general, the process of making simplicia goes through the following stages, plant collection, wet sorting, washing, plowing, drying, dry sorting and storage (Ministry of Health of the Republic of Indonesia, 1985).

Characterization of Simplisia

Characterization of simplicia includes organoleptic tests, drying shrinkage, total ash, acid-soluble ash, water-soluble juice, ethanol-soluble juice (Ministry of Health of the Republic of Indonesia, 2000).

Extract Creation

The simplicia that had been smoothed and weighed was then extracted by stratified maceration with n-hexane solvent and ethyl acetate. First the simplicia is weighed as much as 200 grams and then put in a dark bottle and soaked with *n-hexane* solvent until the sample is submerged. Soaking is done 1 x 24 hours while occasionally the bottle is stirred and shaken. The maceration results are filtered using filter paper to separate the pulp. Maceration is repeated up to 3 times. After three times the pulp is aerated for half an hour. Then the pulp is again put in a dark bottle and soaked with ethyl acetate solvent for 24 hours and then filtered with filter paper. This process is repeated 3 times. The macesan results from n-hexane solvents and ethyl acetate are concentrated with a *vaccum rotary evaporator* until a viscous extract is obtained and then the percent amendment is calculated (Larasati, 2021).

Extract Characterization

Characterization of extracts includes organoleptic tests, moisture content, total ash, acid-soluble ash (Ministry of Health of the Republic of Indonesia, 2000).

Cytotoxic Activity Test

Ethyl acetate extract was weighed as much as 50 mg and then dissolved in 5 ml of ethyl acetate. This solution is used as a mother liquor with a concentration of 10,000 µg/ ml (Meyer *et al.*, 1982). The test was carried out by means of 3 concentration variations namely 1000 µg / ml, 100 µg / ml, 10 µg / ml and each concentration was made 3 repetitions. The test solution is made by pipeting the mother liquor by 500 µl, 50 µl, 5 µl after which the solution that has been made is evaporated solvent by covering the vial with aluminum foil and then making holes in the aluminum foil[8].

Ethyl acetate extract that has been evaporated solvent then re-dissolves with dimethyl sulfoxide (DMSO) as much as 50 µl, add approximately 2 ml of seawater, then enter 10 shrimp larvae add sea water to the limit mark of 5 ml. The treatment lasted for 24 hours for 3 repetitions, the number of observed larvae was calculated and the mortality was determined[8].

RESULTS AND DISCUSSION

In this study, cytotoxic activity of ethyl acetate extract from breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg was carried out using the *Brine Shrimp Lethality Test* (BSLT) method. This research was conducted at the Laboratory of the College of Pharmaceutical Sciences (STIFARM) Padang. Plant identification has been carried out at the Herbarium Laboratory of the Department of Biology FMIPA, Andalas

University (ANDA). The results of the sample identification showed that the plant used was breadfruit (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) with the family Moraceae.

The breadfruit plant (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) in this study was obtained from the Kalak Hantu area, Padang Sarai Village, Koto Tangah District, Padang City, West Sumatra. The part of the plant used is the leaf part. The leaves taken are dark green and fresh leaves as much as 1.5 kg, cleaned of dirt by washing with clean water. Furthermore, breadfruit leaves are dried or at room temperature. Drying aims to reduce moisture content so as to prevent the emergence of fungi that can reduce the quality and efficacy of breadfruit leaves (Freitas, 2018). The dried breadfruit leaves are then mashed and powdered using a blender and the result is in the form of a powder weighing 200 grams.

The breadfruit leaf simplicia powder that has been obtained is then carried out standardization tests based on the Indonesian Herbal Pharmacopoeia standard (Ministry of Health of the Republic of Indonesia, 2017). The purpose of the simplicia standardization test is to determine the purity of a simplicia from impurities that may be carried away, as well as to find out whether the test carried out is in accordance or not with the standards that have been set based on the Indonesian Herbal Pharmacopoeia. The standardization tests carried out include organoleptic tests, drying shrinkage tests, total ash content, acid insoluble ash content, water-soluble juice content. The results of simplisia characterization obtained organoleptic tests of breadfruit leaf simplisia in the form of powder, green color, characteristic aromatic odor and tastelessness, drying shrinkage 7.13%, total ash 1.34%, acid insoluble ash 0.49%, water-soluble juice 7.19% and ethanol soluble juice 9.24%.

The extract manufacturing process uses the cold method of extraction, namely by multilevel maceration (Ministry of Health of the Republic of Indonesia, 2000). Where in the process of working on this study using two types of solvents with different levels of polarity, namely non-polar solvents (n-hexane), semi-polar solvents (ethyl acetate). This research is a series of research, so researchers focus on ethyl acetate solvents, the reason for choosing ethyl acetate solvents is because they are easy to evaporate so they are good for extraction, not hygroscopic and have low toxicity (Wardhani & Sulistyani, 2012). Ethyl acetate is semi-polar so it is able to attract aglicon and glicon compounds (Tensiska *et al*.,2007). Ethyl acetate can dissolve semi-polar compounds on cell walls such as aglicon flavonoids (Harbone, 1987). The advantage of extraction by cascading maceration is that it can minimize the sample to be used and more specifically draw the compounds contained in the sample. Preliminary maceration is carried out using n-hexane solvent, the simplicia of which is used as much as 200 grams with a solvent of 2 liters. The maceration process is carried out for 24 hours. The procedure is repeated up to 3 maceration processes. The residual pulp of n-hexane maceration was macerated with a semi-polar solvent (ethyl acetate) for 24 hours with a solvent of 2 liters, the maceration process was repeated 3 times. The total solvent of ethyl acetate used for maceration is 6 liters. The maceration results were filtered and the filtrate obtained was concentrated with a *Vacuum Rotary Evaporator* at a temperature of 50 ºC and obtained a viscous extract of ethyl acetate as much as 15.8273 grams with a yield value of 7.91365%, the amendment value is related to the large amount of bioactive content contained in plants. Budiyanto (2015) stated that the higher the yield of extracts, the higher the content of substances that are interested in a raw material.

The viscous extract obtained was then carried out characterization tests including organoleptic, water content, total ash and acid insoluble ash. Organoleptic results show that the extract of ethyl acetate of the leaves has a viscous shape, blackish-brown color, characteristic odor and bitter taste. The moisture content is 5.75%, the total ash is 1.15% and the acid insoluble ash is 0.59%.

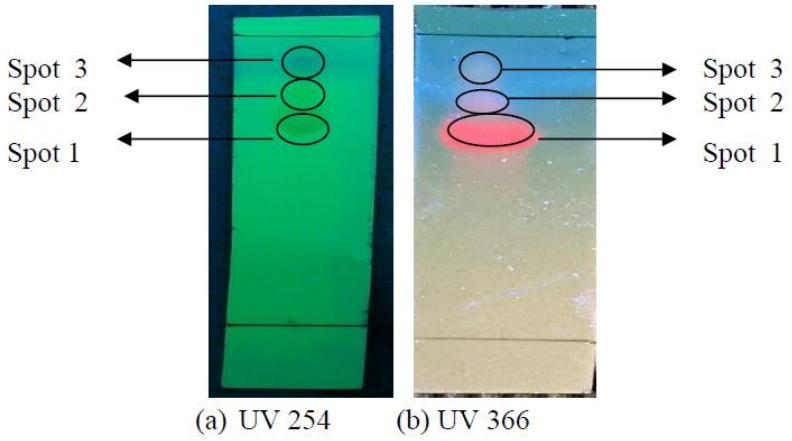

Figure 1. Thin-layer chromatography of ethyl acetate extract of breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg).

Furthermore, thin layer chromatography testing and secondary metabolite content tests were carried out, namely phytochemical screening of ethyl acetate extract of breadfruit leaves. The Thin Layer Chromatography Test aims to see the secondary metabolite compounds contained in the extract based on the degree of polarity between the extract and the solvent used. The results of the KLT examination of ethyl acetate extract of breadfruit leaves were observed directly under the rays of λ 254 and λ 366, namely the appearance of stain spots formed. R f values in ethyl acetate extract of breadfruit leaves R f 1 = 0.63 cm, R f *2* = 0.69 and R *f* 3 = 0.8 cm. The production of different R*f* values can be due to the different content of secondary metabolite compounds, so that the separation distance of the stains formed is different.

The results of the phytochemical screening test of ethyl acetate extract of breadfruit leaves showed that the extract contains alkaloids, flavonoids, phenols, saponins, steroids. Phytochemical screening aims to determine the presence of secondary metabolite groups contained in the extract qualitatively.

Cytotoxic tests were performed on ethyl acetate extract of breadfruit leaves. The extract was made a test solution with several concentrations of 1000,100, 10 µg/ml from a mother solution of 10,000 µg/ml. All test solutions are carried out by three repetitions. The extract is weighed as much as 50 mg, then the extract is dissolved with the appropriate solvent. This solution is used as a mother liquor for extracts. The test solution is prepared by pipetting an extract of 500, 50.5 µl from the mother liquor, after which the test solution is introduced in the desiccator until all its solvents evaporate. This is so that the death of the larvae is not affected by their solvent. After all the solvents have evaporated, 50 µl of DMSO is added and a volume of up to 5 ml is set with seawater. Dissolving extracts with seawater often causes problems due to differences in the level of polarity, extracts are difficult to dissolve with seawater so DMSO is used to help dissolve them. DMSO is one of the solvents that can dissolve almost all compounds, both polar, semi-polar and non-polar, has no effect on cell proliferation and DMSO is not toxic (Yulia & Hidayana, 2017). The extract that has been dissolved with seawater in a vial bottle was added with 10 larvae of Artemia salina. The results of observations for 24 hours found that Artemia salina performed a behavioral response showing symptoms of excitation characterized by loss of balance with an erratic swimming position. The results of the cytotoxic test of ethyl acetate extract of breadfruit leaves were then analyzed and obtained a Lc_{50} value of 11,180 µg/ml (highly toxic) Appendix 2, Table I).

Table I. Results of cytotoxic tes (Lc_{50}) ethyl aetate of breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg).

Sample (extract)	Number of deaths of shrimp larvae with concentration and 3 x repetition				Percentage of mortality of shrimp larvae (%)	Probit value	Lc_{50} (µg/ml)
	Concentration (µg/ml)	Repetition					
		I	II	III			
Ethyl acetate extract of breadfruit leaves	1000	10	10	10	100%	8,09	**11,180**
	100	10	7	8	83,33%	5,954	
	10	6	6	5	56,66%	5,151	

The presence of cytotoxic activity in ethyl acetate extract of breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A. Zorn) Fosberg) is thought to be because the extract contains alkaloid compounds, flavonoids, phenols and saponins. It is known that the alkaloid group derived from plants has a cytotoxic mechanism, which acts as an inhibitor. In the cell cycle process, alkaloids are related to tubulin, which is a protein that makes up microtubules. The binding of tubulin to alkaloids results in the polymerization of proteins into microtubules will be inhibited so that the formation of *myotic spindles* will also be inhibited and the cell cycle will stop at the metaphase stage because they cannot carry out cell division, the cells will then undergo apoptosis (Bertomi, 2011). Flavonoids have strong antioxidant activity to dampen the effects of free radicals that can cause various impacts, one of which is cell or tissue damage that can trigger the onset of cancer (Wayan *et al.*, 2018). The role of antioxidants in preventing cancer is shown through its ability to inhibit oxidation and initiation stages to prevent carcinogen activation and inhibit the promotion and progression stages by suppressing cell proliferation (Wayan *et al.*, 2018). Phenols have strong antioxidant properties and can prevent oxidative stress triggering cancer (Dai, 2010). Saponins have anticancer potential. Cytotoxicity of saponins occurs through the induction of apoptosis or non-apoptosis. Some of them are cell death due to autophagocytes, cell cycle inhibition, decreased production of cytoskeleton disintegration. Extrinsic apoptosis pathways are triggered through the activation of special pro-apoptosis receptors on the cell surface stimulated by special molecules i.e., pro-apoptotic ligands. Intrinsic apoptosis pathways occur by means of cytochrome-c release, depolymerization of mitochondrial membranes, downregulation of *Bcl-2*, stimulation of p53 or homeostasis disorders Saponins also play a role in inhibiting angiogenesi Ca^{2+} (Podolak *et al*,. 2010).

CONCLUSION

From the results of the study, it can be concluded LC_{50} that the value obtained in ethyl acetate extract of breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) is 11,180 µg / ml. Where ethyl acetate extract of breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg) has cytotoxic activity included in the highly toxic category using the *Brine Shrimp Lethality Test method.*

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