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

Aried Eriadi[1*], Dwi Dinni Aulia Bakhtra[1] , Alifti Nursi Fitri[1], Ully Chairunisa[1]

School of Pharmaceutical Sciences (STIFARM Padang), Padang, Indonesia
**Correspondence author: aried.eriadi@gmail.com*
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ABSTRACT

Breadfruit plant (Artocarpus altilis (Parkinson ex F.A Zorn) Fosberg) is a plant that is widely used and has economic value and has a high nutritional content. Breadfruit leaves contain many antioxidants such as flavonoids, xanthones, triterpenoids. Cytotoxic is a medicinal compound that can kill and inhibit the growth of developing cells. This researchaims to determine the value and cytotoxic activity of n-hexane extract of breadfruit leaves LC_{50} Artocarpus altilis (Parkinson ex F.A Zorn) Fosberg) by the Brine Shrimp Lethality Test (BSLT) method. The results of n-hexane extract of breadfruit leaves have cytotoxic activity with a value of 5.7557 µg / ml with a highly toxic category. Phytochemical screening showed n-hexane extracts of breadfruit leaves contained alkaloids, flavonoids, and steroids. This research can be concluded that breadfruit leavesLC_{50} (Artocarpus altilis (Parkinson ex F.A Zorn) Fosberg) have the potential to produce cytotoxic compounds.

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

INTRODUCTION

Indonesian people have been using traditional Indonesian medicinal herbs since ancient times as an effort to maintain health, prevent disease, and health care. These traditional Indonesian medicinal herbs can come from plants, animals, and minerals, but generally those used are of plant originMinistry of Health of the Republic of Indonesia, 2017). Among the many plants that are believed to be efficacious as medicine, one of them is the breadfruit plant. Breadfruit or (*Artocarpus altilis* (Parkinson ex F.A Zorn) Fosberg) is a type of plant that is widely used and has economic value because it produces fruits with high nutritional content(Adinugraha et al., 2014)

The breadfruit plant (*Artocarpus altilis* (Parkinson ex F.A Zorn) Fosberg) has long been used as a traditional medicinal plant by rural communities(Harmanto, 2012). Breadfruit leaves contain many antioxidants such as flavonoids, xanthones, triterpenoids. Of the several antioxidants above, the most researched antioxidants are flavonoids that have anti-inflammatory activity. In addition to anti-inflammatory activity, other effects found are anticancer, antiplatelet, and antisclerotic effects. Based on research Fakhrudin et al., 2015conducted ethyl acetate extract from breadfruit leaves has a high antioxidant activity with a result obtained of IC_{50} 66.5 µg / ml.

Brine Shrimp Lethality Test (BSLT) is a toxicity test method that is widely used as the first stage (*prescreening*) in screening antitumor bioactive compounds.The toxicity level of an extract is commonly known by calculating the percentage of naupli mortality of artemia as a test animal in BSLT(Fajarningsih et al., 2006). BSLT testing is an acute toxicity test conducted in determining toxic effects after dosing for 24 hours. This acute toxicity test will provide preliminary information on its correlation to anti-cancer and will provide clues about the appropriate dosage and which should be used, and also as a clue to the target organs that may be damaged and their specific toxic effects.(Lestari et al., 2019)

Based on previous research, that breadfruit leaf ethanol solvent (*Artocarpus altilis* (Parkinson ex F.A Zorn) Fosberg) has a value ofLC_{50} >1000 µg/ml(Ramadhani, 2009). Methanol extract tested by MTT method against MCF-7 breast cancer cells of breadfruit leaves has a value of LC_{50} 120.85 µg/ml which is toxic(Widowati, 2017). Research that has been carried out by(Rosmawaty & Tehubijuluw, 2013) states that breadfruit leaf extract from chloroform has a value of LC_{50} 387,436 µg / ml is toxicfrom n-butanol breadfruit leaf extract ofLC_{50} 670.5 µg / ml is toxic(Iswahyudi et al., 2015). In the study conducted, the value of breadfruit leaf methanol extract(Gustina, 2017)*Artocarpus altilis* (Parkinson ex F.A Zorn) Fosberg) against T47D breast cancer cells wasLC_{50} 234.4 µg / ml. In the study ethyl acetate extract from breadfruit leaves (Artocarpus altilis (Parkinson ex F.A.Zorn) Fosberg) by having cytotoxic activity with a value of LC_{50} 11,180 µg/ml belongs to the very high category(Eriadi et al., 2022)

* *Corresponding author.*
E-mail address: aried.eriadi@gmail.com

METHOD

TOOLS and Materials

The tools used in this study were *rotary evaporator* (Ika ®), micro pipette 1000 µg (Hamilton), micro pipette 50 µg (Hamilton), aerator (air bubble forming), Uv lamp (Camag), aquarium / larval breeding container, desikator (Duren), oven (Menmert), glassware that supports 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), N-Heksan (PT. Novalindo), seawater, dimethyl sulfoxide (DMSO) solution (PT. Brataco), mercury (II) chloride (PT. Brataco), potassium iodide (PT. Brataco), hydrochloric acid (PT. Brataco)

WORK PROCEDURE

Sampling

A sample of fresh breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A. Zorn) Fosberg) of 1.5 kg was 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

Proses the manufacture of simplicia through stages; plantcollection, wet sorting, washing, plowing, drying, dry sorting and storage.(Ministry of Health of the Republic of Indonesia, 1985)

Characterization of Simplisia and Extract

Characterization of simplicia and extracts includes organoleptic assays, 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 extraction process used is a maceration extraction method with n-hexane solvent. A total of 200 grams of simplician powder in a maserator along with 2 liters of n-hexane solvent were soaked for the first 6 hours while stirring once in a while, allowed to stand for 18 hours. The casting process is carried out at least twice with the same type and amount of solvent. The obtained maserion is evaporated with a *rotary evaporator* until a viscous extract is obtained. .(Ministry of Health of the Republic of Indonesia, 2000)

Phytochemical Screening

1. Alkaloids

N-hexane extract with two drops of Mayer reagent, Wagner reagent and Dragendrof reagent. The presence of alkaloids is characterized by the formation of white deposits and brown deposits and orange deposits on each test tube.

2. Flavonoids

N-hexane extract of breadfruit leaves with concentrated hydrochloric acid (concentrated HCl) and Zn powder to taste. The presence of flavonoids is characterized by red, orange and green colors.(Astuti et al., 2014)

3. Phenol

N-hexane extract of breadfruit leaves with $FeCl_3$1%. Formed bluish-black color indicates the presence of phenols.(Hanani, 2017)

4. Terpenoids and steroids

N-hexane extract with glacial and concentrated COOH. The solution is gently shaken and left for a few minutes. Test positive for steroids if they produce blue or green, while terpenoids produce red or purple$CH_3H_2SO_4$(Harborne, 1987)

5. Saponins

N-hexane extract of breadfruit leaves with tube pemanasan as well aswhipped Positive results were shown by the formation of a stable foam for 15 minutes.(Astuti et al., 2014)

Cytotoxic Activity Test

N-hexane extract with 3 concentration variations; 1000 µg/ ml, 100 µg/ ml, 10 µg/ ml as well as 3 repetitions. The test solution is made by pipetting the mother solution 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.(Meyer et al., 1982)

The n-hexane extract that has been evaporated solvent is dissolved with dimethyl sulfoxide (DMSO) of 50 µl, add approximately 2 ml of seawater, then add 10 shrimp larvae add seawater 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 (Meyer et al., 1982)

RESULTS AND DISCUSSION

In this study, cytotoxic activity of n-hexane extract from breadfruit leaves (*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg was carried out using the *Brine Shrimp Lethality Test* (BSLT) method. 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 part of the plant used is the leaf part with a dry weight of 200 grams of powder. The results of the characterization of simplisia based on the standards of the Indonesian Herbal Pharmacopoeia obtained breadfruit leaf simplisia in the form of powder, green color, characteristic aromatic odor and tastelessness, drying shrinkage 7.88%, total ash 3.93%, acid insoluble ash 2.14%, water-soluble juice 7.74 % and ethanol soluble juice 7.37%(Ministry of Health of the Republic of Indonesia, 2017). The extraction process using the maceration method with n-hexane solvent for 3 days for 3 repetitions obtained the extract weight of 90.6651% grams of amendment value which is 6.0443%. A high yield indicates that the value of the extract produced will be more and more, but on the contrary, the quality of the extract produced is inversely proportional to the amount of amendment produced, namely the lower the yield value produced, the good quality obtained.

The characterization test of n-hexane thick extract of breadfruit leaves showed that it has a viscous shape, blackish-brown color, characteristic odor and bitter taste, water axle 5.7 3%, total ash 1.98% and acid insoluble ash 0.59%.

In the KLT test for n-hexane extract samples, eluents are used, namely ethyl acetate and n-hexane, where ethyl acetate is semi-polar while n-hexane is non-polar, this aims to determine the separation of stains formed based on the degree of polarity of compounds that are retained in the stationary phase or will actually be attracted by the motion phase. For n-hexane extracts used ethyl acetate and n-hexane eluents in a ratio of 1 ml: 1 ml (n-hexane: ethyl acetate). It aims to determine the separation of stains formed based on the degree of polarity of compounds that are retained in the stationary phase or attracted by the motion phase. Compounds that are polar in nature will be retained by the stationary phase of the KLT plate, while compounds that are semi-polar and non-polar will separate or be attracted to form stain spots that rise to the top of the plate carried by the motion phase. Until a ratio of eluents suitable for n-hexane extract of breadfruit leaves is obtained, namely ethyl acetate and n-hexane 1ml: 1 ml. This KLT observation was seen directly under UV 366 light, where stain spots appeared. The values of Rf 1 = 0.56 and *Rf* 2 = 0.52, as shown in figure 1.

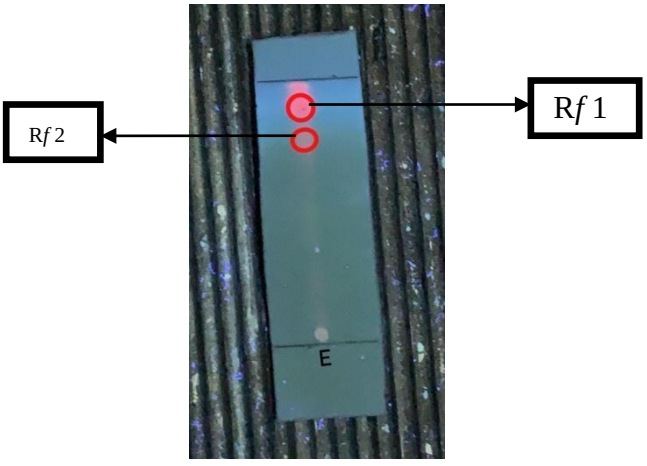

Figure 1. Thin-layer chromatography of n-hexane extract of breadfruit leaves (*Artocarpus altilis* (Parkinson's ex F. A Zorn) Fosberg)

The phytochemical screening test results of breadfruit leaf n-hexane extract showed that the extract contains alkaloids, flavonoids, and steroids.

Cytotoxic testing using the shrimp larval mortality test method using a variation of 3 concentrations, namely 1000 µg / ml, 100 µg / ml, 10 µg / ml, with three repetitions. Dimethyl sulfoxide (DMSO) is used to dissolve the extract. DMSO is one of the solvents that can dissolve almost all compounds, both polar, semi-polar and non-polar compounds and has no activity. At a concentration of 1000 µg/ml, the highest mortality rate was obtained, which caused 100% mortality to *Artemia*salina, and the lowest mortality rate at a concentration of 10 µg/mL. In this study, the percentage of deaths increased by increasing the concentration of the test solution. The higher the concentration of the extract, the higher the percentage of death of *A. salina* Leach larvae.

The value obtainedLC_{50} from the n-hexane extract is 5.7557 µg/ml with a highly toxic category. If a plant extract is toxic according to the valueLC_{50} of the shrimp larval death test method, then the plant can be developed as an anticancer drug, then breadfruit leaves can con tinue their research as an anticancer drug in the future.

This study showed uniform data with research on(Eriadi et al., 2022) ethyl acetate extract of breadfruit leaves (*Artocarpus altilis* (Parkinson ex

F.A.Zorn) Fosberg) against cytotoxic with a value of LC_{50}11,180 µg / ml and research(Inayah, 2007) on cytotoxic test of semipolar fraction of breadfruit bark acetone extract (*Artocarpus communis*) against Myeloma cells had a cytotoxic effect on Myeloma cells with IC_{50}148.64 µg / ml.

Table 1. Cytotoxic test results (LC_{50}) n-hexane extract of breadfruit leaves (*Artocarpus altilis* (Parkinson's ex F. A Zorn) Fosberg)

Sample	The number of deaths of shrimp larvae with a concentration and 3 repetitions				Percentage of shrimp larvae mortality	Probit value	LC_{50}
	Concentration	Repetition					
		I	II	III			
N-hexane extract of breadfruit leaves	1000	10	10	10	100%	8,09	5.7557 µg/ml
	100	10	10	8	93,33%	6,476	
	10	7	6	7	66,66%	5,413	

The content of secondary metabolite compounds that have the potential to be in breadfruit leaf plants contains alkaloids, flavonoids, and saponins. The flavonoid compound group has a mechanism as an anticancer because flavonoids as antioxidants are through the mechanism of cancer cell apoptosis pathways. The mechanism of cell apoptosis in this theory is due to DNA fragmentation. This fragmentation begins with the release of the proximal chain of DNA by reactive oxygen compounds such as hydroxyl radicals. Another effect is flavonoids as an inhibitor of tumor or cancer polyferation, one of which is by inhibiting the activity of protein kinase to inhibit the signal transmission pathway from the membrane to the core cells. Flavonoids inhibit the activity of tyrosine kinase receptors, because the activity of kinase receptors increases to play a role in the malignancy of cancer cells(Ren et al., 2003)

Furthermore, the alkaloid group derived from plants has a cytotoxic mechanism, which acts as a tubulin inhibitor. In the cell cycle process, alkaloids bind to tubulin, which is a protein that makes up microtubules. The binding of tubulin to alkaloids results in the polymeration of proteins into microtubules will be inhibited so that the formation of mitotic spindles will also be inhibited, and the cell cycle will stop at the metaphase stage. Being unable to perform cell division, the cell will then undergo apoptis. Then in the steroid group has a higher toxic activity(Smets, 1994). Such toxic activities have the potential to be anticancer and antitumor(Azah, 2019).

CONCLUSION

From the results of the research that has been carried out, it can be concluded that the value obtained inLC_{50} the n-hexane extract of breadfruit leaves (*Artocarpus altilis* (Parkinson ex F. A Zorn) Fosberg) is 5.7557 µg / ml. *N*-hexane extract of breadfruit leaves (*Artocarpus altilis* (Parkinson ex F. A Zorn) Fosberg) has cytotoxic activity belonging to the category of highly toxic using the *Brine Shrimp Lethality Test* method

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