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Cytotoxic Test of Fig Leaf Ethanol Extract Fraction (Ficus *carica* L.) Using Brine Shrimp Lethality Test Method

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Abstract

Cytotoxic test of fig leaf ethanol extract fraction (*Ficus carica* L.) has been conducted with the *Brine Shrimp Lethality Test* (BSLT) method. This study aims to determine the cytotoxic activity of fig leaf ethanol extract using the *Brine Shrimp Lethality Test* (BSLT) method. Fig leaves are extracted by maceration using 70% ethanol, then proceed with fractionation based on polarity, fractionation using N-hexane solvent, ethyl acetate and water. The results of phytochemical screening of fig leaf ethanol extract fraction stated that, n-hexan fraction contains flavonoid, phenolic, triterpenoid and saponin compounds. The ethyl acetate fraction contains phenolic compounds, triterpenoids and saponins. While the water fraction contains flavonoids, phenolics and saponin compounds. The results of cytotoxic testing of the fraction of fig leaf ethanol extract obtained LC50 values in the n-hexane fraction were 41.78 ppm (toxic), ethyl acetate fraction 33.57 ppm (toxic), and water fraction 16.36 ppm (very toxic).

Keywords: Uji Sitotoksik, Ficus carica, Brine Shrimp Lethality Test

1. Introduction

Tin Plant (*Ficus carica* L.) has long been used traditionally as one of the medicinal plants that have many properties, for the treatment of metabolic disorders, anti-inflammatory, gastrointestinal disorders (colic, loss of appetite and diarrhea), respiratory disorders (sore throat, cough and bronchial disorders). In medicine, figs are used as a mild laxative and expectorant (Duke *et al*., 2002).

Fig plants contain many bioactive components such as flavonoids, vitamins, enzymes, *nicotinic acid*, tyrosine, bergaptene, stigmasterol, psoralen, taraxasterol, beta-sitosterol, rutin, sapogenin, calotropenyl acetate, lepeolacetate and oleanolic acid systosterol which are widely found in tin leaves. Bioactive compounds such as 6-O-acyl-β-d-glucosyl-β-sitosterol, moeity acyl, palmitoyl and linoleyl with small amounts of stearyl and oleyl, have been isolated as strong cytotoxic agents from fig plant sap (*Ficus carica* L.) (Rubnov *et al*., 2001).

BSLT (*Brine Shrimp Lethality Test*) is one of the prescreening or preliminary tests to obtain simple biological activity to determine the level of acute toxicity of a compound or extract using *Artemia salina* as a test animal. *Artemia salina used in toxicity testing* is Artemia salina *which* is in the nauplii stage or larval stage. This is because *Artemia salina* at the nauplii stage is very similar to human cells (Meyer, 1982).

The chemical content of fig leaves is protein, fat, crude fiber, ash, and carbohydrates. In addition, it also contains secondary metabolites, including pentosan, carotene, bergapten, coumarin, flavonoids,

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glycosides, steroids, stigmasterol, sitosterol, triterpenoids, taraxasterol, betasitosterol, sapogenin, tyrosine, phenol, natural benzaldehyde, alkaloids, saponins, karotropenil acetate, fukusin (Joseph &; Raj, 2011).

Cytotoxic is a compound or drug candidate that works to kill and inhibit the growth of developing cells (Mayer &; Gustafon, 2004). Cancer cells are a class of diseases characterized by uncontrolled cell division and the ability of these cells to attack other biological parts (Astana, 2009).

2. Method

Extraction of fig leaves (*Ficus carica* L.) is carried out by maceration method. Cytotoxic fraction test of fig leaf ethanol extract (*Ficus carica* L.) was carried out by BSLT method.

Tools and Materials

The tools to be used in this study are tweezers, micro pipettes (Hamilton), drip pipettes, test tubes (Pyrex), test tube racks, volumetric pipettes, scales (Precisa), tissues, maserators, erlenmeyer (Pyrex), hotplate (Cimarec), beaker glass (Pyrex), measuring cups (Pyrex), rotary evaporator (Hahnvapors model HS-2361N5), split funnel (Pyrex), spatel, vials, larval breeding containers, aerators (air bubble formers) and camera. The materials to be used in this study are fig leaf samples (*Ficus carica* L.), *Artemia salina* eggs, 70% ethanol (Brataco), ethyl acetate (Brataco), n-hexane (Brataco), Aquadest, seawater, Mg powder, Hcl, Mayer, FeCl3 (Merck), Acetate anhydride (Merck) and DMSO (Merck).

Work Procedure

Sampling

2 kg fig leaf samples were taken from Jln. Rakik, Kurao Pagang, Nanggalo District, Padang City, West Sumatra. Then it is put in a clean plastic container and taken to the laboratory.

Plant Identification

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

Simplisia setup

Making simplisia is carried out through the following stages: simplisia collection, wet sorting, washing, knitting, drying, dry sorting (Ministry of Health of the Republic of Indonesia, 1985).

Extract Creation

600-grams of fig leaf simplisia powder is put into the macerator, plus 10 parts 70% ethanol, soaked for 6 hours while stirring occasionally, then let stand for 6 hours while stirring occasionally, then let stand for 18 hours. Maserat is separated by filtration and the process is repeated twice with the same type and amount of solvent, then concentrated with a *rotary evaporator* until a thick extract is obtained (Ministry of Health of the Republic of Indonesia, 2000).

Fractionation of Extracts

A total of 30 g of viscous extract was dissolved in 150 mL of water. The solution is further fractionated by adding 300 mL of n-hexane. Shake in a separator flask and let stand for 10-15 minutes until there are two layers (water in the bottom layer and n-hexan in the top layer). The two layers formed are then separated. The process of adding n-hexan to the separated bottom layer (water) is repeated twice. The top layer (n-hexane) formed during three fractionations is combined and referred to as the n-hexane fraction. The remaining water portion of the n-hexane fractionation process is then further diffracted with ethyl acetate and continued with water. The process that occurs is similar to the fractionation process with n-hexane solvent. The ethyl acetate layer that will be formed during three fractionations is combined and is called the ethyl acetate fraction, as well as the water layer which is referred to as the water fraction and the rest of the water layer is referred to as the water fraction. The fractionation results are thickened using a rotary evaporator (Wala, *et al*., 2015).

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Test of Fraction Chemical Content of Tin Leaf Extract

Water Phytochemicals

Drip fraction, add 1 ml of hydrochloric acid 2 N and 9 ml of water, heat on a water bath for 2 minutes add Mayer's reagent formed a white or yellow precipitate that dissolved in methanol P indicates the presence of alkaloids (Ministry of Health of the Republic of Indonesia, 1995).

Test flavonoid

Steam to dry 1 ml of extract solution, the rest is dissolved in 1 ml to 2 ml of ethanol (95%), add 0.5 grams of zinc powder and 2 ml of hydrochloric acid 2 N let stand for 1 minute. Add 10 drops of concentrated hydrochloric acid, if within 2 to 5 minutes an intensive red color indicates the presence of flavonoids (Ministry of Health of the Republic of Indonesia, 1995).

Test saponin

Take 1 ml of extract add 10 ml of water and then shake strongly for 10 minutes, if stable foam forms for not less than 10 minutes as high as 1-10 cm and in addition of 1 drop of hydrochloric acid 2 N foam does not disappear indicating the presence of saponins (Ministry of Health of the Republic of Indonesia, 1995).

Test fenol

The extract added 2 drops of 1% FeCl3 reagent (w/v). Phenol-containing extracts give it a blue-black or blackish-green color (Harboune, 1987).

Test terpenoid

The extract is added Liberman-Buchard reagent (acetic acid anhydra 10 drops and concentrated H2SO4 as much as 2 drops). The solution is shaken gently and leave a few minutes. The content of terpenoid compounds causes a red or purple color (Harboune, 1987).

Test of Cytotoxic Activity Fraction of fig leaf ethanol extract with *Brine Shrimp Lethality Test* (BSLT) Method.

Each fraction of fig leaf ethanol extract was weighed 30 mg, then dissolved in 3 ml ethanol and this was the parent solution of the sample. The test was carried out by means of 3 variations in concentration, namely 1000, 100 and 10 ppm, and each concentration was made in duplicate 3. The test solution is prepared by pickpocketing 500, 50 and 5 µL respectively from the mother liquor, after which the master solution test is inserted in the desiccator until all the solvents evaporate. As a control prepared 3 vials filled with only 50 µL of DMSO solution, then 2 mL of seawater was added. A total of 10 shrimp larvae were included in the vial, then the volume was sufficient 5 mL with sea water. The number of larvae that live is calculated after 24 hours, then it can be known the number of larvae that die, the LC50 value is calculated using the curve method (Kamu *et al.*, 2010).

RESULTS AND DISCUSSION

No	Sample (Fraction)	Number of deaths of shrimp larvae				Percentage of death of shrimp larvae (%)	Probit value	LC_{50} (ppm)
		Concentration	Repetition					
			I	II	III			
1.	Faction n-hexan	1000	10	10	10	100	8,09	41,78
		100	5	5	3	43	4,824	
		10	2	2	3	23	4,261	
2.	Fraksi etil asetat	1000	10	10	10	100	8,09	33,57
		100	5	4	6	50	5,00	

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		10	4	3	2	30	4,476	
3.	Faction Water	1000	8	8	9	83	5,954	16,36
		100	6	6	7	63	5,332	
		10	5	4	5	46	4,925	

Information:

Value (ppm)	Toxicity
<30	Highly toxic
30-1000	Toxic
>1000	Non-toxic

(MC Laughlin, 1991)

Identification conducted at the Herbarium of Andalas University (ANDA) Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, showed that the plant sample used was *Ficus carica* L. from the family Moraceae. The weight of the fraction obtained from the fractionation of 30-grams of fig leaf ethanol extract (*Ficus carica* L.) is n-hexan fraction of 9.72 grams, ethyl acetate fraction of 6.41 grams, and water fraction of 8.24 grams. Phytochemical screening test of fig leaf ethanol extract fraction (*Ficus carica* L.) showed that; The n-hexan fraction contains flavonoids, phenolics, triterpenoids and saponins. The ethyl acetate fraction contains phenolics, triterpenoids and saponins. The water fraction contains flavonoids, phenolics and saponins. Based on the results of cytotoxic tests of fig leaf ethanol extract fraction *(Ficus carica* L.) using the BSLT method on *Artemia salina larvae with the* Brine Shrimp Lethality Test *method* showed that the LC50 value of the N-hexane fraction was 41.78 ppm (toxic), the LC50 value of ethyl acetate fraction was 33.57 ppm (toxic), and the LC50 value of the water fraction was 16.36 ppm (very toxic).

DISCUSSION

Cytotoxic test fractions of fig leaf ethanol extract (*Ficus carica* L.) with various concentrations of 1000 ppm, 100 ppm, and 10 ppm as much as three times, with the sample solvent used, DMSO (dimethylsulfoxide). Then a cytotoxic activity test was carried out, on *Artemia salina larvae* and calculated using the probit log table. Cytotoxic assay of fig leaf ethanol extract fraction (*Ficus carica* L.) caused the death of *different Artemia salina* larvae, the smaller the LC50 the better the cytotoxic activity.

The LC50 value obtained from the n-hexane fraction is 47.78 ppm, the ethyl acetate fraction is 33.57 ppm, and the water fraction is 16.36 ppm. According to Mclaughin (1991), a compound is categorized as toxic if the LC value is$_{50}$ 0-30 ppm (very toxic), 30-1000 ppm (toxic) and more than 1000 ppm (non-toxic). The n-hexane and ethyl acetate fractions have toxic activity, while the water fraction has highly toxic activity. Cytotoxic activity in the water fraction is due to the presence of flavonoids, phenolics, and saponin compounds.

According to Harborne (1987), flavonoids are a class of phenol compounds which are polar compounds because they have a number of hydroxyl groups, so they will dissolve in polar solvents. Saponins are complex glycoside compounds, which are compounds resulting from condensation of a sugar with an organic hydroxyl compound which when hydrolyzed produces sugar (glycone) and non-sugar (aglicon). Saponins consist of two groups, namely triterpenoid saponins and steroid saponins. Both types of saponins are soluble in water (Robinson, 1995).

Flavonoid, phenolic and saponin compounds are anticancer. The mechanism of flavonoids as anticancer because flavonoids as antioxidants is through the mechanism of activating the apoptosis pathway of cancer cells. 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 inhibitors of tumor or cancer proliferation, one of which is by

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inhibiting the activity of protein kinases so as to inhibit the signal transduction pathway from the membrane to the nuclear cell. Flavonoids inhibit the activity of tyrosine kinase receptors, because the activity of tyrosine kinase receptors increases to play a role in cancer cell malignancy (Ren, *et al.*, 2003).

While phenolic compounds are widely found in nature, including polyphenols and phenolic acids, especially derivatives of 4-hydroxy benzoic acid and 4-hydroxycinnamic acid. Phenolic acids play a role in preventing cancer (Kampa *et al*., 2003). Saponin compounds are anticancer, inhibit the growth of carcinoma cells in breast cancer, with the mechanism of reducing cyclin protein levels and inhibiting the inhibition of E2F release (Birudu &; Naik, 2014).

CONCLUSION

The fraction of n-hexan contains flavonoids, phenolics, triterpenoids, and saponins. The fraction of ethyl acetate contains phenolics, triterpenoids, and saponins. The water fraction contains flavonoids, phenolics and saponins. The n-hexane, ethyl acetate, and water fractions of fig leaf ethanol extract (*Ficus carica* L.) have cytotoxic activity against *Artemia salina* larvae by *the Brine Shrimp Lethality Test method.*

SARAN

It is recommended that the next researcher conduct a cytotoxic test of the fraction of fig leaf ethanol extract (*Ficus carica* L.) using the BSLT method, replace the other fraction and test the fig sample.

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