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Toxicity Test of Basil (*Ocimum sanctum*) Leaf against *Artemia salina* Leach Using *Brine Shrimp Lethality Test*

Surahmaida*, Cicik Herlina Yulianti, Alfi Nur Diana, Alif Nuriyah
Academy Pharmacy of Surabaya, Indonesia

Abstract:
Background*: Basil (Ocimum sanctum) is a member of Lamiaceae family which is known as a medicinal plant. This research aims to determine the toxicity (LC_{50}) of basil leaves. The extraction was carried out by maceration using methanol as a solvent. Toxicity test using the Brine Shrimp Lethality Test method. In this research there were 4 concentration i.e 50 ppm, 100 ppm, 300 ppm, 500 ppm and control, each of which was repeated three times. Each concentration used 10 Artemia salina larvae and the mortality of larvae was calculated after 24 hours. The calculation of the LC_{50} value was identified by the percentage of shrimp larvae mortality using probit analysis. The results showed that the LC_{50} value of the methanol extract of basil leaves was 53.6630 ppm. The extract is toxic if the LC_{50} value is < 1000 ppm. This indicates that the methanol extract of basil leaves is toxic and has the potential as an anti-cancer compound.*
Key Word: *Basil (Ocimum sanctum) Leaf; Toxicity; Brine Shrimp Lethality Test; LC_{50}*

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I. Introduction

The development of science and technology is currently increasingly modern and advanced, but Indonesian people tend to choose "back to nature" which is to use plants as traditional medicine. This is because traditional medicine has relatively small side effects if used properly and without abuse. In addition to the benefits that have been used for generations by the community, these medicinal plants are also cheaper and easier to obtain [1,2].

One of the medicinal plants used as traditional medicine is basil (*Ocimum sanctum*). This plant belongs to the genus Ocimum and belongs to the family Lamiaceae. Basil (*Ocimum sanctum*) is one type of plant that is easily available, spread almost throughout Indonesia and can be grown wild or cultivated. Basil is used by the people of Indonesia as a traditional medicinal plant to treat various diseases because it contains many chemical compounds that are efficacious for human health [3].

The parts of the basil plant which are generally used as ingredients for traditional medicine are the leaves and seeds. Basil leaves have biological activity as analgesic, antiamnestic, anti-cataract, anti-fertility, antihelmintic, antibacterial, anti-inflammatory, antioxidant, antithyroid, immunodulator, antidiabetic, antitumor and anticancer [4]. Basil leaves contain flavonoids, saponins, tannins, steroids, essential oils (consisting of eugenol and methyl eugenol), ursolic acid, champesterol, stigmasterol and -sitosterol [5,6]. The chemical compounds eugenol and water-soluble flavonoids (orientin and vicenin) have antioxidant effects, scavenge free radicals and prevent the growth and spread of cancer by blocking the supply of oxygen and nutrients [7]. Compounds suspected of having anticancer activity should be tested on experimental animals first. One of the widely used toxicity test methods is the *Brine Shrimp Lethality Test* (BSLT). The *Brine Shrip Lethality Test* method is a bioassay method that is used as a preliminary toxicity test, and also to detect fungal toxins, toxicity of plant extracts, heavy metals, pesticides and cytotoxicity testing of dental materials [8].

Toxicity test is a test method used to determine the toxic level of a compound that is determined in a short time after administration of plant extracts. Toxicity test using the BSLT method has a broad spectrum of pharmacological activity, the procedure is simple, fast and does not require large costs (cheap) and the results are reliable. This method using shrimp larvae (*Artemia salina* Leach) as experimental animals. *Artemia salina* Leach is an organism that has a fairly high sensitivity to toxic. The results of this toxicity test have been shown to have a positive correlation with the cytotoxicity of anticancer compounds. If the toxicity test shows the LC_{50} below 1000 ppm, it means that the natural material has potential as an anticancer [9].

In previous studies, the toxicity test of *Ocimum sanctum* basil leaf extract was tested, but in that study, ethanol was used as the solvent. In this study, the toxicity test of basil leaf extract was carried out using methanol as the solvent. It is hoped that the results of this study can provide information that basil leaf extract (*Ocimum sanctum*) has potential toxicity to cancer cells.

II. Material And Methods

Plant Material

The sample used in this study was basil (*Ocimum sanctum*) leaves. A total of 1 kg of fine powder basil leaves (*Ocimum sanctum*) was obtained from Materia Medika Batu Malang, East Java, Indonesia. *Artemia salina* larvae egg were found in Lamongan, East Java.

Extract Preparation

The extraction method used is the maceration method. A total of 300 grams of fine powder basil leaves were put into a glass jar, then macerated for 3 days using 1 liter of methanol solvent. During the maceration process, stirring is carried out periodically (every 24 hours) so that the sample does not solidify and accelerates the penetration of the solvent into the sample.

After 3 days of maceration, then filtered with a cloth to obtain the filtrate. The maceration filtrate was then evaporated using a *rotary evaporator* to remove the solvent at a temperature of 50 ^{0}C to obtain a thick methanolic extract of *Ocimum sanctum* basil leaves.

Preparation of Artemia salina Leach

The hatching of *Artemia salina* larvae eggs was carried out by soaking 1 mg of *A. salina* eggs in a container containing 1 liter of seawater under a 5 watt lamp. *A. salina* eggs will hatch and become larvae after 24 hours [10]. A good larvae of *A. salina* used for the BSLT test is 48 hours old, because if it is more than 48 hours it is feared that the death of *A. salina* is not caused by toxicity but by the limited food supply [11].

Preparation of Test Extract Concentration

The concentration of the test extract for BSLT was 0 ppm (control), 50 ppm, 100 ppm, 300 ppm, 500 ppm. For the preparation of stock solution, methanolic extract of basil leaves was weighed as much as 0.1 gr, then dissolved in 100 ml of sea water, to obtain a stock solution concentration of 1000 ppm. Furthermore, dilution was carried out to make concentrations of 50 ppm, 100 ppm, 300 ppm, 500 ppm. The concentration of 0 ppm (negative control) was carried out without the addition of extract, containing only sea water and 10 larvae of *A. salina*.

Toxicity Test Procedure Using BSLT

This toxicity test was carried out by modifying the research conducted by [12,13]. In this study, testing the toxicity of the methanol extract of basil leaves (*O. sanctum*) against larvae of *A. salina* with a concentration of 0 ppm (as a negative control), 50 ppm, 100 ppm, 300 ppm, 500 ppm

Each test tube contains the test extract with a predetermined concentration + 10 *Artemia salina* larvae that are actively moving (48 hours old), then add up to 10 ml of seawater. While in the control, the test tube contained 10 *Artemia salina* larvae and 10 ml of seawater without the addition of extract. Each concentration was repeated 3 times and compared with the control. So, the total number of samples required is 130 *Artemia salina* larvae.

Statistical analysis

Observations and calculations of the number of dead A. salina shrimp larvae were carried out after 24 hours. Calculate the percent mortality of test larvae after 24 hours of treatment. The effects of toxicity were analyzed from observations with percent mortality [14].

$$\%\, mortality = \frac{number\ of\ dead\ A.salina\ larvae}{number\ of\ A.salina\ larvae} x\ 100\%$$

The sample toxicity test is determined by looking at the value of the LC_{50} which can kill *A. salina* up to 50% and statistical calculations are carried out using probit analysis (probability unit). After knowing the % mortality of *A. salina* larvae, then looking for the probit value through the probit table and regressing linearly, with the equation Y = a + bX

Description: Y = probit value of % death
a = y - intercept
b = regression slope
X = log concentration

The LC_{50} value is calculated from the antilog value of X when Y=5. Toxicity category of basil leaf extract was determined by the value of LC_{50} concentration [15], as presented in Table 1.

Table 1: Chemical Compound Toxicity Value and Categories

No	Toxicity Category	LC_{50} Value (ppm)
1	Very toxic	< 30
2	Toxic	30 - 1000
3	Non toxic	> 1000

III. Result and Discussion

The test sample must be in the form of a fine powder because to facilitate the extraction process, where the higher the level of fineness, the larger the surface of the sample, making it easier to take the active substance in the sample. The choice of maceration method is because its implementation is easier and does not require specific equipment. In addition, the maceration method can be used for both heat-resistant and non-heat-resistant compounds [16].

Toxicity Assay with *Brine Shrimp Lethality Test* (BSLT) Method

Toxicity assay was carried out using the BSLT method using *Artemia salina* larvae. This study was conducted to determine the toxicity ability of the methanolic extract of basil leaves (*Ocimum sanctum*) in killing *A. salina* larvae which had been treated with concentrations of 50 ppm, 100 ppm, 300 ppm, 500 ppm and a control solution containing only seawater. After 24 hours, the mortality of *A. salina* larvae was calculated. Larval mortality is calculated if there is no movement of the larvae. The following are the results of the toxicity test of basil leaf methanol extract against *A. salina* using the BSLT method, which are shown in Table 1.

Table 2: Effect of various concentrations of basil leaf methanol extract on shrimp larvae of *A. salina*

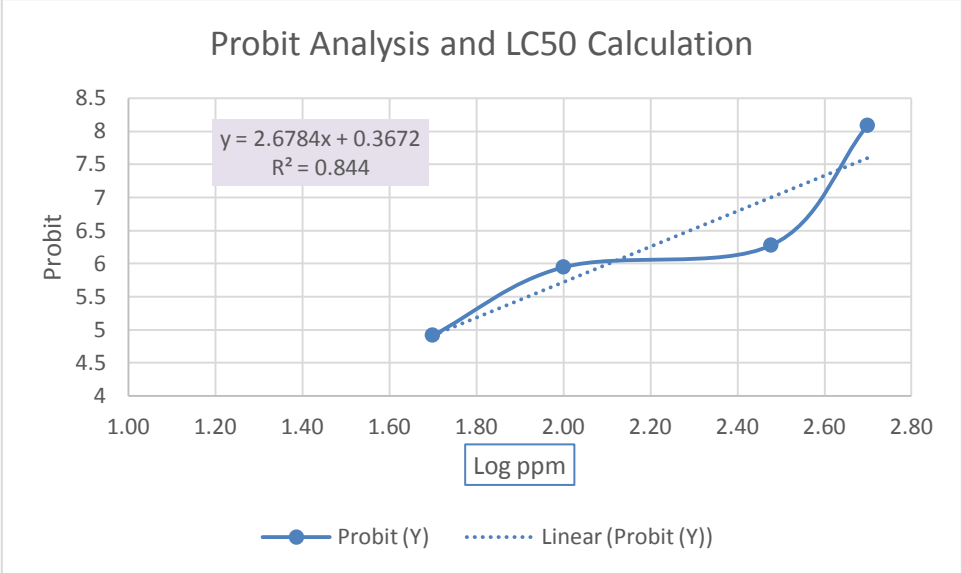
Replication	0 ppm (Control)	Number of Larva Deaths per Concentration			
		50 ppm	100 ppm	300 ppm	500 ppm
1	0	2	7	8	10
2	0	5	9	9	10
3	0	7	9	10	10
Total Larvae Mortality	0	14	25	27	30
Average	0	4,67	8,33	9	10
% Mortality	0	46,7 %	83,3 %	90 %	100 %

Based on Table 2, it can be seen that the higher the concentration, the greater the mortality rate of shrimp larvae, where the highest mortality rate was obtained at a concentration of 500 ppm and the lowest mortality at a concentration of 50 ppm. After getting the percentage of deaths, the LC_{50} value was calculated using the probit method using Microsoft Excel. The results of the calculation of the LC_{50} value are presented in Table 3 below:

Table 3: Calculation of the LC50 value using the Probit Method

Concentration (ppm)	Log Consentration (X)	Probit (Y)	% Mortality	Total Of Mortality	Total of Larvae Test
50	1,70	4,92	47%	14	30
100	2,00	5,95	83%	25	30
300	2,48	6,28	90%	27	30
500	2,70	8,09	100%	30	30

From the table above, a straight line can be drawn with the equation Y=aX+b so that a linear graph can be obtained as follows:



Picture 1: Toxicity test linear regression curve

The calculation results obtained LC_{50} of 53.6630 ppm, so that the methanol extract of basil leaves (*Ocimum sanctum*) is toxic according to BSLT and has the potential as an anticancer. According to [11], LC_{50} is an indication of the toxicity of a substance or chemical compound that can cause 50% death in test animals. The greater the value of LC_{50} means that the toxicity is getting smaller and conversely the smaller the value of LC_{50}, the greater the toxicity. A compound can be toxic if in a short period of time ($LC_{50\text{-}24\ hours}$) it is able to kill 50% of *A. salina* larvae.

The mechanism of death of *A. salina* larvae is related to the secondary metabolite compounds of the extract which are toxic which can inhibit the feeding power of shrimp larvae. When these compounds are ingested by the larvae, their digestive organs will be disturbed. In addition, this compound inhibits taste receptors in the mouth area of shrimp larvae so that shrimp larvae cannot eat and eventually die [11].

In this study it was found that the methanolic extract of basil leaves has potential toxicity. This is thought to be related to the chemical compounds contained in the methanolic extract of basil leaves (*Ocimum sanctum*), namely alkaloids, flavonoids, tannins, saponins, essential oils and terpenoids [17]. These chemical compounds cause the death of the larvae by inhibiting the ability to eat (antifeedant). The way of these compounds work is by acting as *stomach poisoning* so that it will interfere with the digestive system. In addition, this compound will inhibit taste receptors in the mouth area of the larvae. This resulted in the larvae failing to get a taste stimulus so they were unable to recognize the food so that the larvae starved to death [18].

Based on previous research, the toxicity test of 70% ethanol extract of basil leaves (*Ocimum sanctum*) with concentrations of 1200 ppm, 2400 ppm, 5000 ppm, and 10,000 ppm obtained an LC_{50} value of 5901.815 ppm. These results indicate that basil leaves are non-toxic [12]. Meanwhile, in the study of [13], the extract of the methanol fraction of basil leaves (*Ocimum sanctum*) with concentrations of 10 ppm, 100 ppm, and 1000 ppm had an LC_{50} value of 21.87 (very toxic).

This is different from the results of this study, where the methanol extract of basil leaves with concentrations of 50 ppm, 100 ppm, 300 ppm, 500 ppm produced an LC_{50} value of 53.6630 ppm and indicated that the basil leaf extract was toxic. So based on the LC_{50} value obtained by the BSLT method, the basil plant (*Ocimum sanctum*) can be carried out further research to be developed as an anticancer drug.

IV. Conclusion

1. The results of the calculation of the LC_{50} value of the methanolic extract of basil leaves (*Ocimum sanctum*) using probit analysis is 53.6630 ppm so it is classified as toxic
2. Methanol extract of basil (*Ocimum sanctum*) leaves has potential toxicity to Artemia salina larvae using the *Brine Shrimp Lethality Test* method and has potential as an antitumor or anticancer compound.

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