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Determination of Used Cooking Oil's Toxicity Level by Adding Nutmeg Leaves (*Myristica Fragrans Houtt*) Extract

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ABSTRACT

The nutmeg plant (*Myristica fragrans Houtt*) is one of the plants in the *Myristicaceae* family. The leaves of the nutmeg plant are rich in benefits, one of which has toxic indicating properties. The purpose of this study was to determine the level of used cooking oil toxicity by adding nutmeg leaf extract. This study used the maceration extraction method and the yield was 20%. Toxicity testing was carried out using the Brine Shrimp Lethality Test (BSLT) method. The research variable used was the concentration of nutmeg leaf extract added into refined used cooking oil at 10 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm, and 300 ppm. Based on the research results, the optimum concentration of nutmeg leaf extract to determine the level of toxicity in used cooking oil was 250 ppm with an LC_{50} value of 5.2389 ppm.
Keywords: Brine Shrimp Lethality Test (BSLT), nutmeg (Myristica fragrans Houtt) leaves, used cooking oil, toxicity.

1. Introduction

Nutmeg (*Myristica fragrans Houtt*) is one of the plants in the *Myristicaceae* family. Due to their high value as a spice, nutmeg fruit and seeds have been an important trading commodity since Roman times. Nutmeg leaf is one part of the plant that has not been widely used. Compounds contained in nutmeg leaves include alkaloids, triterpenoids, tannins, and 2 flavonoids. The flavonoid components found in nutmeg leaves indicate the presence of phenolic compounds. This phenolic component is important considering its large role in the treatment and prevention of disease, including as antioxidants and has cytotoxic properties [1].

Cooking oil is one of the most vital household necessities and is increasing. The function of cooking oil is as an ingredient in food processing. Repeated use of cooking oil can cause bad effects on health. This is because cooking oil is damaged due to hydrolysis, oxidation, polymerization, and browning reactions when used for frying. The oxidation and polymerization processes damage some vitamins and essential fatty acids in the oil so that it can cause poisoning in the body and various diseases, such as diarrhea, deposition of fat in blood vessels and cancer [2].

One of the efforts that can be done is by reprocessing cooking oil that has been used repeatedly in order to minimize the potential harmful to the body. For example, the use of

leaves of the nutmeg plant (*Myristica fragrans Houtt*) as an indicator in testing the toxicity of used cooking oil. Usually the method of testing the toxicity properties of a material or compound is the Brine Shrimp Lethality Test (BSLT) method [3].

Previous research by Puspa, et al used Phytochemical and toxicity test of nutmeg essential oil (*Myristica fragrans Houtt*) from Lekumutan Island with a comparison of ethyl acetate and methanol solvent using the steam distillation and toxicity testing using the Brine Shrimp Lethality Test (BSLT) method. It was found that the quality of the nutmeg leaf essential oil obtained, namely the refractive index value achieved of 1.475 according to SNI 06-2388-2006 and the results of the toxicity test showed an LC_{50} value of 5.192 ppm. Based on phytochemical test results, namely Nutmeg leaf essential oil contains terpenoids, flavonoids and saponins [4].

2. Material and Methods

2.1. Materials and tools

The materials were cooking oil, nutmeg leaf species *Myristica fragans Houtt*, magnesium, HCl, Tween 80, ethanol 96%, *Artemia salina Leach* larvae, aquades.

The tools were balance sheet, beaker glass, test tube, 1000 ml beaker glass, 25 ml measuring glass, 250 ml measuring glass, 100 ml volumetric flask, funnel, stirring rod, blender, filter, aluminum foil, filter paper, 50 ml beaker glass, evaporator.

2.2. Methods

2.2.1. Maceration

Nutmeg leaves obtained from Parung. The determination process of nutmeg leaves at LIPI, Bogor. Then dehydration process of nutmeg leaves was performed under the sun for 3 days until the color turns brown. The dried leaves were blended until smooth and filtered until a fine powder of nutmeg leaves is obtained. Maceration extraction was 96% ethanol solvent with a ratio of 1: 10 [5]. Maceration extraction was carried out for 5 x 24 hours [6]. Nutmeg leaf extract was concentrated a rotary evaporator to obtain a thick extract [7, 8]. The viscous extract was tested qualitatively. Phytochemical tests for flavonoid compounds and toxicity tests using the Brine Shrimp Lehality Test (BSLT) method [9].

2.2.2. Phytochemical test

1 g of extract had put into a test tube and dissolved by 3 ml of 70% ethanol, and had been shaken to produce an orange solution. Heated on a water bath for ± 1 minute. Filtered to separate the filtrate and residue. The filtrate was added with 0.1 grams of Mg metal and produced an orange solution and there was a yellow precipitate. Then, 2 drops of HCl were added. The red color change in the ethanol layer indicates the presence of flavonoid compounds [10].

2.2.3. Brine Shrimp Lethality Test (BSLT)

200 ml solution of 1000 ppm of used cooking oil after being purified were prepared. 100 ml of 1000 ppm nutmeg leaf extract solution. Was then add Concentrations of the extract within the solution of 1000 ppm, varied respectively 300 ppm, 250 ppm, 200 ppm, 150 ppm, 100 ppm, 50 ppm, and 10 ppm. Each sample was poured into 20 ml beaker glass. The solution of 1000 ppm of used cooking oil was poured into a 20 ml beaker glass. 5 ml of extract solution were dropped into the used cooking oil sample, and then stirred until homogeneous. 10 *Artemia Salina L* shrimp larva were put into each beaker glass containing sample solution of varying concentrations of extract and a sample solution of used cooking oil and extract

mixture. The sample were observed for 1 x 24 hours, and the dead *Artemia Salina L* shrimp larvae were accounted. LC_{50} [11].

2.3. Analytical Method

Yield of the extraction of nutmeg leaves were by calculated according to this formula :

$$Yield = \frac{nutmeg\ leaf\ extract\ weight}{dry\ nutmeg\ leaf\ weight}\ x\ 100\ \%$$

Analysis of the data of the Toxicity test were calculated using the LC_{50} formula.

$$y = a + bx$$
$$LC_{50} = \text{arc log } x$$

description :

a = intercept; y = probit value; x = $\log^{10}$ concentration (ppm); b = slope

The probit value is an analysis that canestimate the effective dose by determining the concentration of mortality. The log concentration of extracts were compared, as well as the number of larvae mortality with the value of the probits depicted in an XY graph. The X axis showed the log concentration of nutmeg leaf extract and the amount of mortality, while the Y axis showed the probits value of each of these concentration logs. So that it was known the optimum conditions where the addition of nutmeg leaf extract works optimally.

3. Results and Discussions

3.1. Results

3.1.1. Nutmeg Leaf Yield

The thick extract of nutmeg leaves was made by the maceration extraction method with 96% ethanol as a solvent achived a yield of 20%.

3.1.2. Phytochemical Test Results

In determining the presence of flavonoid compounds in the nutmeg leaf extract, a qualitative test was carried out. The sample was heated for ± 1-2 minutes, 0.1 grams of Mg to form a precipitate were added, then 2 drops of concentrated HCl were added too. A positive result was confirmed when a red color appeared. Based on the research conducted, nutmeg leaf extract contains flavonoid compounds was indicated by a red color change during the test.

3.1.3. Brine Shrimp Lethality Test (BSLT) Results

Toxicity testing has been done using the Brine Shrimp Lethality Test (BSLT) method. The sample consisted of purified used cooking oil, and thick extract of nutmeg leaves. The test was carried out using artificial seawater media which was added to 10 tail *A. salina shrimp* larvae and counted the number of *A. salina shrimp* larvae that died after being left for 1 x 24 hours during the test.

Table 1. The results of BSLT test for nutmeg leaf extract *Myristica fragans Houtt*

No	Nutmeg leaf extract concentration (ppm)	Mortality (tail)	Total (*A. Salina larva*) (tail)
1	10	2	10
2	50	4	10
3	100	4	10
4	150	6	10
5	200	7	10
6	250	8	10
7	300	8	10

Table 1 and 2 show the results of the toxicity test of used cooking oil with the addition of nutmeg leaf extract using the Brine Shrimp Lethality Test (BSLT) method.

Table 2. BSLT test results of cooking oil mixing and nutmeg leaf extract mixture

No	Cooking oil and nutmeg leaf extract mixture concentration (ppm)	Mortality (tail)	Total (*A. Salina* larva*) (tail)*
1	10	6	10
2	50	7	10
3	100	7	10
4	150	8	10
5	200	8	10
6	250	8	10
7	300	9	10

3.2. Discussion

This research was carried out by utilizing nutmeg plants which were more specific on nutmeg leaves that thrive in the Bogor area, West Java with the species *Myristica fragrans Houtt*. The reason for choosing nutmeg leaves as research raw materials was that in addition to nutmeg plants that are fertile in the area, nutmeg leaves contain flavonoids, saponins, terpenoids and are toxic.

The following is a graph of the probits versus mortality values of nutmeg leaf extract and used cooking oil added with *Myristica fragrans Houtt* nutmeg leaf extract.

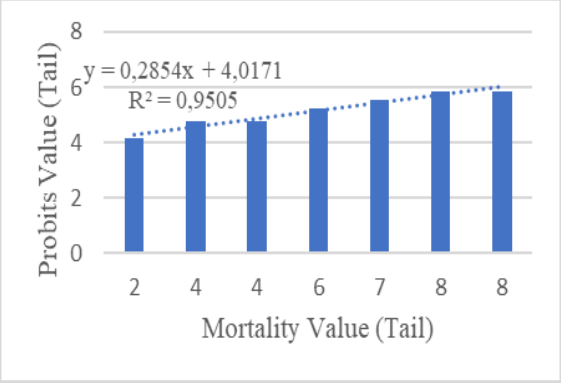

Figure 1. Results of Brine Shrimp Lethality Test nutmeg leaf extract

Based on the graph above, the Brine Shrimp Lethality Test of nutmeg leaf extract at 10 ppm had a mortality of 2 larvas with a probit value of 4.16, 50 ppm had a mortality of 4 larvas with a probit value of 4.75, 100 ppm had a mortality of 4 larvas with a probit value of 4.75, 150 ppm had a mortality of 6 larvas with a probit value of 5.52, 200 ppm had a mortality of 7 larvas with a probit value of 5.52, 250 ppm had a mortality of 8 larvas with a probit value of 5.84, and 300 ppm had a mortality of 8 larvas with the probits value is 5.84.

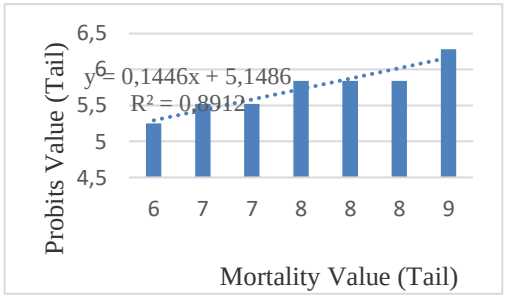

Figure 2. Results of Brine Shrimp Lethality Test for used cooking oil and nutmeg leaf extract mixture

Based on the Fig. 2, the Brine Shrimp Lethality Test of used cooking oil and nutmeg leaf extract mixture at 10 ppm had a mortality of 6 larvas with a probit value of 5.52, 50 ppm had a mortality of 7 larvas with a probit value of 5.52, 100 ppm had a mortality of 7 larvas with probits value of 5.52, 150 ppm had a mortality of 8 larvas with a probits value of 5.84, 200 ppm had a mortality of 8 larvass with a probit value of 5.84, 250 ppm had a mortality of 8 larvas with a probits value of 5.84, and 300 ppm had a mortality of 9 tails with a probit value of 6.28.

Fig. 3 shows the LC_{50} of nutmeg leaf extract and used cooking oil added with nutmeg leaf extract.

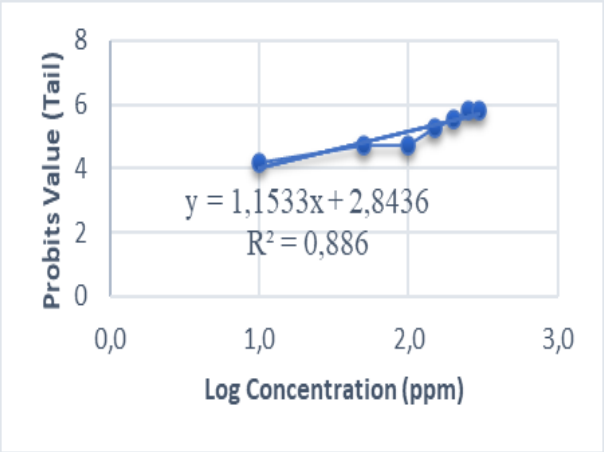

Figure 3. LC_{50} of nutmeg leaf extract

Fig. 4 shows the LC_{50} of used cooking oil and nutmeg leaf extract mixture.

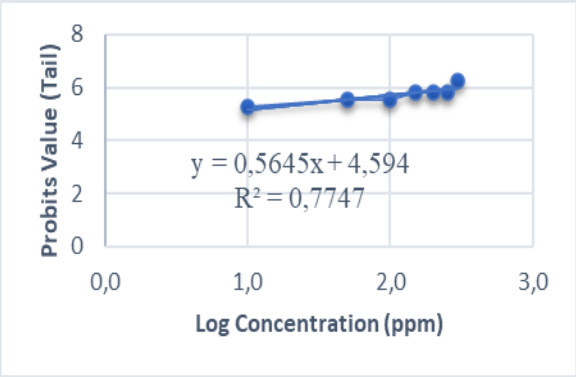

Figure 4. LC_{50} of used cooking oil and nutmeg leaf extract mixture

Based on the analysis of the BSLT test, the LC_{50} value of nutmeg leaf extract (*Myristica fragrans Houtt*) was 74.098 ppm and the LC_{50} value of used cooking oil added to nutmeg leaf extract (*Myritica fragrans Houtt*) was 5.2389 ppm. Thus, the addition of nutmeg leaf extract (*Myritica fragrans Houtt*) can measure the level of toxicity of the used cooking oil.

4. Conclusion

The yield of nutmeg leaf extract using 1:10 maceration extraction method using 96% ethanol solvent was obtained as much as 20%. The optimum condition for the addition of nutmeg leaf extract in the used cooking oil toxicity test was at a concentration of 250 ppm. The results of the BSLT test of used cooking oil added with nutmeg leaf extract obtained an LC_{50} value of 5.2389 ppm. The lower the LC_{50} value, made the mortality rate of larvae *Artemia s L* higher.

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