

## Anti-Cancer Activity Testing of Cumin (*Plectranthus amboinicus*) Ethanol Extract Against *Artemia Salina* Leach by using *Brine Shrimp Lethality Test (BSLT)* Method

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### Abstract

The purpose of this study was to determine the anticancer activity of cumin leaf extract (*Plectranthus amboinicus*), which was applied using the Brine Shrimp Lethality Test (BSLT) method to determine the level of acute toxicity (LC50) of the extract against *Artemia salina* LEACH larvae. Cumin leaf samples were extracted using the maceration method with 96% ethanol. The cumin leaf extract was then tested qualitatively and quantitatively. Toxicity testing used concentrations of 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm, and 2500 ppm, which were given to *Artemia salina* LEACH larvae for 24 hours. Calculate the LC50 value in this study using probit analysis. The results of qualitative and quantitative testing showed the presence of saponins by 5.20%, tannins by 8.21%, flavonoids by 23.93%, and alkaloids by 4.37%. The results of the acute toxicity test of the extract using the BSLT method showed that there was anticancer activity with the acquisition of LC50 of 1000 ppm, which was 697.99 ppm.

**Keywords:** Cumin Leaf Extract; Flavonoids; BSLT Method; Anticancer; LC50

### Introduction

Cancer is a condition in which cells grow out of control and spread abnormally throughout the body (Winarsih H., 2007). There are several varieties of cancer, which are named according to the growth of cancer itself. Cancer is generated by the damage or mutation of proto-oncogene cells that are bound to proteins, which prevents normal cell proliferation (Winarsih H., 2007).

In 2018, there were 17 million cancer cases worldwide, resulting in 9.5 million cancer-related deaths (Robert, *et al.* 2019).

Surgery, chemotherapy, radiation, modification, and immunotherapy are among the cancer treatments used in Indonesia. However, the dangerous side effects of cancer drugs and radiation are still a threat to cancer patients, and not all cancer patients can be cured by surgery (Winarsih H., 2007). This caused traditional medication to be widely used by many people. In addition to being economical and easy to obtain, traditional medicines have also been proven to be effective both empirically and clinically (Iriyanti and Siwi H., 2016). Traditional medicine that has been clinically proven to

treat cancer has been vincristine. Vincristine is a chemical compound of the vinca alkaloid group derived from the *Vinca rosea* that has the working mechanism of inhibiting mitotic cells, causing cells to die.

Traditional medicine is an ingredient derived from plants, animals, minerals, extracts, or mixtures that are used based on experience from generation to generation (BPOM, 2014). Parts of plants that are commonly used as traditional medicines or herbal medicines are roots, leaves, stems, fruits, flowers, and rhizomes (BPOM, 2005). One of the local natural resources that has the potential to treat cancer is the cumin plant (*Plectranthus amboinicus*) (Susanti, 2014).

Cumin is a creeping shrub. In the territory of Indonesia, cumin plants are known as *bangun-bangun* trees or *torbangun* trees, which have a life span of about 3 to 10 years (Muniroh, 2013). Cumin plants are well known in the world of health as well as for research. Cumin is one of the plants that contains compounds such as antioxidants, antibacterial, anti-inflammatory, and anticancer (Muniroh, 2013). The leaves of the cumin plant contain secondary metabolites in the form of flavonoids, saponins, alkaloids, essential oils, and polyphenols (Muniroh, 2013).

The previous research was conducted on the anti-inflammatory effect and acute toxicity of cumin leaf extract (*Plectranthus amboinicus*) in rats induced by arthritis (Lailatul M., *et al.* 2013). In this research journal, mice were tested in-vivo using cumin leaf extract at a rate of 1900 mg/kg, 2800 mg/kg, and 5000 mg/kg, and then its LD<sub>50</sub> was calculated. However, in this study, the maximum results were not found, so the researchers could not calculate the LD<sub>50</sub> and only made observations based on the reactions shown by the mice after the administration of the extract for 24 hours. Meanwhile, Ana Pavla *et al.* (2009) research entitled "in vivo study of the anti-inflammatory and antitumor

activities of leaves from *Plectranthus amboinicus* (Lour.) Spreng (Lamiaceae)" obtained good results where there was inhibition of the growth of sarcoma-180 tumors and Ehrlich's carcinoma ascites in mice. Because the LD<sub>50</sub> value had not been obtained on the examination of cumin leaf extract in mice, it was necessary to test the toxicity of cumin leaf extract in general for cancer with several developments, both in the dose section and the method used.

This study dealt with the toxicity test of the ethanolic extract of cumin leaves on *Artemia salina* using the *Brine Shrimp Lethality Test* (BSLT) method, and then the LC<sub>50</sub> value was calculated. The use of BSLT method was applied in this study because the BSLT method has a positive correlation with the cytotoxic test using cancer cell culture. This is also what makes the BSLT method often used as a screening for anticancer compounds (Anderson, 1991).

## Research Methodology

### Materials

Materials and equipment used are: analytical balance, oven, blender, 80 mesh sieve, a set of maceration tools, filter paper, a set of glassware, *rotary vacuum* evaporator PC 620 select, aquarium, black cloth, perforated bulkhead, AA oxygen regulator-350, lamp, magnifying glass, flacon, UV-VIS spectrophotometric instrument, Agilent carries 60. Meanwhile, the ingredients used are 1000 ml of 96% ethanol, concentrated sulfuric acid, potassium dichromate, aquadest, FeCl<sub>3</sub>, dragendroff, DMSO PA, Mg metal, chloride, magnesium sulfate, magnesium chloride, calcium chloride, potassium chloride, sodium hydrocarbonate, quercetin, magnoflorine, tannic acid, and saponins. All materials used in this study have pro-analytical qualities.

### Making *Simplicia*

First, the cumin (*Plectranthus amboinicus*) leaves used as samples were first determined. The purpose of the determination is to identify the type of plant to be used. The determination was made at Materia Medika, Batu, with letter number 074/599A/102.7/2020.

The preparation of cumin leaf *Simplicia* (*Plectranthus amboinicus*) was carried out by conducting wet and dry sorting, which was then dried using an oven at 60°C. In the process of making *Simplicia*, several characteristic tests were carried out, such as the drying shrinkage test and the moisture content test. The drying shrinkage test is calculated with the following formula:

$$\% \text{ drying shrinkage} = \frac{B}{A} \times 100\% \quad (1)$$

Note:

A = wet leaf weight (grams).

B = dry leaf weight (grams).

While the moisture content test is calculated by the formula (Depkes RI, 2000):

$$\text{Moisture content (\%)} = \frac{A-B}{A} \times 100\% \quad (2)$$

Notes:

A = initial powder weight (grams)

B = the final weight of powder (grams)

### *Production of an ethanol extract of cumin leaves*

The production process of cumin leaf extracts (*Plectranthus amboinicus*) was carried out by the maceration method, using 300 grams of powder soaked in 96% ethanol for 24 hours while stirred several times, then filtered. The powder of cumin leaves that had been macerated was macerated again so that a clear filtrate was obtained. The concentration of the extract using an evaporator was done to obtain a thick extract. In the process of making the extract, several characteristic checks were conducted, such as a yield test and an

ethanol-free test. The yield calculation used the following formula (Hariana A., 2007):

$$\% \text{Yield} = \frac{\text{thick extract weight}}{\text{Simplicia powder weight}} \times 100\% \quad (3)$$

The ethanol-free test was carried out by adding 2 drops of concentrated sulfuric acid and 1 mL of potassium dichromate to the extract. If the orange color turned dark bluish-green, it meant the extract contained ethanol because the orange dichromate ion had been reduced to green chromium ions (Ramadhani, 2009).

### *Qualitative and quantitative Test*

The process of qualitative testing was done by using reagents. The test was conducted as follows:

#### *Saponins*

A total of 1 mL of sample was put into a test tube and 4 mL of distilled water was added. The test tube was then shaken for approximately 15 minutes. Foam that is stable or lasts for 30 minutes indicates the presence of saponins (Harborne J.B., 2006).

#### *Tannins*

1 ml of DMSO was mixed with 0.2 grams of sample. Transfer 1 mL of the solution into a test tube and add 2-3 drops of FeCl<sub>2</sub> 1% solution. Positive results were indicated by the formation of a bluish-black or green-black color (Harborne J.B., 2006).

#### *Alkaloids*

1 ml of DMSO was mixed with 0.2 grams of sample. Put 1 mL of the solution into a test tube, then add Dragendroff's reagent and stir until dissolved. If a red or orange color is formed and orange to yellow precipitate is formed, then the sample contains alkaloid compounds (Dewi S.M. and Venty S., 2005).

*Flavonoids*

1 ml of DMSO was mixed with 0.2 grams of sample. Transfer 1 mL of the solution into a test tube, then add 2-3 drops of Mg metal and concentrated HCl. If an orange or orange color is formed, the sample contains flavonoid compounds (Ergina and Nuryanti, 2014).

Meanwhile, quantitative testing of cumin leaf extract (*Plectranthus amboinicus*) was carried out using a UV-VIS spectrophotometer. The test was conducted by first making a standard solution and then producing a concentration series, followed by preparing a sample and finally determining the concentration using the equation  $y = a + b (x)$  (Chinelo A, *et al.* 2014). For determining the levels of compounds, a standard solution was required in the form of saponins for the saponin compounds test, a standard solution of *tannic acid* for tannin compounds, a standard solution of quinine for alkaloids, and a standard solution of quercetin for flavonoid compounds (Rajendra, 2014). The absorbance measurement using a UV-Vis spectrophotometer dealt with a wavelength of 645 nm for saponins, 620 nm for tannins, 470 nm for alkaloids, and 510 nm for flavonoids (Rajendra, 2014).

*Anticancer Potential Screening with The BSLT Method*

An anticancer potential screening test with the BSLT method was undertaken by inserting 10 larvae into flacons which were already filled with 5 ml of artificial seawater. After that, 1 ml of test solution was added with a concentration of 0 ppm, 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm, and 2500 ppm. Add 1 drop of yeast suspension as a source of larval food, let it stand for 24 hours, then observe its movement for 10 seconds. If the larva of *Artemia salina* LEACH has no movement for 10 seconds, then it can be said that it is no longer alive. Perform three replications in each experiment. Toxicity effects were analyzed from

observations with a mortality percentage. With the formula (Mayer, *et al.* 1982) :

$$\% \text{ Larvae} = \frac{\text{the number of dead larvae}}{\text{the number of tested larvae}} \times 100\% \quad (4)$$

By knowing the mortality of *Artemia salina* larvae, one can then look for the probit number through the table and make a line equation:

$$Y = BX + A \quad (5)$$

Notes:  
 Y = the log concentration  
 X = the Probit number

From this equation, then calculate LC<sub>50</sub> by entering the probit value (50% mortality).

**Results and Discussion**

*The Result of Making Simplicia*

In the process of making Simplicia, several characteristic checks were carried out, such as the drying shrinkage test and moisture content test. The drying shrinkage test was carried out to know how much water content was lost from the drying process (DepKes, 2000). The results of the drying shrinkage test can be seen in the table below:

Table 1. Results of drying shrinkage test

Sample	Initial weight (kg)	Final weight (kg)	Yield (%)
Cumin Leaf	6,667	0.429	6.43

Based on Table 1, it can be concluded that the water content in cumin (*Plectranthus amboinicus*) leaves is very large with a drying shrinkage test result of 6.43%. Therefore, if cumin leaves will be used as a sample that goes through the drying process, many cumin (*Plectranthus amboinicus*) leaves are highly required.

The water content test can be applied as a parameter to see the quality of the simplicia of cumin (*Plectranthus amboinicus*)

leaves used. According to the Indonesian Ministry of Health (2000), the required water content does not exceed 10%. If the water content is by the requirements, it can minimize the water content in the simplicia which can prevent the growth and activity of microorganism enzymes in the simplicia, so that the cumin leaf Simplicia (*Plectranthus amboinicus*) is durable and the active substance content in it does not change (Depkes RI, 2000).

Based on Table 2, it can be concluded that the simplicia used has met the requirements that have been set, which is less than 10%.

Table 2. The results of the water content test of Simplicia powder

Sample	Initial Weight (grams)	Average Final Weight (grams)	Yield (%)
Cumin Leaves	10	9	1.05

#### Results of Making An Ethanol Extract of Cumin Leaves

The extraction process for simplicia powder of cumin leaves (*Plectranthus amboinicus*) was undertaken by using the maceration method. Maceration was chosen because the process is easy and the equipment is quite simple. Besides, the maceration method is good for compounds that are not resistant to heating (Voight R., 1971).


The solvent used in this study was 96% ethanol. A 96% ethanol solvent was used because it is a semi-polar solvent that can attract polar and nonpolar compounds contained in simplicia. In addition, a high ethanol content can prevent the growth of yeast and mold during the soaking process. Additionally, it also has lower toxicity than other organic solvents such as methanol and chloroform (Saifudin A. and Rahayu V., 2011).

In the maceration process, a repetition or *remaceration* of the cumin leaf

residue was conducted to obtain a clear extract. This *remaceration* process aims to obtain a large amount of extract so that the compounds contained in simplicia can be extracted as a whole. The obtained extract was then concentrated using an evaporator to get a thick extract. The use of an evaporator aims to reduce direct contact between the extract and the hot temperature continuously so that the compounds contained are not changed or damaged (Simbolon and Yelmir M., 2018).

In the process of making the extract, several characteristic checks were carried out, such as the yield test and the ethanol-free test. The ethanol-free test of cumin leaf extract (*Plectranthus amboinicus*) has the purpose of ensuring that the resulting extract is free from ethanol because the solvent can kill bacteria or microorganisms, which is suspected to have an impact on the toxicity level of a compound (Suherman S., 2006). Ethanol-free test results can be seen in the table and figure below:

Table 3. Ethanol-free test results

Sample	Treatment	Results	Color Changes
Cumin Leaf Extract	2 Drops of Concentrated Sulfuric Acid + 1 ml of Potassium Dichromate	-	 Blackish Brown

Notes : (+) There is ethanol  
(-) No ethanol

Based on table 3, it can be inferred that the extract is free from ethanol, which is characterized by a color change from orange to a blackish brown. The yield test was used to show how many chemical compounds can be extracted from the extract. The extract yield test was calculated based on the ratio of the final weight of the extract to the initial weight and then multiplied by 100% (Hariana A., 2007).

The yield test results can be seen in the table below:

Table 4: the results of the yield test.

Sample	Simplicia Weight	Extract Weight	% Hasil
Cumin Leaves	300 g	30 g	10 %

Based on table 3, it can be inferred that the yield produced was very limited, so it is required to have a large sample to produce cumin leaf extract (*Plectranthus amboinicus*). The small value of the yield produced is influenced by the particle size, extraction time, and the type and amount of solvent (Saifudin A. and Rahayu V., 2011).

*The Results of The Qualitative and Quantitative Tests*

An examination of the chemical compound content of cumin leaves (*Plectranthus amboinicus*) was carried out to investigate the chemical substances that will be used as test materials, including the examination of the content of saponins, tannins, alkaloids, and flavonoids. The qualitative testing process carried out using reagents obtained results such as the table and figure below.

Tabel 5. Hasil uji senyawa secara kualitatif

Group of compounds	Reagents	Color changes	Result
Saponin	Extract + distilled water	Foam	+
Tannin	Extract + FeCl <sub>2</sub> 1%	Greenish black	+
Flavonoids	Extract + Metal Mg + concentrated HCl	Pink orange	+
Alkaloids	Extract + dragendroff	Orange colored sediment	+

Note : (+) Contains compound  
 (-) No compound

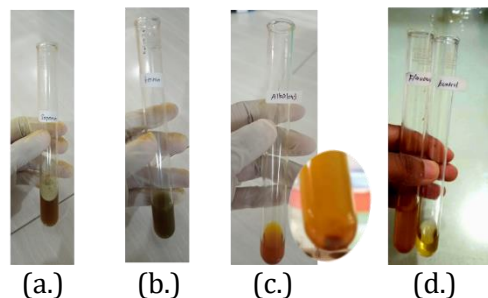


Figure 1. the results of observations of qualitative tests of compounds (a.) saponins, (b.) tannins, (c.) flavonoids, (d.) alkaloids, and control solutions.

Based on table 5 and figure 1, it can be concluded that cumin leaf extract (*Plectranthus amboinicus*) contains saponins, tannins, flavonoids, and alkaloids.

Meanwhile, the analysis of the content of saponins, tannins, flavonoids, and alkaloids of cumin leaf extract (*Plectranthus amboinicus*) was carried out by using a UV-VIS spectrophotometer. This UV-VIS spectrophotometer method is a simple method for determining a very small quantity of substances. Besides, the obtained results are quite accurate, where the readable numbers are directly recorded by the detector and printed in the form of digital numbers or graphs that have been regressed. Determination of the compound content of cumin leaf extract was conducted at the University of Muhammadiyah Malang. The results of the calculation of the standard deviation of compound levels can be seen in the table below:

Table 6. Test results for the content of the extract

Compound	Compound Total (%)		Mean ± standard deviation
	Replication 1	Replication 2	
Saponin	5.07	5.32	5.20 ± 0.17
Tannin	8.07	8.34	8.21 ± 0.19
Flavonoids	23.83	24.03	23.93 ± 0.14
Alkaloids	4.21	4.53	4.37 ± 0.23

Based on table 6, it can be concluded that the content of flavonoid compounds reaches the greatest amount of compounds in cumin leaf extract (*Plectranthus amboinicus*), which is consecutively followed by tannin, saponin, and finally alkaloid compounds.

#### *The Results of A BSLT-Based Screening for Potential Anticancer Agents*

The Brine Shrimp Lethality Test (BSLT) method is a toxicity test method that has a positive correlation with antitumor or anticancer activity (Sukardiman, and Pratiwi, 2004). A positive correlation was shown between the BSLT test and cytotoxicity in cancer cell cultures and had a confidence level of up to 95% (Prawirodiharjo E., 2014) The BSLT method was chosen because it does not take such a long time, comprises an easy, cheap, and accurate method, and requires a small sample (Mayer, *et al*, 1982). The animal used for the testing was *Artemia salina* because it has a response to chemical compounds similar to mammals, such as DNA-dependent RNA polymerase. Besides, this organism has a Na<sup>+</sup> and K<sup>+</sup> dependent ATPase transport system (Mayer *et al*. 1982).

Within the testing process, which used the BSLT method, several steps had to be carried out: the manufacture of artificial seawater at a rate of 5 per mile, shrimp incubation, testing, and LC<sub>50</sub> value calculation.

The manufacturing process of artificial seawater required 5 grams of sodium chloride, 1.3 magnesium sulfate, 1 gram of magnesium chloride, 0.3 grams of calcium chloride, 0.2 grams of potassium chloride, 2 grams of sodium hydrocarbonate, and distilled water (Mudjiman A., 1988). All ingredients were dissolved except sodium bicarbonate, which was dissolved in carbon dioxide-free water, and magnesium sulfate, which was dissolved in hot distilled water and followed by being dissolved with other ingredients. The finished artificial seawater was aerated for 2 hours so that it contained sufficient oxygen for the survival of *Artemia salina* LEACH (Mudjiman A., 1988).

The hatching process of larva eggs was done by putting artificial seawater into a special aquarium, which has two sides, namely the dark and light sides, separated by a screen. Put the larva eggs on the dark side and wait for 24 hours until the larva eggs hatch. The hatched larva will move to the bright part of the aquarium (Prawirodiharjo E., 2014). *Burayak* or nauplius used for this study were those aged 48 hours or 2 days since the nature of burayak or nauplius is more sensitive to incoming substances and their organs are already complete (Prawirodiharjo E., 2014).

The hatched larvae were then divided into six groups, where one group belonged to the control group and the other five groups were the test group. In the test group, the larvae were given ethanol extract of cumin leaves with concentrations of 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm, and 2500 ppm. This concentration was obtained after conducting a preliminary test with levels of 100 ppm, 500 ppm, and 1000 ppm, which then took data on the percentage of larval mortality between 20% and 80%. This percentage was selected since it was able to give a more linear curve and the LC<sub>50</sub> value obtained could describe more accurate results (Ridho, 2011). Observations were made for 24 hours, after which the percentage of larval mortality was calculated. With the help of the probit value table, the percentage of larval mortality was applied to achieve the probit value. The results of the observations can be seen in the table below:

Table 7: Probit value in Microsoft Excel

Concentration (Ppm)	Log 10	Probit	Death (%)	mean ± standard deviation	Mortality
500	2.60	4.82	43	4.33 ± 0.58	13
1000	3.00	5.25	60	6 ± 2	18
1500	3.18	5.61	73	7.33 ± 1.53	22
2000	3.30	5.84	80	8 ± 1	24
2500	3.40	6.75	96	9.67 ± 0.57	29



The table 7 shows us the process of calculating the LC50. The calculation of the LC50 value can be used in two ways. The first method was to calculate the *slope* and *intercept* using Microsoft Excel. *Slope* is the value of the regression coefficient for the X variable, while *intercept* is the average value of the Y variable if the X variable has a value of 0. Then calculate the LC50 equation  $y = ax + b$ , where a is the slope value, while the b value is the value of the *intercept*. The second way to calculate the value of LC50 is by making a graph between the concentration log and the probit value that has been obtained, after which a straight-line equation was made on the curve. The graph can be seen as follows:

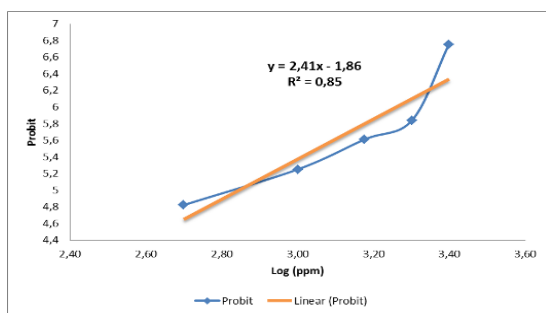


Figure 2. Linear regression graph of the extract against the probit value

The graphic above shows the calculation of the LC<sub>50</sub> value by using the obtained equation. It is  $y = 2.41x - 1.86$ , where the y value contains the probit transformation value. Since this research aims at looking for the LC<sub>50</sub> value, the 50 value within the LC was changed to the probit value first. The value of y that had been changed resulted in a value of 5, so the equation became  $5 = 2.41x - 1.86$  and achieved an LC<sub>50</sub> value of 697,99 ppm.

Based on the calculation of the obtained LC<sub>50</sub>, it can be inferred that the ethanol extract of cumin leaves (*Plectranthus amboinicus*) has toxic properties because, with a concentration of 1000 ppm, it can kill half of the tested larvae. This fact is by Mayer's (1982) statement, which stated that compounds with LC<sub>50</sub> 100 have very toxic properties. However, in the toxic category, it has an LC<sub>50</sub> 1000 and in the practical non-

toxic category, it has an LC<sub>50</sub> > 1000 (Mayer, et al.1982). With the toxic properties of the ethanol extract of cumin leaves (*Plectranthus amboinicus*) on larvae using the BSLT method, it can be said that it has potential as an anticancer due to the positive correlation of the BSLT method with cytotoxic tests using cancer cell culture (Anderson, 1991).

The presence of these toxic characteristics is quite related to the compounds contained in the ethanol extract of cumin leaves (*Plectranthus amboinicus*), such as saponins, tannins, alkaloids, and flavonoids. Based on previous research, those four compounds exhibited toxic properties on microorganisms with anticancer potential. Pawarta (2014) stated that flavonoid compounds function as inhibitors of tumor or cancer proliferation by inhibiting protein kinase activity as well as inhibiting signal transduction pathways from cell membranes into the cell nucleus. Smets (2001) stated that alkaloid compounds act as tubulin inhibitors that inhibit protein polymerization into microtubules so that the cell cycle stops at metaphase, which causes cells to undergo apoptosis. Tiwari dan Kumar (2011) mentioned that saponin compounds undergo an antibacterial mechanism by decreasing digestive enzyme activity and food absorption (*stomach poisoning*), while tannin compounds in anticancer as stomach poison get enzyme activity inhibited by the formation of protein complex bonds in the sulfate enzyme, which can cause digestive disorders and damage cell walls.

## Conclusions

Based on the results and discussion in this study, two conclusions can be drawn, namely the phytochemical screening test for the ethanol extract of cumin leaves (*Plectranthus amboinicus*), which showed the presence of saponins, tannins, alkaloids, and flavonoids with levels of 5.20% for saponins, 8.21% for tannins, 4.37% for alkaloids, and 23.93% for flavonoids. In addition, the screening test for the anticancer potential of the ethanolic extract of cumin (*Plectranthus*



*amboinicus*) using the *Brine Shrimp Lethality Test* (BSLT) method showed potential as an anticancer indicated by the acquisition of an LC value of 50 1000 ppm, which is 697.99 ppm.

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