IN VITRO ACUTE TOXICITY WITH LC50 VALUE AND PHYTOCHEMICAL IDENTIFICATION OF JAMU LANCAR HAID T® USING BRINE SHRIMP LETHALITY TEST (BSLT) METHOD

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ABSTRACT: Toxicity is the ability of a substance to cause toxic effects (poison) at a certain time. The purpose of this study was to determine the secondary metabolites contained in T® smooth menstruation herbal medicine and the LC50 value in Artemia Salina Leach shrimp larvae. T® smooth menstruation herbal medicine is a herbal product commonly used by women to facilitate menstruation but has the potential for misuse. The study used a true experimental design with a post-test only control group design. Phytochemical screening showed that the sample contained alkaloids, flavonoids, saponins, and tannins. The toxicity test was carried out using the Brine Shrimp Lethality Test (BSLT) method at five concentration variations (0, 250, 500, 1000, and 2000 ppm), each with three repetitions. The results of data analysis using linear regression, y = 1.1643x + 1.5472, showed an LC50 value of 932.017 ppm. This value indicates that the T® herbal medicine for smooth menstruation is toxic to Artemia salina larvae.

Keywords: [BSLT, Phytochemicals, T® menstrual flow herbal medicine, LC50, Toxicity Test.]

INTRODUCTION

Toxicity is the ability of a substance or chemical to produce toxic effects on living organisms over a certain period of time. Toxicity testing is an important part of preclinical testing for both pharmaceutical products and traditional medicines to determine toxic risks, biological effects, and safe consumption thresholds (BPOM, 2022). Based on the 2023 BPOM report, 2,442 cases of toxicity were recorded in Indonesia, of which approximately 52% were caused by poisoning from traditional medicines and herbal medicines.(BPOM RI, 2023).

One of the traditional herbal medicines widely used by the public is Jamu Lancar Haid T®, which is said to help regulate menstruation. However, this product is prone to misuse for inappropriate purposes, such as inducing abortion, by consuming excessive doses without medical indication. This raises concerns about the potential toxicity of this herbal medicine.(Rahmawati & Hulukiti, 2016).

This herbal medicine contains natural ingredients such as turmeric (Curcuma domesticae), thousand leaf (Achillea millefolium), sembung leaf (Blumea balsamifera), black cumin (Nigella sativa), and ginger (Zingiber officinale). These ingredients are known to contain secondary metabolites such as alkaloids, flavonoids, tannins, and saponins. Although these compounds have pharmacological benefits such as antioxidant, anti-inflammatory, and menstrual-stimulating properties, excessive consumption can cause toxic effects.(Puspitasari & Prayogo, 2017).

To determine the toxicity level of a product, an acute toxicity test is required. This test measures the toxic effects of short-term exposure to a substance and calculates the LC50 value, which is the concentration of the substance that causes death in 50% of test animals. One commonly used method is the Brine Shrimp Lethality Test (BSLT) using Artemia salina larvae.(Fatimah et al., 2020).

Based on this, this study aims to determine acute toxicity.T® Smooth Menstruation Herbal Medicinethrough the BSLT method and determine its LC50 value. The results of this study are expected to besource of information about the toxicity category of Jamu Lancar Haid T® for its safe use(Sasmito et al., 2017).

METHODS

This type of research is a true experimental study using shrimp larvae (Artemia salina Leach) using the Brine Shrimp Lethality Test (BSLT) method to test the toxicity of Jamu Lancar Haid T®, with evaluation of the results carried out after all treatments were completed. This study was conducted with 5 treatment groups and 3 repetitions each using 10 shrimp larvae in each test.

Time and Place of Research

This research was conducted at the Pharmacology Laboratory of the Cendekia Utama Kudus Health Technology Institute, Jl. Lingkar Timur No. KM.5, Jepang, Mejobo District, Kudus Regency. The research period was from April to May 2025.

Population and Sample

The population in this study was Artemia salina Leach shrimp larvae. The samples were Artemia salina Leach obtained from an ornamental fish retailer in Kudus, with the criteria of being 48 hours old as a test animal and showing no anatomical defects.

Data analysis

Data analysis can be done using Microsoft Office Excel by creating a straight-line graph showing the relationship between the probit value and the log concentration. The LC50 value can be calculated using this straight-line equation by entering the value 5 (probit 50% mortality of test animals) as y, thus finding x as the log concentration value. The antilog of the x value is the LC50 value.

RESULTS AND DISCUSSION

a. Organoleptic Test

Organoleptic testing is a testing method carried out by observing changes in the shape, taste, aroma and color of Jamu Lancar Haid T® which are felt with the five senses.(Roni & Minarsih, 2021).

Table 1. Organoleptic test results

Organoleptic Test	Results
Form	Fine powder
Smell	Typical
Flavor	A bit bitter
Color	Green

Organoleptic Testing Organoleptic testing of Jamu Lancar Haid T® is conducted to determine the quality and suitability of the product based on the five senses. This examination includes observing the physical appearance (color, shape, and homogeneity), aroma, and taste. Good herbal powder has a distinctive color, a strong herbal aroma, and a taste that matches its ingredients, without any foreign objects or signs of damage. This organoleptic examination is the initial step in herbal quality control before further chemical or microbiological analysis is carried out.

b. Water content test

The moisture content of Jamu Lancar Haid T® was analyzed using a moisture balance tool and replicated three times to ensure more accurate results.

Table 2. Results of water content test

Replication to-	Sample Weight (Gram)	Water content (%)
1	1	8.65
2	1	8.87
3	1	8.96
Average		8.83
Devi Standardready		0.16

Water content testing aims to determine the maximum water content in materials, whether in the form of extracts or herbal preparations. This is important because the higher the water content, the greater the likelihood of mold or mildew growth, which can ultimately reduce the stability and biological activity of the herbal preparations during storage.(Anggraini, 2020). The research found an average water content of 8.83%. The percentage of water content of the T® herbal medicine for smooth menstruation shows that it meets the standards of simple drugs, according to Ministry of Health of the Republic of Indonesia (1985)minimum water content for standard simplicia < 10%.

c. Phytochemical screening test

Based on the results of the phytochemical screening test that has been carried out, it shows that SP® male herbal medicine contains the chemical compounds alkaloids, flavonoids, tannins and saponins.

Table 3. Results of phytochemical screening of T® menstrual flow herbal medicine

No	Compound Groups	Reagent	Interpretation	Results
1.	Alkaloid	*-Dragendoff*	Red sediment	(+)
		-meyer	White sediment	(+)
		-wagner	Brown-black sediment	(+)
2.	Flavonoid			
	-Wilstatter test	Mg powder + concentrated HCl	Yellow-orange color	(+)
	-Bate-Smite test	concentrated HCl	Red	(+)
	-10% NaOH test	NaOH 10%	Discoloration	(+)
				(+)
3.	Tannin	Ethanol + $FeCl_3$	Color black Greenish	
4.	Saponin	Boiled distilled water + 2 N HCl	Foam forms for 1 minute	(+)

Source: Processed primary data (2025)

Information:
(+) = Gives a positive result containing the chemical compound group being tested

This phytochemical screening test research was conducted to identify the active ingredients of Jamu Lancar Haid T®, namely alkaloids, flavonoids, saponins, and tannins.

a. Alkaloid Test

From this alkaloid identification, it was found that the T® herbal medicine for smooth menstruation contained alkaloids, as it produced an orange color. This is in line with research conducted by(Nugroho, 2017)The Dragendorff reagent produces an orange precipitate, indicating that the T® herbal medicine for menstrual pain contains alkaloids. The principle of this identification is a precipitation reaction that occurs due to ligand replacement. Nitrogen atoms, which have lone electron pairs in alkaloids, can replace iodo ions in the reagents.

1) Dragendorff's reagent contains bismuth nitrate and potassium iodide in a glacial acetic acid solution of calcium tetraiodibismuthate (III), while Meyer's reagent contains potassium iodide and mercury chloride, where this reagent binds to alkaloids through a coordination bond between the N atom of the alkaloid and the Hg atom of Meyer's reagent, resulting in a non-polar mercury complex compound that precipitates white.
2) Wagner's reagent is a solution made from a mixture of iodine (I_2) and potassium iodide (KI) dissolved in water. In the alkaloid test, this reagent produces a brown precipitate indicating the formation of triiodide ions (I_3^-) as a result of the reaction between iodine (I_2) and iodide ions (I^-) from KI. This reaction occurs due to the interaction between the nitrogen atom (N) in the alkaloid, which has a lone electron pair, with the potassium ion (K^+). This interaction produces a potassium-alkaloid complex compound through a coordinate covalent bond.(Ikalinus et al., 2015)

b. Flavonoid Test

1) Wilstater reagent

The flavonoid test results showed a positive result, indicated by the liquid changing to a yellow-orange color. The flavonoid test was performed using magnesium powder and concentrated HCl, followed by heating. Heating is necessary because most flavonoids are soluble in hot water.(Muthmainnah, 2017), the resulting yellow-orange color indicates the presence of flavonoids due to reduction by concentrated hydrochloric acid and magnesium.

2) Bate-Smith reagent

The results obtained in the flavonoid test using the Bate-Smith reagent were red, indicating that the T® herbal medicine for menstrual pain positively contained anthocyanidin flavonoids.

In line with research conducted by Zirconia et al. (2015), the addition of concentrated hydrochloric acid (concentrated HCl) to hydrolyze and break down anthocyanins into their aglycones, namely anthocyanidins, and with the heating process, the reaction will be accelerated until a red color is formed, indicating a positive result for anthocyanidin flavonoids.

3) 10% NaOH reagent
Identification of flavonoids with 10% NaOH reagent shows that the T® herbal medicine for smooth menstruation contains flavonoids because there is a color change.(Rahayu et al., 2015)This phytochemical test was carried out by adding a few drops of 10% NaOH to cause a color change. The 10% NaOH reagent is a base catalyst that causes the decomposition of the Kristin compound, a derivative of the flavone compound, into acetophenone molecules, indicated by a color change to blackish brown. The occurrence of a color change indicates the presence of flavonoids, a group of phenol compounds.(Theodora et al., 2019).

c. Tannin Test
The results obtained in the tannin test were blackish-blue, which means that the T® herbal medicine powder contains tannins. The addition of FeCl3 aims to ensure the presence of phenolic groups in the sample. After the addition of 1% FeCl3, a greenish-black phenolic group appeared. This is because tannin is a phenolic compound from the polyphenol group. The greenish-black color formed in the sample is caused by the phenolic compounds present in the sample forming a complex compound with Fe3+ ions.(Razoki et al., 2023). In line with previous research conducted by(Manongko et al., 2020)A color change in tannin identification can be caused by the reaction of FeCl3 with one of the hydroxyl groups in the tannin compound. The color change upon addition of FeCl3 is caused by the presence of condensed tannin.

d. Saponin Test
The saponin test results for T® smooth menstruation herbal medicine showed a positive result, indicated by the formation of stable foam in the test tube. This is in line with previous research conducted by(Wang et al., 2018)because of the combination of the structure of the shrinking compound, namely the non-polar sapogenesis chain and the polar side chain, which is soluble in water, so that the foam formed can last for 10 minutes.

d. Toxicity test using the BSLT method

Test Toxicity was carried out in vitro using the Brine Shrimp Lethality Test (BSLT) method, which was carried out by observing the death of Artemia salina Leach shrimp larvae after 24 hours of administering the T® smooth menstruation herbal solution.

Table 4. Percentage of Mortality of Artemia Salina Leach Shrimp Larvae. At Various ConcentrationsT® Smooth Menstruation Herbal Medicine

Test tube	**Extract Concentration in Test Tubes (Mortality Rate of Artemia Salina L Shrimp Larvae From 10 Larvae)**				**Negative Control**
Replication	2000 ppm	1000 ppm	500 ppm	250 ppm	
1	7	6	5	3	0
2	7	5	4	2	0
3	5	5	3	2	0
Average	6.3	5.3	4.0	2.3	0
Average Percentage of Deaths (%)	63	53	40	23	0
Standard deviation waste	1.16	0.58	1	0.58	0

The negative control group, which only used artificial seawater with pH 8, did not experience any larval deaths. This shows that the artificial seawater used as a control or solvent in the test did not have a toxic effect on shrimp larvae, was safe, and did not interfere with the experimental results.

The 250 ppm concentration group showed a mortality rate of 23%, the 500 ppm concentration group showed a mortality rate of 40%, the 1000 ppm concentration group showed a mortality rate of 53%, and the 2000 ppm concentration group showed a mortality rate of 63%. From the data above, it can be seen that the higher the concentration level of T® smooth menstruation herbal medicine, the higher the mortality rate of Artemia salina Leach shrimp larvae.

Shrimp larvae mortality was observed for 24 hours after administration of a solution containing a specific concentration of herbal medicine. The purpose of this observation was to determine whether the larvae were still alive or dead. Assessment was based on simple criteria: if the shrimp larvae still showed movement, they were alive, and if they did not, they were dead.

The data obtained were then graphed to determine the percentage of shrimp larval mortality at various concentrations. The highest mortality rate for Artemia salina Leach larvae was found in the 2000 ppm solution.

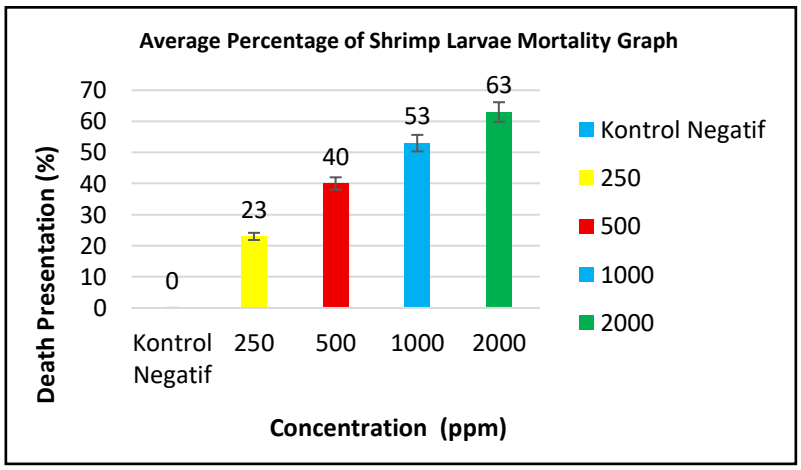

Figure 1. Graph of the average percentage of shrimp larval mortality

The T® smooth menstruation herbal remedy was prepared as a first stock solution with a concentration of 2000 ppm, which was then diluted into solutions with concentrations of 1000 ppm, 500 ppm, and 250 ppm, as well as 0 ppm as a negative control. The negative control contained only artificial seawater, used as a comparison. In the toxicity test, 10 Artemia salina Leach larvae were added to each concentration.

Negative controls were used to determine the effect of seawater and other factors on shrimp larval mortality. This allowed for confirmation of whether the shrimp larval mortality was due to the added herbal medicine or the artificial seawater. The prepared solutions were replicated three times to obtain more accurate and statistically valid results. Ten 48-hour-old Artemia salina Leach shrimp larvae were used for each concentration. Next, test tubes were prepared according to the concentration, and negative controls were added.

Acute toxicity test. The T® smooth menstruation herbal remedy was tested using the in vitro BSLT (Brine Shrimp Lethality Test) method, which uses Artemia salina Leach larvae as test animals. The BSLT (Brine Shrimp Lethality Test) method aims to determine the concentration of T® smooth menstruation herbal remedy that kills half of the initial population of test animals.

Artemia salina Leach, used for toxicity testing, is available in egg form. Therefore, before testing, the aquarium is divided into two sections: a dark section and a light section. Artemia eggs are placed in the dark section; after 24 hours, they will hatch and move to the light section. The light section is provided with a 5-watt light to maintain a warm temperature of 25°-30°C (Saragih et al., 2022)Then, an aerator was added in the dark area to maintain oxygen levels in the water. The aquarium contained artificial seawater by mixing 15 grams of salt (without iodine) in 1 liter of distilled water. Before hatching, a pH test was performed, which resulted in a reading of 8.(Aliyas & Samsia, 2019).

The death of shrimp larvae is thought to be related to the activity of bioactive compounds found in SP® male enhancement herbal medicine, namely flavonoids, alkaloids, saponins, and tannins. These compounds act as appetite suppressants (anfedants). Their mechanism of action is through stomach poisoning, where the larvae's digestive system is disrupted after the compounds

enter their bodies. As a result, the larvae cannot respond normally to taste stimuli, ultimately leading to starvation and death.(Cahya et al., 2023).

e. Data analysis

The data obtained from the research results were analyzed using Microsoft Office Excel and presented in graphical form based on a linear regression equation (Y = bx + a). The analysis was performed by combining probit values and log concentrations to determine the LC50 value. The LC50 value indicates the concentration of a substance capable of causing death in 50% of the test animal population.

Table 5. Calculation of LC50 Value of Jamu Lancar Haid T® Using Probit Analysis

Concentration (ppm)	Log Concentration (x)	% Death	Probit Value (y)
2000	3.3	63	5.33
1000	3.00	53	4.08
500	2.69	40	4.75
250	2.39	23	4.26
Negative Control	0	0	0

The obtained shrimp larval mortality percentages were then used to determine the probit value for each treatment group using a probit table. Afterward, a concentration curve was created by constructing a linear regression equation in the form y = bx + a based on the percentage data.

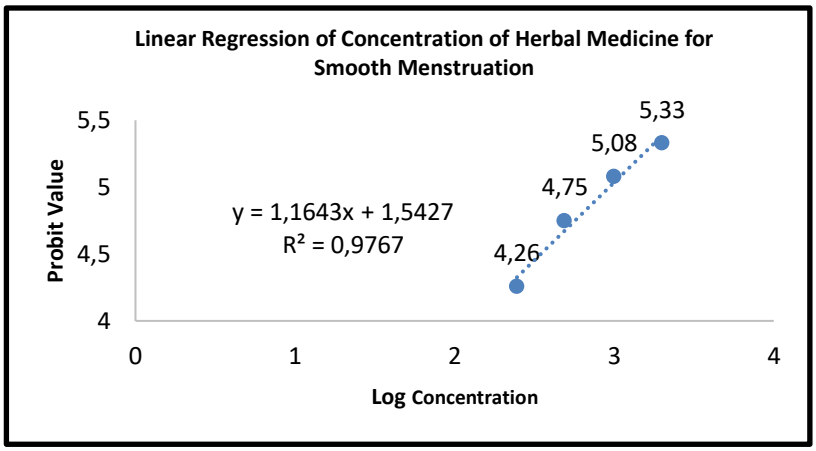

Figure 2. Linear Regression Graph of the Concentration of T® Smooth Menstruation Herbal Medicine

From the graph, the equation y = bx + a and the R-squared value are obtained. The graph of the relationship between concentration and percentage of death is presented in Figure 2. The linear regression equation from the graph is used to find the LC50 value by entering the number 5 as y, so that the value 5 = 1.5427x + 1.1643 is obtained. In the regression image, R^2 and the linear line equation are visible. The R^2 value is a constant, with a value of $R^2 = 0.9767$, which means that the effect of T® smooth menstruation herbal medicine in killing larvae is 99%. The remaining influence is attributed to temperature, pH, and oxygen levels. The y value shows the probit value of larval death, and the x value shows the log concentration. After the number 5 is entered as y, it can be seen that the ability of SP® male strong herbal medicine to kill 50% of the total Artemia salina Leach larvae lies in the concentration932,017ppm.

CompositionT® menstrual flow herbal medicineincluding ginger, turmeric, thousand leaf, sembung leaf, and black cumin. The research results obtained an LC50 valueT® menstrual flow herbal medicine932.017 ppm, which is categorized as toxic. This is in line with research (Suhartanti et al., 2019). The LC50 value of ginger is 37.76 ppm, which is categorized as toxic. The results of the study(Yanita, 2021)stated that the LC50 of turmeric was 15.77 ppm, which is categorized as highly toxic. The results of the study(Yosafat & Ferdinal, 2023)stated that the LC50 of 168.178 ppm of sembung leaves is categorized as toxic. The results of the study(Suryati et al., 2022)stated that the LC50 of black cumin, 568.3292 ppm, is categorized as toxic. This result is also by Efrini (2022),

which states that at LC50 concentrations <1000 ppm, it is toxic. Therefore, the T® herbal medicine has a toxic effect on Artemia salina Leach shrimp larvae at concentrations <1000 ppm.

Studyon products that have been marketed is a post-market research, to evaluate, monitor performance, product safety, effectiveness in products after being launched on the market, as well as assessing long-term safety, which serves to identify side effects or risks that may not appear during pre-marketing trials due to limited sample size or study duration. It is hoped that this research will be a source of information for the public, especially regarding the toxicity of herbal medicines for smooth menstruation, so that they are not misused systematically.(Sasmito et al., 2017).

CONCLUSIONS

Based on the research that has been conducted, the following conclusions were obtained:

a. T® smooth menstruation herbal medicine contains secondary metabolites, flavonoids, tannins, saponins, and alkaloids.
b. The Lethal Concentration 50 (LC50) value of the T® menstrual flow herbal medicine given to Artemia Salina Leach shrimp larvae is 932.017 ppm
c. There is a category of T® menstrual flow herbal medicine that is categorized as toxic using the Brine Shrimp Lethality Test (BSLT) method.

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