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IN VITRO ACUTE TOXICITY OF DANDELION LEAF EXTRACT *(Taraxacum officinale* F.H.Wigg*)* WITH BRINE SHRIMP LETHALITY TEST (BSLT) METHOD

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ABSTRACT

Toxicity is a characteristic feature of substances that can cause harmful effects. This study aimed to determine the toxicity of dandelion leaves (*Taraxacum officinale* F.H. Wigg) to A*rtemia salina* Leach larvae within 24 hours. Dandelion leaves were extracted using a maceration method with 50% ethanol. The yield was 5.88%. The results showed that dandelion leaves contain alkaloids, flavonoids, steroids, triterpenoids, and phenols. Toxicity tests were performed using the Brine Shrimp Lethality Test method, where the final result was the LC50 value. Artemia salina Leach larvae were divided into five groups. Each group had ten larvae that underwent three replications. The 50%ethanolic extract of dandelion leaf concentrations in the treatment media were 50 ppm (P1), 100 ppm (P2), 150 ppm (P3), 200 ppm (P4), and 0 ppm (seawater) as the control. Probit analysis of *Artemia Salina* Leach mortality percentage data. The results indicated that the 50%ethanolic extract of dandelion leaves is harmful to larvae, with LC50 values of 165,223 ppm (toxic) or in the equation y = 2,114x + 0,311, with an R^2 value of 0,9435.

Keywords: *Artemia salina*, BSLT, Dandelion leaf, LC50, Toxicity

INTRODUCTION

Natural products, including organisms (plants, animals, and microorganisms), have been shown to possess health benefits for animals and humans. According to the World Health Organization, in developing countries, 80% of the population still depends on traditional medicines or folk medicines, which are mostly prepared from plants for the prevention or treatment of diseases. Traditional medicine using plant extracts has proven to be more affordable, clinically effective, and has relatively fewer adverse effects than modern drugs (Tran, Pham and Le, 2020; Tee *et al.*, 2021).

Toxicity testing is the first step in determining the safety parameters of a drug before it becomes a product used in humans. A plant compound has been proven to be safe through a toxicity test using experimental animals, so it is very important to know its toxic potential through the lethal concentration and the spectrum of its toxic effects. Moreover, a toxicity test is used to detect the toxic effect of a substance on a biological system and to obtain typical dose-response data from the test preparation. The data obtained can be used to provide information about the degree of danger of the test preparation of human exposure so that the dosage can be determined for human safety (Jabbar *et al.*, 2019).

Most new plants need to be studied scientifically, such as the standardization, biological activity, and toxicity of each plant material. In addition, toxicity experimental assessment has been used as a standard safety study with efficacy tests (Pitakpawasutthi *et al.*, 2021). One such method is the Brine Shrimp Lethality Test (BSLT). This method can be

used to identify the toxicity of natural materials (Saragih *et al.*, 2020). The BSLT method was performed by calculating the mortality of extracts or isolates of *Artemia salina* Leach shrimp larvae for 24 hours. The results obtained as the value of LC50 (Lethal Concentration) is the number of doses that can cause the death of shrimp larvae by 50% after 24 hours (Meyer et al., 1982). Consequently, a lower LC50 value suggested a more significant hazardous effect. The benefits of the BSLT test are that it is simple, quick, straightforward, repeatable, and inexpensive (Sanjaya, Halimah and Tresnawati, 2013). Moreover, the BSLT method is susceptible to harmful chemicals (Hamidi, Jovanova and Panovska, 2014).

Dandelion (*Taraxacum officinale* F.H. Wigg), commonly known as Jombang in Indonesia, is one of 30 medicinal plants processed into Scientific Herbs. Dandelion is known to have various pharmacological activities, including antioxidant, antifibrotic, hepatoprotective, antifungal, antimicrobial, anti-inflammatory, anti-influenza, antidepressant, antiproliferative, and anti-gastric emptying effects (Ali and Halimah, 2020). Dandelion has been empirically used to prevent and treat liver diseases (Muti *et al.*, 2023). Toxicity test research on dandelion plants was conducted by (Sumedhi, 2004), who found that the roots of dandelion plants had an LC50 of 268 ppm. Another study (Akhtar *et al.*, 2022) tested the toxicity of dandelion herbs in various solvents, and obtained the results of dandelion herbs having an LC50 of 7.122 g/mL in methanol solvent, 10.32 g/mL in acetone solvent, and 14.12 g/mL in n-hexane solvent. However, research on the toxicity of dandelion leaf extract has not yet been conducted. Currently, research on dandelion continues to be carried out and developed on plant organs, such as herbs, roots, and leaves. This study aimed to determine the potential toxicity and determine the LC50 value of dandelion leaf extract using the BSLT method.

RESEARCH METHODS

Equipment and Materials

The tools used in this study were analytical balances (Sartorius® 220g), filter paper, blenders (Philips®), rotavapor (rotary evaporator), magnifying glass (Joyko®), test tubes, micropipettes (DLAB®), hatchery containers, styrofoam and laboratory glassware (Erlenmeyer flasks, beaker glass, measuring cups, and glass funnels).

Research materials used in this study include dandelion leaf simplisia, ethanol 96% (Technical, CV Putra Masagus®), aquadest, potassium iodide (Emsure®), Methanol (Emsure®), Magnesium (Emsure®), HCl (Merck), Non-iodine salt, FeCl3 (Emsure®), H2SO4 (Emsure®), Chloroform (Emsure®), NaOH (Emsure®), Aluminum chloride (Emsure®), Iodine (Emsure®), yeast Saccharomyces cerevisiae (Fermipan®), and cyst artemia salina (Supreme Plus®).

Research Procedure

1. Plant Determination

   The determination of *Taraxacum officinale* F.H Wigg was carried out at Balai Besar Penelitian dan Pengembangan Tanaman Obat Tradisional (B2P2TOOT) Tawangmangu, Karanganyar, Central Java

2. Dandelion Leaf Extraction

   Dandelion leaf powder (200 g) was extracted using as much as 2 L of 50% ethanol solvent for 4 × 24 hours with stirring in the first 6 hours. The maserat was filtered and the remaining pulp was remacerated for 3 days. All the filtrates were evaporated using a rotary vacuum evaporator at 50°C. Subsequently, the % yield of the extract was calculated using the following formula:

$$\text{Yield (\%)} = \frac{Extract\ Weighht}{Dry\ simplicia\ weight} \text{ x } 100\%$$

3. Phytochemical Qualitative Analysis

Alkaloids (Mandal et al., 2015)

Mayer Test

The extract was added to a few drops of Mayer's reagent (potasiomercuric iodide). The results of cream-colored deposits indicate the presence of alkaloids

Wagner Test

A few drops of Wagner's reagent (iodine solution in potassium iodide) were added to the extract. The result of a reddish-brown precipitate indicates a positive test

Flavonoids

Test tubes containing 0.5 g of dandelion leaf were filled with 50% ethanol extract before being filled with 5 mL of distilled water. The test tube was filled with concentrated HCl and magnesium particles for moderation. According to (Parbuntari *et al.*, 2018), the finding of a color change from orange to red indicates the presence of flavones, while changes from red to dark red and from red to magenta indicate the presence of flavonols

Phenolics and Tannins

A few drops of 5% iron chloride were added to the extract, and the resulting dark green color indicated the presence of phenolic compounds (Mandal et al. 2015). The presence of tannin compounds is indicated by blue, blue-black, green, greenish-blue, or precipitate (Iqbal et al, 2015).

Steroids and Terpenoids

A few drops of Liebermann-Burchard reagent were added to the extract, and the presence of triterpenoids was characterized by the formation of a dark red color and the formation of brown rings at the intersection of the two layers, as well as at the top. layer changed from green to brown (Iqbal, Salim and Lim, 2015).

Saponins

Saponins were detected after the extract was shaken with aquades in a test tube and heated for 5 minutes (Iqbal, Salim and Lim, 2015).

4. BSLT Toxicity Test (Ningdyah et al, 2015; Aqiila et al, 2017; Surya, 2018).

***Artemia salina* Leach Egg Hatching.**

Artemia salina eggs were prepared for hatching in containers or jars. The container was split into two parts: light and dark. The black cardboard and duct tape covered the dark part. The two parts are bounded with Styrofoam, which is perforated as a path for hatched artemia. The container was filled with one liter of seawater until both holes were filled, and 1 g of Artemia salina eggs was added to the dark parts. The eggs develop into larvae after 24 hours and naturally swim to the light-filled space.

Test Material Preparation

The stock solution was prepared at 2500 ppm by weighing 62.5 mg of 50% ethanol extract and then dissolved in 25 mL of artificial seawater as a solvent. The test solution was prepared by diluting the soluble stock solution to concentrations of 50, 100, 150, and 200 ppm.

Preparation of Control Materials

The control solution contained solvent from the test solution. The control solution was prepared by adding 10 mL of seawater.

Toxicity Test

Test solutions of various concentrations and 10 A. salina larvae were added to each vial. Then, up to 5 ml (Meyer et al., 1982). The test vials were placed under illumination for 24 hours in triplicate. Each dead larva at each concentration was counted and recorded. The standard criterion for measuring shrimp larval mortality is that shrimp larvae do not show movement during 10 seconds of observation.

Data Analysis

The percentage of death of *Artemia salina* Leach larvae at each concentration was calculated for data processing and analysis. The probit value can be determined by converting the percentage of larval death at each concentration, using a manual probit analysis, into a probit value. The probit analysis method was performed using Microsoft Office Excel by graphing the linear equation between the probit value and the logarithm of the extract concentration used in the study. The LC50 value is calculated by entering the value (probable 50% mortality of test animals) as y, and the value of x is returned as the logarithmic value of the extract concentration used in research in linear equations. The LC50 value is the antilog of the x-value.

RESULT AND DISCUSSION

Plant Determination

Plant determination was carried out to determine the correctness of the samples and to prevent errors during data collection. Dandelion (*Taraxacum officinale* F.H. Wigg) was obtained from Balai Besar Penelitian dan Pengembangan Tanaman Obat Tradisional (B2P2TOOT) in Tawangmangu, Karanganyar, Central Java, and determined in the same place. The result of this determination is contained in the letter KM.04.02/2/761/2022, where it states that the sample is a simplisia of *Taraxacum officinale* (L.) Weber ex F.H. Wiggs of the Asteraceae family.

Dandelion Leaf Extraction

The extraction of dandelion leaves was carried out by maceration for 4 × 24 hours and using 50% ethanol solvents. Maceration is the simplest extraction process that uses a solvent with several stirrings in a macerator (Kementerian Kesehatan, 2017). The maceration equipment and procedures are very simple so it is easy to do and does not use heat (Febrina, Rusli and Muflihah, 2015). The fundamental benefit of maceration extraction is that it uses direct methods and unheated equipment to prevent damage to natural materials. Although some compounds have poor solubility in solvents at room temperature, maceration of extracts allows for the extraction of large amounts of compounds (Andasari et al., 2020).

Table I. The Yield of 50%-Ethanolic Extract of Dandelion Leaf

Solvent Extract	Yield (%)
Ethanol 50%	5,8

The yield indicates the number of phytochemical compounds present in the extract. Differences in the solvents used in the extraction process affected the yield of the extract. In this study, it was shown that the yield of the 50%ethanolic extract of dandelion leaves was 5.8%. The more active the chemicals extracted from a substance, the higher the yield value (Wahyuni and Marpaung, 2020). Ethanol is a universal solvent that can dissolve polar and nonpolar compounds (Widyaningrum et al. 2020). According to (Marpaung and Septiyani, 2020), ethanol is used as a solvent in research because it has several advantages, including its low boiling point so that it easily evaporates from extracts, the ability to dissolve active compounds with varying degrees of polarity, and the fact that it is non-toxic and environmentally friendly.

Phytochemical Qualitative Analysis

Phytochemical screening was performed to qualitatively determine the content of secondary metabolites using a color change reaction (Hayat *et al.*, 2020). The results of the phytochemical screening of the 50%-ethanolic extract of dandelion leaf contain alkaloids, flavonoids, phenols, tannins, and terpenoids. According to several studies, dandelion leaves contain phenolic compounds, tannins, flavonoids, terpenoids, and alkaloids (Jedrejek *et al.*, 2019; Lwin, 2019; Marcus and Edori, 2019).

Table II. Phytochemical Screening of 50%-Ethanolic Extract of Dandelion Leaf

Phytochemical Screening	Result	Observations
Alkaloid - Meyer - Wagner	 + +	 A white precipitate formed Brown precipitate formed
Fenol / Tanin	+/+	Formed blackish-green color
Saponin	-	No foam formed
Steroid/Terpenoid	+	A brown ring forms between the two layers
Flavonoid	+	Orange color formed

Note:
Positif (+) : Contains secondary metabolite compounds
Negative (-) : Does not contain secondary metabolite compounds

Brine Shrimp Lethality Test (BSLT)

The purpose of this study was to use BSLT to investigate the toxicity of dandelion. Plant extracts may be screened for biological activity using a simple tabletop bioassay known as BSLT, which has produced promising findings. Brine shrimp is poisonous to a wide range of substances, both natural and synthetic, and its demise upon exposure to different plant extracts provides the basis for toxicity testing. Because bioactive substances are nearly always detrimental at high levels, toxicology may be regarded as pharmacology at higher doses. This assumption has been employed in BSLT to evaluate medicinal plant extracts (Aksono *et al*., 2022).

As shown **Table III**, the BSLT test revealed that the 50%ethanolic dandelion leaf extract was toxic to *Artemia salina*. Based on the investigation results, in P1 and P2, where the extract concentration was 50 ppm and 100 ppm, the mortality rates were 37% and 43%, respectively, because the 50%ethanolic extract of dandelion leaf toxicity level was still low. The mortality rate was 50% at the P3 and 200 ppm extract concentrations. As it could eradicate *Artemia salina* at this dosage, the 50%ethanolic dandelion leaf extract was toxic. The death rate was 60% in P4 when the extract concentration was 200 ppm. This outcome indicates that *Artemia salina* is highly hazardous to 50%ethanolic dandelion leaf extract at this dosage. In the control group, there were no dead larvae, since there was no addition of 50%ethanolic extract of dandelion leaves containing poisonous compounds. This demonstrated that the death of *Artemia salina* was caused by the administration of 50%ethanolic extract of dandelion leaf and not by environmental factors. As a result, the number of *Artemia salina* deaths correlated with the 50%ethanolic dandelion leaf extract concentration.

The lethal concentration 50 (LC50) represents the maximum concentration of extract required to eradicate 50% of the *Artemia salina* population, based on the findings of toxicity tests. Consequently, a lower LC50 value indicates a more serious adverse impact (Utami and Ardiyanti, 2019). According to this study, the content of 50%ethanolic dandelion leaf extract was often correlated with the frequency of deaths linked to *Artemia salina*. Using the probit technique, the LC50 of a 50%ethanolic extract of dandelion leaves was determined to be 165.223 ppm. According to Mshelia *et al*., (2016) and Meyer et al. (1982), LC50 <30 ppm is highlyof toxic, LC50 30-1000 ppm is toxic, and LC50 >1000 ppm is non-toxic. These components resulted in the classification of 50%ethanolic dandelion leaf extract as toxic.

Table III. The Toxicity Test Results of the 50% Ethanolic Extract of Dandelion Leaf on *Artemia salina* Larvae

Group	Concentration Log	Number of Dead Larvae			Average	Percent of Deaths	Probit Value
		1	2	3			
Control	0	0	0	0	0	0%	0
P1 (50 ppm)	2,097	4	5	2	3,67	37%	4,67
P2 (100 ppm)	2,398	3	6	4	4,33	43%	4,82
P3 (200 ppm)	2,699	6	4	5	5	50%	5
P4 (400 ppm)	3	4	8	6	6	60%	5,25

The toxicity of an extract is influenced by the compound content contained in the extract (Di Kusuma et al., 2018), as well as the 50%ethanolic extract of the dandelion leaf. Alkaloids have toxic properties that act as toxins in the mouths of larvae and prevent them from receiving taste stimuli that can hinder their ability to eat. They can kill *Artemia salina* by causing indigestion. Because the larvae cannot recognize the food, they die of hunger. Because alkaloids are in contact with digestive toxins at high concentrations, they can kill larvae instantly by attacking critical organs (Jelita *et al.*, 2020; Hernanda et al., 2021; Kurniawan and Ropiqa, 2021).

Artemia salina larvae can starve to death owing to the ability of flavonoids to reduce the activity of digestive enzymes, inhibit food absorption, and function as gastric toxins (Hernanda et al., 2021). In addition, flavonoids can block taste receptors in the mouth area of larvae. This prevents the larvae from receiving taste sensations and interferes with their ability to detect food, causing them to starve to death. The mouth of *Artemia salina* allows the entry of toxic substances, which are then absorbed by the gastrointestinal tract and through cell membranes. The process of metabolic response damage occurs when the absorption process continues with the entry of harmful substances into the body of *Artemia salina* (Fadli, Suhaimi and Idris, 2019; Jelita *et al.*, 2020; Hartini *et al.*, 2022).

Tannins have a bitter taste, which can inhibit the ability of larvae to eat (Hartini *et al.*, 2022). In addition, tannins have been shown to block taste receptors in larval mouth regions, as well as the ability of insects to digest food (Fadli, Suhaimi and Idris, 2019; Hartini *et al.*, 2022). Growth hormones, known as steroids, affect larval molting. Steroids thicken the chitin cell wall in the larval body to prevent the larvae from growing normally and eventually killing it (Pranata et al., 2021). Terpenoids prevent insects from eating larvae because of their bitter taste (Hernanda et al, 2021).

The formula for the line y = ax + b, which can be used to verify the LC50 value, is shown in **Figure 1**. The outcome of solving the equation y = 2.114 x + 0.311 with probit 5 (y = 5) is x = log 2.218, which is then antilogested to produce a value of 165.223 ppm. The probit analysis results do not accurately represent the true values because they are qualitative estimates. The results of the probit analysis also provided interval estimates. The 50%ethanolic dandelion leaf extract was 94% efficient at killing *Artemia salina*, as shown in **Figure 1**, which also shows that the R^2 value is 0.9435. The complete diversity of the dependent variable Y, which can be attributed to or explained by the variety of independent variable X, includes the coefficient of determination (R^2).

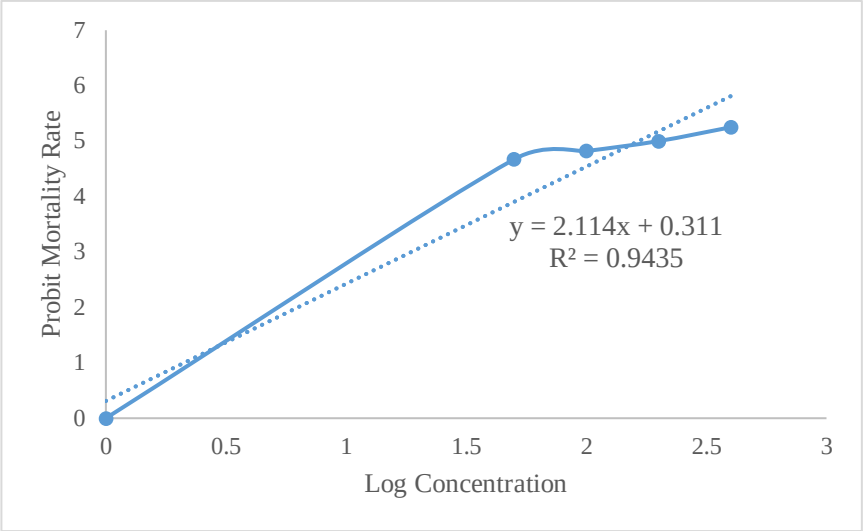

Figure 1. Graph of the Correlation of the Log Concentration of 50%-Ethanolic Extract of Dandelion Leaf with the Probit Mortality Rate of *Artemia salina* Larvae

A significant degree of testing sensitivity was demonstrated for *Artemia salina* larvae. A 48-hour-old *Artemia salina's* mouth and digestive system were designed to take in particular particles based on their shape. Within 24 hours after birth or during the second instar phase, Artemia salina larvae cannot interact with their environment and absorb external chemicals or extracts, even though they have a digestive system (Hamidi et al., 2014).

Salinity, pH, and seawater temperature must be considered for *Artemia salina* egg hatching and growth. A pH drop of less than 7 can be lethal (Hiola et al., 2014). A pH of 8–9, which is somewhat alkaline, is necessary for cyst hatching. In hatcheries, temperatures below 6°C or above 35°C are detrimental to the growth of *Artemia salina*. Temperatures between 26 and 31°C are ideal for *Artemia* proliferation (Martin & Davis, 2001). A lamp was introduced to the *Artemia* larvae during the hatching phase as a light source to maintain the water at the ideal temperature and encourage the larvae to shed their eggshells. Because of their positive phototaxis traits, which attract light, actively moving larvae swim toward the light source (Martin and Davis, 2001). The non-selective filter in the digestive system of *Artemia salina* facilitates the entry of toxic substances into the mouth (Aksono *et al*., 2022).

CONCLUSION

The 50%ethanolic extract of dandelion leaf was determined to be toxic to *Artemia salina* in the BSLT test, with an LC50 value of 165.223 ppm based on probit analysis. Interestingly, this study provides evidence regarding the toxicity of dandelion leaves, which can be further emphasized as a foundation for conducting other toxicity studies for new drug discovery and development.

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