Anticancer Pre–Screening of Sansevieria masoniana C. Using Brine Shrimp Lethality Assay

A. L. Mawardi*, A. R. S. Siregar, Elfrida
Biology Education Department, Faculty of Teacher Training and Education
Samudra University
Nanggroe Aceh Darussalam, Indonesia
*mawardibio@unsam.ac.id, rasuna23@gmail.com

***Abstract*—A lot of detrimental side effects on cancer treatment, causing lately, the focus of cancer treatment now shifted toward natural products such as medical plants. *Sansevieria masoniana* C are medical plants which have a high antioxidant content. This study aimed to evaluate the anticancer potential of *Sansevieria masoniana* C. extract with a fast pre-screening method brine shrimp lethality assay (BSLA). This method measuring the LC50 of the of *Sansevieria masoniana* C. extract to *the Artemia salina* Leach Larvae that analog with the cancer cells. The result from *Sansevieria masoniana* C. extract shows that *Sansevieria masoniana* C. has potential anticancer activity with LC50 107,49 µg/mL. *Sansevieria masoniana* C. are products with excellent cytotoxicity and were promising sources for developing anticancer agents.**

Keywords—Anticancer activity, BSLA, Sansevieria masoniana C.

I. INTRODUCTION

Cancer is still one of the deadliest diseases in the world. Based on data from the World Health Organization, it is estimated that in 2005-2015 there were 84 million people who died cause of cancer [1].

As a developing country, cancer in Indonesia is the sixth disease with the highest mortality rate, the prevalence of cancer sufferers in 2013 in Indonesia was 347,792 people or the equivalent of 1.4% of Indonesia's population [2–4].

Indonesia has the potential for abundant natural wealth, especially in the potential for medicinal plants. Based on the World Conservation Monitoring Center, it is reported that Indonesia has various types of medicinal plants with the number of plants reaching 2,518 plants, of which some medicinal plants have the potential to develop anticancer drugs due to metabolites contained in plants [5]. Plants that have anticancer potential are plants of the genus Sansevieria.

Plants from the genus Sansevieria are one of the ornamental plants that are very popular among the public because they are considered able to ward off free radicals in nature because they have high antioxidant content. Apart from its function as an ornamental plant, Sansevieria can also be used as traditional medicine such as inflammation, cough, and influenza [6]. Sansevieria is thought to have anticancer potential because it contains active compounds in the form of antioxidants that have the potential to have anticancer abilities such as flavonoids, alkaloids, saponins, tannins, steroids/ triterpenoids [7,8]. Species from the genus Sansevieria selected for anticancer activity were Sansevieria masoniana Chahinbecause it is not yet known the information on the anticancer potential of this species, even though this information is very important to know to make the plant of the Sansevieria genus as an anticancer agent plant. The use of Sansevieria masoniana C. plants in research is also based because this species is easy to find in tropical areas such as Indonesia so it is efficient and cheap to be used as research samples.

The test conducted to determine the anticancer potential is the cytotoxic test using the Brine Shrimp Lethality Assay (BSLA) method. BSLA is a method that is widely used in the search for new anticancer compounds from flora and fauna in nature. This method correlates with anticancer activity where the *Artemia salina* Leach larvae test used has a toxic effect similar to the toxic effect that occurs in vitro leukemia cell cultures in mice (9PS) and nasopharyngeal carcinoma cells (9KB) so it can be said that death in *Artemia salina* Leach has leukemia cancer cell death and nasopharyngeal carcinoma. The ability of artemia to monitor cytotoxic effects is very possible because artemia has system information in mammals, namely DNA-dependent RNA polymerase type and an oubaine-sensitive Na + and K + dependent ATPase, so that compounds or extracts that have activity in the system can be detected [9,10].

Artemia salina Leach is quite accurate in representing the cancer cell model, this has been proven by Meyer et.al., in 1982 in their research [11]. The results of his research indicate that a fact shows that a feeling that is cytotoxic, when tested either in cancer cell cultures or on artemia gives the same toxic results or effects. This method is also easy to work with, fast, inexpensive, and accurate confidence level up to 95% [11,12]. The extract indicator is said to be toxic (potentially anticancer) if it has an LC_{50} value of less than 1000 µg/mL [12,13]. If the LC50 value of the extract obtained shows very toxic activity, further research can be carried out by isolating plant cytotoxic compounds as the development of alternative anticancer drugs.

II. METHODS

A. Plant Material

Fresh leaves are collected from several areas in Stabat, North Sumatra Indonesia between Januari and Maret 2020. Seven kilogram (7 kg) of Sansevieria masonina C. fresh leaves were collected. Samples were cleaned and then dried and aired without direct sun exposure for 1 week, the purpose of drying is to prevent enzymatic reactions (microbial activity) and prevent the growth of fungi so that they can be stored longer and are less easily damaged and their chemical composition does not change. Manually the drying process is complete, it is marked when the leaf character can be crushed or broken easily by hand and has a dry light brown color. After drying, the leaves are crushed by using blender and stored in a closed container.

B. Extraction

The extraction method used is the maceration method, 500g of Sansevieria masoniana C. powder was macerated with 2 liters of 96% ethanol in a closed container for 2 x 24 hours with occasional stirring. Then the macerate is filtered and concentrated using a rotary evaporator at 100 ^{o} C, then evaporated with a water bath to form a thick extract.

C. Brine Shrimp Lethality Assay

Artemia salina Leach is used in the second instar stage because it is very susceptible to toxins in the early stages of its growth. The eggs of Artemia salina Leach were hatched for 2 days before treatment. Hatching is done using a mineral water bottle filled with artificial seawater by adding 1 liter of aqua dest, 25 grams of fish salt, and 0.15 grams of sodium bicarbonate, then aerated and placed under a lamp. Then put 1g of artemia eggs into artificial seawater. The eggs will hatch 18-24 hours then wait until the larvae are 48 hours old. After 48 hours, turn off the aerator and wait about 5-10 minutes for the brine shrimp and the hatched eggshells to separate. The shell of the *Artemia salina* Leach is on the surface of the water, the Artemia salina Leach that hatches will move naturally towards the bright area so that the shrimp larvae are separated from the eggshell. Then using a dropper, the larvae are taken by being sucked at the bottom of the bottle. Larvae are ready to be used as test animals [14].

The test was carried out using four different concentration variants and 1 control 0µg/mL, 10µg/mL, 100µg/mL, and 1000µg/mL. The test was carried out by inserting 10 larvae of Artemia salina Leach into glass vials that had been added with 10 mL of seawater and 10 mL of extract at each concentration with four repetitions. The vial was placed under the lamp for 24 hours, after 24 hours the dead larvae were counted (immobile larvae) using hand lens as percent of mortality [13,15]. From data on percent mortality and the concentration variations were plotted on a graph, and LC_{50} was calculated.

D. Phytochemical Screening

The phyto-chemist screening of bioactive compund of the Sansevieria masoniana Chahin including flavonoid, saponin, alkaloid, tanin, steroid, and, triterpenoid.

E. Data Analysis

The toxic effect was obtained from observing the number of deaths of artemia salina leach larvae after 24 hours of treatment.The percentage of deaths is calculated by the following formula; % death = (number of death larvae/total number of larvae)$\times 100\%$ [16]. The effect of toxicity on Artemia salina Leach can be seen from the lethality concentration 50% (LC_{50}) value, where this value is determined based on a probit analysis and makes a linear regression equation y = bx + a where y = probit number, x = log concentration, a = slope value, and b = intercept value. So that the concentration that causes death is 50% in larvae of *Artemia salina*Leach [17].

III. RESULTS AND DISCUSSION

Brine shrimp lethality assays are the initial tests performed to evaluate the potential of Sansevieria masoniana C. as an antiproliferative agent [13,14]. This assays makes it possible to determine whether Sansevieria masoniana C. has the ability to inhibit cell growth so that it can be tested further to become a candidate for anticancer medicine. The data presented in Figure 1 show that there are different effects of various concentrations of extracts on the mortality of Artemia salina Leach larvae. This indicates that each extract concentration has a different toxic effect on shrimp larvae. The mortality of Artemia salina Leach larvae was indicated by the motion of the larvae after 24 hours and the larvae were pale.

The data show that the percentage of larvae mortality was obtained at the concentration of the extract, with the highest number of larvae deaths at a concentration of 800 µg/mL, with a percentage of 100% mortality. The lowest percentage of mortality was at a concentration of 100 µg/mL, with a percentage of 52.5% mortality. The control experiment with 0 µg/mL extract, no dead larvae were obtained so that the death of pure larvae was caused by the extract and not due to the influence of artificial seawater in the test. Thus, the extract of the leaves of the Sansevieria masoniana C. has the potential for toxicity to the larvae of Artemia salina Leach. The results obtained are following the provisions of toxicity, namely the higher the concentration of the extract causes the higher the number of larvae deaths, which shows the higher its toxicity and has potential as an anticancer. From the mortality results, a straight line equation is obtained which can be seen in the relationship curve between Y (the probit value of the percentage of mortality) and X (log concentration) for the three extracts of Sansevieria masoniana C. (Figure 2).

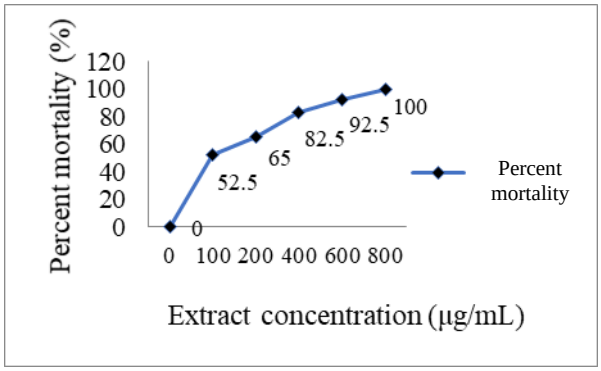

Fig. 1. Effect of concentrationextract against the death of Artemia salina Leach Larvae.

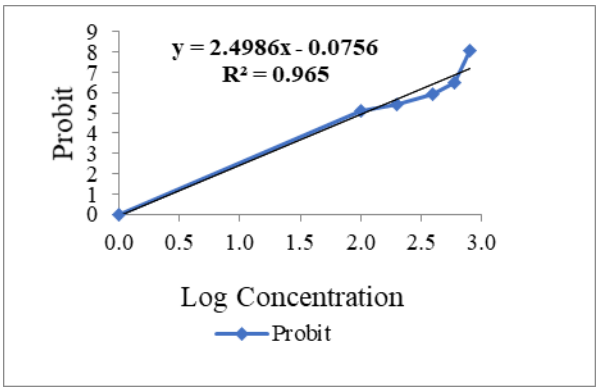

Fig. 2. Linear Regression Curve of Sansevieria masoniana C.Extract Toxicity test.

From the curve in Figure 2, it shows that the greater the log value of the concentration of the *Sansevieria masoniana* C. extract, the mortality probit value obtained from the percent mortality of Artemia salina Leach larvae also increases. It can be seen that the correlation is positive because the R^2 value is 0.965, so it can be said that the log of concentration and probit has a correlation because the R value has a range greater than 0 and less than 1. The LC50 value is determined by substituting the value of 50 in X (Figure 2) so that the Y value is obtained in each equation, then the Y value is interrogated and the LC50 value of the extract is obtained. The probit analysis results obtained an LC50 value of 107,49 µg/mL which means that the extract has a toxic effect and 50% larval mortality at a concentration of 107,49 µg/mL.

The LC_{50} result from *Sansevieria masoniana* C. Show that *Sansevieria masoniana* C. has potential biological (anticancer) activity with LC_{50} 107,49 µg/mL. Extracts that are toxic to the larvae of Artemia salina Leach are considered to show biological activity, so this test can be used as an initial screening for bioactive compounds that are thought to have anticancer properties [18,19]. According to Meyer et.al., in 1982 several extracts those who have an LC50 <1000 µg/mL using the brine shrimp lethality assay are considered very toxic and contain the physiologically active principle.

TABLE I. RESULT FROM PHYTOCHEMICAL SCREENING

Secondary Metabolites	Screening Result
Flavonoid	(+)
Saponin	**(+)**
Terpenoid	**(-)**
Steroid	**(-)**
Alkaloid	**(-)**
Tanin	**(-)**

Sansevieria masoniana C. has flavonoid and saponin substances (table 1). This maybe the main factor for that plants has cytotoxic activity and potential as anticancer agent. Flavonoids have an anticancer mechanism because flavonoids are antioxidants, namely through the activation mechanism of the apoptotic pathway of cancer cells. The mechanism of cell apoptosis in this theory is due to DNA fragmentation. This fragmentation begins with the release of the proximal DNA chain by reactive oxygen compounds such as hydroxyl radicals. Another effect is flavonoids as inhibitors of tumor/cancer proliferation, one of which is by inhibiting protein kinase activity so that it inhibits the signal transduction pathway from the membrane to the nucleus cells. Flavonoids inhibit tyrosine kinase receptor activity because the increased activity of tyrosine kinase receptors plays a role in the growth of cancer cells. Flavonoids also function to reduce tumor resistance to chemotherapy agents. Another reason flavonoids can kill cancer cells is due to the presence of an OH- group on the flavonoids that binds to the integral protein of the cell membrane. This causes the active transport of Na + - K2 to be blocked. Active transport that stops causes uncontrolled entry of Na + ions into the cell, this causes the rupture of the cell membrane. The rupture of the cell membrane causes cell death [20,21].

Saponins has reported can demonstrate significant anticancer activity, such as anti-angiogenesis, anti-proliferation, anti-metastasis, and can reversal of multi-drug resistence (MDR) effects through mechanism that prootion of cell- differentiation and induction of apoptosis and prootion of cell- differentiation [22-24]. Saponins can decrease the activity of digestive enzymes and absorption of food. The mechanism of mortality for Artemia salina Leach larvae is related to the function of flavonoids and saponins which inhibit the feeding power of larvae (antecedent). The way these compounds work is to act as stomach poisoning. Therefore, when these compounds enter the larva's body, their digestive organs will be disturbed. This compound will inhibit the receptors in the mouth area of the larvae. This results in the larvae failing to get the taste stimulus so that they are unable to recognize the food and as a result, the larvae die of starvation. This process took place in just 24 hours which resulted in the mortality of Artemia salina Leach larvae by 50% [25].

IV. CONCLUSIONS

Based on this research, it can be concluded that Sansevieria masoniana C. has cytotoxic activity and potential to becone anticancer agent with the value of LC_{50} 107,49 µg/mL. From this research, it can also be seen that flavonoids and saponins

are the main factors for toxicity activity and are promising compounds to be used as anticancer agents. Based on this research, the researcher can continue to do more in-depth research on the topic. analyze and isolate bioactive contents and testing it to the human cell line in vitro and forward to testing it in vivo and clinical trials.

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