Cytotoxicity Test Of Green Betel Leaf Ethanol Extract (*Piper Betle L.*) Using Shrimp Larvae (*Artemia Salina L.*) Using The Brine Shrimp Lethality Test (BSLT) Method

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Abstract.

The Brine Shrimp Lethality Test (BSLT) method is a preliminary method for screening anticancer compounds by looking at the cytotoxic effect of a sample that will be tested on Artemia salina shrimp larvae. This study aims to evaluate the cytotoxic effect of ethanol extract of green betel leaves (Piper betle L.) on Artemia salina Leach larvae using the Brine Shrimp Lethality Test (BSLT) method. Extraction was carried out by maceration method using 96% ethanol, and phytochemical screening to show the presence of secondary metabolite on green betel leef. Cytotoxicity tests were carried out at five extract concentrations (concentrations of 50 ppm, 100 ppm, 250 ppm, 500 ppm, and 750 ppm). Based on the results of the research carried out, it can be concluded that the results show that the percentage of larvae deaths ranges from 30-80% and the highest mortality was obtained at a concentration of 750 ppm with a total of 26 larval deaths, this death was in line with the increase in concentration. The Lethal Concentration (LC50) value obtained was 121.3317 ppm. In conclusion, green betel leaf extract contains secondary metabolite compounds which cause cytotoxic effects on Artemia salina Leach shrimp larvae with a mortality percentage of 30-80% and the LC50 value is 121.3317 ppm.

Keywords: *Cancer, Green Betel Leaf, Cytotoxicity, Artemia Salina and Lethal Concentration.*

I. INTRODUCTION

Cancer is one of the diseases with the highest mortality rate in almost every country, with data from the World Health Organization (WHO) stating that cancer is the leading cause of global death, resulting in nearly ten million deaths in 2020 [1], [2]. In Indonesia, cancer caused 234,511 deaths in 2020, so addressing this problem requires an active role from individuals and lifestyle changes [3], [4]. Indonesia, as an agrarian country with abundant natural resources, has great potential in the utilization of plants as herbal medicines, including betel leaves (*Piper betle L.*), which have been studied to have various health benefits, including as antibacterials and anticancers [5], [6]. This study aims to evaluate the cytotoxicity effect of green betel leaf ethanol extract on *Artemia salina L*each larvae using the Brine Shrimp Lethality Test (BSLT) method, which is an effective method for screening new anticancer compounds [7]. The BSLT method allows researchers to assess the toxicity of compounds on shrimp larvae, where the higher the death of larvae after administration of the compound, the higher the cytotoxic activity [8]. Previous research has shown that VCO and EVOO can improve metabolism and reduce oxidative stress, as well as reduce fat accumulation in the body of mice [9]–[11]. Green betel leaf (*Piper betle L.*) is a herbal plant that has long been used in traditional medicine in various cultures, including in Indonesia. This plant is known to have various properties, such as antimicrobial, anti-inflammatory, and antioxidant properties, which result from the content of various phytochemical compounds, such as flavonoids, saponins, and tannins [12]–[14].

Research on the bioactive potential of green betel leaves is increasing, especially in the context of natural medicine development. One commonly used method for evaluating the toxicity potential of plant extracts is the Brine Shrimp Lethality Test (BSLT), which uses Artemia salina shrimp larvae as a model. The cytotoxicity test using the BSLT method provides initial information regarding the toxic potential of plant

extracts, which is very important in the drug development process. This method is considered efficient and economical, and is able to provide fast results [15], [16].In this study, an ethanol extract from green betel leaves will be tested to determine the LC50 value, which is the concentration at which 50% of *Artemia salina L*arvae die, providing an indication of the extract's toxicity level [16]. Previously, similar research has been conducted on various types of plants, showing that many plant extracts have significant cytotoxic potential. For example, matoa leaf extract (*Pometia pinnata*) shows a fairly low LC50 value, indicating strong cytotoxic properties [17]. In addition, research on the extract of chicken pox leaves (*Tagetes erecta L.*) also shows similar results, with diverse phytochemical content and LC50 values that indicate potential toxicity [18]. This shows that many plants have potential as anticancer agents, and further research is needed to explore this potential. Therefore, it is important to conduct further research on the cytotoxicity effect of green betel leaf ethanol extract on *Artemia salina L*arvae, as a first step in exploring the therapeutic potential of this plant. The results of this study are expected to contribute to the development of natural medicines and broaden understanding of the therapeutic potential of green betel leaves [12], [13], [19].

II. METHODS

This research is an experimental study with a post-test only group design that aims to test the cytotoxicity of green betel leaf (*Piper betle L.*) ethanol extract against *Artemia salina L*each larvae using the *Brine Shrimp Lethality Test* (BSLT) method, which was carried out at the Phytochemical Pharmacognosy Laboratory, Faculty of Pharmacy, Universitas Muslim Indonesia from October to December 2024. The dependent variable in this study was green betel leaf extract, while the independent variable was the number of larval deaths. The study population consisted of *Artemia salina L*arvae, with inclusion criteria of nauplii stage shrimp larvae and intact green betel leaves, and exclusion criteria for larvae that did not show movement activity and damaged leaves.

Operational definitions include extract concentration in ppm, larval activity, cytotoxicity effects, and phytochemical screening, which are measured using various tools and methods of analysis. Extraction is carried out by maceration using 70% ethanol, followed by phytochemical tests to identify bioactive compounds such as alkaloids, saponins, and flavonoids. Larvae were hatched by immersing Artemia eggs in seawater under lamp light, and a solution of the extract was made to determine its concentration for orientation. A toxicity test was carried out by adding larvae to the extract solution and calculating mortality after 24 hours, where the percentage of mortality was calculated using a predetermined formula. Data analysis was performed by determining the LC50 value using simple linear regression, and research ethics were upheld by including permission from relevant parties [1], [19]–[21]. This study is expected to contribute to the development of natural medicines and broaden understanding of the therapeutic potential of green betel leaves.

III. RESULT AND DISCUSSION

This study used green betel leaves obtained from plant sellers, where a determination was carried out at the Phytochemical Pharmacognosy Laboratory of the Faculty of Pharmacy, Universitas Muslim Indonesia, to identify morphological, anatomical, and biological characteristics to determine the scientific name and proper classification. After that, the samples were dried and ground into powder, with the aim of reducing the moisture content that can become a medium for the growth of microorganisms, so that the quality of the active compound content in the samples is maintained [22]. Green betel leaf simplisia was then extracted using the maceration method with 96% ethanol solvent. This extraction process is carried out without heating, so as to avoid damage to labile compound components [23]. The maceration method was chosen for its ease and practicality, and 96% ethanol was chosen as the solvent because of its abundant availability, efficiency, and safety for the environment, as well as its ability to effectively extract polar and nonpolar compounds. After the maceration process, the extraction results were filtered and evaporated using a rotary evaporator to obtain a thick extract of green betel leaves. This process ensures that the bioactive compounds contained in green betel leaves can be properly extracted, so that they can be used for further research on their therapeutic potential.

Content of Secondary Metabolite Compounds in Green Betel Leaf Extract

The thick green betel leaf extract obtained was then subjected to phytochemical screening to determine the content of secondary metabolite compounds in the extract. The phytochemical test results of the green betel leaf extract showed positive results for alkaloids, saponins and flavonoids.

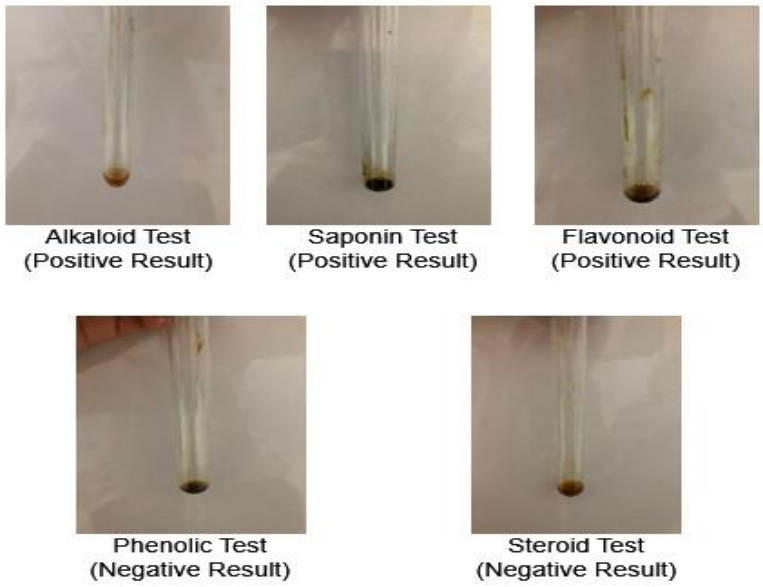
Table 1. Phytochemical Screening Results of Green Betel Leaves (*Piper betle L.*)

No	Content Chemistry	Reactant	Result	Description
1	Alkaloid	Dragendorff	Orange Deposits	Positive
2	Saponin	Vanilin-asam sulfat	Blue Violet	Positive
3	Flavonoid	Sitroborat	Red or Yellow Orange Color	Positive
4	Fenolik	$FeCl^3$	Dark Green Color	Negative
5	Steroid	Liebermenn-Bucherd	Brownish Yellow Color	Negative

Source: Processed Primary Data, 2024

In table 1, it is found that the phytochemical screening results of green betel leaves contain alkaloids, saponins and flavonoids. As for phenolic and steroids, they were not found in the green betel leaf extract. This is also shown in the following figure:

Fig 1. Phytochemical Screening Results of Green Betel Leaf Ethanol Extract



Cytotoxicity Effect of Green Betel Leaf Extract on Shrimp Larvae

The extraction results were then tested for cytotoxicity using the Brine Shrimp Lethality Test (BSLT) method. This test is a preliminary anticancer screening test to see simple biological activity to determine the cytotoxicity of compounds or extracts using shrimp larvae. The parameters measured are to observe and calculate the number of deaths of shrimp larvae as test animals.

Table 2. Death of Shrimp Larvae in Green Betel Leaf Extract at a Concentration of 50 ppm

Concentration 50 ppm		
Green Betel Leaf Extract	**Number of Shrimp Larvae in Each Tube**	**Number of Dead Shrimp Larvae In Each Tube**
Tube 1	10 Shrimp Larvae	2 Shrimp Larvae
Tube 2	10 Shrimp Larvae	3 Shrimp Larvae
Tube 3	10 Shrimp Larvae	4 Shrimp Larvae
TOTAL	**30 Shrimp Larvae**	**9 Shrimp Larvae**
Average		**3**

Source: Processed Primary Data, 2024

Table 2 shows that at a concentration of 50 ppm, the effect of green betel leaf extract was obtained in 3 tubes, each tube containing 10 larvae, for a total of 30 larvae in all tubes. In the first tube, 2 larvae died, in the second tube, 3 larvae died, and in the third tube, 4 larvae died, so that the total number of larvae deaths obtained at a concentration of 50 ppm was 9 larvae deaths. The death of the larvae obtained shows that green betel leaf extract has a cytotoxicity effect on shrimp larvae.

Table 3. Death of Shrimp Larvae in Green Betel Leaf Extract at a Concentration of 100 ppm

Concentration 100 ppm		
Green Betel Leaf Extract	**Number of Shrimp Larvae in Each Tube**	**Number of Dead Shrimp Larvae In Each Tube**
Tube 1	10 Shrimp Larvae	2 Shrimp Larvae
Tube 2	10 Shrimp Larvae	6 Shrimp Larvae
Tube 3	10 Shrimp Larvae	5 Shrimp Larvae
TOTAL	**30 Shrimp Larvae**	**13 Shrimp Larvae**
	Average	**4,33**

Source: Processed Primary Data, 2024

Table 3 shows that at a concentration of 100 ppm, the effect of green betel leaf extract was obtained in 3 tubes, each tube containing 10 larvae, for a total of 30 larvae in all tubes. In the first tube, 2 larvae died, in the second tube 6 larvae died, and in the third tube 5 larvae died, so that the total number of larvae deaths obtained at a concentration of 100 ppm was 13. The death of the larvae obtained shows that green betel leaf extract has a cytotoxicity effect on shrimp larvae.

Table 4. Death of Shrimp Larvae in Green Betel Leaf Extract at a Concentration of 250 ppm

Concentration 250 ppm		
Green Betel Leaf Extract	**Number of Shrimp Larvae in Each Tube**	**Number of Dead Shrimp Larvae In Each Tube**
Tube 1	10 Shrimp Larvae	7 Shrimp Larvae
Tube 2	10 Shrimp Larvae	7 Shrimp Larvae
Tube 3	10 Shrimp Larvae	7 Shrimp Larvae
TOTAL	**30 Shrimp Larvae**	21 Shrimp Larvae
	Average	**7**

Source: Processed Primary Data, 2024

Table 4 shows that at a concentration of 250 ppm, the green betel leaf extract had an effect on 3 tubes, each tube containing 10 larvae, for a total of 30 larvae in all tubes. In the first tube, 7 larvae died, in the second tube, 7 larvae died, and in the third tube, 7 larvae died, so the total number of larvae deaths obtained at a concentration of 250 ppm was 21 larvae deaths. The death of the larvae obtained shows that green betel leaf extract has a cytotoxicity effect on shrimp larvae.

Table 5. Death of Shrimp Larvae in Green Betel Leaf Extract at a Concentration of 500 ppm

Concentration 500 ppm		
Green Betel Leaf Extract	**Number of Shrimp Larvae in Each Tube**	**Number of Dead Shrimp Larvae In Each Tube**
Tube 1	10 Shrimp Larvae	7 Shrimp Larvae
Tube 2	10 Shrimp Larvae	8 Shrimp Larvae
Tube 3	10 Shrimp Larvae	9 Shrimp Larvae
TOTAL	**30 Shrimp Larvae**	24 Shrimp Larvae
	Average	**8**

Source: Processed Primary Data, 2024

Table 5 shows that at a concentration of 500 ppm, the effect of green betel leaf extract was obtained in 3 tubes, each tube containing 10 larvae, for a total of 30 larvae in all tubes. In the first tube, 7 larvae died, in the second tube, 8 larvae died, and in the third tube, 9 larvae died, so that the total number of larvae deaths obtained at a concentration of 500 ppm was 24 larvae deaths. The death of the larvae obtained shows that green betel leaf extract has a cytotoxicity effect on shrimp larvae.

Table 6. Death of Shrimp Larvae in Green Betel Leaf Extract at a Concentration of 750 ppm

Concentration 750 ppm		
Green Betel Leaf Extract	**Number of Shrimp Larvae in Each Tube**	**Number of Dead Shrimp Larvae In Each Tube**
Tube 1	10 Shrimp Larvae	7 Shrimp Larvae
Tube 2	10 Shrimp Larvae	9 Shrimp Larvae
Tube 3	10 Shrimp Larvae	10 Shrimp Larvae
TOTAL	**30 Shrimp Larvae**	26 Shrimp Larvae
	Average	**8,6**

Source: Processed Primary Data, 2024

Table 6 shows that at a concentration of 750 ppm, the effect of green betel leaf extract was obtained in 3 tubes, each tube containing 10 larvae, for a total of 30 larvae in all tubes. In the first tube, 7 larvae died, in the second tube, 9 larvae died, and in the third tube, 10 larvae died, so the total number of larvae deaths obtained at a concentration of 750 ppm was 26 larvae deaths. The death of the larvae obtained shows that green betel leaf extract has a cytotoxicity effect on shrimp larvae.

Table 7. Shrimp Larvae Death in Negative Control

Negative Control		
Green Betel Leaf Extract	**Number of Shrimp Larvae in Each Tube**	**Number of Dead Shrimp Larvae In Each Tube**
Tube 1	10 Shrimp Larvae	10 Shrimp Larvae
Tube 2	10 Shrimp Larvae	10 Shrimp Larvae
Tube 3	10 Shrimp Larvae	10 Shrimp Larvae
TOTAL	**30 Shrimp Larvae**	30 Shrimp Larvae
Average		**10**

Source: Processed Primary Data, 2024

Table 7 shows that in the negative control, which is seawater alone without the addition of green betel leaf extract, no larval mortality was obtained. The absence of larval mortality indicates that the negative control has no cytotoxicity effect on shrimp larvae.

Percentage of Shrimp Larvae Deaths After Treatment

Table 8. Percentage of Shrimp Larvae Deaths at Each Concentration

Concentration	Number of Deaths	Percentage of Deaths
50 ppm	9 Shrimp Larvae	30%
100 ppm	13 Shrimp Larvae	43,33%
250 ppm	21 Shrimp Larvae	70%
500 ppm	24 Shrimp Larvae	80%
750 ppm	26 Shrimp Larvae	80,66%

Source: Processed Primary Data, 2024

In table 8, the presentation of every 30 shrimp larvae per concentrate is obtained, namely at 50 ppm by 30%, at 100 ppm 43.33%, at 250 ppm 70%, at 500 ppm by 80%, and at 750 ppm by 80.66%. And the lowest percentage is obtained at the lowest concentration, also showing a mortality rate of 30% at a concentration of 50 ppm, as well as the highest percentage value obtained at 80.66% at a concentration of 750 ppm. With this, it can be seen that the percentage of larval mortality increases along with the increase in the concentration of green betel leaves. Then, in each presentation of larval mortality, it is converted to a probit value to find out the LC50 value.In table 8, the presentation is obtained for every 30 shrimp larvae per concentrate, namely at 50 ppm by 30%, at 100 ppm 43.33%, at 250 ppm 70%, at 500 ppm by 80%, and at 750 ppm by 80.66%. And the lowest percentage is obtained at the lowest concentration, also showing a mortality rate of 30% at a concentration of 50 ppm, as well as the highest percentage value obtained at 80.66% at a concentration of 750 ppm. With this, it can be seen that the percentage of larval mortality increases along with the increase in the concentration of green betel leaves. Then, in each presentation of larval mortality, it is converted to a probit value to find out the LC50 value.

LC50 Value of Green Betel Leaf Extract (*Piper betle L.*) by the BSLT Method

To determine the LC50 value, it can be calculated based on the percentage of larval mortality, then converted into a probit value. After calculating the percentage of mortality and converting it into a probit value, the data is plotted into a linear regression equation with the value Y = probit value and X = Log C (log concentration).

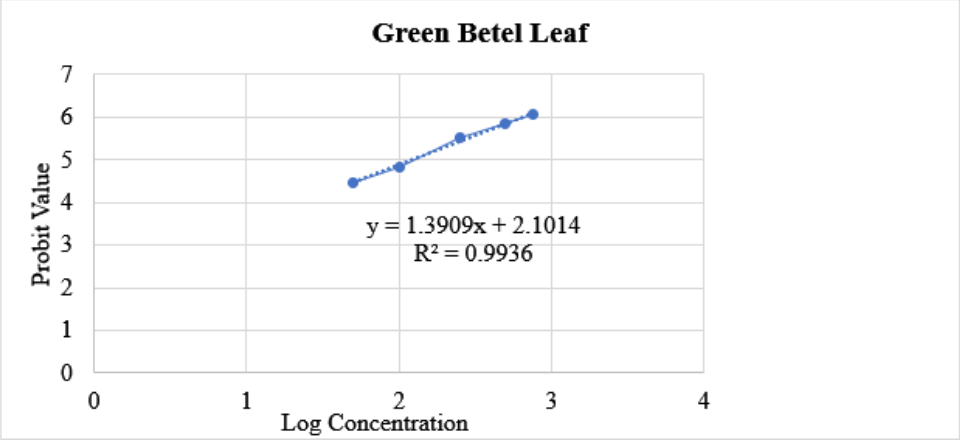
Table 9. Data on the Observation of the Death of Artemia Salina Leach Shrimp Larvae for 24 Hours from Green Betel Leaf Etaol Extract (Piper betlL.) Using the BSLT Method

		Number of Dead Shrimp Larvae in Each Series Concentration of Test Sample Solution				
Seri Concentration	**Replication**	**50**	**100**	**250**	**500**	**750**
Log Concentration		1,69897	2	2,39794	2,69897	2,87506
Green Betel Leaf	**1**	2	2	7	7	7
Ethanol Extract (*Piper*	**2**	3	6	7	8	9

betle L.)	3	4	5	7	9	10
Number of Deaths		9	13	21	24	26
Average		3	4,333333	7	8	8,666667
%		30,000	43,333	70,000	80,000	86,667
Probit		4,48	4,82	5,52	5,84	6,08

Source: Processed Primary Data, 2024

Fig 2. Linear Regression Equation Curve of Green Betel Leaf Ethanol Extract on Shrimp Larvae



Description:
Y = ax + b
Y = 5
a = 1.3909
b = 2.1014
x = 2.083974
LC50 = 121.3317

Figure 2 shows that the increase in mortality is directly proportional to the increase in concentration. The toxicity of a compound can be calculated by summing the mortality of shrimp larvae with the *Lethal Concentration* 50 (LC50) parameter. The toxic nature of an extract can be categorized as very toxic if the LC50 value is <30 ppm, toxic with an LC50 value of 31-1000 ppm and said to be non-toxic if the LC50 value is >1000 PPM [1]. Based on the linear regression equation, the LC50 value can be calculated with the equation Y=ax+b. Then the LC50 value based on the data obtained is Y= 1.3909x + 2.1014 with the Y value (probit value) at 50% larval mortality is 5, and the obtained x value is 2.083974. Furthermore, the LC50 value is the log inverse of the x value, which is 121.3317. Then the LC50 value is categorized as toxic.

Green Betel Leaf Compound Content

In table 1, the content of green betel leaves is found to contain secondary metabolite compounds, namely alkaloids, flavonoids and saponins. As for steroids and phenolics, they are not contained in green betel leaves. Green betel leaves (*Piper betle L.*) are widely known in traditional medicine and have various bioactive compounds that contribute to their therapeutic properties, including alkaloids, flavonoids, and saponins, which can contribute to larval death in cytotoxicity tests [24]–[26]. Previous research has shown that these compounds can interfere with the physiological functions of larvae, with alkaloids acting as toxins through interference with the nervous system, causing the larvae to fail to recognize food and starve to death [1]. In addition, alkaloids also act as tubulin inhibitors, inhibiting the formation of microtubules which are important in the cell cycle, resulting in cell death [27]. *Flavonoids*, on the other hand, work by stopping the growth of larvae by inhibiting signals that enter the cell nucleus and attacking protein kinases, which play a role in the proliferation of cancer cells [13]. *Flavonoids* can also mediate DNA damage, increase proapoptotic proteins, and induce programmed cell death, as well as inhibit the activity of important enzymes such as proteases and lipases [28]. *Saponins*, which have soap-like properties, can lower oxygen levels in larvae by binding to dissolved oxygen, causing the death of larvae due to osmosis which changes the structure of the cell membrane [13], [28].One of the main compounds found in green betel leaf extract is

eugenol, which is known to have antiseptic and anti-inflammatory properties, and can inhibit the growth of various types of pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli* [26], [29].

Besides *eugenol*, other compounds such as carvacrol and chavibetol also contribute to the antibacterial activity of green betel leaves [30], [31]. Green betel leaf ethanol extract can inhibit neutrophil infiltration in a sepsis model, confirming the anti-inflammatory potential of these compounds. Green betel leaf extract has anti-virulence activity by reducing the expression of rhamnolipid genes in Pseudomonas aeruginosa, indicating that the compounds in green betel leaves are not only antibacterial but can also modulate bacterial virulence [26], [32]. In the context of traditional medicine, green betel leaf has been used to treat various diseases, including respiratory tract infections, digestive disorders, and skin problems, the effectiveness of green betel leaf extract as an antibacterial agent in the treatment of skin infections [29], [33].The compounds in green betel leaves also show potential as antioxidant agents, protecting body cells from free radical damage [25], [34]. In addition, green betel leaf ethanol extract can lower blood glucose levels in male mice, indicating therapeutic potential in the management of diabetes [35]. The *flavonoid* and *polyphenol* compounds in green betel play an important role in antioxidant and anti-inflammatory activity, which can aid in the treatment of various inflammatory conditions [25], [36]. In more in-depth research, Mustarichie and Saptarini highlighted that the bioactive compounds in green betel leaves can function as new candidates for the treatment of herpes viruses, demonstrating the antiviral potential of green betel leaf extract [37]. This suggests that further research into these compounds could pave the way for the development of new and more effective drugs.

Cytotoxicity Effect of Green Betel Leaf Extract on Shrimp Larvae

This study shows that green betel leaf ethanol extract (*Piper betle L.*) has a significant cytotoxicity effect on shrimp larvae (*Artemia salina L.*) using the *Brine Shrimp Lethality Test* (BSLT) method, which is in line with previous research, namely by testing cytotoxicity in Chinese betel leaf (*Peperomia pellucida*) [13]. The content of secondary metabolite compounds such as alkaloids, flavonoids, and saponins can interfere with the physiological functions of larvae and contribute to larval mortality [13]. Green betel leaves contain similar compounds, although the results of this study show differences in the content of the compounds, which may be due to variations in the concentration or reagents used. Alkaloids are known to cause larval death through disturbances to the nervous system, resulting in the larvae being unable to recognize food and starving to death [1]. In addition, alkaloids function as tubulin inhibitors, inhibiting the formation of microtubules which are important in the cell cycle, resulting in cell death [27]. *Flavonoids* play a role in stopping larval growth by disrupting signals entering the cell nucleus and attacking protein kinases, which function in cancer cell proliferation [13]. *Saponins*, which have soap-like properties, can lower oxygen levels in larvae, causing larval death due to osmosis which changes the structure of the cell membrane [28].Another major compound in green betel leaf extract is *eugenol*, which has antiseptic and anti-inflammatory properties and can inhibit the growth of pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli* [26], [29]. Other compounds such as *carvacrol* and *chavibetol* also contribute to the antibacterial activity of green betel leaves.

In addition, green betel leaf extract can inhibit neutrophil infiltration in a sepsis model, confirming the anti-inflammatory potential of these compounds. Green betel leaf extract has anti-virulence activity by reducing the expression of the *rhamnolipid gene* in *Pseudomonas aeruginosa*, which shows that the compounds in green betel leaf are not only antibacterial but can also modulate bacterial virulence. In the context of traditional medicine, green betel leaves have been used to treat various diseases, including respiratory tract infections, digestive disorders, and skin problems, the effectiveness of green betel leaf extract as an antibacterial agent in the treatment of skin infections.The compounds in green betel leaves also show potential as antioxidant agents, protecting body cells from free radical damage [25], [34]. Green betel leaf ethanol extract can lower blood glucose levels in male mice, indicating therapeutic potential in the management of diabetes. *Flavonoid* and *polyphenol* compounds in green betel leaf play an important role in antioxidant and anti-inflammatory activity, which can aid in the treatment of various inflammatory conditions [25]. Bioactive compounds in green betel leaves can serve as new candidates for the treatment of herpes viruses, demonstrating the antiviral potential of green betel leaf extract [37].

Percentage of Shrimp Larvae Death After Treatment

This study shows that the ethanol extract of green betel leaf (*Piper betle L.*) has a significant cytotoxic effect on shrimp larvae (*Artemia salina L.*), with the percentage of larval death being directly proportional to the increase in the concentration of the extract given, as shown in table 8. This is in line with the theory that the higher the extract concentration, the more larvae that die, which is also supported by previous research showing an increase in the percentage of larval mortality as the concentration of rattan extract increases [38]. The *Brine Shrimp Lethality Test* (BSLT) method used in this study has been widely documented in scientific literature as an effective tool for evaluating the cytotoxicity of plant extracts, known for its simplicity and ability to provide fast and reliable results [39], [40]. The BSLT results show that green betel leaf ethanol extract can cause the death of shrimp larvae in significant proportions, which indicates the presence of bioactive components in the extract. Previous research has also shown that various plant extracts, including those from *Citrus maxima* and *Phyllanthus niruri*, exhibit cytotoxic activity that can be measured using BSLT, with LC50 values varying depending on the concentration and type of extract used [11], [41].

BSLT is an effective tool for the initial screening of bioactive compound toxicity, providing early indications of the potential toxicity of these compounds to non-target organisms [39], [42]. In this context, the death of shrimp larvae after treatment with green betel leaf ethanol extract can be used as an initial indicator to evaluate the safety of using the extract in medical or therapeutic applications. Various Mikania species have a significant cytotoxic effect on shrimp larvae, with results showing that larval death can be influenced by the concentration of extract used Comparative Cytotoxicity of Selected Mikania species using Brine Shrimp Lethality Bioassay and Sulforhodamine B (SRB) Assay [41]. These findings support the hypothesis that green betel leaf extract can cause significant shrimp larvae mortality, indicating potential toxicity that needs to be considered in the context of its use.BSLT is a useful method for evaluating the toxicity of plant extracts, and the results can provide insight into potential side effects of using these extracts [42]. These findings are relevant to this study, where the percentage of shrimp larvae mortality after treatment with green betel leaf ethanol extract indicates that the extract has potential toxicity that needs further evaluation. The LLST can be used to assess the cytotoxic effect of plant extracts on shrimp larvae, providing important information about the potential use of these extracts in drug development [43].

LC50 value of Green Betel Leaf Extract (*Piper betle L.)* using the BSLT Method

The LC50 value is used to assess the level of toxic effect of a compound and predict its potential as an anticancer, with the results of this study showing an LC50 value of 121.3317 ppm which is categorized as toxic. The smaller the LC50 value, the higher the toxicity level, according to theory [44]. Cytotoxicity testing of green betel leaf ethanol extract (*Piper betle L.*) using the *Brine Shrimp Lethality Test* (BSLT) method is a common approach to evaluating the toxicity potential of various plant extracts. This method involves the use of Artemia salina shrimp larvae as test organisms, which allows researchers to determine the LC50 value of the extracts tested. Previous research has shown that extracts from various plants have significant cytotoxic activity, which can be measured using the BSLT method [11], [41]. The results emphasize that BSLT is an effective tool for the initial screening of bioactive compound toxicity, providing early indications of the potential toxicity of these compounds to non-target organisms [39]. In this context, the death of shrimp larvae after treatment with green betel leaf ethanol extract can be used as an initial indicator to evaluate the safety of using the extract in medical or therapeutic applications.

Extracts from Chrozophora senegalensis have high cytotoxic activity, confirming that the BSLT method can be used to assess the potential toxicity of plant extracts in an efficient and effective manner [45]. In addition, various extracts from *Cinnamomum iners* also have a significant cytotoxic effect on shrimp larvae, in line with the finding that the LC50 value can vary depending on the type of extract and concentration used [46]. The cytotoxic potential of plant extracts is often correlated with the concentration of active compounds contained in them [47]. BSLT is a reliable method for assessing the toxicity of bio-based pesticides, showing that extracts containing active compounds such as eugenol can have a significant cytotoxic effect [48].Extracts from various plants, including Ficus racemosa, have cytotoxic activity that can be measured using the BSLT method [49].

This finding supports the use of the BSLT method to evaluate green betel leaf extract, given the similarity in the composition of active compounds that may contribute to cytotoxic effects. BSLT has a strong correlation with cytotoxicity in human cancer cells, which indicates that extracts that show cytotoxic activity in BSLT may also have potential as anticancer agents [50]. Toxicity testing using BSLT can provide valuable insights into the potential toxicity of plant extracts [51].In Figure 2, after calculating the percentage of larval mortality, it is then converted to a probit value, and then a linear regression value is obtained in this study, namely Y = 1.3909x + 2.1014. This calculation gives a Y value of 5, which represents the LC50 value in order to obtain several concentrations from the LC50. And the LC50 calculation result obtained is 121.3317 ppm and is categorized as toxic and potentially anticancer. It was also stated in the study that LC50 results obtained <1000 ppm are said to be toxic and potentially anticancer, which can stop the growth of cancer cells [13], [52]. The toxic value obtained in this study provides an overview of the effectiveness of green betel leaves in inhibiting the growth of cancer cells. This study is important as a reference in the development of anticancer drugs by utilizing natural ingredients such as green betel leaves so that it can be a solution to minimize the side effects caused by chemical drugs.

IV. CONCLUSION

Based on the research conducted, it can be concluded that green betel leaves contain secondary metabolite compounds in the form of alkaloids, saponins, and flavonoids, and have cytotoxicity effects on *Artemia salina L*each shrimp larvae, as evidenced by the death of larvae at each concentration tested. The percentage of larval mortality at five concentrations ranged from 30-80%, indicating that the higher the concentration given, the more shrimp larvae died. The LC50 value obtained was 121.3317 ppm, indicating that green betel leaf has a cytotoxic effect, making it a potential anticancer and antioxidant agent. For further research, it is recommended that a pharmacology and pharmacokinetics test be carried out so that it can proceed to the clinical trial stage.

V. ACKNOWLEDGMENTS

We would like to express our sincere gratitude to all individuals and institutions that contributed to the successful completion of this research on the cytotoxicity test of green betel leaf ethanol extract (*Piper betle L.*) using shrimp larvae (*Artemia salina L.*) through the Brine Shrimp Lethality Test (BSLT) method. First and foremost, we extend our heartfelt thanks to our academic advisors and mentors for their invaluable guidance, support, and encouragement throughout the research process. Their expertise and insights were instrumental in shaping our study and enhancing our understanding of the subject matter. We also wish to acknowledge the contributions of our colleagues and fellow researchers who provided assistance and collaboration during the course of this project. Their constructive feedback and support were crucial in refining our methodology and analysis. Additionally, we are grateful to the laboratory staff at the Faculty of Medicine, Universitas Muslim Indonesia, for providing the necessary resources and facilities that enabled us to conduct our experiments effectively.

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