Journal of Experimental and Clinical Pharmacy (JECP)
Doi: 10.52365/jecp.v3i2.674
http://jurnal.poltekkesgorontalo.ac.id/index.php/JECP/
2023, 3(2), 116-131

Research Article

Toxicity Activity and Total Phenolic Content of Soursop Leaves from Three Regions in South Sulawesi, Indonesia

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ARTICLE INFO

Submitted : 01 May. 2023
Revised : 16 May. 2023
Accepted : 02 Aug. 2023

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ABSTRACT

Abstract: The fruit plant known as the soursop (*Annona muricata* L.) is indigenous to the Caribbean, Central America, and South America. The leaves of soursop contain substances with the potential to be cytotoxic, such as phenols and anonaceous acetogenins. Numerous variables, including geographic location, climatic conditions, and pest or disease disturbance factors, might impact a compound's phenol levels and toxicity. This study aims to investigate whether there are variations in the phenolic content and toxicity of the ethanol extract of soursop leaves that are grown in Gowa, Takalar, and Pinrang, three distinct regions in South Sulawesi, Indonesia. The Folin-Ciocalteu reagent was used to evaluate the total phenolic content, whereas Artemia salina Leach larvae were used for the BSLT method of the toxicity test. The results showed that the extracts of soursop leaves from Gowa, Pinrang, and Takalar had total phenolic contents of 3.7588% mg GAE/g, 0.9686% mg GAE/g, and 1.3832% mg GAE/g, respectively. Gowa, Pinrang, and Takalar have LC50 values of 38.19 µg/mL, 34.35 µg/mL, and 70.95 µg/mL, respectively, indicating their toxicity. The conclusion is that there were differences in the phenolic content and toxicity level of the ethanol extract of soursop leaves. All of these areas could source raw materials of soursop for the development of herbal medicines with cytotoxicity, i.e., anticancer, because they have LC50< 1,000 µg/mL.

Keywords: Soursop leaves (*Annona muricata* L.); phenolic; toxicity

eISSN 2775-1368

INTRODUCTION

Indonesia is rich in sources of natural medicinal materials that have been used as traditional medicinal herbs for generations. Medicinal plants are expected to be utilized in the development of public health (Badaring et al. 2020). One of the plants that has potential as traditional medicine is soursop (*Annona muricata* L.). Soursop leaves come from Central America and the Caribbean region. Soursop requires warm and humid tropical temperatures to grow well. Soursop can also grow in tropical lowlands up to 1,000 m altitude. The phytochemical content of soursop is known to have selective anticancer properties (Zuhud 2011). Soursop contains natural phenolic compounds that have potential as antioxidants, have bioactivity as drugs, and contain annonaceous acetogenins, which have cytotoxic potential (Puspitasari et al. 2016). Cytotoxic compounds are compounds that can be toxic to inhibit and stop the growth of cancer cells (Handayani, Kurniawati, and Abdul Rasyid 2020).

One of the preliminary tests of cytotoxicity extract plants is the Brine Shrimp Lethality Test (BSLT) method. The BSLT toxicity tests were carried out using Artemia salina Leach larvae as test animals (Zebua 2011). BSLT is a method that is often used as an initial screening of active compounds from several compounds contained in plants because it is relatively cheap and fast (Fidyasari, Istiana, and Eka 2017).

Soursop leaves contain several compounds such as flavonoids, alkaloids, saponins, tannins, quinones, and phenols (Rahman, Haniastuti, and Utami 2017). Many studies revealed that phenol from plants has activities in vitro and in vivo as a potential anticancer. Phenol levels can be measured using UV-Vis spectrophotometry and Folin-Ciocalteu reagents. (Fendy 2013).

Research conducted by Wulandari showed that the test of total phenolic content (mg GAE/g extract) from the three regions was different; the total phenolic content of 5.72% b/b GAE in samples from Jember was the highest when compared to samples from Bangkalan (2.95% b/b GAE) and Batu (1.78% b/b GAE) (Wulandari, Nugraha, and Siswanti 2019). This difference is influenced by several factors, including the geographical location of the plants, climatic factors including temperature, air, and humidity, and essential factors such as light, water, and soil nutrients. As well as pest, disease, and weed interference factors. Other research on soursop from three different areas has yielded very strong antioxidants; all areas have IC50s less than 50 µg/mL, and the higher value was from the Makassar, South Sulawesi area (Aminah et al. 2016).

Based on statistical data from the Central Bureau of Statistics of South Sulawesi Province, soursop production in South Sulawesi Province by Regency/City in 2020, it is known that there are three largest soursop producing areas in South Sulawesi Province, which are Gowa, Takalar, and Pinrang districts. Soursop can grow on all types of soil with a degree of acidity (pH) between 5-7 and in places with altitudes between 100 and 1,000 m. In mountainous areas, soursop plants grow well. The climate required by soursop plants includes rainfall, air temperature, and wind. The appropriate air temperature for soursop plants is around 22–32°C. Rainfall required by soursop plants is between 1,500 and 3,000 mm per year. Rainfall should be evenly distributed throughout the year (Sunarjono 2005). Takalar area has a land elevation of 0-1,000 masl, rainfall ranges per year of 2,300–3,000 mm, a temperature of 21°C–33°C, and an RH of 81% (BPS Takalar 2022). The Gowa area has a land elevation of 10–2800 masl, and rainfall per year ranges from 2,000–3,000 mm. Temperature ranges from 22–33°C, RH 81% (BPS Gowa 2021). Pinrang area has a land elevation of 0-1,000 masl, and annual rainfall ranges from 1,073 mm to 2,910 mm. The normal average temperature is between 27°C and 28°C with 82%–85% humidity (BPS Pinrang 2019). These three regions could be used as a source of raw materials for soursop in developing herbal medicine because it has the potential to be an anticancer.

Based on the description above, research was conducted on aoursop leaves from Takalar, Gowa, and Pinrang to determine phenolic content and toxicity levels using the BSLT method based on the place of growth.

MATERIALS AND METHODS

Material

Glassware (pyrex), incandescent lamp (Philips), a set of egg-dropping tools, micropipette (Memmert), a volume pipette (pyrex), KLT plate silica gel 60 F254, a set of maceration tools, a set of rotavapor tools, thermometer (ASTM), analytical scales (Caratseries), water bath (Memmert) and vial.

Seawater, gallic acid (Sigma), distilled water, soursop leaf extract, ethanol 96%, ethanol p.a, ethyl acetate, $FeCl_3$ 1%, Folin-Ciocalteu, concentrated HCl, HCl 2 N, Whatman paper, Artemia salina Leach larvae, NaOH, Na_2CO_3 7%, Bouchardat reagent, Dragendorf reagent, Mayer reagent, magnesium powder and yeast suspension.

Method

Collection of the Plants

Soursop old leaf sampling was taken in three areas, first in Tamarunang Village, Somba Opu Subdistrict, and Gowa Regency. The second is in the region of Pa'batangan Village, Mappakasunggu Subdistrict, Takalar Regency, and the last is in Laleng Bata Village, Paleteang Subdistrict, Pinrang Regency in South Sulawesi Province.

Preparation of Extract

Soursop leaf powder that has been crushed is weighed and then put into a maceration container and added with 96% ethanol solvent until the powder is soaked. The experiment was carried out at room temperature for 3x24 hours, with results obtained as much as Gowa 34.097 g, Pinrang 18.414 g, and Takalar 17.884 g.

Phytochemical Screening test

Alkaloid Test

Each extract was dissolved in an ethanol solvent, and the results obtained were filtered to obtain the filtrate. The filtrate is divided into 3 parts of 5 ml each and then added with Mayer reagent to form a white or yellow precipitate that dissolves in methanol, Dragendorf reagent to form an orange-brown precipitate, and Bourchardat reagent to form a brown to black precipitate. Positive alkaloids are formed when two or three parts form sediment (Arifuddin and Bone 2020).

Flavonoid Test

A total of 5 mL of extract was dissolved in ethanol, then Mg powder and 5 drops of concentrated HCl were added. If the result is red, yellow, or orange, it is positive for flavonoids (Arifuddin and Bone 2020).

Tannin Test

A total of 5 mL of extract dissolved in ethanol is added to the $FeCl_3$ reagent. Extracts containing tannin will be blue or blackish green. (Arifuddin and Bone 2020).

Saponin Test

The ethanol extract of each sample was added to 10 ml of hot distilled water and dissolved first while heated in a water bath and then shaken vigorously. If no foam is formed, it is negative, but if it remains bubbly

after standing for 10 minutes and then adding 2 N HCl, the foam does not disappear, and it is positive for saponins (Arifuddin and Bone 2020).

Quinone Test

The solution of each extract in ethanol solvent is added with a few drops of 1 N NaOH solution. If a red color is formed, it indicates the presence of quinones (Arifuddin and Bone 2020).

Phenolic Test

A total of 1 mg of extract was added to 2 drops of $FeCl_3$ 1%. The extract is positive for phenol when it produces a solid green, red, purple, blue, or black color (Kurang and Adang 2018).

Qualitative Test for Phenolic Compound

The phenol identification test with TLC refers to Listiawati et al. (2022), by making a mobile phase consisting of methanol:ethyl acetate (3:4), put into the chamber, and close until saturated. Prepare silica gel 60 F254 (20 cm x 20 cm), then cut into 1 cm x 7 cm and marked 0.5 cm at the top and 1 cm at the bottom of the silica. Dot the extract that has been dissolved with 96% ethanol above the bottom mark of silica gel 60 F254 then insert it into the chamber. Elution until the top mark of silica gel 60 F254. Then take and dry and do things for the gallic acid comparator. Phenolic detection is seen from the positive reaction indicated by the formation of a blue-black stain after being sprayed with a 1% $FeCl_3$ specific reagent.

Total Phenolic content

The total phenolic content was determined using the Folin-Ciocalteau colorimetric method with gallic acid as the standard. Slight modifications to the technique described by Fawwas et al. (Fawwaz, Nurdiansyah A, and Baits 2017). Using a UV-Vis spectrophotometer (728 nm), sample and standard measurements were taken against the reagent blank. 10, 20, 40, 50, and 60 µg/mL of gallic acid were generated from a stock solution of 1,000 µg/mL. A sample was made by diluting 5 mg with 10 mL of ethanol p.a. In brief, 1 mL of each of the standard and samples were mixed with 0.4 mL of 10% Folin-Ciocalteu reagent and allowed to react for 4–8 minutes. The mixture was then combined with 4 mL of 7.5% Na_2CO_3 solution and shaken until homogenous. Aquadestilata was added to 10 mL and incubated in the dark at room temperature for 2 hours (Fawwaz, Nurdiansyah A, and Baits 2017). Using UV-Visible spectrophotometry, absorbance was subsequently measured at 728 nm using distilled water as a blank. Gallic acid was utilized to develop a standard curve. The total phenol content was

expressed as mg/g equivalent gallic acid (GAE). All analyses were performed in triplicate.

Toxicity Test with Brine Shrimp Lethality Test (BSLT)

The soursop extracts from three areas Gowa, Takalar, Pinrang were examined for their toxicity to brine shrimp larvae. In 10 mL seawater, solutions containing 1% DMSO (v/v), the toxicity of compounds was evaluated at concentrations of 10 µg/mL, 50 µg/mL, 250 µg/mL, 500 µg/mL, and 1,000 µg/mL. In this experiment, ten larvae were used per test, and the number of survivors after twenty-four hours was determined. At each concentration, three replicates were established. As a control, distilled water is used. Using probit analysis, the lethal concentration (LC50) was determined. Calculating the LC50 value employs a regression line derived by plotting the concentration of the substance towards the percentage of death on a probit scale (Handayani, Kurniawati, and Abdul Rasyid 2020).

RESULTS

The liquid extracts from each region are then evaporated and yielded from Gowa were 5.2456%, Pinrang 5.8459%, and Takalar 6.3871% as shown in table 1.

Table 1. Data on Percent Yield (%) Extract Ethanol of Soursop Leaf

Sample	Regions	Simplisia Weight (g)	Extract Weight (g)	Yield Percent (%)
Ethanol Extract of Soursop Leaves	Takalar	280	17.884	6.3871
	Pinrang	315	18.414	5.8459
	Gowa	650	34.097	5.2456

The function of $FeCl_3$ is to hydrolyze tannin so that it will produce a blue-black color change (Sangi et al. 2008) as shown in table 2.

Table 2. Phytochemical Screening Result of Ethanol Extracts of Soursop Leaves from Three Regions.

Chemical compound	Ethanol Extracts Of Soursop Leaves		
	Gowa	Takalar	Pinrang
Alkaloid :			
Mayer	+	+	-
Dragendorf	+	+	+
Bouchardat	+	+	-
Phenolic/Tannin	+	+	+
Saponin	+	-	+
Quinone	+	+	+
Flavonoid	+	+	+

Table 3. Qualitative Test Results Using TLC

Sample	Eluent	Specific Reagent	Rf value	Color
Gallic Acid	Methanol : Ethyl Acetate (3:4)	$FeCl_3$	0.800	Black
Gowa			0.781	Blackish blue
Pinrang			0.872	Blackish blue
Takalar			0.745	Blackish blue

The results obtained in testing the determination of the total phenolic content of soursop leaf ethanol extract are a sample from Gowa with a value of 3.7588% mg GAE/g, which has the highest value of total phenolic content compared to the values of Pinrang (0.9686% mg GAE/g) and Takalar (1.3832% mg GAE/g.

Table 4. Results of Total Phenolic Content of Three Regions

Region	Y	A	B	Average	Content (%)
Gowa	1.119	0.024	0.0144	37.588	3.7588
	1.090	0.024	0.0144		
	1.057	0.024	0.0144		
Pinrang	0.306	0.024	0.0144	9.686	0.9686
	0.311	0.024	0.0144		
	0.292	0.024	0.0144		
Takalar	0.451	0.024	0.0144	13.832	1.3832
	0.443	0.024	0.0144		
	0.402	0.024	0.0144		

Table 5. Observation Data of Mortality of Artemia salina Leach Larvae for 24 Hours from Ethanol Extract of Soursop Leaves from Takalar by BSLT Method

Sample (Takalar)	Replication	Number of Dead Shrimp Larvae Per Concentration Series of Test Sample Solution				
		10 µg/mL	50 µg/mL	250 µg/mL	500 µg/mL	1,000 µg/mL
Ethanol Extract of Soursop Leaves	1	1	4	7	8	9
	2	2	6	8	9	9
	3	2	3	8	8	8
Mortality Total		5	13	21	24	26
Mortality Rate (%)		16.67	43.33	76.77	83.33	86.67
Probit Value		4.05	4.82	5.74	5.95	6.13

Table 6. Observation Data of Mortality of Artemia salina Leach Larvae for 24 Hours from Ethanol Extract of Soursop Leaves from Pinrang by BSLT Method

Sample (Pinrang)	Replication	Number of Dead Shrimp Larvae Per Concentration Series of Test Sample Solution				
		10 µg/mL	50 µg/mL	250 µg/mL	500 µg/mL	1,000 µg/mL
Ethanol Extract of Soursop Leaves	1	4	5	7	8	9
	2	3	6	8	9	9
	3	3	5	9	9	10
Mortality Total		10	16	24	26	28
Mortality Rate (%)		33.33	53.33	80	86,67	93.33
Probit Value		4.56	5.08	5.84	6.13	6.48

Table 7. Observation Data of Mortality of Artemia salina Leach Larvae for 24 Hours from Ethanol Extract of Soursop Leaves from Gowa by BSLT Method

Sample (Gowa)	Replication	Number of Dead Shrimp Larvae Per Concentration Series of Test Sample Solution				
		10 µg/mL	50 µg/mL	250 µg/mL	500 µg/mL	1,000 µg/mL
Ethanol Extract of Soursop Leaves	1	3	5	8	10	10
	2	3	6	8	9	9
	3	3	4	7	8	10
Mortality Total		9	15	23	27	29
Mortality Rate (%)		30	50	76.67	90	96.67
Probit Value		4.48	5.00	5.74	5.28	6.88

DISCUSSION

The first stage in this research is the collection of soursop leaves, where the soursop leaf sample is taken in three regions and identified by the unit of determination of plants in the laboratory of Phytochemistry and Pharmacognosy, Faculty of Pharmacy, Universitas Muslim Indonesia. Soursop leaves were then macerated using a 96% ethanol solvent. The maceration method was chosen because the process is easy, fast, and relatively inexpensive. It could also extract the content of compounds contained in samples that are not resistant to heating without damaging these compounds. The solvent was ethanol because it has lower toxic properties compared to other solvents, and ethanol can also dissolve non-polar or polar compounds so that all components of non-polar or polar compounds contained in sSoursop leaves can be dissolved in the extraction process.

The next step after extract preparation is phytochemical screening. The screening carried out in this research includes analyses of alkaloids, phenols, flavonoids, quinones, saponins, and tannins. In the alkaloid test,

positive results are indicated by the formation of a precipitate. With the Mayer reagent, a white-to-yellow solution is obtained, and in the Dragendorff test, it forms a covalent bond with K^+, which is a metal ion, so that an orange precipitate is formed. Positive results in the Mayer reagent are indicated by the presence of a white-to-yellow precipitate. The Dragendorff reagent is indicated by the presence of an orange precipitate, and the Bouchardat reagent is indicated by the presence of a black-brown precipitate. Phenol is a polar compound in the form of a colorless crystalline substance that has a distinctive odor. The chemical formula is C_6H_5OH, and the structure has a hydroxyl group (-OH), which binds to the phenyl ring (Marliana, Suryanti, and Suyono 2005). According to Harborne (1987), qualitative evidence showing the presence of phenol can be obtained using a 1% $FeCl_3$ reagent. Phenol will form a concentrated green, red, purple, blue, or black color due to the reaction with iron (III) chloride (Dirjen POM 1979).

Flavonoid testing is done by taking a little extract, adding magnesium powder and concentrated HCl, and then homogenizing. The addition of concentrated HCl in the flavonoid test is used to hydrolyze flavonoids into their aglycones. According to Robinson (1995), flavonoids are compounds that contain two aromatic rings with more than one hydroxyl group. The reduction of magnesium and concentrated HCl produces a red, yellow, or orange color in flavonoids. The quinone test is carried out using a 1 N sodium hydroxide (NaOH) solution reagent. The 1 N NaOH reagent serves to deprotonate the phenol group on quinones so that phenolic ions are formed. This phenolic ion can absorb certain light and cause a red color. Saponins contain glycosyl groups that act as polar groups, while steroid and triterpenoid groups function as non-polar groups that are active on the surface, so that when shaken with water, saponins will form micelles, where the polar structure will face out while the nonpolar group will face in, so that under these conditions it will form and look like foam. In the tannin test, the addition of $FeCl_3$ will react with one of the hydroxyl groups present in tannin.

The screening results of soursop leaf ethanol extracts from the three regions show that soursop leaf ethanol extracts from Gowa contain alkaloids, flavonoids, tannins, saponins, and quinones. The ethanol extract of soursop leaves from Pinrang contains flavonoids, tannins, saponins, and quinones, and the ethanol extract of soursop leaves from Takalar contains alkaloids, flavonoids, tannins, and quinones. The different chemical content is caused by several factors, based on several previous studies showing that altitude is one of the factors that affect the growth of a plant. The phytochemical content of secondary metabolites, such as flavonoids, from a plant will be different in each region because it is influenced by several

environmental factors, including light, temperature, pH, and altitude of the growing place, which will affect the phytochemical content of the plant. Besides this, the processing of raw materials can also affect the extracted chemical content.

The effect of altitude on plants is closely related to environmental factors, such as temperature. Higher air temperatures in the lowlands cause the capacity of water vapor to increase, so that the relative humidity of the air decreases, especially during the day. In addition to the sunlight intensity factor, the low value of sunlight intensity can be caused by the presence of shading such as clouds, trees, or other forms of shade. In addition, the harvest age of the sample can also affect the content of compounds in the sample (Lallo et al. 2022).

The Thin Layer Chromatography (TLC) test shows the color changes formed for gallic acid: strong black, Gowa, Pinrang, and Takalar blue-black, which indicates positive phenol content. Then the calculation of the Rf value was obtained by gallic acid 0.8, Gowa 0.781, Pinrang 0.872, and Takalar 0.745. The results of the TLC test of soursop leaf extract from the three regions are shown in table 3. The phenol test results showed a positive result marked by a blue-black color change caused by phenol reducing Fe^{3+} to Fe^{2+}, marked by a blue-black color (iron (III) hesasianoferate) (Hanani 2017).

Gallic acid is used as a standard solution because it is one of the most natural and stable phenols and is relatively cheap compared to others. Gallic acid is reacted with the Folin-Ciocalteu reagent to produce a yellow color, indicating that it contains phenol, after which it is added to the Na_2CO_3 solution to produce a blue color (Ahmad et al. 2015). Phenolic compounds react with the Folin-Ciocalteu reagent only in an alkaline atmosphere in order to dissociate protons in phenolic compounds into phenolic ions, so Na_2CO_3 solution is added (Alfian and Susanti 2012).

The measurement of total phenolic compounds in each region was made in as many as three replications for data accuracy purposes. So, from the results of this study, total phenolic content in soursop leaf ethanol extracts from Gowa was 3.7588% w/b, Pinrang was 0.9686% w/b, and Takalar was 1.3832% w/b calculated against gallic acid phenol compounds (GAE). The results of total phenolic content can be seen in table 4, where the total phenolic content of each extract is expressed in GAE (Gallic Acid Equivalent). GAE is the equivalent number of milligrams of gallic acid in 1 gram of sample (Lee et al. 2003). The results obtained in testing the determination of the total phenolic content of soursop leaf ethanol extract are a sample from Gowa with a value of 3.7588% mg GAE/g, which has the highest value of total phenolic content compared to the values of Pinrang (0.9686% mg GAE/g) and Takalar (1.3832% mg GAE/g). According to

previous research conducted by Wulandari, Nugraha, and Siswanti (2019), soursop leaf powder from Jember, Bangkalan, and Batu has been tested to determine the total phenolic content using UV-Vis spectrophotometry after reacting with Folin-Ciocalteu preaction with gallic acid comparison. The results showed that the test of total phenolic content (mg GAE/g extract) from the three regions was different, the total phenolic content of 5.72% b/b GAE in samples from Jember was the highest when compared to samples from Bangkalan (2.95% b/b GAE) and Batu (1.78% b/b GAE). This indicates that the sample from Jember has the highest potential source of phenolic compounds.

The Brine Shrimp Lethality (BSL) test is one of the screening methods to determine the toxicity of a material or is the initial stage to determine whether a compound has potential as an anticancer, which can be carried out with cytotoxic tests. The cytotoxic test can be known from the number of deaths of Artemis salina Leach shrimp larvae due to the effect of extracts or compounds of natural ingredients at the concentration given (Purwanto, Rismawati, and Sadiyah 2015). This test was carried out for 24 hours in each group, i.e., the test group (ethanol extract) aims to determine and compare how the concentration of the extract given affects the number of deaths of Artemia salina Leach shrimp larvae (Setyowati and Cahyanto 2016), and the negative control (seawater) aims to ensure that the death of Artemia salina Leach larvae is caused by exposure to bioactive components of the extract, and each test group and negative control were given 1 drop of yeast as a source of nutrition. This study was conducted in replication, aiming to make the data obtained more accurate and reliable. In this study, soursop leaf ethanol extract was used with testing concentrations of 10 µg/mL, 50 µg/mL, 250 µg/mL, 500 µg/mL, and 1,000 µg/mL and aims to see the variety of responses given LC50 values <1,000 µg/mL that are declared toxic and have potential toxicity. Testing was also carried out for the control of each solvent to see if the response to the death of test animals was caused by chemical components of the sample or solvent used (Chasani, Fitriaji, and Purwati 2013).

As seen in tables 5, 6, and 7, the total mortality was obtained by adding up the dead Artemia salina leach larvae at each concentration (Oratmangun, Fatimawali, and Bodhi 2014). The mechanism of action of larval mortality is thought to be that the compounds contained in soursop leaf extract can inhibit larval feeding power (antifeedant) by acting as stomach poisoning. Therefore, if these compounds enter the bodies of shrimp larvae, their digestive systems will be disrupted. The compounds in the extract can enter through the mouth of Artemia salina and are absorbed into the digestive tract through the cell membrane, then proceed with the distribution process of toxic compounds into the body of Artemia salina,

and there is a process of damage to metabolic reactions. The anatomical structure of the Artemia salina body at the nauplii stage is still very simple, consisting of the skin layer, mouth, anthena, digestive tract, and prospective thoracopods. Changes in the concentration gradient between the inside and outside of the cell cause toxic compounds to spread rapidly to the body of Artemia salina. The effect of metabolic damage that is caused occurs rapidly and can be detected within 24 hours, causing 50% death in Artemia salina. After observation for 24 hours, the data obtained were then analyzed using probit analysis to determine the LC50 value (Kurniawan and Ropiqa 2021). Probe analysis is a type of regression used to analyze variations in binominal responses. Probit analysis is commonly used in toxicology to determine the relative toxicity of chemicals to living organisms (Vincent 2008).

The LC50 result obtained from the Soursop leaf extract from Takalar is 70.95 µg/mL, the soursop leaf extract from Pinrang is 34.35 µg/mL, and the Soursop leaf extract from Gowa is 38.19 µg/mL, from the three regions has an LC50 value <1,000 µg/mL, which indicates that the extract has potential cytotoxic activity and potential as an anticancer. Previous research conducted by Sumiati et al. (2016), showed that the ethanol extract of soursop leaves from Bogor showed toxic potential because it had an LC50 value of 86.45 µg/mL. Furthermore, research by (Foudubun and Nugroho 2019) shows that the ethanol extract of mountain soursop leaves from Malang shows toxic potential because it has an LC50 value of 366.24 µg/ml (Meyer et al. 1982).

Based on the LC50 value and total phenolic content obtained from Takalar, Pinrang, and Gowa districts, it can be concluded that all of the districts are better to use as a source of medicinal plant raw materials because they have lower LC50 values. The LC50 value and total phenolic content obtained from the three regions are different, as are those that can affect them. The environment is a factor that is very instrumental in the biosynthesis of metabolites in plants. Plants can regulate the production of metabolites in accordance with the changing factors that exist in the environment, especially light, which is very instrumental in the biosynthesis of its compounds (Setyorini et al. 2016). In addition, things that can affect the levels of a compound in plants include the geographical location of the plant and climatic factors, including temperature, air, and humidity. In addition, there are essential factors such as water and soil nutrients, as well as pest, disease, and weed interference factors (Aminah et al. 2016).

CONCLUSIONS

Soursop leaf ethanol extract derived from three regions has different total phenolic content and LC50 values, where the total phenolic content in soursop leaf ethanol extract originating from Gowa is the highest with a value of 3.7588% mg GAE/g, Takalar 1.3832% mg GAE/g and Pinrang 0.9686% mg GAE/g, and soursop leaf ethanol extract originating from Pinrang had the highest LC50 value with a value of 34.35 µg/mL, Takalar had an LC50 value of 70.95 µg/mL, and Gowa had an LC50 value of 38.01 µg/mL.

ACKNOWLEDGEMENT

Author gratefully acknowledge the financial support from LP2S Universitas Muslim Indonesia, and Faculty of Pharmacy Universitas Muslim Indonesia for providing the laboratory facilities to carried out the research.

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