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Article

Toxicity of Extracts and Fractions of Bangkal Leaves (*Nauclea subdita* (Korth.) Steud.) and Genjer (*Limnocharis flava* (L.) Buch) on the Bioindicator *Artemia salina* Leach

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Abstract

This study investigated the secondary metabolite content and cytotoxic activity of Bangkal leaves (*Nauclea subdita* (Korth.) Steud.) and Genjer plants (*Limnocharis flava* (L.) Buch) using *Artemia salina* Leach larvae as bioindicators. The objectives were to identify the compounds present in the extracts and to evaluate their toxicity levels. Extraction was performed by maceration with 96% ethanol, followed by fractionation using n-hexane, ethyl acetate, and n-butanol solvents according to polarity. Toxicity testing employed the Brine Shrimp Lethality Test (BSLT), with mortality observed after 24 hours and LC_{50} values calculated using the Reed and Muench method. Phytochemical screening of Bangkal leaf extract revealed the presence of steroids, triterpenoids, phenols, quinones, and saponins. Toxicity assays showed LC_{50} values of 418.79 ppm (ethanol extract), 716.14 ppm (n-hexane), 263.03 ppm (ethyl acetate), and 70.79 ppm (n-butanol). For Genjer extracts, LC_{50} values were 490.23 ppm (ethanol extract), 551.31 ppm (n-hexane), 425.01 ppm (ethyl acetate), and 46.02 ppm (n-butanol). These findings indicate that the n-butanol fractions of both Bangkal leaves and Genjer plants exhibit the strongest cytotoxic activity compared to other fractions, suggesting potential bioactive properties worthy of further investigation.

Keywords: Bangkal; Genjer; Metabolites; IC_{50}; *Artemia salina*

1. INTRODUCTION

Traditionally, medicinal herbs are derived from various plant parts, including roots, bark, wood, leaves, flowers, and seeds. To validate the efficacy of traditional medicine, scientific investigations such as bioactivity studies, pharmacological assessments, and the identification and isolation of active phytochemicals are required [1].

Initially, the use of plants as medicine was based on hereditary experiences. So far, traditional medicine, especially herbal medicine, is considered quite potent to treat various diseases, especially by those who have proven its efficacy. However, the low knowledge of the content of medicinal compounds raises doubts in those who have not proven it [2]. The wealth of medicinal plants in tropical countries is very diverse, with Indonesia having a biodiversity of 40.000 types of flora. Approximately 15.000 plants possess medicinal properties, with around 7.500 species utilized as raw materials for medicine, and as many as 283 species used in the traditional medicine industry [3].

Using medicinal plants to overcome diseases is the right solution. Along with the development of herbal products, it is currently one of the options. Some of the plants that have been widely known and widely used by the community are the skeletal plant bangkal (*Nauclea subdita* (Korth.) Steud.) and genjer plants (*Limnocharis flava* (L.) Buch. Bangkal is one of the plants traditionally used by the local people. Part of the shank plant that is widely used is the leaves or bark of the stem, made in the form of cold powder, which is believed to whiten the skin, give a yellowish white impression, remove dark spots, prevent acne, and clean dead cells on the skin of the face [4]. Extracts of the leaves, bark, and seeds of these plants are traditionally used to treat abdominal pain, cough, fever, and diarrhea [5,6]. The leaves and bark of the stubborn stem have antioxidant, antimicrobial, genotoxic, and clastogenic effects [7,8,9], water extract as an antibacterial against *Propionibacterium acne* and *Staphylococcus aureus* [10], infusion of stubborn leaves is effective as an antidiabetes [11]. The content of secondary metabolites is polyphenol, alkaloids, flavonoids, and saponins, while the leaf extract of the bangkal plant contains compounds of the polyphenol group, tannins, alkaloids, flavonoids, and quinones [7,10].

Genjer plant (*L. flava*) is a wild plant that lives in swamps or muddy ponds with a lot of water. It is said to have originated in America, especially in parts of countries with tropical climates. In the Southeast Asian region, genjer plants can be found in Malaysia, Thailand, Burma, and Indonesia. In Indonesia, genjer plants are widely found on the islands of Sumatra and Java, found in lowlands [12]. Based on previous research, it was found that the leaves of genjer flower contain antioxidant compounds, flavonoids, saponins, quinones, phenols, hydroquinones, tannins, terpenoids, alkaloids, and steroids [13-16]. In addition, genjer plants are thought to contain primary metabolites of amino acids and reduced sugars [17,18].

Antioxidant activity test on leaf, stem, and genjer flower extracts using the DPPH method showed a leaf IC_{50} result of 122.0 ppm. The parts of genjer plants that have the best antioxidant activity are the leaves, with a medium category [19], have antioxidant activity, antibiotics, accelerate wound healing, anemia, anti-inflammatory, jengkolates poisoning, maintain healthy skin, and help lower cholesterol [15]. In addition, the leaves have antilipooxygenase and antioxidant activity [20].

The toxicity test is one of the tests carried out to assess the safety of a chemical compound, including compounds contained in cosmetics and food ingredients. Toxicity is defined as the study of nature and mechanism of the toxic effects of various substances on living things and other biological systems [25]. Cytotoxic tests are used to predictions the presence of antineoplastic activity. Brine Shrimp Lethality Test (BSLT) is one of the methods for screening cytotoxic compounds using *Artemia salina* Leach. Meyer [26] explained that compounds that have cytotoxic effects can also be detected by the Brine Shrimp Lethality Test (BSLT) method. Based on the functional approach of the compound group mentioned above, it is necessary to conduct cytotoxicity studies of bangkal and genjer leaves on the bioindicators of *A. salina in vitro* and identification of secondary metabolite groups contained in the extracts, including: ethanol extract, n-hexane, ethyl acetate and n-butanol fraction to obtain scientific data that can be used to support research on verification and development of effects specific pharmacological measures.

2. MATERIALS AND METHODS

2.1. Material

The material used in this study is bangkal leaves (*Nauclea subdita* (Korth.) Steud.) obtained from the East Kutai area, and genjer leaves (*Limnocharis flava* (L.) Buch obtained from Paser Regency, East Kalimantan, aquades water, aluminum foil, filter paper, Dragendorff reagent ($BiNO_3$ + Ki), Meyer reagent ($HgCl_2$ + KI), H_2SO_4 (P), CH_3COOH, CHC_{13}, $FeCl_3$, $K_3[Fe(CN)_6]$, Mg powder, HCl (P), NaOH, solvents: 96% ethanol, n-hexane, ethyl acetate, and n-butanol, DMSO (Dimethyl Sulfo Oxide), *Artemia salina* Leach shrimp eggs, yeast, seawater, and Tween 80.

2.2. Instrument

The equipment used is: Macerator, rotary evaporator (Buchi®), analytical tube (Dan gf 300®), split funnel, chemical cup (Pyrex®), measuring flask (Pyrex®), drip pipette, Erlenmeyer (Pyrex®), oven (Memmert), water bath (Wisebath®), test tube, tube clamp, vortex (Health H-VM-400®), mixing rod, vial bottle, measuring cup, volume pipette (Pyrex®), micropipette (Swiss® Sacores), spatula, aerator and incandescent lamp and glass vial.

2.3. Method

2.3.1. Collection of Bangkal leaves (*Nauclea subdita* (Korth.) Steud.)

The collected bangkal leaves are fresh and dark green in color, which are located on the 4th to 6th leaves of the top of the branches/twigs. It is then cleaned from the grid, then washed with water until clean (wet sorting), then weighed, then dried by drying - ventilating the room temperature 27°C, the drying process is continued by being in the oven at 40°C for 24 hours, until the moisture content at simplicia is not more than 10% b/v [27].

2.3.2. Collection of Genjer leaves (*Limnocharis flava* (L.) Buch

Genjer leaves (*Limnocharis flava* (L.) Buch) was chosen, which was dark green, taken by cutting the green part (leaves and stems), then washed with water until clean. After collecting, then cutting into small pieces, then drying in the open air at a room temperature of 27°C, the drying process is continued by being in the oven at 40°C for 24 hours, until the moisture content at simplicia is not more than 10% b/v [27].

2.3.3. Manufacture of Ethanol Extract from Bangkal and Genjer Leaves

Samples of dried bangkal leaves weighed as much as 381.614 grams, and dried genjer leaves as much as 250 grams, then put into a macerator container, given 96% ethanol refining liquid of 8.0 each, soaked for 5 days while stirring. The fiber is obtained, filtered, then evaporated using an evaporator at a temperature of 40ºC, then each ethanol extract is vaporized over a water bath until 35.367 g of dried ethanol extract of bangkal leaves is obtained, and 43.20 grams of ethanol extract of genjer leaves. According to the FDA, the residual solvent content in the extract is 1.046, and according to the Ministry of Health of the Republic of Indonesia is 1% [28]. The dried ethanol extract obtained is then weighed, stored in a tightly sealed container, and kept away from light [29].

2.3.4. Fractionation of Ethanol Extract of Bangkal and Genjer Leaves

The crude ethanol extract of bangkal and gejer leaves were each weighed 20.0 grams, then each was put into a separate funnel, added 50 mL of n-hexane solvent and 50 mL of aquadest (one fractionation cycle), whipped until homogeneous, then the n-hexane soluble extract and water-soluble extract were accommodated. This process is carried out repeatedly, and the separation process is completed, marked with a solution of colorless n-hexane extract. Furthermore, the water-soluble extract is added with 50 mL of ethyl acetate solvent, then beaten until homogeneous. The soluble ethyl acetate extract is accommodated, carried out repeatedly until the ethyl acetate solvent is saturated. The water-soluble extract is then added to 50 mL of n-butanol solvent and then filtered repeatedly until the n-butanol solvent is saturated and colorless. All extracts of Bangkal leaf fraction and genjer leaves were vaporized using an evaporator at a temperature of 40°C - 50ºC.

All fractional extracts obtained fractions (n-hexane, ethyl acetate, and n-butanol) were weighed and stored in a tightly closed container and kept away from light [29].

2.3.5. Secondary Metabolite Identification and BSLT Testing

2.3.5.1. Identification of Secondary Metabolites of Bangkal Extract

The secondary metabolite groups of bangkal leaves were identified using specific chemical reagents, namely for the detection of compound groups of steroids and terpenoids using Lieberman-Burchard, alkaloid groups using chemical reagents Dragendorf and Mayer, Mg powder and hydrochloric acid for flavonoid group detection, and FeCl3 2% reagent for phenolic/polyphenol group. The test was carried out by a qualitative test method based on chemical reactions by looking at the deposits and the color change of the solution in the tube. Meanwhile, the saponin test was carried out by the formation of foam using aqudest and HCl. Secondary metabolite test was carried out in the natural materials laboratory of the Faculty of Pharmacy Universitas Mulawarman with reference to the phytochemical test method as described by Harborne [1].

2.3.5.2. Brine Shrimp Lethality Test (BSLT) Assessment

BSLT testing refers to an expanded test method according to [30,31]. Egg hatching is carried out in a clear container, such as a beaker or jar, using seawater media. To further stimulate hatching, the eggs that are being hatched are given light at the beginning after the eggs bubble. Irradiation is carried out continuously during the hatching process using the power of 40 watts of incandescent/fluorescent light so that the hatching temperature of 25-30°C is maintained in temperature room. Artemia salina Leach eggs will hatch perfectly into larvae (nauplii) in 24-36 hours. Active shrimp larvae (naupli) that were 48 hours old were used as test animals in the study [26,32]. Furthermore, the larvae were captured from as many as 10 fish in 2500 μL of seawater. The salinity of the seawater used was 35 ppt (equivalent to 35 grams of salt per seawater), with a pH of 7.5 – 8 [46]. Ethanol extracts, n-hexane fractions, ethyl acetate, and n-butanol of bangkal leaves are made into a test stock solution with a concentration of 1500 ppm, then from all test stock solutions are diluted with a series of successive concentrations: 100, 200, 300, 400, 500, and 1000 ppm. The determination of this test concentration range is based on preliminary test results using a concentration of 1000 ppm with a maximum mortality of >95% of the bioindicator, which is the highest concentration commonly used in in vitro studies using Artemia bioindicators. According to Mayer [26] an extract is considered toxic if its LC_{50} value is <1000 μg/mL.
As for ethanol extract, n-hexane, ethyl acetate, and n-butanol, Genjer leaves are made into a stock solution of 1000 ppm, then diluted with a concentration series: 100, 300, 500, 700, and 900 ppm. Testing of *A. salina* larvae was carried out with 5 replicates each per group amounting to 10 larvae, and 1 container containing 2 drops of 1 percent DMSO as a negative control. The incubation process at room temperature for 24 hours was observed, the activity of the extracts and test fractions was carried out, and the number of dead and still alive larvae was calculated from each vial and then calculated by probit analysis to determine the cytotoxic effect (LC_{50}) [26, 31, 32].

3. RESULT AND DISCUSSION

Results and discussion contain research findings and discussion. Write down the findings obtained from the results of the research that has been carried out and must be supported by adequate data. Research results and findings must be able to answer questions or hypotheses.

3.1. Simplicia Extreaction

The drying process of simplicia is carried out with the aim of reducing the moisture content; the requirement for a good simplicia moisture content is less than 1%. Simplicia with with the moisture content can maintain the quality degradation of simplicia or damage the storage process over a long period of time and prevent enzymatic reactions from occurring [27]. The use of the maceration method in the extraction process with damping without heating aims to avoid damage to the components of the compound that are labile and not resistant to heat. The purpose of extraction is to extract the chemical

content of the simplicia by using organic solvents. The solvents used for the extraction of Bangkal leaves are polar and non-polar solvents, namely: ethanol, n-hexane, ethyl acetate, and n-butanol. The use of solvents with polarity differences aims to extract compounds based on the polarity properties of compounds, based on the like and this like principle.

3.2. Identification of Secondary Metabolites of Bangkal Leaves

Identification of secondary metabolites is carried out using chemical reagents specific to the plant compound group, which are compounds that are not essential for the growth of organisms. Most plants that produce secondary metabolite compounds utilize these compounds to defend themselves and compete with other living things in the vicinity [30]. Secondary metabolite testing is carried out as an initial stage to detect the class of secondary metabolite compounds present in biological materials. Identification of secondary metabolites of ethanol, n-hexane, ethyl acetate, and n-butanol extracts of scarlet leaves using chemical reagents sprayed on specific compound groups, including for the detection of alkaloid groups using Mayer and Dragendorf reagents. Lieberman Bourchard for the detection of terpenoids-sterols. Mg powder and hydrochloric acid for flavonoid group detection, and Iron (III) chloride and potassium hexacyanoferrate (III) ($K_3Fe(CN)_6$) for polyphenol and tannin detection, 10% sodium hydroxide (10% NaOH) solution for Quinon compound group detection, and aquades-HCl for saponin detection [33]. **Table 1**. Demonstrated the results of the identification of secondary metabolite groups of ethanol, n-hexane, ethyl setate, and n-butanol extracts of bangkal leaves using a variety of specific diagnostic reagents.

Table 1. Secondary metabolites of ethanol, n-hexane, ethyl acetate and n-butanol extracts of Bangkal leaves

Secondary Metabolite	**Extracts/fractions**			
	ethanol	**n-hexane**	**ethyl acetate**	**n-butanol**
Alkaloids	-	-	-	-
Steroids/Triterpenoids	+	-	+	-
Flavanoids	-	-	-	-
Phenolics/polyphenols	+	+	-	+
Quinon	+	-	-	+
Saponina	+	-	-	+

Description:
(-): Unidentified secondary metabolites
(+): Secondary metabolites identified

The results of the identification of secondary metabolites of ethanol extract leaves contain a group of steroid/terpenoid compounds, phenolics/polyphenols, quinones, and saponins. In the n-hexane fraction, the polyphenolic/phenolic compound group was detected, the ethyl acetate fraction was detected to contain the secondary metabolites of steroid or polyterpenoids, and the n-butanol fraction contained the phenolic/polyphenolic compound group, quinones, and saponins. The results of this study are in line with those reported by Nazarni Rahmi et al. [10,34]. Steroid and triterpenoid screening relies on the ability of these compounds to produce a distinctive color when reacted with concentrated sulfuric acid (H_2SO_4) in an acetate anhydride solvent medium [35]. The expected colors are usually red, orange, or purple for triterpenoids, and blue for steroids. Ethanol extract of bangkal leaves gives positive results, evidenced by a pronounced reddish-purple discoloration, which unequivocally confirms the presence of triterpenoid compounds.

In the flavanoid test, a positive reaction was shown by the change of the extract solution to red after the addition of magnesium powder (Wilstater method). This process was due to the response of the Mg^{2+} complex with orthodihydroxy and ketone hydroxy in the flavonoid compound ring, causing a shift in the red batochromic wavelength [37,38]. A positive reaction test of metabolites of the phenolic compound group using a $FeCl_3$ diagnostic reagent, the final reaction was addressed with a black solution [36,37]. Phenolic compounds will form a black complex with the addition of $FeCl_3$, with the $FeCl_3$ ion mechanism reacting with the aromatic –OH group [38,39]. The colored complex formed is thought to be iron (III) hexophenolite undergoing a bathochromic shift towards greater wavelengths [37,39].

3.3. Cytotoxicity of Bangkal and Genjer leaf extract

3.3.1. Cytotoxicity of ethanol extract and n-hexane, ethyl acetate, and n-butanol fraction of bangkal leaves

Secondary metabolite compounds are chemical compounds that generally have bioactivity capabilities and function as a protection for the plant body from pest and disease disturbances for the plant itself and the environment [21]. Some of the groups of compounds that have cytotoxic activity are the groups of flavonoids, alkaloids, and phenolic compounds [22]. The principle that a plant can be used as an anticancer is that the plant contains cytotoxic compounds [23]. Cytotoxic compounds are compounds or substances that can damage normal cells and cancer cells or can be used to inhibit the growth of malignant tumor cells [24]. In a study conducted by Aprilyanie *et al.* (2023), it was stated that toxicity testing can be carried out both *in vitro* and i*n vivo* [43]. One of the in vitro tests is the Brine Shrimp Lethality Test (BSLT), which is a method used to determine the toxicity to cells (cytotoxic) of a compound produced by plant extracts using *Artemia salina* Leach shrimp larvae [44]. The secondary metabolites of bangkal leaves contain a group of compounds of polyphenols, alkaloids, flavonoids, saponins, tannins, and quinones [7,10].

The results of the cytotoxic bioactivity of ethanol extract, n-hexane, ethyl setate, and n-butanol fractions of bangkal leaves were obtained after 24 hours through the incubation process of test bioindicators. In this test, DMSO was used as a negative control (solvent control). The DMSO concentration used was 1% and served as a solubility enhancer for the test extract. The test results showed that DMSO did not cause death to the test bioindicator, so its activity data was not included in the analysis of extract toxicity calculations. Data on the mortality of the number of dead shrimp larvae for each extract and test fraction, as well as the LC_{50} value of the activity of each extract and fraction on the mortality of the *A. salina* larval bioindicator can be seen in **Table 2** below.

Table. **2** Data on the number of shrimp larval deaths from each extract and leaf fraction of Bangkal

Const. (ppm)	Log Cons.	Ethanol Extracts		n-hexane fraction		ethyl acetate fraction		Const. (ppm)	Log Cons.	n-butanol fraction	
		AM	AH	AM	AH	AM	AH			AM	AH
50	1.699	-	-	-	-	2	48	10	1.000	0	66
100	2.000	2	38	1	66	5	40	20	1.301	0	56
150	2.176	-	-	-	-	8	33	30	1.477	2	46
200	2.301	6	30	3	57	11	26	40	1.602	4	38
250	2.398	-	-	-	-	16	19	50	1.699	7	30
300	2.477	11	24	5	49	22	14	60	1.778	10	23
350	2.544	-	-	-	-	28	10	70	1.845	15	16
400	2.602	17	19	7	41	35	6	80	1.903	21	11
450	2.653	-	-	-	-	43	3	90	1.954	27	7
500	2.699	23	15	10	33	52	1	100	2.000	34	3
600	2.778	30	11	13	26	-	-	-	-	-	-
700	2.845	37	8	17	19	-	-	-	-	-	-
800	2.903	45	5	22	13	-	-	-	-	-	-
900	2.954	53	3	28	8	-	-	-	-	-	-
1000	3.000	62	1	34	4	-	-	-	-	-	-

The LC_{50} value table, show that exposure of ethanol extract, n-hexane fraction and ethyl acetate fraction of Bangkal leaves to the test larvae gave 50% of the larvae mortality in the concentration range of 50 ppm (logC 1.699) – 700 ppm (logC 2.845). The results of this test proved that ethanol extract, n-hexane fraction, ethyl acetate fraction and n-butanol fraction of shank leaves can kill 50% of *A. salina* test animals. Based on the results of the calculation analysis using the Reed and Muench method, the LC_{50} value of ethanol extract was obtained of 418.79 ppm, LC_{50} for the n-hexane fraction was obtained a value of 716.14 ppm, ethyl acetate fraction of 263.03, and n-butanol fraction of 70.790 ppm. These LC_{50} values

show that the cytotoxic activity of ethanol extract and the n-hexane fraction is relatively weak with an LC_{50} value between 400-700 ppm, while for the LC_{50} value of the ethyl acetate fraction is moderately classified with an LC_{50} value of 263.03 ppm, for the n-butanol fraction an LC_{50} value of 70.79 ppm, this value is obtainedshowed that the n-butanol fraction had a strong cytotoxic effect with an LC_{50} value of less than 100 ppm.

3.3.2. Cytotoxicity of ethanol extract and n-hexane, ethyl acetate and n-butanol leaf Genjer extract

The secondary metabolites of genjer leaves contain a group of compounds: flavanoids, amino acids, phenols, hydroquinones, reducing sugars, tannins, terpenods, saponins and alcloids, steroids [15-18]. The results of the research on the cytotoxic bioactivity of ethanol extract, n-hexane, ethyl setate and n-butanol genjer leaves were obtained after 24 hours through the incubation process of test bioindicators. Data on the mortality of the number of dead shrimp larvae for each extract and test fraction, as well as the LC_{50} value of the activity of each extract and fraction on the mortality of the *A. salina* shrimp larval bioindicators can be seen in **Table 3** below.

Table. **3** Data on the number of shrimp larval deaths from each extract and leaf fraction of Genjer

Const. (ppm)	**Log Cons** .	**Ethanol Extracts**		**Etil asetat fraction**		**n-Heksan fraction**		**Const. (ppm)**	**Log Cons.**	**n-Butanol fraction**	
		AM	AH	AM	AH	AM	AH			AM	AH
100	2.000	12	38	14	36	13	37	10	1.000	12	38
300	2.477	19	31	21	29	-	-	30	1.470	20	30
350	2.544	-	-	-	-	17	33	50	1.690	27	23
500	2.699	24	26	27	22	-	-	70	1.850	32	18
600	2.778	-	-	-	-	27	23	90	1.954	41	9
700	2.845	32	18	33	17	-	-	-	-	-	-
850	2.930	-	-	-	-	33	17	-	-	-	-
900	2.954	41	9	44	6	-	-	-	-	-	-
1100	3.040	-	-	-	-	42	8	-	-	-	-

The LC_{50} value table shows that exposure of ethanol extract, n-hexane fraction, and ethyl acetate fraction of genjer leaves to the test larvae gave 50% of the larvae mortality in the concentration range of 50 ppm (logC 1.699) – 600 ppm (logC 2.778). The results of this test prove that ethanol extract, n-hexane, ethyl acetate, and n-butanol fraction of genjer leaves can kill 50% of *A. salina* test animals. Based on the results of the calculation analysis using the Reed and Muench method, the LC_{50} value of ethanol extract was obtained as 490.23 ppm, the LC_{50} for the n-hexane fraction was obtained as 551.31 ppm, ethyl acetate fraction was 425.01, and n-butanol fraction was 46.02 ppm. These LC_{50} values show that the cytotoxic activity of ethanol extract, n-hexane, and ethyl acetate fractions is relatively weak with an LC_{50} value between 450 ppm-560 ppm, while for the LC_{50} value of the n-butanol fraction a LC_{50} value of 46.02 ppm, this value shows that the n-butanol fraction has a very strong cytotoxic effect with an LC_{50} value of less than 50 ppm.

Table 4. LC_{50} Value bangkal t and genjer leaf extrac

No.	**Sample**	**Toxic concentration [LC_{50} (ppm)]**	
		Bangkal	**Genjer**
1.	Extract ethanol	418.79	490.23
2.	N-hexane fraction	716.14	551.31
3.	Ethyl acetate fraction	263.03	425.01
4.	Fraksi n-butanol	70.79	46.02

Based on the data in **Table 4**, the cytotoxicity value (LC_{50}) of each extract and test fraction can be found, and the fraction that shows the strongest activity kills the bioindicator of the *A. salina* test. According to Meyer et al. (1982), which states that if the test results of an extract using the Brine Shrimp Lethality Test (BSLT) method can cause the death of 50% of *A. salina* larvae at concentration of 50<1000 ppm within 48 hours indicates that the sample has potential as an anticancer, antibacterial, antifungal and so on [26]. The cytotoxicity value of each extract and fraction of bangkal leaves and genjer leaves in a row is n-butanol fraction with a value of 70.79 ppm for bangkal, and 46.02 ppm for genjer leaves, followed by ethyl acetate fraction with LC_{50} in the range of 250 – 450 ppm, ethanol crude extracts with LC_{50} values in the range of 410 – 495 ppm, and n-hexane fraction with range of 550 - 720 ppm. The toxicity of ethanol crude extracts, n-hexane, and ethyl acetate fractions showed weak cytotoxic activity, while the cytotoxic activity of each n-butanol fraction was strong with LC values of 50 ppm < 50 ppm. According to Rajabi at al. (2015), a lethal concentration of 50% (LC_{50}) of less than 50 ppm indicates very strong cytotoxic activity [45].

4. CONCLUSION

Based on the results of the research obtained, it can be concluded that: bangkal leaves contain secondary metabolites of the steroid/terpenoid compound group, phenolics/polyphenols, quinones and saponins, with cytotoxic activity of ethanol extract, n-hexane fraction, ethyl acetate with LC_{50} values ranging from 200 - 700 ppm indicating that cytotoxic activity is weak, while the n-butanol fraction has a strong cytotoxic effect with a value of LC_{50} < 100 (70.79 ppm). Forg genjer leaves, the cytotoxic activity of the ethanol extract, the n-hexane, ethyl acetate fraction, with an LC_{50} value ranging from 400 ppm - 600 ppm, indicates that the cytotoxic activity is very weak, while for the n-butanol fraction, the cytotoxic effect is very strong with an LC_{50} value of < 50 (46.02 ppm).

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