Cytotoxic activity assay from leaves and fruit extracts of *Ficus aurata* (Miq.) using brine shrimp lethality test method

Nurhamidah¹, H Nurdin², Y Manjang², A Dharma², and Suryati²

¹ Department of Education Chemistry, Faculty of Teacher Training and Education, Bengkulu University, Raya Kandang Limun Street, Bengkulu 38371, Indonesia  
² Department of Chemistry, Faculty Mathematics and Science, Andalas University, Indonesia

*e-mail: dailami_nurhamidah@yahoo.com*

Abstract. This research aimed to cytotoxic activity assay from n-hexane, ethyl acetate, and methanolextracts of *Ficus aurata* (Miq.) leaves and fruits using *Brine Shrimp Lethality Test* method. Extraction process was gradually held with eluents n-hexane, ethyl acetate, and methanol. The strength of the cytotoxic activity is determined by the value of LC50 of each extract tested. The results showed that hexane extract from both leaves and fruits had stronger cytotoxic activity than ethyl acetate and methanol extract, where the hexane extract from the leaf had the strongest cytotoxic activity with an average LC50 of 13.74 μg/mL, followed by hexane extract from fruit with an average LC50 value of 33.10 μg/mL.

1. Introduction

The natural potential of Indonesia is very rich in biological resources that have not been fully utilized and are the assets and foreign exchange of the State. One such biological resource is a plant, where it is a chemical source (chemical resources) that continues to produce throughout the year through a natural process [1]. Chemicals contained in these plants include secondary metabolite compounds such as classes of steroid, terpenoid, alkaloid, flavonoid, phenolic and coumarin that have some biological activity [2].

Leaves *Ficus variegate* Blume widely used by the community as a vegetable because it is believed to facilitate breastfeeding in breastfeeding mothers and the results showed that the leaf extract *Ficus variegate* Blume has a high total phenol content and powerful antioxidants and cytotoxics [3]. Ethyl acetate extract from *Ficus aurata* (Miq.) has strong antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* bacteria with KHM values equal to cefadroxil positive control of 0.1563 μg/mL [4]. Species of Ficus in India have medicinal properties such as anticancer [5]. *Ficus carica* has many activities such as anticancer [6].

Cancer is a disease due to abnormal growth of cells in the tissues of the body undergoing mutations and changes in the biochemical structure. One of the causes of cancer is the mutation of genes, where the cells undergo changes as a result of exposure to ultraviolet light, chemicals or materials derived from nature [7]. Treatment of cancer has been widely done one of them is chemotherapy and drugs treatment, but taking drugs and chemotherapy have side effects such as inflammation of the lining of the mouth, pain and discomfort (mucositis), diarrhea, skin peeling, swelling of the body, pain in the oral part, and the lack of platelets or platelets of blood cells that play an important role in the blood
clotting process (thrombocytopenia). Therefore, not a few Indonesian people switch to using traditional drugs based on natural [8].

Indonesia rich in plant diversity has identified several potential anticancer plants such as soursop species (*Annona muricata*), mangosteen (*Garcinia mangostana*), jarak plant (*Jatropha curcas*), star fruit (*Averrhoa carambola*), jackfruit (*Artocarpus heterophyllus*), walnut (Canadian indicum), mango (*Mangifera indica*), *Morinda citrifolia*, coffee (*Coffea sp.*), breadfruit (*Artocarpus altilis*), mundu plant (*Garcinia dulcis*), and melinjo plant (*Gnetum gnemon*)[9].

Toxicity is a relative measure of the degree of toxicity between chemicals to other chemicals in organisms similar to the ability of toxins (molecules) to cause damage when entering into the body and the location of susceptible organs [10].

Brine Shrimp Lethality Test is a compound test in general that can detect some bioactivity in an extract. Positive correlations were found between BSLT and cytotoxic toxicity of P-388 leukemia cells in vivo [11].

2. Research Methods

2.1. Plant Materials

*Ficus aurata* (Miq.) Miq. taken from the campus environment of Andalas University, Limau Manis Village, Pauh District, Kotamadia Padang, West Sumatera. Plant identification was done at Herbarium Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University (YOU), Padang with identification number 204/ K-ID / YOU/ VI/ 2014.

2.2. Chemical material

Chemicals for the purposes of extraction are used which are of technical quality and distillate ie: hexane, ethyl acetate and methanol. While the material for cytotoxic antivitas test used DMSO and sea water.

2.3. Animals for Experiments

The cytotoxic activity test of BSLT method from Ficus leaf and fruit extract, using animal *Arthemia salina* shrimp larvae test.

2.4. Extraction

Ficus leaf and fruit extraction was performed by maceration (immersion) using hexane, ethyl acetate and methanol. Dry powdered leaves and fruits *Ficus aurata* (Miq.) Miq. inserted each into the maserator (dark bottle volume of 2.5 L) then soaked in hexane and shaken. After 3 days the immersion was filtered and accommodated with dark bottles, the solvent evaporated by rotary evaporator at 40°C. The immersion is repeated until the evaporation of the solvent leaves no viscous hexane extract.

The hexane condensed extract obtained is stored in the cooler stream until subsequent use. The maceration is continued using an ethyl acetate solvent. The residual residue of the hexane mixture is immersed in ethyl acetate and shaken. After 3 days the immersion was filtered and accommodated with dark bottles, the solvent evaporated by rotary evaporator at 40°C. The immersion is repeated until the solvent evaporation results leave no viscous ethyl acetate extract.

The obtained ethyl acetate acid extract is stored in the coolant stream until subsequent use. The maceration is followed by methanol solvent. The residue of ethyl acetate maceration is immersed in methanol and shaken. After 3 days the immersion was filtered and accommodated with dark bottles, the solvent evaporated by rotary evaporator at 40°C. The immersion is repeated until the evaporation of the solvent leaves no viscous methanol extract. The obtained methanol condensed extract is stored in the coolant stream until subsequent use.

2.5. Testing cytotoxic activity by BSLT method

Test of cytotoxic activity by Brine Shrimp Lethality Test method of *Ficus aurata* (Miq.) Miq extract. This uses a test procedure performed that is modified [12]. The concentration of test solution used was 1000, 100 and 10 μg / mL. The preparation of a 1000 μg / mL test solution was carried out by taking
20 mg of each n-hexane extract, ethyl acetate and methanol and then dissolved in 20 mL of methanol. For the preparation of the 100 μg/mL test solution was performed by taking 2 mL of 1000 μg/mL concentration solution dissolved in 20 mL of methanol, while for the preparation of 10 μg/mL test solution was carried out by taking 2 mL of 100 μg/mL concentration solution dissolved in 20 mL methanol, so that the test solution obtained with the concentration of 1000, 100 and 10 μg/mL.

Taken 5 mL each concentration of test solution, inserted into the vial and given a limit, then let the solvent evaporate. After drying is reconstituted by adding into each vial 50 μL DMSO, then seawater is added to the limit. A total of 10 shrimp larvae were inserted into each vial and added seawater to the calibration limit, then observed the death of shrimp larvae after 24 hours, this treatment was done three times. The LC50 values of the n-hexane, ethyl acetate and methanol fractions were determined using the regression curve between the concentration log (X) and the probit value (Y). The probit value (Y) is obtained by transforming the percent of deaths in the probit table.

3. Results and Discussion
Test Result Cytotoxic activity The hexane and ethyl acetate extract of leaf and hexane, ethyl acetate and methanol extract from *Ficus aurata* fruit using Brine Shrimp Lethality Test (BSLT) method can be seen in table 1, 2, 3, 4 and 5 below.

**Table 1. BSLT Test Results of Hexane Leaf Extraction Samples**

| Repetition | Concentration (μg/mL) | Log Concentration | Total Larvae Test | Dead Larvae | % Mortality | Probit Value | LC 50 (μg/mL) |
|------------|-----------------------|------------------|-------------------|-------------|-------------|--------------|---------------|
| I          | 10                    | 1                | 10                | 5           | 50.000      | 5            | 18.2011       |
|            | 100                   | 2                | 10                | 7           | 70.000      | 5.524        | 9.768         |
|            | 1000                  | 3                | 10                | 10          | 100.000     | 9.768        |               |
| II         | 10                    | 1                | 10                | 5           | 50.000      | 5            | 11.5027       |
|            | 100                   | 2                | 10                | 7           | 70.000      | 5.524        |               |
|            | 1000                  | 3                | 10                | 9           | 90.000      | 6.282        |               |
| III        | 10                    | 1                | 10                | 5           | 50.000      | 5            | 11.5027       |
|            | 100                   | 2                | 10                | 7           | 70.000      | 5.524        |               |
|            | 1000                  | 3                | 10                | 9           | 90.000      | 6.282        |               |

| 1          | 2                     | 3                     | Average | SD | LC 50   |
|------------|-----------------------|-----------------------|---------|----|--------|
|            | 18.2011               | 11.5027               | 13.7355 | 3.8673 | 13.7355±3.8673 |

**Table 2. BSLT Test Results Leaf Ethyl Acetate Extract**

| Repetition | Concentration (μg/mL) | Log Concentration | Total Larvae Test | Dead Larvae | % Mortality | Probit Value | LC 50 (μg/mL) |
|------------|-----------------------|------------------|-------------------|-------------|-------------|--------------|---------------|
| I          | 10                    | 1                | 10                | 1           | 10.000      | 3.178        | 943.6262      |
|            | 100                   | 2                | 10                | 2           | 20.000      | 4.158        |               |
|            | 1000                  | 3                | 10                | 5           | 50.000      | 5            |               |
| II         | 10                    | 1                | 10                | 1           | 10.000      | 3.178        | 943.6262      |
|            | 100                   | 2                | 10                | 2           | 20.000      | 4.158        |               |
|            | 1000                  | 3                | 10                | 5           | 50.000      | 5            |               |
| Repetition | Concentration (ug/mL) | Log Concentration | Total Larva Test | Dead Larvae | % Mortality | Probit Value | LC 50 (ug/mL) |
|------------|-----------------------|-------------------|------------------|-------------|-------------|--------------|---------------|
| I          | 100                   | 2                 | 10               | 2           | 20.000      | 4.158        | 35.7114       |
|            | 1000                  | 3                 | 10               | 5           | 50.000      | 5            | 35.7355       |

### Table 3. BSLT Test Results Hexane Fruit Extract

| Repetition | Concentration (ug/mL) | Log Concentration | Total Larva Test | Dead Larvae | % Mortality | Probit Value | LC 50 (ug/mL) |
|------------|-----------------------|-------------------|------------------|-------------|-------------|--------------|---------------|
| I          | 100                   | 2                 | 10               | 4           | 40.000      | 4.747        | 574.5132      |
|            | 1000                  | 3                 | 10               | 5           | 50.000      | 5            | 545.1299      |

| Repetition | Concentration (ug/mL) | Log Concentration | Total Larva Test | Dead Larvae | % Mortality | Probit Value | LC 50 (ug/mL) |
|------------|-----------------------|-------------------|------------------|-------------|-------------|--------------|---------------|
| I          | 100                   | 2                 | 10               | 3           | 30.000      | 4.476        | 545.1299      |
|            | 1000                  | 3                 | 10               | 5           | 50.000      | 5            | 545.1299      |

### Table 4. BSLT Test Results Ethyl Acetate Fruit Extract

| Repetition | Concentration (ug/mL) | Log Concentration | Total Larva Test | Dead Larvae | % Mortality | Probit Value | LC 50 (ug/mL) |
|------------|-----------------------|-------------------|------------------|-------------|-------------|--------------|---------------|
| I          | 100                   | 2                 | 10               | 3           | 30.000      | 4.476        | 545.1299      |
|            | 1000                  | 3                 | 10               | 5           | 50.000      | 5            | 545.1299      |

| Repetition | Concentration (ug/mL) | Log Concentration | Total Larva Test | Dead Larvae | % Mortality | Probit Value | LC 50 (ug/mL) |
|------------|-----------------------|-------------------|------------------|-------------|-------------|--------------|---------------|
| I          | 100                   | 2                 | 10               | 3           | 30.000      | 4.476        | 545.1299      |
|            | 1000                  | 3                 | 10               | 5           | 50.000      | 5            | 545.1299      |

| Repetition | Concentration (ug/mL) | Log Concentration | Total Larva Test | Dead Larvae | % Mortality | Probit Value | LC 50 (ug/mL) |
|------------|-----------------------|-------------------|------------------|-------------|-------------|--------------|---------------|
| I          | 100                   | 2                 | 10               | 3           | 30.000      | 4.476        | 545.1299      |
|            | 1000                  | 3                 | 10               | 5           | 50.000      | 5            | 545.1299      |
Table 5. BSLT Test Results of Methanol Fruit Extract

| Repetition | Concentration (μg/mL) | Log Concentration | Total Larvae Test | Dead Larva | % Mortality | Probit Value | LC 50 (μg/mL) |
|------------|-----------------------|-------------------|-------------------|------------|-------------|--------------|---------------|
| I          | 100                   | 2                 | 10                | 2          | 20.000      | 4.747        | 189.9328      |
|            | 1000                  | 3                 | 10                | 7          | 70.000      | 5.524        |               |
| II         | 100                   | 2                 | 10                | 4          | 40.000      | 4.747        | 189.9328      |
|            | 1000                  | 3                 | 10                | 7          | 70.000      | 5.524        |               |
| III        | 100                   | 2                 | 10                | 4          | 40.000      | 4.747        | 189.9328      |
|            | 1000                  | 3                 | 10                | 7          | 70.000      | 5.524        |               |

Table 6. Probit Value

| %  | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|----|---|---|---|---|---|---|---|---|---|---|
| 0  | 2,674 | 2,946 | 3,119 | 3,249 | 3,355 | 3,445 | 3,524 | 3,595 | 3,659 |
| 10 | 3,178 | 3,773 | 3,825 | 3,874 | 3,920 | 3,964 | 4,006 | 4,046 | 4,085 | 4,122 |
| 20 | 4,158 | 4,194 | 4,228 | 4,261 | 4,294 | 4,326 | 4,357 | 4,387 | 4,417 | 4,447 |
| 30 | 4,476 | 4,504 | 4,532 | 4,560 | 4,597 | 4,615 | 4,642 | 4,668 | 4,695 | 4,721 |
| 40 | 4,747 | 4,733 | 4,798 | 4,824 | 4,849 | 4,900 | 4,925 | 4,950 | 4,950 | 4,975 |
| 50 | 5,000 | 5,030 | 5,050 | 5,075 | 5,100 | 5,126 | 5,151 | 5,182 | 5,202 | 5,277 |
| 60 | 5,253 | 5,279 | 5,305 | 5,332 | 5,358 | 5,385 | 5,413 | 5,446 | 5,468 | 5,496 |
| 70 | 5,524 | 5,553 | 5,583 | 5,613 | 5,643 | 5,674 | 5,706 | 5,772 | 5,722 | 5,806 |
| 80 | 5,842 | 5,878 | 5,919 | 5,854 | 5,994 | 6,036 | 6,080 | 6,125 | 6,175 | 6,227 |
| 90 | 6,282 | 6,341 | 6,405 | 6,476 | 6,576 | 6,645 | 6,751 | 6,881 | 7,054 | 7,326 |
| 100| 9,768 |       |       |       |       |       |       |       |       |       |

The value of LC50 of each extract is determined based on the equation of straight line with X is the antilog of sample concentration and Y probit value. The probit value obtained from the average percent of deaths (the treatment carried out triplo) is transformed to the probit value table. By entering the value of Y = 5 (50% death), then the value of LC50 can be determined. From the LC50 values listed in tables 1, 2, 3, 4 and 5 showing n-hexane extracts from leaves and fruits had stronger cytotoxic activity, where hexane extracts from leaves had the strongest cytotoxic activity with an average LC50 of 13.74 μg/mL, followed by hexane extract from fruit with average LC50 value of
33.10 μg / mL, fruit methanol extract with LC50 value 189.93 μg / mL, extract of ethyl acetate fruit with LC50 value 554.92 μg / mL and which has the weakest cytotoxic activity is the ethyl acetate extract from the leaf with LC50 943.63 μg / mL.

The results of this study showed that the active component that has cytotoxic activity, more extracted on n-hexane solvent, this indicates that chemical compounds that have cytotoxic activity are stronger in Ficus aurata (Miq.) Miq. is a non-polar compound.

4. Conclusion
From the research result, it can be concluded that hexan extract from leaf and fruit is the most potent as cytotoxic compound, wherein hexan extract from leaf is stronger than fruit with LC50 value 13.74 μg / mL and fruit 33.10 μg / mL, followed by fruit methanol extract with LC50 189.93 μg / mL, ethyl acetate extract of fruit with LC50 value 554.92 μg / mL and which has the weakest cytotoxic activity is extract of ethyl acetate from leaf with LC50 943.63 μg / mL.

References

[1] Ersam T 2005 *Pemberdayaan Keanekaragaman Hayati Hutan Tropika: Fenolat Terprenilasi dari Artocarpus dan Garcinia (Nangka dan Manggis)* in Prosiding Seminar Nasional Kimia (Surabaya: Indonesia) pp 22-24
[2] Kristianti A N et al 2008 Fitokimia (Surabaya: Universitas Airlangga)
[3] Lushaini S et al 2015 JKK. 4 1
[4] Nurhamidah 2017 Aktifitas Antibakteri Ekstrak Daun dan Buah Ficus aurata (Miq.) Miq. Menggunakan Metoda Resazurine Microtiter Assay in Prosiding Semirata BKS-PTN Wilayah Barat (Jambi: Indonesia) pp 2366-2371
[5] Dhungana P et al 2013 *Int. J. Pharm. Life Sci.* 4 2314
[6] Joseph B and Raj S J 2011 *Int. J. PharmTech Res.* 3 8
[7] Wijaya C H 1999 *Telaah Ringkas Rempah-rempah Tradisional. Andaliman, Rempah Tradisional Sumatera Utara dengan Aktivitas Antioksidan dan Antimikroba. Buletin Teknologi dan Industri Pangan* (Medan: Universitas Sumatera Utara)
[8] Nurul K S 2014 *Uji Toksisitas Akut Ekstrak Metanol Daun Laban Abang (Aglaiaelliptica Blume) Terhadap Larva Udang (Artemia Salina Leach) Dengan Metode Brine Shrimp Lethality Test (Bslh)* (Jakarta: UIN Syarif Hidayatullah)
[9] Saputro et al 2015 Pros Sem Nas Masy Biodiv Indon 1 477
[10] Soemirat J 2005 *Tokskologi Lingkungan* (Yogyakarta: Gadjah Mada University Press)
[11] Nuria M 2016 *Uji Toksisitas Dengan Metode Bslh Senyawa Steroid Fraksi Petroleum Eter Mikroalga Chlorella sp* (Malang: UIN Maulana Malik Ibrahim)
[12] Meyer B N et al 1982 *Planta Medica* 45 31