

Cytotoxic Activity of Selected Medicinal Plants from Papua, Indonesia

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Abstract: Some medicinal plants from Papua which have been used traditionally as anticancer agents for many ethnic in Papua Islands. But, scientific evidence for this study were still limited. **Objective:** The present study was conducted to test for *in vitro* Brine Shrimp Lethality Test (BSLT) of the seven selected medicinal plants from Papua, namely, *Drymis piperita* (local name : Kayu Akway), *Myrmecodia beccarii* (Sarang semut), *Biophytum petersianum* (Rumput kebar), *Vernonia amygdalina* (Daun Afrika), *Villebrunea rubescens* (Daun Jilat), *Laportea aestuans* (Daun Gatal) and *Breynia cernua* (Katuk hutan). Seven ethanolic extracts were evaluated for their cytotoxic activity against *Artemia salina* Leach with concentration extracts from 10;100; and 1000 [g/mL. Cytotoxicity was evaluated in terms of LC₅₀ value from the 24 hour counts using the probit analysis method. **Results:** Ethanolic extracts of *Myrmecodia beccarii* showed the highest cytotoxicity with LC₅₀ was 8.33 [g/mL against brine shrimp, but all selected medicinal plants showed the active potential cytotoxic activity because the LC₅₀ value were <1,000 [g/mL. From this research, *M.beccarii* indicated the possible potential use of medicinal plants from Papua as anticancer agents.

1 INTRODUCTION

Papua Province is rich in biodiversity. The indigenous Papuans utilize most of endemic plants as food, board and medicines. Several Papua endemic plants such as *Drymis piperita*, *Myrmecodia beccarii*, *Biophytum petersianum*, *Vernonia amygdalina* *Villebrunea rubescens*, *Laportea aestuans* and *Breynia cernua* often used as a medicinal plant for the Papuan people (Dinkes Papua, 2016)

Drymis piperita Hook.f. (local name : Kayu Akway) is a woody, evergreen and aromatic plan that was a member of Winteraceae. This plant is used by Sougb tribe living in Sururey village, District of Manokwari, West Papua to heal malaria and to enhance the vitality of body (Cepeda, 2015).

Myrmecodia beccarii known as “sarang semut” (Rubiaceae) from Merauke Region, Papua which

have been used traditionally for human vitality enhancer, muscle pain, inflammation, and antioxidants for supporting anticancer agents from natural products (Dirgantara, 2013).

Biophytum petersianum (Oxalidaceae) commonly known as “rumput kebar”, is valuable medicinal plant from Kebar District from Manokwari Region and the whole plant has been used in mouthwashes, antidotes and laxatives (Sambodo, 2018).

Laportea aestuans (Urticaceae) is an indigenous plant of Papua which has been widely used for pain relief as traditional medication for Papua community and the local name is “daun gatal” (Simaremare, 2017).

Villebrunea rubescens as original plants, known as “Lick Leaves” in English and “Daun Jilat” in the Papua local name, of the family urticaceae is a medicinal herb with a long history of used. *V. rubescens* has been used for the treatment of pain

relieved or bruised in the local community widely. These leaves are picked, and warmed over a fire and then attached into the part of body pained and licked it will give the effect of bleeding from the body. The local people believed that the body felt better after used the leaves (Gunawan, 2018).

Vernonia amygdalina (local name Daun Afrika) from Asteraceae family has been used for Papua community to medicate malaria epidemic and blood sugar disease (Handjojo, 2018).

Breynia cernua (local name Katuk Hutan) is an Indonesian medicinal plant (family: Euphorbiaceae) originated from Papua which have been used traditionally as alternative treatment for breast and cervical cancer (Dirgantara, 2018).

Typically any research on natural substances suspected of potentially as drugs or empirically has been used by the community as a drug, beginning with a pre-clinical test of toxicity to predict its safety level, followed by other pharmacological tests. Toxicity test method can be done *in vitro* and *in vivo*. One of the most commonly used methods of *in vitro* toxicity is the *Brine Shrimp Lethality Test* (BSLT) method. BSLT method is one of the fastest and cheapest way to screen for toxicity from plant extract by using marine animals namely *A.salina* shrimp larvae. Toxicity test with BSLT method has a broad spectrum of pharmacological activities, simple procedure, fast and does not require a large cost, and the results can be accounted for. In addition, this method is often associated with a method of screening anticancer compounds. For these reasons, this test is best used in the initiation of natural materials research for anticancer agents (Meyer *et al.*, 1982).

2 MATERIAL AND METHODS

2.1 Sample Preparation

Plant material such as *Drymis piperita*, *Myrmecodia beccarii*, *Biophytum petersianum*, *Vernonia amygdalina*, *Villebrunea rubescens*, *Laportea aestuans* and *Breynia cernua* were collected from some public forests in Papua and West Papua Province, Manokwari; Merauke: Sentani; Serui and Jayapura City. All plants were washed with running tap water and then rinsed by distilled water to remove any adsorbed contaminant from sample surface. The cleaned sample was dried and mashed to the ground to fine powder 30 mesh.

2.2 Extraction of Plant Material

Fine powder 100 gram of plant material was macerated with 300 mL 96% ethanol at room temperature for 3 x 24 hours, repeated until maceration obtained translucent color. Extract of obtained was concentrated to obtain further combined and filtered using filter paper, and then the solvent removed using a rotary vacuum evaporator at 40 °C to obtain a concentrated ethanol extract of *Drymis piperita*, *Myrmecodia beccarii*, *Biophytum petersianum*, *Vernonia amygdalina* *Villebrunea rubescens*, *Laportea aestuans* and *Breynia cernua*.

2.3 Toxicity Tests Using the Method of *Brine Shrimp Lethality Test* (BSLT)

Method of Meyer (1982), is used to study the toxicity of the general sample using shrimp eggs (*A. salina* Leach). *Brine Shrimp Lethality Test* (BSLT) is one of the methods bioactive compounds present in natural materials using shrimp larvae (*A. salina*). Known toxicity properties based on the number of larvae mortality (Mc Laughlin, 1983). An extract is said to be toxic to *A. salina* if it has a value of LC50 (lethal concentration to 50% larval shrimp) less than 1,000 µg/ml.

2.4 The Hatching of Shrimp larvae

Prepared shrimp vessel for hatching eggs which have been filled with sea water 1,500 ml, with pH of 7.0, place the lamp to warm temperatures in vessel of hatching and fed air by using the aerator. Inserted into the sea water of 4.0 g shrimp eggs for hatching. Vessel hatching eggs covered with aluminum foil, and the lights turned on for 48 hours to incubate the eggs. After 48 hours of shrimp eggs will hatch into larvae and ready for use. Shrimp larvae that will be used for testing were taken using a pipette.

2.5 Preparation of Sample Solution that will be Tested

Ethanol extract of *Drymis piperita*, *Myrmecodia beccarii*, *Biophytum petersianum*, *Vernonia amygdalina* *Villebrunea rubescens*, *Laportea aestuans* and *Breynia cernua* that will be made alternative dilution procedure developed by McLaughlin *et al* (1983) were adopted in the preparation of the different dilutions of the plant extracts for BSLT where 20 mg of each extract was dissolved in 2 mL of the solvent. The final

concentrations were 0, 10, 100, and 1000 $\mu\text{g}/\text{mL}$ in sea water. When the ethanol extract insoluble added 2 drops of DMSO (dimethyl sulfoxide 0.2%). There were three (3) replicates in each concentration. A control test was also prepared.

2.6 Procedure of Toxicity Test Methods using BSLT

The seawater was put in a small plastic container (hatching chamber) with a partition for dark (covered) and light areas. Shrimp eggs were added into the dark side of the chamber while the lamp above the other side (light) will attract the hatched shrimp. Two days were allowed for the shrimp to hatch and mature as nauplii (larva). After two days, when the shrimp larvae are ready, 4 mL of the artificial seawater was added to each test tube and 10 brine shrimps were introduced into each tube. Thus, there were a total of 30 shrimps per dilution. Then the volume was adjusted with artificial seawater up to 5 mL per test tube. The test tubes were left uncovered under the lamp. The number of surviving shrimps were counted and recorded after 24 hours. Using probit analysis, the lethality concentration (LC_{50}) was assessed at 95% confidence intervals. LC_{50} of less than 100 $\mu\text{g}/\text{mL}$ was considered as potent (active). As mentioned by Meyer and others, LC_{50} value of less than 1,000 $\mu\text{g}/\text{mL}$ is toxic while LC_{50} value of greater than 1,000 $\mu\text{g}/\text{mL}$ is non-toxic. The percentage mortality (%M) was also calculated by dividing the number of dead nauplii by the total number, and then multiplied by 100%. This is to ensure that the death (mortality) of the nauplii is attributed to the bioactive compounds present in the plant extracts.

Observations were made after 24 hours to calculate the percentage of mortality shrimp larvae *A. salina*. Mortality data are used to calculate the value of Lethal Concentration 50 (LC_{50}). After 24 h of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial was counted and noted. From this data, the percentage of mortality of the nauplii was calculated for each concentration and LC_{50} values with 95% confidence limits were determined using Probit analysis Finney (Meyer, 1982).

3 RESULTS AND DISCUSSION

The Brine Shrimp Lethality Test (BSLT) represents a rapid, inexpensive and simple bioassay

for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxicity and anti-tumor properties (Krishnaraju, 2005). Presently there is an increasing interest world wide in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases (Lobo, 2009).

Toxicity tests of crude ethanol extract of *Drymis piperita*, *Myrmecodia beccarii*, *Biophytum petersianum*, *Vernonia amygdalina* *Villebrunea rubescens*, *Laportea aestuans* and *Breynia cernua* conducted to determine the level of toxicity of the extracts against larvae shrimp *A. Salina*. The test results showed that seven ethanolic extracts from Papua were potent cytotoxic activity because the LC_{50} value less than 1,000 $\mu\text{g}/\text{mL}$ that at different concentration levels will have an impact on mortality and larval toxicity of this case is shown in Table 1.

Based on results, mortality of *A. salina* in ethanol extract of plant *M. beccarii* showed the highest potential cytotoxic activity with low LC_{50} value with 8.33 $\mu\text{g}/\text{mL}$ with minimum concentration (10 $\mu\text{g}/\text{mL}$) can reach 50% mortality after 24 hours of treatment. Mortality of *A. salina* in ethanol extract of *L. aestuans*, *V. rubescens* and *D. piperita* showed high mortality with LC_{50} 34.95; 61.82 and 79.59 $\mu\text{g}/\text{mL}$ respectively. Then, the cytotoxic activity for *B. cernua* (255.76 $\mu\text{g}/\text{mL}$), *B. petersianum* (463.61 $\mu\text{g}/\text{mL}$) and *V. amygdalina* (865.58 $\mu\text{g}/\text{mL}$) showed active cytotoxic effect with LC_{50} more higher than another plant extracts. But, all plants extracts from this research showed the active potential activity

Toxicity testing results of crude extracts showed the percentage of *A. salina* larvae mortality increased along with the increase in concentration of the extract. The results reveal that crude ethanol plant extract of *M. beccarii* showed that the compound contained therein are active and possess a high bioactivity, which means that at low concentrations has toxic and lethal larvae of *A. salina*. *M. beccarii* has been reported to have high potential antioxidant activity (Dirgantara, 2013) with several active compounds such as flavonoid, tannin and triterpenoid/steroid (Dirgantara, 2015).

Results from this study indicate that while plant species with LC_{50} values < 1000 $\mu\text{g}/\text{mL}$ may used for local wisdom community, this study calls for further work aimed at isolating the cytotoxic compounds responsible for the observed activity and to search new compound for anticancer therapy.

4 CONCLUSION

From this research, *M.beccarii* showed the highest cytotoxic activity with LC_{50} 8.33 $\mu\text{g/mL}$ and indicated the possible potential use of medicinal plants from Papua as anticancer agents.

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