Acute toxicity of papaya leaf extract on *Artemia salina* Leach larvae

W H Nugrahaningsih¹,⁎, A Titi² and N K Dewi¹

¹ Biology Department, Mathematic and Natural Science Faculty of Universitas Negeri Semarang, Indonesia
² Student of Biology Department, Mathematic and Natural Science Faculty of Universitas Negeri Semarang, Indonesia

⁎Correspondence: nugrahaningsihwh@mail.unnes.ac.id

Abstract. Papaya leaf has long been used as a natural medicine. It is beneficial for curing malaria, enhancing appetite, removing acnes, boosting the quality and quantity of breast milk and healing toothache. Most of the using based on their empirical experience. A preliminary test to examine papaya leaf extract’s ability as a medicine needs to be conducted, and in order to do that, a safety test must be implemented to determine its toxicity value. This research aims to determine the value of LC₅₀ of papaya leaf aqueous extract. The Brine Shrimp Lethality Test (BSLT) method was conducted to determine acute toxicity. The 48 hours-old *Artemia salina* Leach larvae were observed for 24 hours in sea water mixed papaya leaf extract on concentrations: 0 µg/ml, 1.000 µg/mL, 2.000 µg/mL, 5.000 µg/mL and 10.000 µg/mL. The LC₅₀ value obtained was 88.5 mg/mL. According to BSLT, papaya leaf extract has potentially low toxicity on *Artemia salina* Leach larvae.

1. Introduction

Indonesians have been using medicinal herbs for a long time, especially the papaya. Papaya leaf extract is rich with secondary metabolite and substances such as Ca²⁺, Mg²⁺, Na⁺, K⁺, Cl⁻ and Li, potentially beneficial to cure many diseases [1]. The ethanolic extract of *C. papaya* has established as the anti-inflammatory of arthritis animal model [2]. The bioactive compounds (alkaloids, flavonoids and polyphenols) showed antinociceptive effect probably mediated centrally and peripherally; and involving mild anti-inflammatory mechanisms [3]. The potential of Carica papaya against pathogenic bacterial may be used for the treatment of gastroenteritis, urethritis, otitis media, typhoid fever and wound infections [4]. The leaves extract of C. papaya is promising as good larvicidal and pupicidal properties of against chikungunya vector, A. aegypti [5]. Papaya leaf and seed extract effective as larvicides against Anopheles larvae mortality too [6]. A case study reported increasing of platelet count of dengue fever patient [7,8] .

The development of phytopharmacca or the standardized herbal medicine should comply with safety requirements, beside effectiveness and has a standard dose of consumption. It is necessary to conduct scientific tests in the fields of pharmacology and toxicology. Seed and leaf were part of plant used to medicine. The previous study suggested the different effect of polar and nonpolar solvent to get the extract. A preliminary test on papaya leaf aqueous extract’s capacity as medicine must be performed by means of a safety test to determine its toxicity value. Mortality test using *Artemia salina* Leach. has been
proven to be an effective research tool. Regardless, brine shrimp test is an easier toxicity screening procedure. This method is simpler—a small amount of substances is already sufficient to conduct a micro-scale test. This method is easy to perform, affordable, fast and quite accurate, but only need to pay attention to the solvent factors [9]. This research aims to determine the LC$_{50}$ value based on the toxicity test of papaya leaf aqueous extract on *Artemia salina* Leach larvae.

2. Methods
The acute toxicity test was conducted in the Laboratory of Biological Animals’s Physiology of Universitas Negeri Semarang. The acute toxicity test was conducted by practicing the Brine Shrimp Lethality Test (BSLT). Consecutively, the following activities are parts of the research procedures: *Artemia salina* Leach. Larvae preparation, toxicity test using BSLT, and LC$_{50}$ analysis.

2.1 *Artemia salina* Leach larvae preparation
*A. salina* Leach. eggs and sea water with 30% salinity were provided by the Balai Besar Pengembangan Budidaya Air Payau (BBPBAP) of Jepara, Jawa Tengah, Indonesia. The *A. salina* Leach. eggs were hatched by soaking them in seawater within a petridish at room temperature with adequate lights. *A. salina* Leach. eggs eligible for the test were those sinking in salt water, while those floated were not used. The eggs hatched after being in larvae phase for 24 hours and those categorized as actively moving 48 hours-old larvae were chosen for the toxicity test [10].

2.2 Toxicity Test
The toxicity test of papaya leaf extract was conducted by BSLT modified how Meyer *et al.* did [11]. The first prepared 25 flacons, divided into five groups for five repeated of each concentration. The control group (K) were added papaya leaf extract solution in the seawater, with a concentration of 0 µg/mL until the final volume reached 5 mL. The concentration of *Carica papaya* leaf extract on experiment groups were 1,000 µg/mL (P1), 2,000 µg/mL (P2), 5,000 µg/mL (P3) and 10,000 µg/mL. The *A. salina* Leach larvae were added to flacon, ten larvae each flacon. The BSLT toxicity test was conducted for 24 hours at room temperature in a well-lit room. After 24 hours of test, *A. salina* Leach. Larvae were observed and the number of dead larvae in each concentration was counted. Finally, the mortality rate and the LC$_{50}$ value were determined.

2.3 LC$_{50}$ Analysis
The data obtained was analyzed by employing Probit Analysis with a confidence level of 95% to determine the LC$_{50}$ value. The LC$_{50}$ value was counted for the total number of dead 48 hours-old *A. salina* Leach. larvae within 24 hours after they were exposed to the test material. All analysis were conducted in Microsoft excel function.

3. Results and Discussion
The result of papaya leaf extract toxicity test on the 48 hours-old *A. salina* Leach. larvae is presented in Table 1. Total mortality rate was obtained by summing the number of dead *A. salina* Leach. larvae in each papaya leaf extract concentration, while the average larvae mortality was obtained by dividing the total mortality of larvae in each concentration with the number of replications conducted. Furthermore, the larvae mortality percentage was derived from the mortality average rate in each concentration.
The LC50 value obtained from papaya leaf extract toxicity test on A. salina Leach. larvae was 88.5 mg/mL. The lower limit is 15.8 mg/mL, while the upper limit is 1.275E+180 µg/mL.

Papayas are plants widely spread in tropical and several sub-tropical regions. It is commonly known that papaya leaves are good for health. Papaya leaf extract is rich with metabolite compounds, such as alkaloid, saponin, flavonoid and free terpenoid. The secondary metabolites found in the papaya leaf liquid extract were tannin, flavonoid, saponin, phenol, steroid and alkaloid [12]. The toxicity test using BSLT, which is a preliminary safety test performed on a certain medicine, produced insights on the LC50 value of papaya leaf extract when tested on A. salina Leach. larvae. The A. salina test may expedite toxicity experiments and decrease costs, and therefore, may be considered an alternative to the in vitro cell culture assay [13]. The result of BSLT can be function as a preliminary research for the separation of compounds having the potential to be toxic. The BSLT method uses the 48 hours-old A. salina Leach. larvae as test animals because they have characteristics similar with those of mammals, e.g. having the DNA-dependent RNA polymerase DNA. The thin skin of A. salina Leach. larvae sensitive to its environment, they are commonly used in toxicity tests.

The result of the toxicity test of papaya leaf extract on A. salina Leach. larvae showed the high value. In comparison with seed, the LC50 value of leaf extract was higher than seed. The 96-h LC50 of pawpaw seed powder to adult tilapia is 4.2 mg/L with 95% confidence limit of 31.86 – 93.81 mg/L [14].

In comparison with ethanolic extract, the LC50 of aqueous extract was lower. The high value of LC50 indicated the safety to consume. The similar study showed that papaya leaf ethanolic extract effective in killing larvae of Anopheles sp, LC50 value were 422.311 ppm, 1399.577 ppm (LC90) [6]. Another study suggested acute toxicity leaf extract at 2000 mg/kg BW administered orally to Sprague Dawley rats did not caused any death or acute adverse effect on the clinical observation and mortality [15]. Orally given for 28 days did not produce treatment related changes in body weight, food intake, water level, hematological parameters and serum biochemistry [16].

Solvent selection is based on the specific characteristics of the targeted bioactive compound [17]. Water is used as a solvent during this research’s extraction process because it is not easily evaporated, stable, not highly flammable and widely available. This research is very important because water solvent is very easy to use shall it is meant for immediate implementation within the community. Water solvent (polar) within an extract could dissolve alkaloid, triterpenoid, steroid, flavonoid, saponin and tannin compounds.

### Table 1. Number of dead A. salina Leach. larvae due to exposure to papaya leaf extract calculated using BSLT

| Replication number | Number of dead A. salina Leach. larvae in each papaya leaf extract concentration (µg/mL) |
|--------------------|------------------------------------------------------------------------------------------|
|                    | 0 µg/mL | 1000 µg/mL | 2000 µg/mL | 5000 µg/mL | 10000 µg/mL |
| 1                  | 0       | 0          | 0          | 3          | 1           |
| 2                  | 0       | 0          | 1          | 0          | 1           |
| 3                  | 0       | 0          | 0          | 1          | 0           |
| 4                  | 0       | 0          | 0          | 1          | 1           |
| 5                  | 0       | 0          | 0          | 3          | 1           |
| Total mortality rate | 0      | 0          | 1          | 8          | 4           |
| Average            | 0       | 0          | 0.2        | 1.6        | 0.8         |
| Mortality percentage | 0 %     | 0%         | 2%         | 16%        | 8%          |

Table 1. Number of dead A. salina Leach. larvae due to exposure to papaya leaf extract calculated using BSLT.
Metabolite compounds such as alkaloid, saponin and tannin were assumed to be the cause of digestive system disorder and cause poison in the larvae’s stomach. Bioactive compounds such as saponin and tannin might obstruct growth and trigger anti feedant mechanism. As toxic substances contained within the extract, bioactive compounds can enter the larva’s body through the mouth as they usually find food in the environment they live in. A larva would die because of its inability to detoxify harmful compounds that penetrate into its system.

The chemical compounds such as alkaloid, flavonoid and saponin within papaya leaf extract plays important roles in health. Saponin in papayas can heal wounds by boosting collagen production, an important protein for the healing process of wounds. The carpaine alkaloid is a distinct compound found in papayas, is toxic for microbes, and serve as a detox agent within the body. Flavonoid functions as an antioxidant, and as an antibiotic through its interfere with microorganism functions. Furthermore, it also serves as an antivirus for viruses such as HIV/AIDS and herpes.

Based on the LC50 value obtained from this research, papaya leaf extract cannot be used as an anti-cancer medicine. This is because the LC50 value in the research is above 1000 µg/ml. An extract is considered as having the potential to be used as an anti-cancer medicine if it has a toxicity with LC50 value lower than 1000 µg/mL. Meanwhile, for pure compounds, the value should be less than 200 µg/mL [11]. Researches on bioactive compounds had been conducted for the sake of human being’s health, ranging from researches on how they can be used as supplements to how they can be used as medicines. Based on the substances found within it, it can be concluded that papaya leaf extract studied in this research can be utilized to cure other diseases.

4. Conclusion

Carica papaya leaf widely used to prevent and cure against diseases. People eat the papaya leaf in way boiled, fresh, juice and infuse, those are use water as solvent. By the BSLT test, we concluded that aqueous extract of Carica papaya leaf had wide range dose in safety. The high value of LC50 implicated the potency of extract to prevent any disease included heal wounds and infection diseases. But, it is proven that the extract not potential to develop as anti-cancer medicine. The anti-cancer usually has low value of LC50.

References

[1] Vyas SJ, Khatri TT, Ram VR, Dave PN, and Joshi HS. 2014 Int. lett. nat. sci. 12 p 16.
[2] Owoyele BV, Adibukola OM, Funmilayo AA, and Soladoye AO 2008 Inflammopharmacology 16 p 168
[3] Anaga AO and Onehi EV 2010 Afr. J. Pharm. Pharmacol. 4 p140
[4] Nirosha N and Mangalanayaki R.2013 IJAPBC 2 p 473
[5] Kovendan K, Murugan K, Kumar AN, Vincent S, and Hwang JS 2012 Parasitol Res 110 p 669
[6] Sesanth H, Arsunan AA, and Ishak H. 2014. IJSRP 4 p 6
[7] Siddique O, Sundus A, and Ibrahim MF 2014 Pak Med Assoc 64 p 364
[8] Ahmad N, Fazal H, Ayaz M, Abbasi BH, Mohammad I, and Fazal L 2011 Asian Pac J Trop Biomed 1 (4) pp 330-333
[9] Wu C 2014 J Adv Pharm Technol Res 5 (1) pp 57-58
[10] Riisgård HU, Zalacáin D, Jeune N, Wiersma JB, Lüskow F, and Pleissner D 2015 J Crustacean Biol 35 (1) pp 650-658
[11] Karchesy YM, Kelsey RG, Constantine G and Karchesy JJ 2016 SpringerPlus 5 p 510.
[12] Bamisaye FA, Anjani EO, and Minari JB 2013 J. Med. Plants. Stud. 1 p 171
[13] Rajabi S, Ramazani A, Hamidi M, and Naji T 2015 J Pharm Sci 23
[14] Ayotunde EO and Ofem BO 2008 Afr. J. Biotechnol. 7 (13) pp 2265-2274
[15] Halim SZ, Abdullah NR, Afsan A, Abdul RBA, Jantan I, and Ismail Z 2011 J. Med. Plants Res 5 (xx) pp 1867-1872
[16] Afsan A, Abdullah NR, Halim SZ, Abdul R BA, Semail RHR, Abdullah N, Jantan I, Muhammad
H, and Ismail Z 2012 *Molecule* 17 (4) p 326

[17] Cos P, Vlietinck AJ, Berghe DV, and Maes L 2006 *J Ethnopharmacol* 106 p 290