

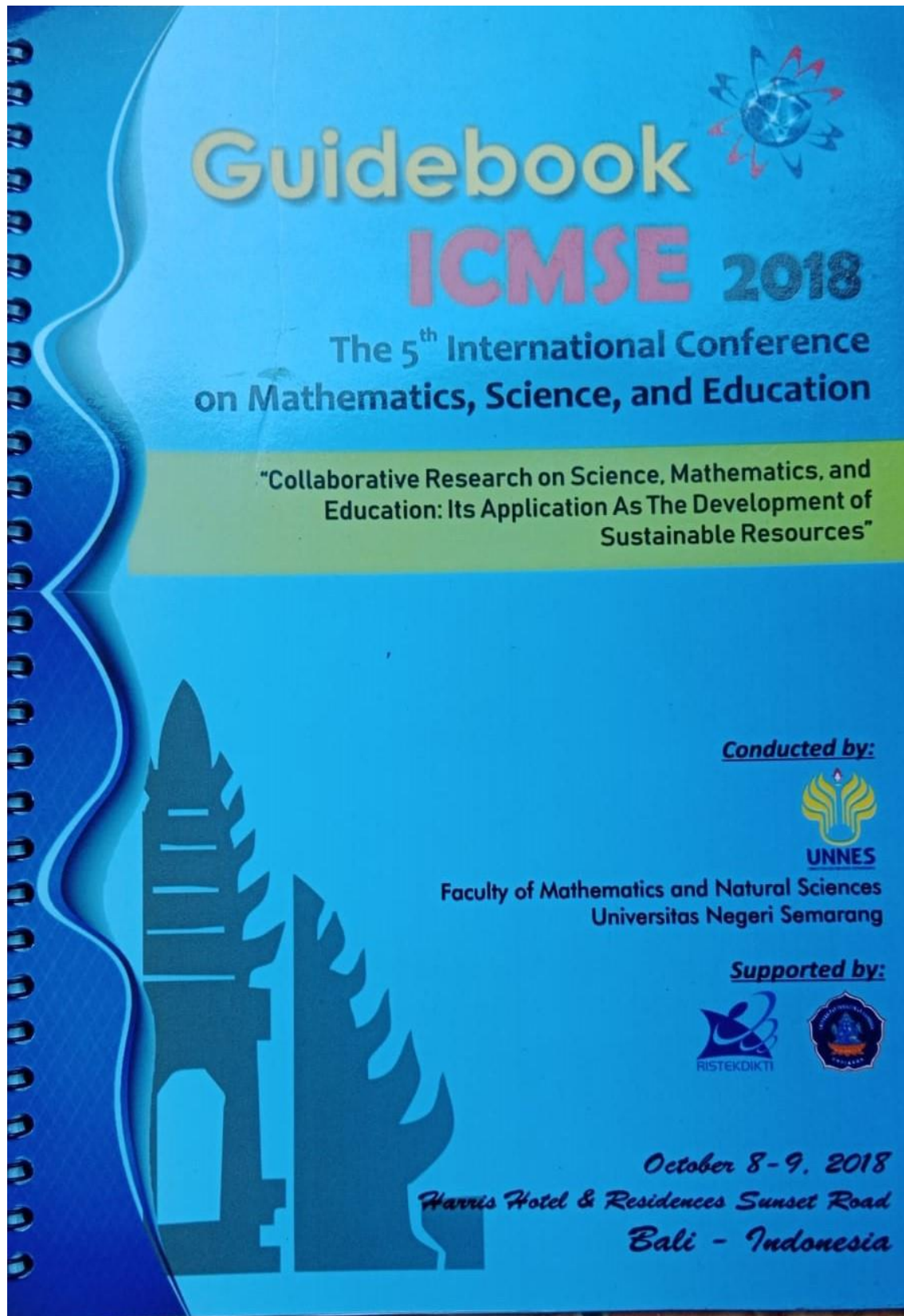
Judul Artikel: Acute toxicity of papaya leaf extract on Artemia salina leach larvae, Penulis: W H Nugrahaningsih1,* , A Titi2 and N K Dewi1, Nama Seminar/Konferensi/Simposium: 5th International Conference on Mathematics, Science and Education 2018 (5th ICMSE2018), Penyelenggara Seminar/Konferensi/Simposium: UNNES, Waktu Pelaksanaan Seminar/Konferensi/Simposium: 8–9 October 2018, ISBN/ISSN: 17426588, 17426596

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Komentar dari Reviewer : *Penulis 1/3 Sesuai bidang keilmuan Bahasa dan sistematika tulisan baik, IMRADC ada dan jelas, unsur kelengkapan sesuai kaidah ilmiah Informatif mengenai toksisitas akut daun pepaya Terbitan prosiding terindeks Scopus dengan SI 11% dimohon **melampirkan cover, daftar isi, bukti korespondensi, ethical approval, sertifikat pembicara/daftar hadir, tidak dinilai dulu***

Yth Penilai PAK
Jabatan Fungsional

Artikel berjudul Acute toxicity of papaya leaf extract on Artemia salina leach larvae merupakan hasil penelitian untuk menguji ekstrak daun pepaya terhadap larva Artemia salina. Larva Artemia salina merupakan jenis udang-udangan yang termasuk dalam invertebrate, dan juga bukan merupakan host/agen dari suatu penyakit sehingga tidak memerlukan ethical approval. Berikut kami lampirkan cover dan daftar isi, bukti korespondensi dan sertifikat presenter.



Guidebook

ICMSE 2018

The 5th International Conference
on Mathematics, Science, and Education

“Collaborative Research on Science, Mathematics, and Education: Its
Application As The Development of Sustainable Resources”

Organized by:



Faculty of Mathematics and Natural Sciences
Universitas Negeri Semarang

Supported by:



October 8-9, 2018
Harris Hotel & Residences Sunset Road
Bali - Indonesia

**The 5th International Conference on Mathematics, Science, and
Education (ICMSE 2018)**

Time (WITA)	Event
07.00-08.30	Registration
08.30-09.30	Opening Ceremony & The Address of Rector Universitas Negeri Semarang Prof. Dr. Fathur Rokhman, M.Hum.
09.30-10.00	Speech of Minister of Research, Technology and Higher Education of Republic of Indonesia Prof. H. Mohamad Nasir, Ph.D.
10.00-10.15	Coffee Break
	Plenary Session
10.15-11.45	Prof. Dominic Reeve, Ph.D. Coastal Engineering & Chartered Mathematics, Swansea University, United Kingdom Assoc. Prof. Dr. Zaiton Abdul Majid Physical Chemistry – Universiti Teknologi Malaysia, Malaysia Assoc. Prof. Dong Fengming, Ph.D. Mathematics – National Institute of Education, Nanyang Technological University, Singapore Moderator: Cepi Kurniawan, M.Si., Ph.D.
11.45-12.30	Editorial Information for Publication
12.30-13.30	Lunch Break
13.30-17.20	Paralel Session

Parallel Session DAY 1, October 8th 2018

Rooms	R-01	R-02	R-03	R-04	R-05	R-06	R-07	R-08	R-09	R-10	R-11	R-12	R-13
Chairman	Ch-01	Ch-02	Ch-03	Ch-04	Ch-05	Ch-06	Ch-07	Ch-08	Ch-09	Ch-10	Ch-11	Ch-12	Ch-13
13.30-13.50	M-01	M-16	M-31	M-46	M-61	SE-01	SE-16	SE-31	P-01	P-16	C-01	B-01	CS-01
13.50-14.05	M-02	M-17	M-32	M-47	M-62	SE-02	SE-17	SE-32	P-02	P-17	C-02	B-02	CS-02
14.05-14.20	M-03	M-18	M-33	M-48	M-63	SE-03	SE-18	SE-33	P-03	P-18	C-03	B-03	CS-03
14.20-14.35	M-04	M-19	M-34	M-49	M-64	SE-04	SE-19	SE-34	P-04	P-19	C-04	B-04	CS-04
14.35-14.50	M-05	M-20	M-35	M-50	M-65	SE-05	SE-20	SE-35	P-05	P-20	C-05	B-05	CS-05
14.50-15.05	M-06	M-21	M-36	M-51	M-66	SE-06	SE-21	SE-36	P-06	P-21	C-06	B-06	CS-06
15.05-15.20	M-07	M-22	M-37	M-52	M-67	SE-07	SE-22	SE-37	P-07	P-22	C-07	B-07	CS-07
15.20-15.35	M-08	M-23	M-38	M-53	M-68	SE-08	SE-23	SE-38	P-08	P-23	C-08	B-08	CS-08
15.35-15.50	M-09	M-24	M-39	M-54	M-69	SE-09	SE-24	SE-39	P-09	P-24	C-09	B-09	CS-09
15.50-16.05	M-10	M-25	M-40	M-55	M-70	SE-10	SE-25	SE-40	P-10	P-25	C-10	B-10	CS-10
16.05-16.20	M-11	M-26	M-41	M-56	M-71	SE-11	SE-26	SE-41	P-11	SE-46	C-11	B-11	M-107
16.20-16.35	M-12	M-27	M-42	M-57	M-72	SE-12	SE-27	SE-42	P-12	SE-47	C-12	B-12	M-108
16.35-16.50	M-13	M-28	M-43	M-58	M-73	SE-13	SE-28	SE-43	P-13	SE-48	C-13	C-16	M-109
16.50-17.05	M-14	M-29	M-44	M-59	M-74	SE-14	SE-29	SE-44	P-14	SE-49	C-14	C-17	M-110
17.05-17.20	M-15	M-30	M-45	M-60	M-75	SE-15	SE-30	SE-45	P-15	SE-50	C-15	C-18	M-111

Parallel Session DAY 2, October 9th 2018

Rooms	R-01	R-02	R-03	R-04
Chairman	Ch-14	Ch-15	Ch-16	Ch-17
08.00-08.15	SE-51	SE-67	M-76	M-91
08.15-08.30	SE-52	SE-68	M-77	M-92
08.30-08.45	SE-53	SE-69	M-78	M-93
08.45-09.00	SE-54	SE-70	M-79	M-94
09.00-09.15	SE-55	SE-71	M-80	M-95
09.15-09.30	SE-56	SE-72	M-81	M-96
09.30-09.45	SE-57	SE-73	M-82	M-97
09.45-10.00	SE-58	SE-74	M-83	M-98
10.00-10.15	SE-59	SE-75	M-84	M-99
10.15-10.30	SE-60	SE-76	M-85	M-100
10.30-10.45	SE-61	SE-77	M-86	M-101
10.45-11.00	SE-62	SE-78	M-87	M-102
11.00-11.15	SE-63	SE-79	M-88	M-103
11.15-11.30	SE-64	SE-80	M-89	M-104
11.30-11.45	SE-65		M-90	M-105
11.45-12.00	SE-66		M-112	M-106

Chairman and Co-Chairman

DAY	Code Room	Name	DAY	Code Room	Name
DAY 1	Ch-01 R-01	Chairman <i>ex officio</i>	DAY 1	Ch-10 R-10	Chairman: Ida Bagus Putu Mardana
		Co-Chairman: Adi Nur Cahyono			Co-Chairman: Muhammad Zuhair Zahid
	Ch-02 R-02	Chairman <i>ex officio</i>		Ch-11 R-11	Chairman: I Wayan Muderawan
		Co-Chairman: M Kharis			Co-Chairman: Nanik Wijayanti
	Ch-03 R-03	Chairman: I Gusti Nyoman Yudi Hartawan		Ch-12 R-12	Chairman: Ida Ayu Putu Suryanti
		Co-Chairman: Arief Agoestanto			Co-Chairman: Endah Peniati
	Ch-04 R-04	Chairman: Ratih Ayu Apsari		Ch-13 R-13	Chairman: I Nyoman Sukajaya
		Co-Chairman: Amidi			Co-Chairman: Putriaji Hendikawati
	Ch-05 R-05	Chairman <i>ex officio</i>		DAY 2	Ch-14 R-01
Co-Chairman: Ardhi Prabowo		Co-Chairman: Margareta Rahayuningsih			
Ch-06 R-06	Chairman <i>ex officio</i>	Ch-15 R-02	Chairman <i>ex officio</i>		
Co-Chairman: Andreas Priyono Budi Prasetyo		Co-Chairman: Langlang Handayani			
Ch-07 R-07	Chairman: I Wayan Mudianta	Ch-16 R-03	Chairman: I Wayan Puja Astawa		
	Co-Chairman: Ella Kusumastuti		Co-Chairman: Adi Nur Cahyono		
Ch-08 R-08	Chairman <i>ex officio</i>	Ch-17 R-04	Chairman <i>ex officio</i>		
Co-Chairman: Aji Purwinarko			Co-Chairman: Endang Sugiharti		
Ch-09 R-09	Chairman: Supriyadi				
	Co-Chairman: Suharto Linuwih				

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B-02	The Effects of Temperature and Acidity on Soybean Extraction to Obtain Highest Concentration of Cysteine <i>Dewi Mustikaningtyas, Sri Widyarti, Muhaimin Rifa'i, and Nashi Widodo</i>
B-03	Influence of Variation Concentration of Green Dry Mushroom (<i>Trichoderma harzianum</i>) on Making of Waste Composting Sawdust <i>Ibnul Mubarak</i>
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B-05	Acute Toxicity of Papaya Leaf Extract on <i>Artemia salina</i> Leach. Larvae <i>Nugrahaningsih W H, Titi Alfath, Nur Kusuma Dewi</i>
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B-09	Tempe yogurt: an alternative and more preferable plant derived yogurt with high nutritional and antioxidant value <i>Siti Harnina Bintari, Sarjana Parman</i>
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Korespondensi

Tanggal	Kegiatan
25 Juli 2018	Submit abstrak melalui sistem
4 Agustus 2018	Abstrak accepted
8-9 Oktober 2018	Pelaksanaan conference
18 Oktober 2018	Upload fulltext
20 Januari 2019	Hasil review diunduh
24 Januari 2029	Upload hasil revisi

History submit abstrak

The screenshot shows a web browser window with the URL `icmse.unnes.com/registration_new/index.php?mod=author&content=abstract_list`. The page displays the author's submission history for Paper ID B-1200. The submission process is shown as a vertical timeline with four steps:

- 1. Abstract Submission**: Completed on 25 Jul 2018 14:17:47. Includes a file icon and an "update file" button.
- 2. Abstract Review**: Includes a "Review history" section with a "Reviewer:" box containing the text: "Please adjust the format according to the template of IOP Proceeding" and "accepted, 04 August 2018 22:38:56".
- 3. Decision: Accepted**
- 4. Payment**: Includes a link for "Payment information click here".

On the right side, there is a "Registration" section with the following details:

- Type Author: Speaker
- Publication: Journal of Conference Series (IOP)
- Buttons: "Edit" and "Delete B-1200"

The left sidebar contains navigation options: Home, Submission (1), Co-Author, Payment, and Template.

History submit fulltext

The screenshot shows the submission interface for paper B-1200. The paper title is "Acute Toxicity of Papaya Leaf Extract on *Artemia salina* Leach, Larvae". The submission history table is as follows:

#	User	Date	Activity	Score
1	author	18 Oct 2018 07:18:34	Nugrahaningsih WH submit fullpaper	0.00 B
2	reviewer	20 Jan 2019 22:31:47	General comment: in general, articles are good enough, some things that need to be improved, especially the result and discussion section	Review result
3	author	24 Jan 2019 09:29:49	Nugrahaningsih WH revise fullpaper	0.00 B

The status of the submission is "Under reviewed".

Hasil Review

The screenshot shows a Microsoft Word document titled "1_B-1200_rev-Nugrahaningsih-WH-vSuVf&BjP.C.docx" in Protected View. The document content is as follows:

Acute Toxicity of Papaya Leaf Extract on *Artemia salina* Leach, Larvae

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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 88507.768 µg/ml. According to BSLT, papaya leaf extract has potentially low toxicity on *Artemia salina* Leach larvae.

Keywords: Acute toxicity; *Artemia salina*; BSLT; *Carica papaya*

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 anticancer effect probably mediated centrally and peripherally; and

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. Furthermore, this method is easy to perform, affordable, fast and quite accurate [9]. This research aims to determine the LC₅₀ value based on the toxicity test of papaya leaf aqueous extract on *Artemia salina* Leach larvae.

2. Material and 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 LC₅₀ 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/Center for Brackish Water Culture Development) 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 LC₅₀ value were determined.

2.3. LC₅₀ Analysis

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What does it mean?

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.

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

Replication number	Number of dead <i>A. salina</i> 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

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Results and discussion

Total mortality rate	0	0	1	8	4
Average	0	0	0.2	1.6	0.8
Mortality percentage	0 %	0%	2%	16%	8%

The LC₅₀ value obtained from papaya leaf extract toxicity test on *A. salina* Leach. larvae was 88,507.768 µg/mL. The lower limit is 15,822.484 µg/mL, while the upper limit is 1,275E+180 µg/mL.

4. Discussion

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 papawpaw 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 (LC₅₀) [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 (leaf) with an extract could distribute the bioactive substances.

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 carpine 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 antiviral 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.

5. 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.

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