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The Toxicity of *n*-Hexane, Ethyl Acetate and Ethanol Extracts of SarangBanua (*Clerodendrumfragrans* Vent Willd) Leaves by Brine Shrimp Lethality Test (BSLT) Method

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Abstract.Sarangbanua traditional medicinal plant is found in Simalungunand NorthTapanuli Regencies, Sumatera, Indonesia. The result of plant determination, sarangbanuais *Clerodendrumfragrans* Vent Willd, including the Verbenaceae family.This study aims to determine the toxicity of the three types of leaf extracts of *C. fragrans* Vent Will. The Brine Shrimp Lethality Test (BSLT) method was used to determine the toxicity of the extracts. The mortality data were then analyzed by Probit SAS to obtain LC_{50} values.The results showed that the three types of *C. fragrans* leaf extracts had LC_{50} values below 1000 µg/mL, so they were classified as toxic and potentially bioactive. The $LC5_0$ values of each of the ethanol, ethyl acetate and ethanol extracts were 26.25; 37.50 and 41.97 µg/mL**Keywords:** *Clerodendrumfragrans* Vent Willd, toxicity, brine shrimp lethality test (BSLT), Indonesian medicinal plants.

1. Introduction

The high biodiversity of Indonesian plant hasthe potential to be developed as raw material for natural medicine. One of the plants that have been used by the people of the Simalungun area, Sumatera as traditional medicine is the sarangbanua. Sarangbanuais *Clerodendrumfragrans* Vent Willd, including the Verbenaceae family according to Herbarium Botani LIPI Cibinong, in June, 2017.This plant has been used as a traditional medicinal plant for stomach aches, fever, high blood pressure medicine, and diabetes.

Secondary metabolites alkaloids, triterpenoids, flavonoids, saponins, tannins, quinones are present in the ethanol extract of *C. fragrans* leaves.The alkaloids, steroids, saponins, tannins are present in ethylacetate extracts and in the extract *n*-hexane there are alkaloids, triterpenoids, flavonoids [1]. These secondary metabolites found in *C. fragrans*are potential for these plants to become medicinal plants. Another plant in the same family is the *C fragrans* namely*Clerodendrumphilippinum*Schauerhas antibacterial activity because it contains relatively high secondary metabolites [2].

Plant secondary metabolites can be obtained by extraction using solvent. Ethanol, ethyl acetate and *n*-hexane solvents have different dielectic constant (20^0C) values, namely 22.40; 6.02 and 1.90 which causes the difference in the polarity of the three solvents.The ethanol, ethyl acetate and *n*-

hexane solvents respectively are referred to as polar, polar and non-polar solvents. [3]. The choice of solvent for the extraction process is based on the solubility principle "like dissolve like". The nonpolar compounds dissolve only in nonpolar solvents and vice versa.The choice of solvent in the extraction process is an important factor[4-6]. The secondary metabolites found in *C. fragrans*are potential for *C. fragrans*as medicinal plants.

The Brine Shrimp Lethality Test (BSLT) is a method to determine the bioactivity of a compound from natural ingredients**.** Larvae *Artemiasalina*L is widely used for environmental studies, toxicity, and screening of bioactive compounds from plant extracts [7-8]. The BSLT test has a spectrum of pharmacological activity that is easy to perform, simple, fast, and does not require large costs with a 95% confidence level. The toxicity of compounds is expressed by the LC_{50} value. The LC_{50} value is an indication of the concentration of compounds that cause shrimp larvae death to 50% of the population. A sample is said to be toxic if it has a value of LC_{50} <1000 µg/ml. The BSLT test can be used as a sedative, toxicity, insecticide test, and as a preliminary test for cytotoxic or anti-cancer compounds [9]. By using BSLT method, of 30 medicinal plants traditionally used in Bukota Tanzania, as many as 28 plants are safe to use with LC_{50} valuesbetween 30-100 µg/mL [10].

Based on the description above, the authors were interested in testing the toxicity of *C. fragrans* leaf extracts which were extracted using ethanol, ethyl acetate and *n*-hexane as solvents.This study aims to determine the toxicity of secondary metabolites contained in *C. fragrans*leafextract as the basis to the development of *C. fragrans* plants as raw material herbal medicine.

2. Methods

2.1. Preparation of plant samples

The 4.1 kg of fresh leaves of *C. fragrans* were taken from the Simalungun area, North Sumatra.The leaves are separated from the stems, washed, drained, and dried in the room. The dry leaves were mechanically milled to obtain a crude leaf of powder *C. fragrans*as much as 520 g.

2.2. *Plant extract preparation.*

The 0.5 kg of *C.fragrans* leaf simplicia powder was extracted with *n*-hexane for 48 hours, then filtered and concentrated with a rotary evaporator. Part of the waste is macerated with ethyl acetate solvent for 48 hours, filtered and concentrated, obtained ethyl acetate extract concentrated. Part of the waste is extracted again with ethanol for 48 hours, filtered, and concentrated to obtain concentrated ethanol extract. The extraction process is carried out by maceration.

2.3. Toxicity brine shrimelethality test (BSLT)

The three types of *C. fragrans* leaf extracts were tested for their toxicity using the BSLT method with the following activity stages :

2.3.1.Culture of shrimp larvae.A total of 50.0 mg of eggs is*Artemiasalina*Leach put into a hatchery filled with filtered seawater. After being aerated and irradiated with a 20 watt TL lamp for 24 hours, the eggs that have hatched into *nauplii*can be used as test animals.

*2.3.2.Test sample preparation.*As much as 40.0 mg of each extracted sample was dissolved in seawater to 20.0 mLin order to obtain a sample solution of 2000 µg/mL.The samples of *n*-hexaneand ethyl acetate extracts which were rather difficult to dissolve in seawater. The extracts were added with 1% dimethyl sulfoxide (DMSO) of 1.0 µL. A total of 2 mL of sample solution of 2000µg/ml was diluted to 20 mL in order to obtain a sample solution of 200 µg/mL.The 2 mL of sample solution of 200 µg/mL was diluted to 20 mL and a sample solution of 20 µg/mL concentration was obtained.

2.3.3. Test implementation. A total of 5.0 mLof the test sample from each concentration was put into a container that had diaries of 10 mL. Then, seawater was added to the container containing 10 shrimp

larvae that were 2 days old, and seawater was added to 10 mL, so that the final sample concentrations in each container were 1000, 100, and 10 µg/mL. Each concentration was made three times (triple). The experiment container was placed under sufficient light for 24 hours.

2.3.4. Experimental data analysis. Observation of the number of *Artemiasalina*of dead from each sample concentration was carried out after 24 hours of the experiment. The mortality or mortality rate (%) was calculated by comparing the number of *Artemiasalina*that died with the total number of *Artemiasalina*tested. LC_{50}value is calculated by plugging the concentration and probit logs into the regression equation line. A substance is said to be active or toxic if the LC_{50} value is<1000 µg/mL. The LC_{50} value iscalculated by entering the log of concentration and probit into the regression equation line [8].

3. Results and discussion

The toxicity test results of leaf extract *C. fragrans*using the BSLT method are presented in Table 1. The results of the toxicity test showed that giving ethanol extract caused the highest mortality rate of the tested animals (100%), followed by ethyl acetate extract (96.67%) and *n*-hexane extract (93.30%). (Table 1).

Table 1. Toxicity test results of leaf extract *C. fragrans*with BSLT method

No	Sample Test	Extract Concentration (µg/mL)	Number of Dead Larvae				Mortality (%)	LC_{50} (µg/mL)
			I	II	III	Total		
1	Extract of *n*-hexane leaves *C fragrans*	10	2	2	3	7	23.33	41.97
		100	8	9	9	26	86.67	
		1000	9	9	10	28	93.30	
		Control	0	0	0	0	0	
2	Extract of Ethyl Acetate leaves *C fragrans*	10	2	3	3	8	26.67	37.50
		100	9	8	9	26	86.67	
		1000	9	10	10	29	96.67	
		control	0	0	0	0	0	
3	Extract of Ethanolleaves *C fragrans*	10	3	3	4	10	30.00	26.25
		100	8	9	9	26	86.67	
		1000	10	10	10	30	100.00	
		control	0	0	0	0	0	

Note: The total number of tested animals are 30 animals.

The high mortality rate causes the LC_{50} value of the ethanol extract to be the lowest, namely 26.25 µg/ mL.This is probably because ethanol as a polar solvent easily attracts the active compounds contained in the sample, such as phenolic compounds, alkaloids, and terpenoids,were toxic to the zological system. Secondary metabolites flavonoids, triterpenoids, alkaloids, quinones and saponins are found in the ethanol extract of *C. fragrans*.

Plant extracts that had an LC_{50} value of less than 20 µg/mL showed anticancer properties[9]. The BSLT test results of the ethanol extract of rantihitamfruit *(S. blumei*) which have an LC_{50} value of 21.10 µg/mL [12] have an anticancer activity of leukemia cells L_{1210} [13] and contain the alkaloid steroid glycoside β2-solanine compound [14]. The BSLT test results of methanol/water extract (70/30) from another plant of the genus Clerodendrum in Kenya, namely *Clerodendrummyricoides,* had lower mortality than *C. fragrans*. At a sample of *C. myricoides*10 µg/mL had a 16% mortality, at 1000 µg/ mL it had a 76% mortality. The LC_{50} value of *C. myricoides* was 204.66 <1000 (µg/mL) and the plant

was potentially bioactive [15]. Meanwhile, oral administration of *C. capitatum* extract to Wistar rats did not show hematological and biochemical side effects with an LD50 of 5g/Kg BW[16].

A substance is said to be active or toxic when the LC_{50} value<1000 (µg/mL).Based on this, the three *C. fragrans* leaf extracts were toxic and potentially bioactive with LC_{50}values of 26.25 µg/mL (ethanol extract), 37.50 µg/mL (ethyl acetate extract) and 41.97 µg/mL (*n*-hexane extract).The LC_{50} valuesof the three types of leaf extracts *C. fragrans*were in the range less than 1000 µg/mL, so the three extracts had the potential to be bioactive.

4. Conclusion

The leaf extracts*C fragrans*are toxic and potentially bioactive with LC_{50} values of 26.25 µg/mL (ethanol extract), 37.50 µg/mL (ethyl acetate extract) and 41.97 µg/mL (*n*-hexane).The extracts of *C. fragrans*has the highest toxicity with an LC_{50} valueof 26.25 µg/mL compared to the ethyl acetate extract (LC_{50} 37.50 µg/mL) and the extract *n*-hexane (LC_{50} 41.97 µg/mL). The LC_{50} valuesof the three types of leaf extracts *C. fragrans*were in the range less than 1000 µg/mL, so the three extracts had the potential to be bioactive. Leaf extract *C fragrans* have the potential to be developed as herbal medicine and it is necessary to research the isolation of bioactive compounds from leaf extract *C. fragrans* to develop the potential of Indonesian medicinal plants.

References

[1] Simorangkir M, Nainggolan B and Silaban S 2018 Secondary metabolites phytochemical analysis of *n*-hexane, ethyl acetate and ethanol extracts of Sarang banua (*Clerodendrumfragrans* Vent Willd) leaves, *AISTSSE,* October 18-19, Medan, Indonesia.

[2] Venkatanarasimman B, Rajeswari T and Padmapriya B 2012 Antibacterial potential of crude leaf extract of *Clerodendrum philippinum* Schauer*International Journal of Pharmaceutical & Biological Archives* **3** 2 307-310

[3] Smallwood M 1996 *Handbook of Organic Solvent Properties* John Wiley & Sons Inc, New York, pp 7, 65, 227

[4] Zang Q 2015 Effects of extraction solvents on phytochemicals and antioxidant activities of Walnut (*Juglans regia* L.) green husk extracts *European Journal of Food Science and Technolog***3** 5 15-21

[5] Widyawati PS, Budianta TDW, Kusuma FA and Wijaya EL 2014 Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indicia* Less leaves extracts *International Journal of Pharmacognosy and Phytochemical Research* **6** 4 850-855.

[6] Rajagukguk J, Simamora P, Saragih CS, Abdullah H, Gultom NS, Imaduddin A. Superparamagnetic Behaviour and Surface Analysis of Fe3O4/PPY/CNT Nanocomposites. *Journal of Nanomaterials*. 2020 Oct 22;2020.

[7] Wahyuono S dan Rachman A 1995 Uji toksisitas beberapa tumbuhan obat Indonesia dengan b*rine shrimp lethality test* (BSLT) *Majalah Farmasi Indonesia***6** 4 108-112

[8] Sunaryono S, Chusna NM, Mufti N, Munasir M, Rajagukguk J, Taufiq A. Investigation of magnetic properties and anti-microbial activity of Mn0. 25Fe2. 75O4/Ag composites. *AIP Conference Proceedings* 2020,. **2251**, **(1)**, p. 040001). AIP Publishing LLC.

[9] Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nicholas DE and Mclaughlin JL 1982 Brine shrimp : a conventient general bioassay for active plant constituents *Planta Medika* **45** 31– 42

[10] Moshi MJ, Innocent E, Magadula JJ, Otieno DF, Weisheit A, Mbabazi PK and Nondo RSO 2010Brine shrimp toxicity of some plants used as traditional medicines in Kagera region, north western Tanzania *Tanzania Journal of Health Research***12** 1 1-6

[11] Moshi MJ, Mbwambo ZH, Nondo RSO, Masimba PJ, Kamuhabwa A, Kapingu MC, Thomas P and Richard M 2006 Evaluation of ethnomedical claims and brine shrimp toxicity of some plants used in Tanzania as traditional medicines *Africn*

Journal of Traditional, Complementary and Alternative Medicines **3** 48 - 58
[12] Simorangkir M, Surbakti R, Barus T dan Simanjuntak P 2014 Toksisitas ekstrak *n*-heksana, etil asetat dan etanol dari buah ranti hitam (*Solanum blumei* Nees ex Blume) dengan metode *brine shrimp lethality test* (BSLT) *Prosiding Seminar Nasional Kimia Pascasarjana Universitas Sumatera Utara*, 2014, Medan, Indonesia 226-229
[13] Simorangkir M, Silaban S, Surbakti R, Barus T and Simanjuntak P 2017 Aktivitas antikanker ekstrak etanol buah ranti hitam (*Solanum blumei* Nees ex Blume) terhadap sel leukimia L_{1210} *Chimica et Natura Acta* **5** 1 31-35
[14] Simorangkir M, Barus T, Surbakti R and Simanjuntak P 2016 Isolation and toxicity of steroidal alkaloid glycoside from fruits of ranti hitam (*Solanum blumei* Nees ex Blume) *Asian Journal of Chemistry***28** 1 203-206
[15] Kamanja IT, Mbaria JM, Gathumbi PK, Mbaabu M, Kabasa JD, Kiama SG 2018 Cytotoxicity of selected medicinal plants extracts using the brine shrimp lethality assay from Samburu county, Kenya *The Journal of Medical Research* **4** 5 249-255
[16] Idoh K, Agbonon A, Potchoo Y and Gbeassor M 2016 Toxicological assessment of the hydroethanolic leaf extract of *Clerodendrum capitatum* in Wistar rats *Pan African Medical Journal* **24**pp 1-13

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