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Effective Fraction of n-Hexane and Identification of Active Larvasida from Sirzak *(Annona muricata.* linn*)* Due to Larva of *Aedes aegypti*

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ABSTRACT

Effective fraction of *n*-hexane and identification of active larvasida from zuurzak (*Amonia muricata*, Linn) was due to larva of *Aedes aegypti* as infector of yellow fever. Extraction was carried out with maser-action method by using ethanol solvent. Two kilograms maser-acted result of zuurzak seed dry pollen produced 189.8 g of ethanol extract. Initial test showed that ethanol extract of active zuurzak seed was as agent of larva ide with LC_{50} of 244.27 ppm. Python-chemical test presented that extract of zuurzak seed contained of secondary metabolite included group of sapponin, alkaloid, and titerpenoid. The ethanol extract was suspension into the mixture of ethanol- water (7 : 3), then it was succession with *n*-hexane, ethyl-acetate, and *n*-buthanol. Evaporation of solvent from each extract was produced 14.7 kg extract of n-hexane fraction, 0.8 g extract of ethyl acetate fraction, and 4,6 g extract of *n*-buthanol fraction. Toxicity test of bio-larva ide was carried out to mosquito larva of *Aedes aegypti* in star 3-4 with variation of concentration (10, 100, 500, 1nd 100 ppm) with 3 times return during 24 hours of observation to the three fractions. The results was n-hexane fraction with LC_{50} of 73.77 ppm, ethyl acetate fraction with LC_{50} (value of dead concentration) of 340.71 ppm, and *n*-buthanol fraction with LC_{50} of 725.18 ppm. Activity test on larva of *Aedes aegypti* presented that *n*-hexane was effective and had the highest toxicity with LC_{50} of 73.77 ppm. Analysis and identification of *n*-hexane fraction with GC-MS method showed that there were 9 peaks of compound which 3 of them were dominant with relative retention time was close and had high enough of abundance percentage which was as the compound group of organic fatty acid such as methyl palmitate, methyl linoleic, and methyl oleate.
Keywords: fraction, identification, larvasida, *Aedes agypti*, *Annona muricata*. Linn

INTRODUCTION

Manado city was as an endemic area of yellow fever. There was increasing cases on the year of 2010 whereas 998 dead cases with 25 death and 2.5% of CFR. Comparing with January to April 2010 which there were only 832 cases, it was seemed a sharp enough increasing of cases. Hence, this disease could be any time changed and it would suddenly increase [1]. Nowadays, it had not found the virus killing vaccine of yellow fever. One of the preventives of infection was by controlling and fighting the vector for cutting the transmissions of disease [2].

Aedes aegypti was a main vector of yellow fever in Indonesia. *Aedes aegypti* was also disturbed human life because the hen mosquito bit and sucked blood mainly during 08.00 – 12.00 am and 03.00 – 05.00 pm [3]. These mosquitoes were life domestically. It was meaned that they preferred to live indoor than outdoor [4]. Potency places for developing larva were water storage like drum, barrel, and bath pool. The other places were place of animal drinking, flower vase, ant places, and water storage of used things [2]. The method for fighting yellow fever which developed byWHO, was the same as malaria. It was carried out by exterminating infection domain of mosquito larva [5].

Nowadays, research about bio-active compound in family of *annonaceae* had been developed. One of *annonaceae* family which had been observed about active compound percentage was named as *Annona muricata* Linn. This type was popular as local zuurzak. This vegetation could be used as traditional and insecticide food [6]. Zuurzak was included insecticide compound and it was observed with different procedure it would be produced the different active compound too.

Rupprecht *et. al*. [7] had isolated asetogenin of *annonaceae* family and he expressed that ethanol extract was active. Rieser *et al*. [8] also reported that a compound producted by isolation and elusidation tructure of zuurzak seed was named as muricasatine and the result of methanol extract was active pesticide and anti tumor. Soediro [9] had isolated the compound of *annonasin* and *annonasinon* from endosperm of zuurzak seed. It produced active extract of ethyl acetat due to larva of shrimp and had activity as anti cancer. Extract of zuurzak seed had potency to hold the growth rate of *Heliothis (helicoverpa) armigera* larva. Vegetation pesticide of

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zuurzak leaf was effective to control pest trip. It was also effective to control brown locuse. Vegetation pesticide of zuurzak leaf and tobacco were effectve used to control grasshoppers and carterpilars.

MATERIALS AND METHODS

Materials

Chemical materials used in this research were ethanol, *n*-hexane, ethyl acetate, *n*-buthanol, zuurzak seed [9], larva of *Aedes aegypty*.

Tools

Tools used in this research were blender, analytical balance, beker glass, separated funnel, Erlenmeyer, micro pipette, vacuum cycle evaporator, desiccator, drop pipette, test tube, watch glasses, and GC-MS.

Work procedure

The main material used in this research was old zuurzak seed (*Annona muricata*.Linn). These seeds were collected from household and restaurant at Manado city and it was the side result of zuurzak juice. Preparation of research material was included determination of vegetation, collecting of zuurzak seed, cleaning, drying by windings (not under direct sun shine), and grinding zuurzak seed with blender.

Two kilograms of zuurzak seed in the form of pollen was extracted by macerating during 1 x 24 hours using ± 15 l of ethanol until the whole components was extracted. Production of ethanol extract was evaporated with vacuum cycle evaporator until it became coagulated. Coagulated extract ethanol was solution with 200 ml of ethanol-water with comparison of 7 : 3. Then, extract of ethanol-water was de-fractionated with *n*-hexane (3 x 200 ml) so that was produced extract of ethanol-water and extract of *n*-hexane. Extract of ethanol-water was evaporated until the whole ethanol evaporated and then the rest extract of water was de-fractionated sequencely with 3 x 200 ml of ethyl acetate and 3 x 200 ml of *n*-buthanol. Each extract such as *n*-hexane, ethyl acetate, and *n*-buthanol was tested biologically due to larva of *Aedes aegypti*, but extract of water was not carried out for this test.

Media of larva of *Aedes aegypti* was made by filling plastic dish with water and inside wall was lining with filter paper. Filter paper was functioned as the place of hen mosquito for sticking its egg. Then sticked eggs at filter paper were dried on room temperature and were kept in closed dished. For hatching eggs, filter paper was immersed into plastic tray which was filled water and after ± 24 hours, eggs would hatch and grow to become larva of instar I. Larva of instar I would go through development level into larva of instar II, III (4 days) and instar V (2 days). Every 2 days larva was fed spotted fish of 1-2 grams. Media of larva growing was changing water every 2 days. Larva would grow into pupa during 8 days. Larva of instar III/IV was used in testing.

Larvasida test was carried out in vial and was prepared test of 13 samples. There were used 4 treatments and 3 return treatments for each sample and it was needed 12 vials and 1 vial as control. Each sample was balanced of 200 mg and then was solutioned with ethanol of 20 ml. Solution was pipitted with micro pipette of 2000 µl; 1000µl; 200µl; 20 µl and each of them was entering into vial and then the solvent was evaporated during 24 hours. After that, to enter water of 10 ml, larva of *Aedes agypti* of 25 and then extract solution was added water until the volume became 20 ml in the concentration of 1000; 500; 100; and 10 ppm. As a control, it was entered water of 10 ml, larva *Aedes agypti* of 25, and then it was added water until the volume became 20 ml. Observation was carried out during 24 hours due to the death of the larva. Analysis of data was carried out for finding death concentration (LC_{50}).

RESULTS AND DISCUSSION

Extraction

Extract production of zuurzak seed of 2 kg was produced by maceration using ethanol of ± 15 l. The result was extract of coagulated ethanol of 190 g with colour of dark brown.

Fractionation

After the action as above, extract of coagulated ethanol was solution by ethanol-water of 100 ml with comparison of 7 : 3. After the mixture was fractionated sequencely using the solvents of *n*-hexane, ethyl acetate, and *n*-buthanol, it was produced coagulated extract and fraction of *n*-hexane of 14.7 g with black brown colour, coagulated extract of ethyl acetate fraction of 0.8 g with yellow brown colour, and coagulated extract of n-buthanol of 4.6 g with yellow brown colour..

Toxicity test of Bio-larvasida

Activity test of zuurzak seed extract as bio-larva ide was conducted at Laboratory of Chemical Sciences, Faculty of Mathematic and Natural Sciences, Public University of Manado, Tondano. It was conducted during 3

weeks due to the larva of *Aedes aegypti* for each extract of fractionated extract of *n*-hexane, ethyl acetate, and *n*-buthanol.

Toxicity test of each extract (Figure 1) with the method of Probity Analysis (*Finney Method*) by using Minitab 14 software was intended to find the concentration of $LC_{50.}$ Result showed that coagulated extract of effective *n*-hexane and it was the most toxic due to the larva of *Aedes aegypti* with the value of LC_{50} was 73.77 ppm, but the coagulated extract of ethyl acetate extract was toxic due to the larva of *Aedes aegypti* with the value of LC_{50} was 340.71 ppm, and coagulated extract of *n*- buthanol extract was not too toxic due to the larva of *Aedes aegypti* with the value of LC_{50} was 725,18 ppm.

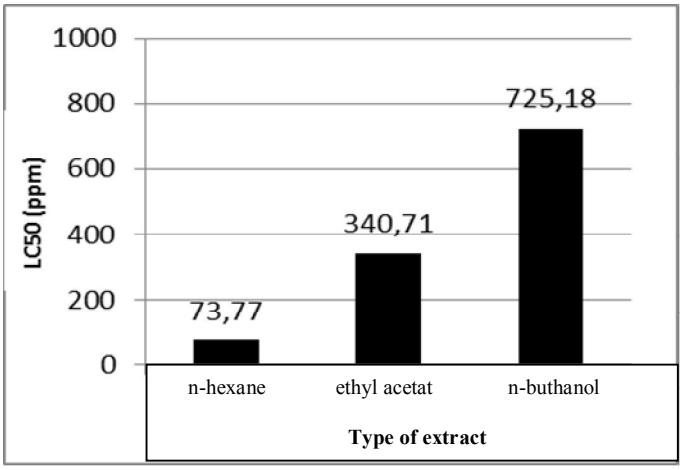

Figure 1 Extract value of LC_{50} of *n*-hexane fraction, ethyl acetate, and *n*-buthanol on larva of *Aedes agypti* after Treatment of 24 hours

Then, extract of the most toxic of *n*- hexane was analysed with GC-MS for knowing the inside percentage compound.

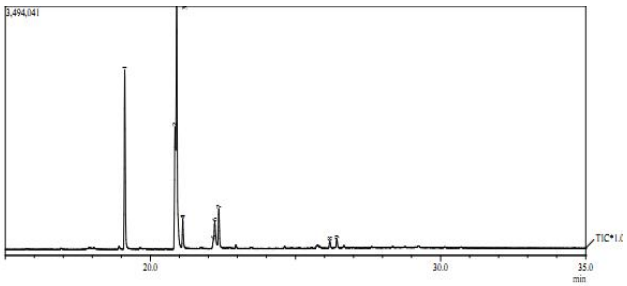

Figure 2 Chromatogram fraction of GC: n-hexane of zuurzak seed extract

Result of chromatogram GC of *n*-hexane fraction showed that was 9 peaks of compounds which the 3 peaks were the most dominant and gave big enough intensity with time of retention was relative close. Based on the information of chromatogram GC (Figure 2), it showed that 4 compounds had abundance < 4%, they were 4, 5, 8, and 9 with the value sequence were 3.90; 1.16; 1.01; and 1.22%. But compound of 6 and 7 had abundance 4.28 and 5.27%. The rest one was the compound of 1, 2, and 3 had abundance of 24.73; 19.09; and 39.35% sequence so that was as the most dominant component in the fraction of *n*-hexane. From 9 peaks of produced chromatogram, it indicated that fraction of *n*-hexane was not relative mere that these compounds was active larva ide due to the larva of *Aedes agypti*.

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Name
1	14.362	14.325	14.450	10156804	4.76	3607393	
2	15.988	15.925	16.083	16020484	7.51	4456003	
3	16.887	16.833	16.967	7040838	3.30	2893909	
4	19.182	19.058	19.258	57946336	27.16	13026868	
5	20.917	20.833	21.058	25826972	12.10	5942174	
6	21.148	21.075	21.225	17767460	8.33	6103366	
7	22.393	22.317	22.467	16520935	7.74	5027860	
8	25.174	25.100	25.258	7688911	3.60	2141963	
9	26.262	26.183	26.333	10867388	5.09	3086993	
10	26.678	26.600	26.767	9028950	4.23	2514095	
11	28.853	28.742	28.942	22711418	10.64	4414197	
12	29.337	29.258	29.425	11781492	5.52	3207986	
				213357988	100.00	56422807	

Figure 3 information of GC chromatogram

Result of GC-MS for *n*-hexane fraction from zuurzak seed extract was presented as in Table 1 below.

Table 1 Composition of compound at *n*-hexane fraction of zuurzak seed with GC-MS

Peak	Time of retention (minute)	Abundance (%)	Possibility of compound
1	19.119	24.73	Methyl palmitate
2	20,853	19.09	Methyl linoleate
3	20.909	39.35	Methyl oleate
4	21.118	3.90	Methyl stearate
5	22.175	1.16	2-(2- hydroxy-6-(-2hydroxy-2-nitrocyclopentyl)phenyl)acetic acid
6	22.219	4.28	Benzyl 2-(3,4-dihydrocidfenil) acetate
7	22.360	5.27	10-nonadecanol
8	26.184	1.01	Methyl lignocerat
9	26.427	1.22	2-(((deca-1,4,6,8-tetraenylamino) methoxy)carbonyl) benzoic acid

Based on Table 1 as above, it could show that fraction of *n*-hexane of zuurzak seed fraction (*Annona muricata.* Linn) contained the compound of organic fatty acid, phenolic, and triterpenoid with the main component was fatty acid of methyl oleat. The structure of fatty acid of methyl oleat was as in Figure 4 below.

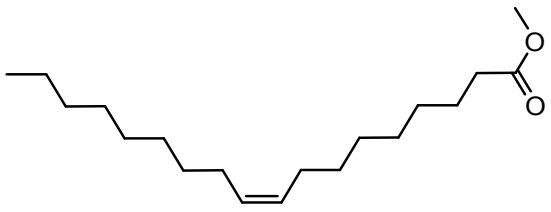

Figure 4 Molecule structure of fatty acid Methyl Oleat.

CONCLUSION

Based on the observation and analysis as above, it was concluded as follow. Result of python-chemical test showed that extract of zuurzak seed contained the compound of secondary metabolite as the group of saponin, alcaloid, and triterpenoid. Result of initial test showed that ethanol extract of zuurzak seed as the agent of larva ide with the value of LC_{50} was 244.27 ppm. Result of biological activity test on the larva of *Aedes aegypti* was coagulated extract of b-hexane with the value of LC_{50} was 73.77 ppm, for methyl acetate was 340.71 ppm, and for buthanol was 725.18 ppm. Result of biological activity test showed that *n*-hexane had the most toxic with the value of LC_{50} was 73.77 ppm. Identification of active fraction of anti larva ide with GC-MS contained 3 components of chemical compound which were as organic fatty acids such as methyl palmitate, methyl linoleat, and methyl oleat. The compounds above were guessed as anti active anti larva ide due to the larva of *Aedes agypti*.

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