

Composition of Water Extract from Wild Bitter Gourd
(*Momordica charantia* L.) Fruit for application as
Antifeedant and Mortality Test on Armyworm Larvae
(*Spodoptera litura* Fab.)

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Abstract

This study was to determine the fruit maturity level of wild bitter gourd and the composition of water extract which is effective as antifeedant and appropriate for mortality test on armyworm larvae. Results obtained show that water extract of wild bitter gourd fruit contains phenolic compounds, flavonoids and triterpenoids. Terpenoid compounds contained in water extract of fruits at maturity level 4H, 8H and 12H were momordicoside L, momordicoside K, compound 3β , β 7, 25-trihydroxycucurbita-5.23(E)-diena-19-al:R1=H, R2= H and momordicine 1. Fruit maturity level 4H 50% and 4H 60% resulted to the highest antifeedant index, ie 40.08% and 44.20%. LC₅₀ at fruit maturity level 4H observed on day 7 was 40%.

Keywords: Wild bitter gourd, Water extract, Antifeedant, Mortality, Triterpenoids, Armyworm larvae

1. Introduction

Armyworm (*Spodoptera litura* Fab.) is a polyphagous insect, which has many kinds of host plants. Armyworm is an important pest in many kinds of plants, such as weeds like grinting and reeds, horticultural crops such as tomatoes, chili, beans, cabbage, onions, spinach, kale, potatoes, crops such as rice, corn and soybeans, as well as plantation crops such as citrus, cotton, sugarcane. Armyworm can reduce the production of soybean up to 80%, resulting in crop failure if the pest is not controlled (Marwoto and Suharsono, 2008).

Efforts to control pests can be done with mechanical/physical, biological or chemical methods. The control of this pest has so far, been done mostly by the use of chemical method through application of synthetic insecticides, but this method results in a negative impact on insects, environment and human health. The negative impact on insects is the occurrence of pest resistance to synthetic insecticides, the pest resurgence or the killing of natural enemies of pests. The negative impact on the environment can be the accumulation of synthetic insecticide residue on the farm and in the crops harvested. The negative impact on humans could be an accident on the users of synthetic insecticides and various diseases in humans such as skin irritation, disruption of the nervous system and cancer likelihood as a result of intake of food contaminated with synthetic insecticides (Rogers, 2010).

Pesticide residue is pesticide remains on farmland and in agricultural yield that are not decomposed. Some studies indicated that there are pesticide residues on land and agricultural production. Karyadi, *et al.*, (2011) observed an increase in heavy metals levels (Pb) on onion crop land in Kendal, Centre Java province due to the use of pesticides. This happened because the farmers in Kendal used seven kinds of pesticides containing heavy metal Pb. Pb levels increased by 43 071.60 mg/Ha compared to the land condition before planting and after onions harvest. Spraying frequency, the pesticides dose, and variable content of Pb in pesticides significantly affected the content of Pb in the soil. One season of onion planting

can increase the Pb content in the soil as much as 2 991.26 mg/Ha. Pesticide residues have also been found on the agricultural yields. Mutiatikum and Sukmayati (2009) found insecticide residues on the samples of local and imported rice from the cities of Cianjur, Semarang and Surabaya. In Surabaya and Semarang, residues of insecticides (lindane, aldrin and heptaklor) were found on rice in low levels. Although there were only small amounts of residues, it should be a cause for concern because they have a long half-life. Similar results were also found in various vegetables, like red peppers, lettuce, and onions (Miskiyah and Munarso, 2009); spinach (*Amarantus indica*), water spinach (*Ipomoea aquatica*), mustard (*Brassica juncea*) and beans (*Vigna sinensis*) (Tuhumury, *et al.*, 2012), as well as in carrots (*Daucus carota*) (Ohorella, *et al.*, 2013). Pesticide residues were also found in red peppers, lettuce, and onion which were obtained from farmers, traders, and markets in vegetable production centers at Central Java and West Java (Miskiyah and Munarso, 2009).

Because of the negative impact of synthetic insecticides applied as the most common way to control pests on plants, there is need for efforts towards the control of pests with methods that are more friendly to the environment and human health. This can be effective by the use of botanical insecticides which are biodegradable and safe for the environment and human health. Botanical insecticides do not kill insect pests directly, but they can reduce the incidence of pest attacks by acting as insect repellents or interfering with the development of eggs, larvae and pupae of insects; inhibit skin turnover insects; disturb insect communication; inhibit the ability of eating insects provisionally or permanently (as antifeedant); inhibit the reproduction of insects or can attract insects (as attractant) (Ware, 1983).

Bitter gourd is one of the plants in *Cucurbitaceae* family which has been widely studied as a botanical insecticide. The parts of its plant which have been studied are the leaves (Yasui, 2002; Ling, *et al.*, 2008; Devanand and Rani 2008; and Abe and Matsuda, 2000). Bitter gourd fruit has also been studied by Singh, *et al.*, 2006 and Maurya, *et al.*, 2009, but the age of its fruit which is most effective as botanical insecticide has not been studied.

Wild bitter gourd (*Momordica charantia* L.) is an annual plant that can grow well at an altitude 0-1000 m asl. Wild bitter gourd plant morphology is similar to the characteristics of bitter gourd cultivated for food purposes, but it has special characteristic. The wild one has small rash resembling a thorn in the entire skin surface of the fruit. The other characteristic is that the size of the fruit is much smaller than the green bitter gourd, which is around the size of a thumb about 2-5 cm in fruit length.

Wild bitter gourd plants are vines or climbing, it has tendrils strong stems and smelled dreadful. The stems can reach 2 to 3 m. The plant roots is the taproot type. The leaves arranged alternate, the leaves width can reach 10 cm. Pare leaves are oval and have fur on their surface. Leaf blade pare split almost to the leaf base. The leaves are shaped like a finger bone. The plant is monoceous. The flowers are yellow and grow from the armpit. Flowers began to appear around the age of 45-55 days after sowing. There are male (*staminate*) and female flowers (*pistilate*) on the same plant. The female one become fruit after pollination. The fruit is elliptical. Unripe fruit is green. The ripe one turn to orange or orange yellowish and brake into three pieces. The seeds is light brown to black. It is flat round shape and

jagged edge of a flat section. The seeds are about 5-9 mm length and 2.5 to 6 mm width (Holm, *et al.*, 1997).

Bitter gourd has function as a botanical insecticide. Yasui (2002) showed that the methanol extract of bitter gourd leaves effectively hinder eating larvae of *Spodoptera litura* and *Pseudolamia separata*. Ling, *et al.* (2008) stated that in the extract of bitter gourd leaf there are triterpenoids compounds which are effectively hinder the eating of *Plutella xylostella* larvae.

The ethanol extract of bitter gourd leaves inhibited the oviposition of *Liriomyza trifolii* imago on bean leaves treated with the extract. The concentrations of 2000-4000 mg/ml ethanol extract of bitter gourd leaves showed antifeedant and antioviposition activity in *L. sativae* Blanchard imago (Diptera: agromyzidae) significantly. Antifeedant index (AFI) of cyclohexane extract of bitter gourd leaves with a concentration of 1000 µg/ml for 2 days in imago *L. sativae* was 11.08%. While at the same concentration, AFI of ethyl acetate extract and n-butanol extract of bitter gourd leaves at the same concentration was 34, 89%, and 22.99%, respectively. The AFI lowest value was water extract that was 0%. Ethyl acetate extract of the bitter gourd leaves had the highest bioactivity (Ling, *et al.*, 2009).

Acetone extract of bitter gourd leaves showed toxic activity and greatly impede the ability feeding of the *Spodoptera litura* Fab. larvae instar 3. Tests was conducted by feeding that has been treated acetone extract of bitter gourd leaves with a dose of 100 mg / 21cm². LD₅₀ value of the acetone extracts of bitter gourd leaves was 72.60 mg/21 cm² (Devanand and Rani, 2008). Meanwhile, the methanol extract of bitter gourd leaves hindered eating four species of Cucurbitaceae family beetles, namely the *Aulacophora femoralis*, *A. nigripennis*, *Epilachna admirabilis* and *E. boisduvali* (Abe and Matsuda, 2000).

Besides the leaves, bitter gourd fruit also has a botanical pesticide potency. Bitter gourd fruit juice and hexane extract of bitter gourd fruit serves as a larvicide in mosquito larvae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* (Singh, *et al.*, 2006), likewise with petroleum ether extract, carbon tetrachloride extract and methanol extract of bitter gourd fruit. Those extracts were also capable of controlling larvae of *A. stephensi* and *C. quinquefasciatus* (Maurya, *et al.*, 2009). Petroleum ether extract of bitter gourd fruit was more effective than the karbonetertraklorida extract in controlling larvae of *A. stephensi* and *C. quinquefasciatus*. Petroleum ether extract has LC₅₀ lower than the carbon tetrachloride extract. LC₅₀ petroleum ether extract of bitter gourd fruit on the larvae of *Anopheles stephensi* was 27.60 ppm (treatment for 24 hours) and 17.22 ppm (treatment for 48 hours), and LC₉₀ petroleum ether extract of bitter gourd fruit on the larvae of *Anopheles stephensi* was 154.99 ppm and 94.79 ppm respectively for treatment 24 hours and 48 hours. While LC₅₀ of carbon tetrachloride extract of bitter gourd fruit for larvae of *A. stephensi* was 49.58 ppm (treatment for 24 hours) and 16.15 ppm (treatment for 48 hours), and LC₉₀ for the treatment of 24 and 48 hours also was 521.02 ppm and 369.99 ppm (Maurya, *et al.*, 2009).

Study results showed that botanical insecticide active compounds that had been isolated from the leaves extract of bitter gourd was terpenoids (Mekuria, *et al.*, 2005), (Yasui, 2002), (Ling, *et al.*, 2008), (Abe and Matsuda, 2000) and (Devanand and Rani, 2008). Terpenoids in plants

was part of plant self-defense mechanism against pests and diseases, so it was used much as insecticides, fungicides and herbicides in agriculture, and as antimicrobial and antifungal in pharmaceutical field. Terpenoids provide benefits for the plant by refusing (repellent) or killing predators or disease. However, terpenoids can also be counterproductive for plants because it can act as attractants. The effectiveness of terpenoids for the plant defense was affected by the total number of existing terpenoids, the number of existing specific terpenoids and was also influenced by the composition of two or more existing terpenoids. *Chrysothamnus nauseosus* plants during the summer was protected from pests due to the high content of terpenoids, which was about 80 ug/g dry weight. Terpenoids contained by the plant consists of α - and γ -muurolen, β -humulen and E- β -farnesen. In winter, the content of these terpenoids declined so that deer can eat these plants (Tellez, *et al.*, 2002).

Yasui (2002) found two kinds of active compounds obtained via silica gel chromatography purified by HPLC in bitter gourd, namely monoglukosida triterpenoids (momordisin II) and diglukosida triterpenoids (momordisin I). Triterpenoids identified as 3,7,23-trihidroksicucurbita-5,24-dien-19-al (momordisin I) which were isolated from the leaves of bitter gourd inhibit oviposition of *Liriomyza trifolii* on host plant leaves treated with 33.60 g/cm² leaf surface (Mekuria, *et al.*, 2005). Ling, *et al.*, 2008 also found that momordisin I and momordicine II were also active in inhibiting eating ability of *Plutella xylostella* larvae instar 2 and 3. Momordisin II showed antifeedant effect significantly on *P. separata* on artificial food with concentrations of 0, 0.02; 0.1 and 0.5% momordisin II (Yasui, 2002). Each momordisin I and II did not affect the eating ability of *Epilachna admirabilis* and *E. boisduvali*, but the eating ability was inhibited by mixture of momordisin I and II or II momordisin mixtures with other components (Abe and Matsuda, 2000).

Armyworm (*Spodoptera litura* Fab.) was a polyfagous pest which was widespread in Asia and Africa. It was classified into insect which undergo perfect stage of metamorphosis, namely egg-larva-pupa-adult insect (imago) stage. The larvae hid during the day and searched for food at night. The eggs laid by the female imago in groups on the underside of the leaf surface at night. It was covered by an orange brown like cotton layer. The eggs laid by the female imago average can reach until 400 eggs in 3-4 groups each time laying. Each group totaled 80-150 eggs, so total eggs laid were up to 1 500-2 500 eggs within 6-8 days. The eggs were round, slightly flattened with a diameter of 0.4-0.7 mm. The incubation eggs period lasted in 3-5 days. The larvae newly hatched had measuring 2.00 to 2.74 mm (Alyokhin, *et al.* 2012).

Larvae grew through 5-6 instar periods. At instar 1-3 it remained on the underside of leaves surface. At instar 4-6, it will dropped to the ground, loosened the ground, and prepared the clay for the cocoon. The shape of last period instar was fat and smooth with a length of about 40-50 mm. The larva period lasted about 20-28 days (Sullivan, 2007).

Color of the pupa was maroon and it lied in the ground. The length was about 18-22 mm. The last segment of the stomach was shaped like two hooks. Pupal period lasted 7-11 days. The imago had a yellowish-white body. The front wings were dark brown with light shadow lines and stripes. Hind wings were white with violet sheen satin and brown stripe. The head was

like having light and dark brown tufts. The body length ranged from 14-18 mm. Wing span reached 28-38 mm (Sullivan, 2007). According to Alyokhin, *et al.* (2012) the armyworm life cycle took place within 30-40 days.

The wild bitter gourd had not been studied as botanical insecticide until now, either. So, this study used water solvent to extract the wild bitter gourd fruit because it was universal solvent which easily to find, economical and friendly to environment.

2. Research Methods

2.1 Materials and Tools

The research was conducted at Biology Laboratory, Agriculture Faculty, Widyagama Malang University, Indonesia for toxicity test of water extract of wild bitter fruit on armyworm larvae instar 2. This was also conducted at Chemical Laboratory of Polytechnic Malang for active compound test. Materials used in this study were three levels of fruit maturity of wild bitter gourd, and armyworm larvae instar two. The tools used in this study were grinder, sifter 60 mesh, centrifuge, waterbath, plastic cups, glassware, digital scales and LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry).

This research consisted of two studies: first was the test using HPLC-MS/MS to determine the chemical compounds in the water extract and second was the toxicity test of the extract on armyworm as antifeedant and mortality tests. HPLC-MS/MS test was conducted according to the determination procedure conducted by Ma, *et al.*, 2012.

2.2 Water Extracts of Wild Bitter Gourd Fruit Making

Wild bitter gourd fruits on this research were from Tawang Argo Village, Karangploso subdistrict, Malang, Indonesia. The village had a hilly topography with a height of 700 m asl. Flower of wild bitter gourd was observed first to know the life span from anthesis to riping fruit. Based on data which was 14-15 days, then the three maturity levels fruit of wild bitter gourd were determined:

- 4H: fruit maturity level 4 days after anthesis;
- 8H: fruit maturity level 8 days after anthesis;
- 12H: fruit maturity level 12 days after anthesis.

The female flower of wild bitter gourd was marked with a label. The fruit was harvested in accordance with the age of the treatment which was 4, 8 and 12 days after anthesis, then was sliced thinly and dried in the winnowing and covered with black cloth on the shady conditions for 5 days. The harvest of fruit maturity level 4H was as many as 770 pieces weighing 271.20 grams, while fruit maturity levels 8H and 12H were as many as 652 pieces weighing 1 143.93 grams, and 727 pieces weighing 2 773.44 grams. After that, the fresh wild bitter gourd fruit was dried for 5 days, blended into powder and sieved with 16 mesh sieve. The powder obtained from the fruit maturity levels 4H, 8H and 12H respectively were as much as 31.19 grams, 30.64 grams, and 30.03 grams dry weight, while the water contents on the powder at fruit maturity levels 4H, 8H and 12H were 9.97%, 10.1% and 10.3%, respectively.

The powder of wild bitter gourd fruit maturity levels 4H, 8H and 12H was extracted by maceration with water solvent based on the procedure performed by Balafif, *et al.* (2013). The powder as much as 30 grams was extracted by maceration with distilled water for 3 x 24 hours. On day 1 the powder was macerated with 180 ml of distilled water, as well as day 2 and day 3. Total distilled water was added to 540 ml. The filtrate result from day 1, day 2 and day 3 was centrifuged twice each at a speed of 3 000 rpm for 5 minutes. The filtrates obtained from treatments 4H, 8H and 12H were respectively 117.5 ml, 115.0 ml and 116.3 ml. The filtrates subsequently were thickened with a water bath at a temperature of 100 °C up to one-third volume, which was to be 38.3 to 39.2 ml.

2.3 The Phytochemical Test

Water extract of wild bitter gourd fruit contained saponin if there was stable foam when it was given hot water and shaking vigorously after cooling. The extract contained alkaloids if there was white sediment after water extract sample was treated with Mayer and there was brown sediment after extract sample was reacted with Dragendorff reagent. Water extract contained any steroids if appeared green or blue color after the sample was reacted with the Liebermann-Burchard reagent. This was indicated by the onset of a yellow color after the sample reacted with 2 N H₂SO₄ solution, the incidence of red color after the sample was treated with NaOH 10% solution and the incidence of yellow color accompanied with foam after the sample water extract was treated with concentrated HCl concentrated, and Magnesium. The water extract contained phenolic compounds indicated. by the appearance of a black color after the sample was treated with 5% FeCl₃ solution. Water extract contained triterpenoids if appeared brownish red color after the sample was reacted with the Liebermann-Burchard reagent.

2.4 The Bioactive Compounds Test with LC-MS/MS

Operating Conditions of Liquid Chromatography-Tandem Mass Spectrometry were as follows. Column used had as specifications Hypersil Gold (50mm x 2.1 mm x 1.9 m). UHPLC brands ThermoScientific 1250 ACCELLA type which consisted of vacuum degasser, quartener pump, an autosampler thermostatically controlled with a personal computer through a program called x-calibur 2.1. A mobile phase consisted of 0.1% formic acid in aquabidest, phase B consisted of 0.1% formic acid in Acetonitrile, phase C consisted of 0.1% formic acid in methanol. A linear gradient at a rate of 300 mL/minute with a mobile phase was set as follows: a) 0-0.6 (minute 65% A, 25% B, 10% C), 2-3.5 minute 90% B, 10% C), 4-5 minutes which was equal to 0-0.6 minutes. Injection volume on LC was 10 µL. The column was controlled at 30 °C, and autosampler compartment was set at 10 °C. The usage of MS/MS Triple Q (quadrupole) mass spectrometer TSQ Quantum ACCESS MAX from Thermo Finnigan with ionization source ESI (electro spray ionization) was controlled with software TSQ Tune-operated with positive mode.

2.5 Antifeedant Test on Armyworm Larvae

The aim of this test was to determine the level of fruit maturity and concentration of water extract of wild bitter gourd fruit which was effective as antifeedant test on each level of fruit

maturity. The tests were carried out with five concentrations of water extract of wild bitter gourd fruit and control. Each treatment concentrations of water extract and controls was repeated 4 times using a randomized block design. Pest target was 20 armyworm larvae instar two in each treatment unit. The concentrations of water extracts used were 20%, 30%, 40%, 50% and 60%. To make 20%, 30%, 40%, 50% and 60% concentration, 2, 3, 4, 5 and 6 ml concentrated solution of water extract were taken and put in a 10 ml flask and then was added distilled water until the solution volume reached 10 ml.

The treatment was conducted by dipping larvae feed into each extract water according to maturity level and treatment concentration. Feed given to the armyworm larvae was cabbage which is the common feed used in mass breeding of armyworm. Cabbage was purchased from traditional markets and was washed first with water and then dried. The cabbage was cut circular with diameter of 3 cm. Pieces of cabbage were then dipped in water extract and air-dried for five minutes. They were put into a plastic cup with 9 cm diameter on the top side, diameter 7 cm on below side and height of 7 cm. For the control treatment, the pieces of cabbage were dipped in distilled water only. Into each plastic cup was inserted one hungry armyworm larvae. Filter paper soaked in 0.5 ml water was placed on the base of plastic cups to maintain moisture. The treatments were conducted over 24 hours.

The tests were carried out with non-choice method (Pavela, 2009). Observations were carried out 24 hours after treatment. The rest of leaves that were not eaten by the armyworm larvae was calculated to obtain leaf area eaten. Percentage antifeedant index (AF) was calculated using the formula:

$$AF (\%) = (C - T) / (C + T) \times 100\% \text{ (Pavela, 2009),}$$

where C: leaf area eaten by larvae on control, T : leaf area eaten by larvae on treatment. Water extract which had the greatest value of antifeedant index was the most active extracts for antifeedant test.

2.6 Mortality Test

The aim of this test was to determine the level of maturity of bitter gourd fruit and concentration of water extract which was effective as a mortality test. The method used was the same as the antifeedant test method. Observations were made every 24 hours after treatment up to 6 x 24 hours, ie at 24, 48, 72, 96, 120, 144 hours after the treatment. Number of armyworm larvae that died in every 24 hours and the percentage mortality was observed. Percentage armyworm larvae that died was then calculated based on the following formula:

$$(X-Y) / X \times 100\% \text{ (Abbott, 1987),}$$

X: live armyworm larvae on control

Y: live armyworm larvae on the treatments

2.7 Data Analysis

The data on antifeedant and mortality test was analyzed by F test at error level $\alpha = 5\%$, and if there were significant results, the analysis was continued with HSD test (Steel and Torrie, 1960).

3. Result and Discussion

3.1 Phytochemicals test of Water Extract of Wild Bitter Fruit

The phytochemical test result are stated in Table 1.

Table 1. Secondary metabolites in water extract of wild bitter gourd fruit

No.	Secondary Metabolite	Reagent	Result
1.	Saponin	Hot water	-
2.	Alkaloid	Mayer reagent	-
		Dragendorf reagent	-
3.	Steroid	Liebermann-Burchard reagent	-
4.	Phenolic	FeCl ₃ 5%	+
5.	Flavonoid	H ₂ SO ₄ 2N	+
		NaOH 10%	+
		Concentrated HCl + Mg	+
6.	Triterpenoid	Liebermann-Burchard reagent	+

This was consistent with the research conducted by Horax, *et al.* (2005) which states that there was phenolic compounds in the extract of bitter gourd fruit. Horax, *et al.* examined the total phenolic content and acid phenolic components in 4 bitter gourd varieties which were India White, India Green, China White and China Green. Total phenolic content obtained from samples dried with oven was much higher than from samples that went through freeze drying. Total phenolic content obtained from bitter gourd fruit pulp extract was 5.36 to 8.90 mg CAE/g dry weight. Total phenolic content of the bitter gourd seeds extract was lower than that contained in the bitter gourd fruit which was 4.67 to 8.02 mg CAE/g dry weight. Major phenolic acids found were gallic acid, gentisic acid, catechin, epicatechin and chlorogenic acid. Meanwhile, protocatecheuic acid, sirinic acid and benzoic acid were also found in the extract but in small amounts, ie less than 10 mg/100 g material dry weight. Similar results were also demonstrated by Ghaima, *et al.* (2013) who found phenolic compounds in bitter gourd fruit extract.

Water extract of wild bitter gourd fruit contained flavonoids. This was in line with the observation by Tan, *et al.*, 2014, that the bitter gourd fruit extract contains flavonoid compounds. Total flavonoid contained in the water extract was very small, ie only 5.4% compared with the flavonoids content presented in the acetone extracts. Mada *et al.* (2012) suggested that flavonoids were also found in the water extract of bitter gourd leaves.

Wild bitter gourd fruit water extract contained triterpenoids. This was in accordance with Sundari, Padmawati and Ruslan (1996) who reported that bitter gourd flesh contained steroid/triterpenoid. Nagarani, *et al.* (2014) also stated that other parts of bitter gourd plants contained cucurbitane triterpenoids, phenolic compounds, glucoside and several types of peptides. However, Nagarani, *et al.* (2014) regretted that there was still very little information about bioactive compounds in wild bitter gourd.

3.2 Bioactive Compounds in Water Extract of Wild Bitter Gourd Fruit

Preparation and procedures for testing the active compound content was like that conducted by Ma, et al, 2012. The results of analysis of the active compounds in water extract of wild bitter gourd fruit maturity levels 4H, 8H and 12H are presented in Table 2.

Table 2 shows that there were 4 kinds of triterpenoid compounds in water extract of bitter gourd fruit maturity level 4H, 8H and 12H, namely momordicoside L, momordicoside K, 3 β , 7 β , 25-trihydroxycucurbita-5,23(E)-diena-19-al: R1=H, R2=H and momordicine 1. The structure of those compounds are shown in Figure 1.

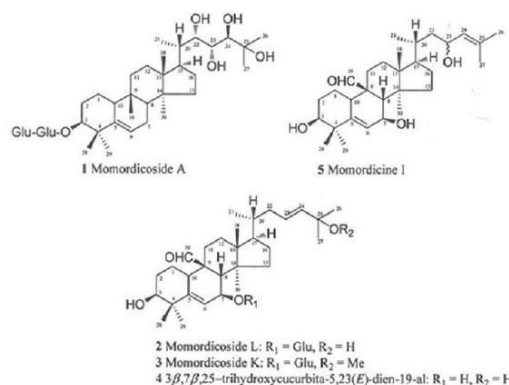


Figure 1. The Structure of Momordicoside L, Momordicoside K, 3 β , 7 β , 25-trihydroxycucurbita-5,23(E)-diena-19-al:R1=H, R2=H and momordicine 1

(Ma, et al., 2012).

Tabel 2. Active Compounds and the Area Found in the Water Extract of Wild Bitter Gourd Fruit Maturity Levels 4H, 8H and 12H

Fruit Maturity Level	Active Compound	Area
H	Momordicoside L	340 986
	Momordicoside K	11 906
	3 β ,7 β ,25-trihydroxycucurbita-5,23(E)-diena-19-al:R1=H,R2=H and Momordicine 1	35 290
8H	Momordicoside L	125 490
	Momordicoside K	706
	3 β ,7 β 25-trihydroxycucurbita-5,23(E)-diena-19-al:R1=H,R2=H and Momordicine 1	1 513
12H	Momordicoside L	85

		484
	Momordicoside K	4 243
	3 β ,7 β , 25-trihydroxycucurbita-5,23(E)-diena-19-al:R1=H,R2=H and momordicine 1	341

The compounds area found in the water extract of fruit maturity level 4H was the widest, so it can be stated that active compound in the water extract of fruit maturity level 4H was the highest. In water extract of fruit maturity level 4H the content of momordicoside L was 2.73 and 3.99 higher than those contained in the water extract of fruit maturity levels 8H and 12H. Momordicoside L content in water extracts of fruit maturity level 8H were 1.46 times higher than those contained in water extract of fruit maturity level 12H. Meanwhile, momordicoside K content in water extracts of fruit maturity level 4H was also the highest when compared to the content of water extracts of fruit maturity levels 8H and 12H. The amount of momordicoside K in water extract of fruit maturity level 4H was respectively 16.86 and 2.81 times higher than that contained in the water extract of the fruit maturity levels 8H and 12H. While momordicoside K contained in the water extract of fruit maturity level 8H was less than that contained in water extract of fruit maturity level 12H, ie 0.17 times. The amount of compound 3 β , 7 β , 25-trihydroxycucurbita-5.23(E)-diena-19-al:R1=H, R2=H and momordicine 1 at fruit maturity level 4H was respectively 23 times and 103 times higher than at fruit maturity level 8H and 12H. These results are in line with that expressed by the Maharani (2013) that different age affected content and types of constituent compounds in the different plant parts.

Momordicoside L in water extracts of fruit maturity level 4H, 8H and 12H can be seen on Figure 2-7.

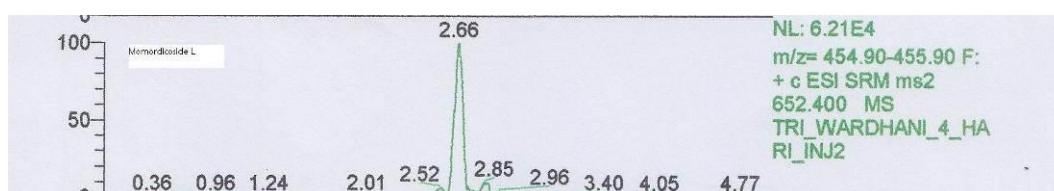


Figure 2. Momordicoside L in Water Extract of Fruit Maturity Level 4H of Wild Bitter Gourd

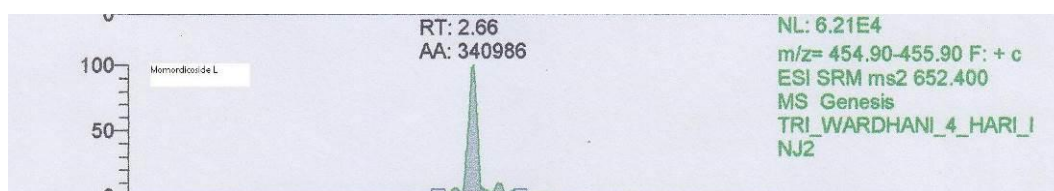


Figure 3. Area of Momordicoside L in Water Extract of Fruit Maturity Level 4H of Wild Bitter Gourd

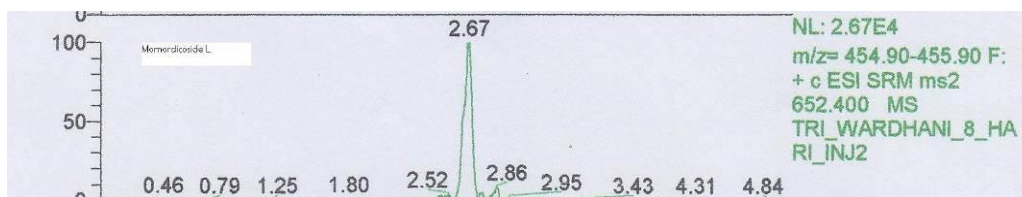


Figure 4. Momordicoside L in Water Extract of Fruit Maturity Level 8H of Wild Bitter Gourd

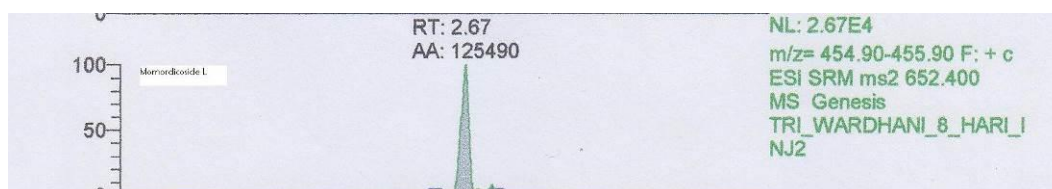


Figure 5. Area of Momordicoside L in Water Extract of Fruit Maturity Level 8H of Wild Bitter Gourd

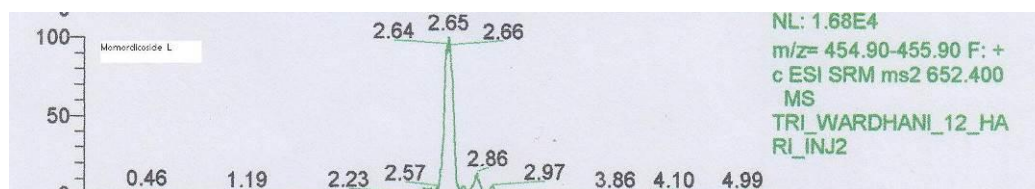


Figure 6. Momordicoside L in Water Extract of Fruit Maturity Level 12H of Wild Bitter Gourd

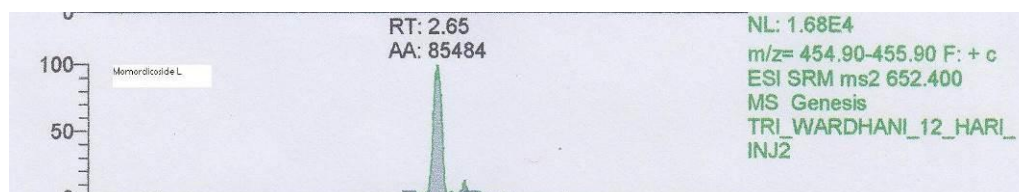


Figure 7. Area of Momordicoside L in Water Extract of Fruit Maturity Level 12H of Wild Bitter Gourd

The comparison of momordicoside L compound which found in water extracts with fruit maturity levels 4H, 8H and 12H. The figures can be seen on Figure 8-13.

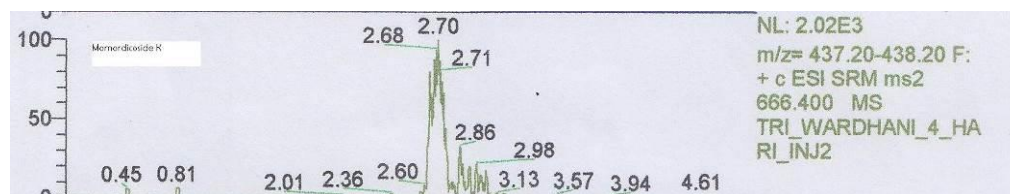


Figure 8. Momordicoside K in Water Extract of Fruit Maturity Level 4H of Wild Bitter Gourd

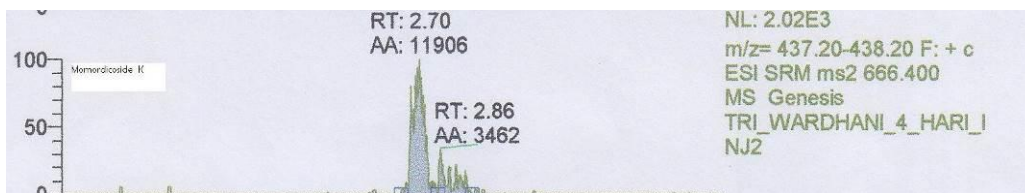


Figure 9. Area of Momordicoside K in Water Extract of Fruit Maturity Level 4H of Wild Bitter Gourd

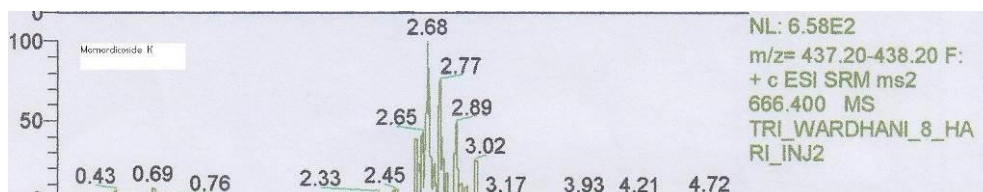


Figure 10. Momordicoside K in Water Extract of Fruit Maturity Level 8H of Wild Bitter Gourd

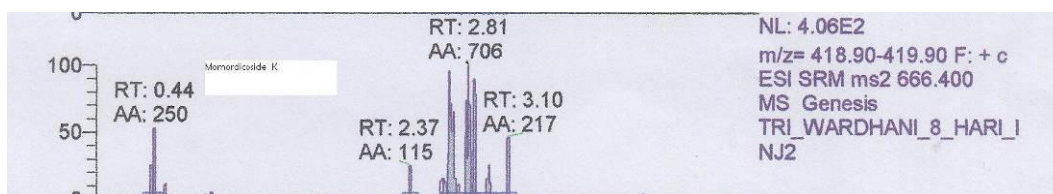


Figure 11. Area of Momordicoside K in Water Extract of Fruit Maturity Level 8H of Wild Bitter Gourd

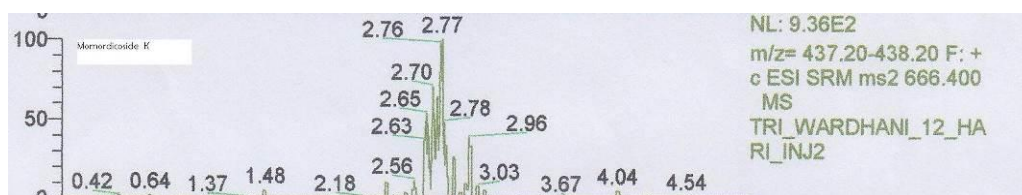


Figure 12. Momordicoside K in Water Extract of Fruit Maturity Level 12H of Wild Bitter Gourd

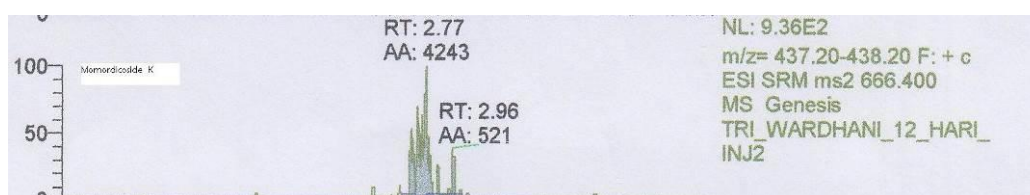


Figure 13. Area of Momordicoside K in Water Extract of Fruit Maturity Level 12H of Wild Bitter Gourd

Comparison of compound 3β , 7β , 25-trihydroxycucurbita-5.23(E)-diena-19-al:R1=H, R2=H and Momordicine 1 among the water extracts with fruit maturity levels 4H, 8H and 12H (Figure 14 -19).

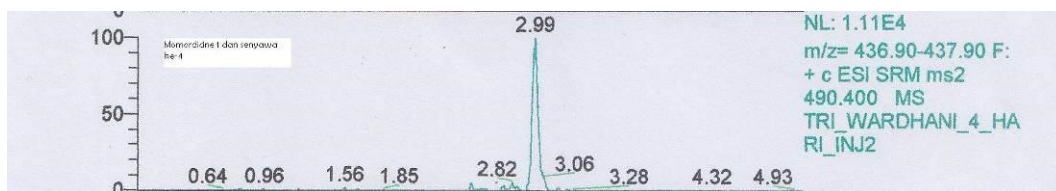


Figure 14. 3β , 7β , 25-trihydroxycucurbita-5,23(E)-diena-19-al:R1=H, R2=H and Momordicine 1 in Water Extract of Fruit Maturity Level 4H of Wild Bitter Gourd

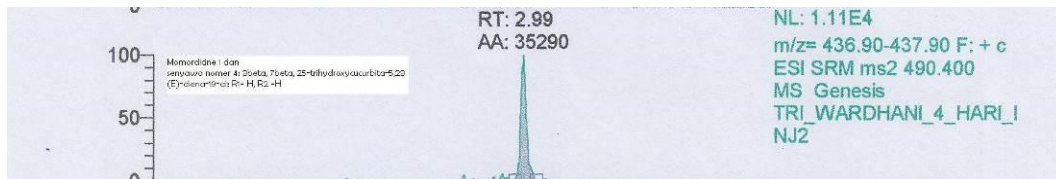


Figure 15. Area of 3β , 7β , 25-trihydroxycucurbita-5,23(E)-diena-19-al:R1=H, R2=H and Momordicine 1 in Water Extract of Fruit Maturity Level 4H of Wild Bitter Gourd

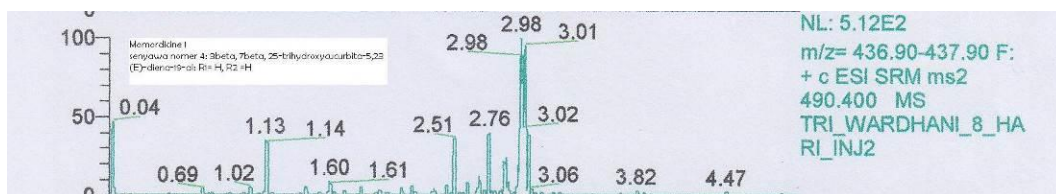


Figure 16. 3β , 7β , 25-trihydroxycucurbita-5,23(E)-diena-19-al:R1=H, R2=H and Momordicine 1 in Water Extract of Fruit Maturity Level 8H of Wild Bitter Gourd

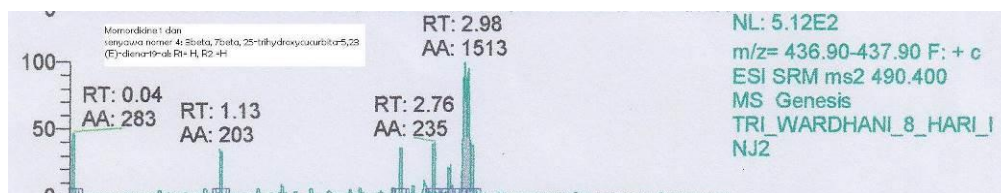


Figure 17. Area of 3β , 7β , 25-trihydroxycucurbita-5,23(E)-diena-19-al:R1=H, R2=H and Momordicine 1 in Water Extract of Fruit Maturity Level 8H of Wild Bitter Gourd

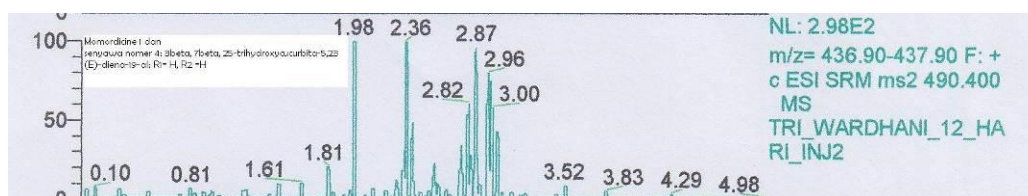


Figure 18. 3β , 7β , 25-trihydroxycucurbita-5,23(E)-diena-19-al:R1=H, R2=H and Momordicine 1 in Water Extract of Fruit Maturity Level 12H of Wild Bitter Gourd

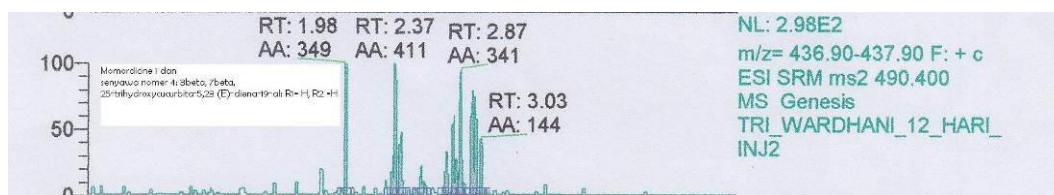


Figure 19. Area of 3 β , 7 β , 25-trihydroxycucurbita-5,23(E)-diena-19-al:R1=H, R2=H and Momordicine 1 in Water Extract of Fruit Maturity Level 12H of Wild Bitter Gourd

3.4 Antifeedant Test

Based on the analysis of variance, the interaction between fruit maturity levels and the concentrations of water extract affected antifeedant index significantly, so the analysis was continued with honestly significant difference to find the different treatments. Analysis of variance was based on arcsin transformation of antifeedant index data. The antifeedant index can be seen on Table 3.

Table 3. Interaction Between Fruit Maturity Levels and Concentrations of Water Extract of Wild Bitter Gourd Fruit on Antifeedant Index (%)

Treatments	Antifeedant Index (%)
4H 20%	28,10 cd
4H 30%	29,96 de
4H 40%	34,22 f
4H 50%	40,08 g
4H 60%	44,20 h
8H 20%	26,35 cd
8H 30%	28,52 cd
8H 40%	28,70 de
8H 50%	32,62 ef
8H 60%	33,05 ef
12H 20%	20,75 a
12H 30%	22,03 ab
12H 40%	26,29 cd
12H 50%	25,09 bc
12H 60%	28,22 d

Note: Numbers followed by the same letter on the same column were not significantly different by HSD test with $\alpha = 5\%$

At fruit maturity level 4H of wild bitter gourd, concentrations 20% and 30% did not differ on the antifeedant index. Concentrations 20% and 30% caused the lowest antifeedant index, when compared to the concentrations 40%, 50% and 60%. This showed that the water extract of bitter gourd fruit at a concentration 20% and 30%, had the lowest ability to inhibit eating ability of armyworm larvae instar two. At fruit maturity level 4H of bitter gourd, water

extract concentration 60% gave the highest antifeedant index value, which meant that this concentration had the highest inhibition on eating ability of armyworm larvae.

At fruit maturity level 8H of wild bitter gourd, a similar phenomenon occurred with the fruit maturity level 4H of wild bitter gourd. The antifeedant index values at concentration 20% and 30% were not different. Both concentrations led to the same inhibition of the eating ability of armyworms larvae instar two. Antifeedant index values of the concentration 50% and 60% did not differ with the antifeedant index at concentration 40%, either, but they were higher than the antifeedant index at concentration 20% and 30%. Concentration 50% and 60% of water extract of fruit maturity level 8H were able to inhibit eating ability of armyworm larvae instar two greater than concentration 20% and 30%. The inhibition of eating ability of armyworm larvae instar two at concentration 50% and 60% were 32.62% and 33.05%.

In the water extract of fruit maturity level 12H, concentration 20% and 30% also had lower antifeedant index value than concentrations 40% and 60%. Antifeedant index on concentration 40% was not different with concentration 50% and 60%, but different with concentration 20% and 30%. The highest antifeedant index was at the concentration 40% and 60%.

Comparing among the fruit maturity levels 4H, 8H and 12H, antifeedant index at concentrations 20% and 30% at fruit maturity level 4H was not different with the antifeedant index at fruit maturity level 8H, but in contrast with fruit maturity level 12H. Antifeedant index on extracts of fruit maturity level 4H was higher than on extracts of fruit maturity level 12H. Antifeedant index on extracts from fruit maturity level 8H was also higher than the antifeedant index of the extract with the fruit maturity level 12H.

Among high concentrations, ie 50% and 60%, it was found that the water extract of 4H maturity level of wild bitter gourd fruit had higher antifeedant index value than fruit maturity levels 8H and 12H. Antifeedant index of water extract of maturity level 8H of wild bitter gourd fruit was also higher than fruit maturity level 12H treatment.

This occurred because the water extract of younger wild bitter gourd fruit, i.e. in fruit maturity level 4H had more content of triterpenoid compounds than in fruit maturity level 8H and 12H. Based on the chemical compounds analysis, in the fruit maturity level 4H, 8H and 12H, triterpenoid compounds found were momordicoside K, momordicoside L, compound 3 β , 7, 25-trihydroxycucurbita-5.23(E)-diena-19-al:R1=H, R2=H and Momordicine 1. In water extract of fruit maturity level 8H and 12H there were also found such triterpenoid compounds but in lower contents than in fruit maturity level 4H. Momordicoside L content in fruit maturity level 4H was 2.73 and 3.99 times higher than that found in 8H and 12H fruit maturity level. Momordicoside K content in the water extract of 4H fruit maturity level 4H was also the highest. The compound was 16.86 and 2.81 times higher than that contained in fruit maturity levels 8H and 12H. While 3 β , 7, 25-trihydroxycucurbita-5.23(E)-diena-19-al:R1=H, R2=H and Momordicine 1 found in water extract of fruit maturity level 4H was 23,20 and 103,0 and times higher than in fruit maturity level 8H and 12H.

This was in line with Dewi (2013) who said that different ages of fruit had different contents of secondary metabolite compounds. The research result on 3 levels of fruit maturity indicated that the content of secondary metabolites in fruits with different ages were different. Dewi (2013) working on the bel fruit (*Limonia acidissima*) showed that the methanol extract of ripe bel fruit had higher bioactivity than the methanol extract of old and young bel fruit. The secondary metabolite content in bel fruit, ie flavonoids, increased with the increasing age of bel fruit. Rahardjo, Darwati and Shusena (2006) demonstrated similar results as well, that the organs of plants at different ages produced secondary metabolites with different contents. Secondary metabolites contained in canopy of purwoceng (*Pimpinella pruatjan* Molkenb) at nine months of age was higher than for the age of three and six month. Tellez *et al.* (2002) stated that the effectiveness of secondary metabolites for plant defense was affected by the total number of existing compounds, and the content and composition of the compounds.

3.5 Mortality Test

Analysis of variance test results indicated that the interaction between the treatment of fruit maturity level of wild bitter gourd and concentration of the water extract did not significantly affect the percentage of armyworm larvae mortality. In the fruit maturity levels 4H, 8H and 12H of wild bitter gourd, on the observations of day 1 to day 6, the concentration of water extracts did not significantly affect the percentage of armyworm larvae mortality. On the observations on day 7, extract concentration on the fruit maturity level 4H significantly affected the percentage of armyworm larvae mortality, while the extract concentration on the fruit maturity levels 8H and 12H were not significant. Mortalities of armyworm larvae can be seen on Table 4.

Table 4. Armyworm Larvae Mortality at Water Extract Concentrations of Fruit Maturity Level 4H (%)

Concentration	Observation Day-						
	1	2	3	4	5	6	7
20%	0.00	1.25	1.25	5.00	8.75	25.00	30.26a
30%	0.00	0.00	0.00	2.50	7.50	18.42	35.53a
40%	0.00	3.75	5.00	11.25	13.75	28.95	44.74ab
50%	0.00	1.25	2.50	5.00	10.00	42.11	59.21ab
60%	0.00	2.50	5.00	10.00	11.25	47.37	68.42b

The numbers in the same column followed by the same letter do not differ by HSD test with level $\alpha = 5\%$

Fruit maturity level 4H on the observation day 7 showed that armyworm larvae mortality at concentrations 20% and 30% were lower than at 60% concentration treatment. Meanwhile, the armyworm larvae mortality at concentration of 40% treatment did not differ with concentrations 50% and 60%.

At fruit maturity level 8H observation day 7, armyworm larvae mortality at concentration 20%, 30%, 40%, 50% and 60, respectively, was 27,63%, 34,21%, 40,79%, 47,37%, and

48,68%. While at fruit maturity level 12H observation day 7, armyworm larvae mortality at concentration 20%, 30%, 40%, 50% and 60, respectively, was 26,32%, 27,63%, 28,95%, 31,58%, and 36,84%.

Based on probit analysis to determine LC_{50} values, at fruit maturity level 4H observations day 7, probit model obtained was as follows:

$$Y = 0.844 + 2.121 x,$$

where x = concentration based on \log_{10} transformed. At the fruit maturity level 4H, the estimated LC_{50} was the concentration 40%.

4. Conclusion

Water extract of wild bitter gourd fruit contained phenolic, flavonoids and triterpenoids, but did not contain saponin, alkaloids, and steroids.

The active compounds contained in the water extract of wild bitter gourd fruit at maturity level 4H, 8H and 12H are momordicoside L, momordicoside K, compound 3β , $\beta 7$, 25-trihydroxycucurbita-5.23(E)-diena-19-al:R1=H, R2= H and momordicine 1.

Interaction between fruit maturity levels 4H, 8H and 12H and the concentration of the water extract resulted in a significantly different of antifidant index at non-choice method. Fruit with a maturity level 4H 50% and 60% resulted the highest antifeedant index, ie 40.08% and 44.20%.

In mortality test, there was no interaction between fruit maturity level and extract concentration. Fruit maturity level gave no significant result on mortality armyworm larvae, except on observation day 7 at fruit maturity level 4H. LC_{50} at fruit maturity level 4H on observation day 7 was 40%.

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