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## The Ability of Essential Oil and Non Volatile Fraction from The Stem of Wild Zingiberaceae *Amomum apiculatum* as Natural Insecticide against *Drosophila melanogaster*

<sup>1</sup>Addin Akbar, <sup>2</sup>Abdi Dharma and Mai Efdi

<sup>1</sup>Department of Chemical Engineering, Politeknik ATI Padang

<sup>2</sup>Department of Chemistry Andalas University

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### ABSTRACT

Insecticidal activity of essential oil and non-volatile fraction from the stem of wild zingiberaceae *Amomum apiculatum* against *Drosophila melanogaster* have been assayed. The assay consists of mortality, antifeedant, and repellent. Essential oil concentrations varied 0 (as control) ; 0,1; 0,5; 1%. The assay result showed that the higher percentage for mortality, antifeedant, and repellent are 35,33; 96,61; 73,33 % respectively. The concentrations of non-volatile fraction also varied 0; 0,1; 0,5 %. The assay result showed that the higher percentage for mortality, antifeedant, and repellent for methanol crude extract are 17,80; 95,02; 26,67 % respectively. For n-hexane fraction the higher percentage for mortality, antifeedant, and repellent are 42,20; 96,03; 80 % respectively. For ethyl acetate fraction the higher percentage for mortality, antifeedant, and repellent are 60; 96,35; 73,33 % respectively. The essential oil and non-volatile fraction from the stem of *Amomum apiculatum* also inhibit regeneration process of *Drosophila melanogaster*. The assay result showed that in the 5<sup>th</sup> day of assay eggs and larva appears in control medium, but eggs and larva did not appear in medium of essential oil and non-volatile fraction. This result conclude that the essential oil and non-volatile fraction from the stem of *Amomum apiculatum* had the potential as natural insecticide.

**Key words** : natural insecticide, insecticidal activity, *Amomum apiculatum*, *Drosophila melanogaster*, essential oil, non-volatile fraction

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### INTRODUCTION

Most of the agricultural land use of pesticides, both to protect agricultural products as well as to prevent the spread of pests and plant diseases. The resulting negative effect is that it can cause damage to land and water ecosystems, as well as the accumulation of a chronic illness that can cause health problems in humans such as nerve damage, aging and other diseases. Chemical-based pesticides have had a negative impact on the environment and humans[2][10]. Currently pest control principles developed is environmental based pest management. One concept is to develop and use a biological pesticide (bio pesticide) secure environment, mainly from local plants. Gingers *Zingiberaceae* group has various phytochemicals content of the base material for a natural insecticide. But attention has been given only to function as a source of medicinal plants and herbs. Anai Valley Nature Reserve according to the journal "Checklist of *Zingiberaceae* of Malesia" listed as one of the "collection site" or holotype specimen collection locations for the five types of *Zingiberaceae* in Sumatra. One type of *Zingiberaceae* which is a collection of Anai

Valley Nature Reserve is *Amomum apiculatum*. This plant group is one of the widest among the *Zingiberaceae* species numbers between 150-180 species. These plants spread across south east Asia from the Himalayas to northern Australia. The part rod was taken then extracted essential oils contained therein which have the potential to be used as a natural insecticide. In addition to essential oils from the rod, extract non-volatile fraction also has the potential to be used as a natural insecticide<sup>[1][6][7][8]</sup>. Volatile and non-volatile fraction is tested for natural insecticidal activity against an insect *Drosophila melanogaster*. Compounds that contained in *Amomum apiculatum* both of volatiles such as essential oils and non-volatile fraction tested for natural insecticide activity against *Drosophila melanogaster* by calculating the percentage of mortality (Death), antifeedant (decrease appetite), and repellent (rejection). Expected later *Amomum apiculatum* can be used as the raw material for natural insecticides manufacture that have high capability and environmentally friendly[12][14][15].

## MATERIALS AND METHODS

### 3.1 Materials

*Amomum apiculatum* stem, fruit flies (*Drosophila melanogaster*), aluminum foil, gauze, cotton, yarn, distilled water, methanol, n-hexane, ethyl acetate, agar, acetone, medium *Drosophila melanogaster* (banana, rice flour, flour agar). Steam distillation apparatus, an analytical balance, autoclave, oven, micro pipette, separating funnel, GC-MS Varian Saturn 2000, UV light ( $\lambda$  254 nm and 356 nm), rotary evaporator, jam jars and laboratory glassware.

### 3.2 Method

Stem of *Amomum apiculatum* distilled to obtain essential oils. The oil later varied concentration at 0 (control), 0.1, 0.5, and 1%. Dregs waste products of distillation and then extracted with methanol. Furthermore concentrated methanol extract varied concentration of 0%, 0.1% and 0.5%. Concentrated methanol extract then fractionation by polarity by using solvent n-hexane and ethyl acetate. Then each fraction also varied concentration of 0%, 0.1% and 0.5%. Essential oils and non-volatile fraction will be tested for insecticidal activity with observation of mortality, antifeedant and repellent. Essential oils then analyzed by GC-MS to see the active compounds contained in the oil acts as a natural insecticide.

## RESULTS AND DISCUSSION

### 2.1 Isolation Essential Oils from *Amomum apiculatum*

Essential oils from rof of *Amomum apiculatum* isolation with direct steam distillation method. Essential oils are derived from stem *Amomum apiculatum* is old yellow.

### 1.2 Extraction and Fractination of samples

*Amomum apiculatum* dregs that have been distilled and then macerated using methanol. Results maceration then concentrated by rotary evaporator. Concentrated methanol extract then fractionated by polarity. The solvent used is n-hexane and ethyl acetate. The result is three fractions i.e. the fraction of n-hexane, ethyl acetate fraction and a methanol fraction. Results fractionation then also concentrated by rotary evaporator.

### 1.3 Test of Insecticidal Activity for Essential Oils of *Amomum apiculatum*

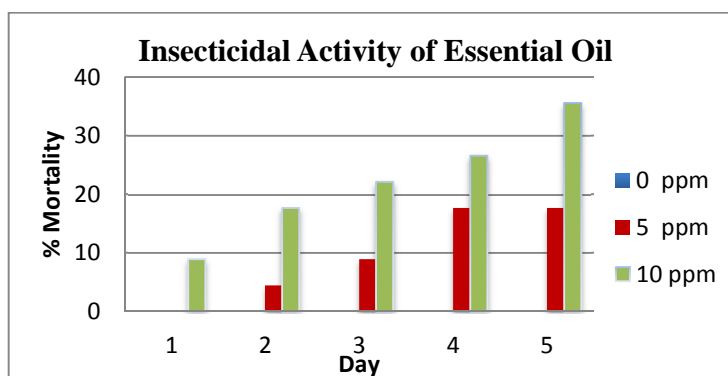


Fig 1. Mortality result for Insecticidal Activity of Essential Oils from *Amomum apiculatum*

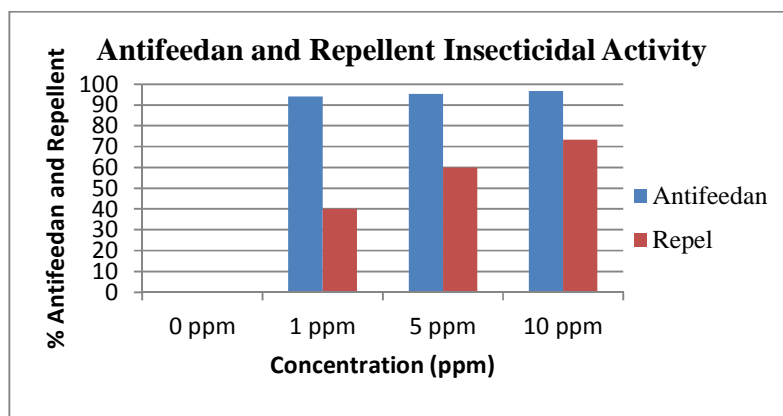


Fig 2. Antifeedan and Repellent result for Insecticidal Activity of Essential Oils from *Amomum apiculatum*

It is seen that as the concentration increase so the percentage of mortality are also increase. The highest percentage of mortality at a concentration of 10 ppm on fift day with 35.53%. This result proves that the essential oil from *Amomum apiculatum* contain active compound that can kill insects on a daily basis. The antifeedan and repellent result also the function of concentration where as the concentration increase so the % antifeedan and repellent also increase. The result conclude that *Amomum apiculatum* contain specific compound that decrease the appetite of an insects.

#### 1.4 GC-MS result for *Amomum apiculatum* essential oils

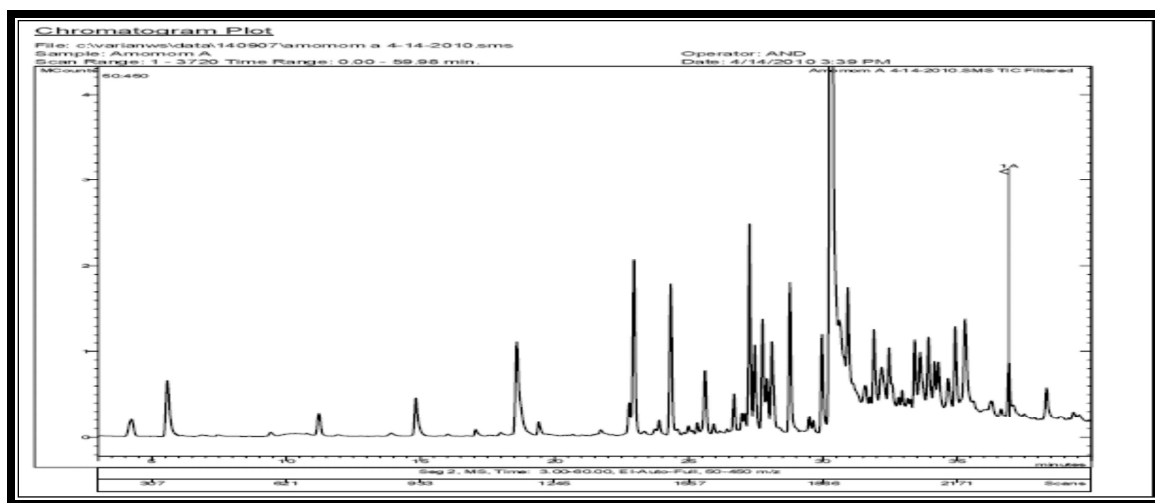


Fig 3. GC-MS result for *Amomum apiculatum* essential oils

From measurements using GC-MS, essential oils stem of *Amomum apiculatum* generate 30 peak. Essential oils usually contain monoterpenes and sesquiterpenes volatile. From the spectrum obtained and have been tested using MS, monoterpenes consist are are d-limonene, 1s, cis-calamene, l-linalool, cubenol, and guaiazolene. Also known that d-limonene and l-linalool has the ability as a natural insecticide.

#### 1.5 Insecticidal Activity of non-volatile fraction of *Amomum apiculatum*

In this research is not only essential oils are thought to have potential as a natural insecticide, the distillation residue extracted with methanol suspected to contain compounds that have the potential as a natural insecticide. In this study concentrated methanol extract is divided into fractions namely n-hexane and ethyl acetate fraction. To see the potential as a natural insecticide, then tested the biological activity.

1.5.1 Methanol Crude Extract Activities

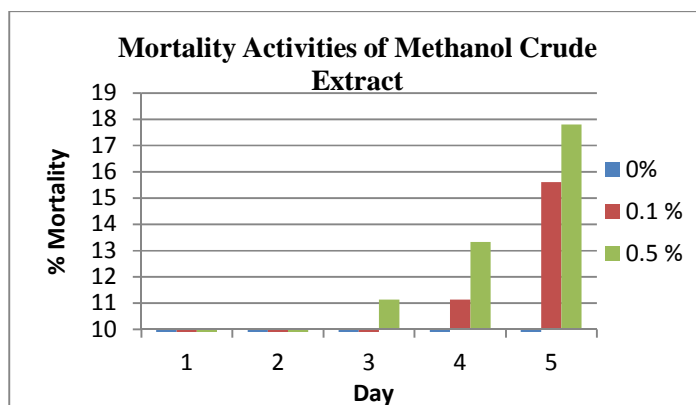


Fig 4. % Mortality methanol Crude Extract Insecticidal Activities

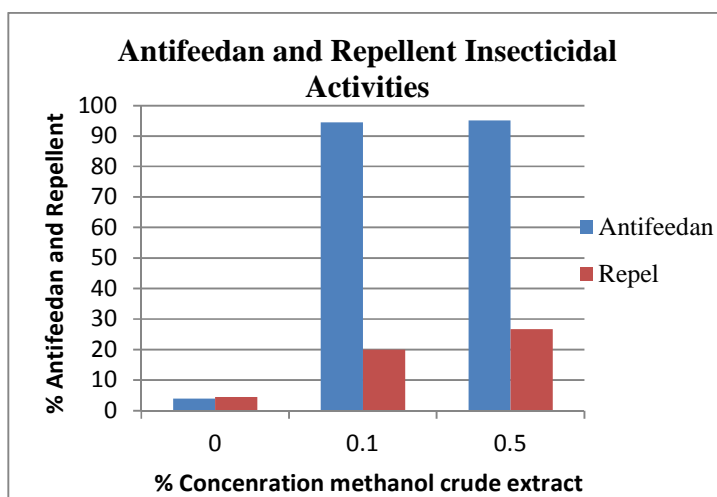


Fig 5. Antifeedan and Repellent Insecticidal Activities of methanol crude extract

From the result can be seen that non volatile fractions also have insecticidal activities. Especially methanol crude extract which all of non volatile compound dissolved. This proves that this type of ginger has the strong active point to become natural insecticide. The value of % mortality, antifeedan and repellent is the function of concentration which also as well as the concentration increase. The slight number of value from control can be considered as a behavioral effects of *Drosophila melanogaster*.

1.5.2 Ethyl Acetate and n-Hexane Fraction Activities

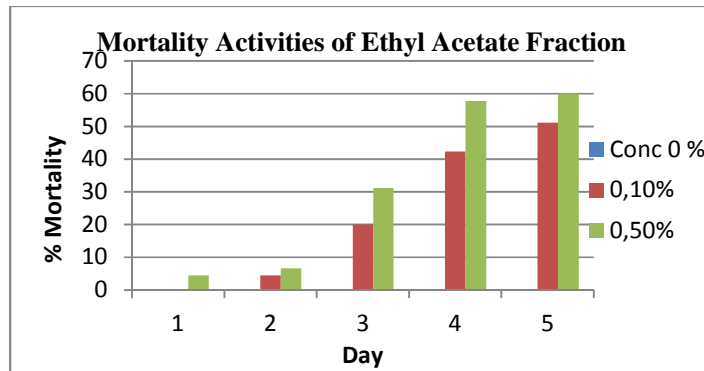


Fig 6. % Mortality ethyl acetate fraction Insecticidal Activities

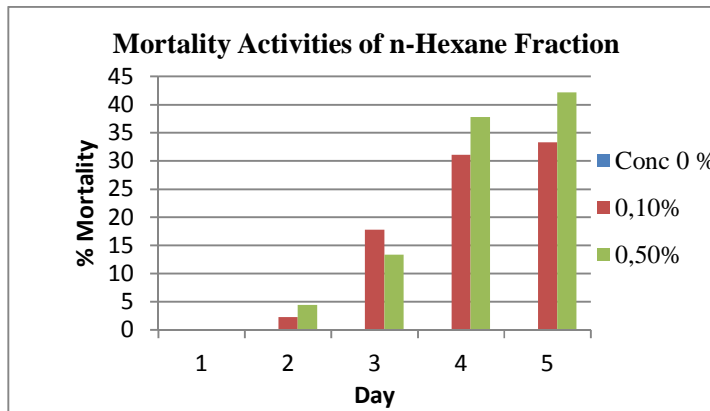


Fig 6. % Mortality n-hexane fraction Insecticidal Activities

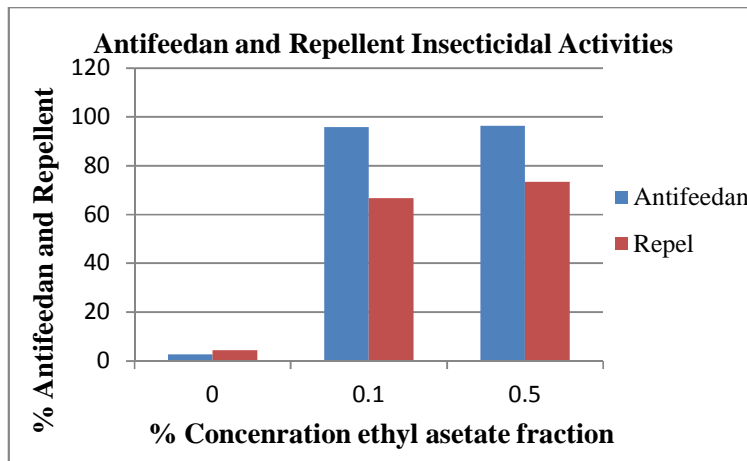


Fig 7. Antifeedan and Repellent Insecticidal Activities of ethyl acetate fraction

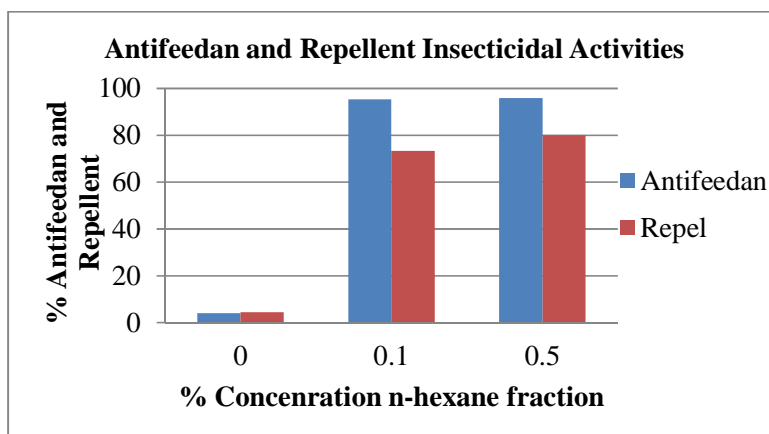


Fig 8. Antifeedan and Repellent Insecticidal Activities of n-hexane fraction

The two fractions showed very good insecticidal activity when compared to controls. On the results of the test of mortality is known that ethyl acetate fraction has a higher value than the n-hexane. This shows that the active compounds act as natural insecticides more contained in the semi-polar and polar fraction. The assay result showed that in the 5<sup>th</sup> day of assay eggs and larva appears in control medium, but eggs and larva did not appear in medium of essential oil and non-volatile fraction. This result conclude that the essential oil and non-volatile fraction from the stem of *Amomum apiculatum* had the potential as natural insecticide.

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