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Analytical and Phytochemical Exploration of Bioinsecticide Granules Mixed Betel Leaf Extract (*Piper betle*) and Srikaya Seed Extract (*Annona squamosa*)

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ABSTRACT

One alternative in controlling *Aedes aegypti* larvae is with the use of bioinsecticides that are environmentally friendly, safe, and inexpensive. Betel leaf and srikaya seeds contain chemical compounds that can be used as bioinsecticide. The following paper deals with detection of phytochemical in the Granules Mixed Betel Leaf Extract and Srikaya Seed using Thin Layer Chromatography (TLC) and FTIR. The TLC analysis was performed Rf value for 0.91 flavonoid compound; alkaloids 0.49; saponins 0.29 and 0.65; tannin 0.89; and anthraquinone 0.22 and 0.45. The FTIR analysis was performed represented the presence of various functional groups which includes alcohol, alkane, nitrile, and ether.

Keywords: Phytochemical, Bioinsecticide, Granula, TLC, FTIR

INTRODUCTION

Background

Insecticide is one type of pesticide that can kill larvae or insects pests. Insecticide can be used to overcome the increase of Dengue Hemorrhagic Fever (DHF) incidence is to break the spreading chain of *Aedes aegypti* mosquito as a vector of dengue fever. DHF is a viral disease that is dangerous because of its high morbidity and mortality.

Insecticides commonly used by communities are temepos (organophosphates) that can cause death in non-target organisms. Therefore, since 1985 WHO recommends to seek natural insecticides that do not cause many negative impacts on non-target organisms. This natural insecticide is called bioinsecticide. One of bioinsecticides to kill *A. aegypti* mosquito larvae is betel leaf and srikaya seeds. The use of betel leaf as an alternative bioinsecticide because betel leaves easy to grow and easily obtained in almost all regions in Indonesia, the price is relatively cheap, and does not cause environmental damage⁽¹⁾. While the use of Srikaya seed as an alternative bioinsecticide because Srikaya seeds are not widely used and become waste so that other uses are needed to increase the economic value of Srikaya seed. The research has been done to produce granule of toxic compound mixture of betel leaf and srikaya seed extract to *A. aegypti* mosquito larva and proven to kill 95% larvae of *A. aegypti* mosquito with 1 g/10 L water dosage in 105 minutes.

Betel leaf and Srikaya seeds contain chemical compounds that can be used as bioinsecticide⁽²⁾. Various research results show that betel leaf contains phenol and its derivative compounds such as kavikol and eugenol, containing alkaloids, tannins, flavonoids, saponins and essential oils that are as larvicidal⁽³⁾. The extracts of srikaya seeds contain chemical compounds of acetogenin group consisting of annonain, squamosin and asimisin⁽⁴⁾. In addition, srikaya seed extract also contains alkaloid compounds, tannins, saponins, flavonoids and fatty oils composed of metal palmitate, metallic stearate and methyl linoleate⁽⁵⁾.

With so many chemical constituents that have many of these properties, it causes the desire to know more about the chemical compounds in the plant. Therefore it is necessary to do research on chemical compound content in granule of betel leaf extract and srikaya seed extract of srikaya seeds.

Purpose

This research aimed to Analytical and Phytochemical Exploration of Bioinsecticides Granules Mixed Betel Leaf Extract (*Piper betle*) and Srikaya Seed (*Annona squamosa*) used thin layer chromatography (TLC) and Fourir Transform Infrared Spektroskopi (FTIR) methods.

METHODS

This research was true-experimental research (Posttest Only Without Control Group Design) conducted in laboratory of Biology and Chemistry, faculty of Pharmacy, Jember University. The granule sample used "Bio Ae" product. Bio Ae was a granule being contained a mixture of betel leaf extract and srikaya seeds produced by the University of Jember in collaboration with the District Health Office of Jember as the user agency and PT Agaricus Sidomakmur Sentosa as the industrial side who would do the production. Bio Ae products had been proposed to obtain HKI and had reached the publication stage with the publication number 2015/01902.

The materials were glassware, butanol, acetic acid, water, ethyl acetat, methanol, n-hexane, chloroform, Plat silica Gel UV-254. Instrumen used is Bruker's alpha FTIR system with OPUS version 7.0.122

Detection Using Thin Layer Cromatography (TLC)

1. TLC Study of Flavonoid

The solvent system of butanol: acetic acid: water (4:1:5) was used as eluent. The favonoids were detected under UV-254 nm light. The Rf values were calculated and noted.

2. TLC Study of Alkaloid

The solvent system of ethyl acetat: methanol: water (9:2:2) was used as eluent. The favonoids were detected under UV-254 nm light. The Rf values were calculated and noted.

3. TLC Study of Sapogeni

The solvent system of n-hexane: ethyl acetat (4:1) was used as eluent. The favonoids were detected under UV-254 nm light. The Rf values were calculated and noted.

4. TLC Study of Polifenol

The solvent system of chloroform: ethyl acetat (1:9) was used as eluent. The favonoids were detected under UV-254 nm light. The Rf values were calculated and noted.

5. TLC Study of Antrakuinon

The solvent system of toluena: ethyl acetat: acetic acid (75:24:1) was used as eluent. The favonoids were detected under UV-254 nm light. The Rf values were calculated and noted.

Detection Using FTIR

Samples were inserted into sample containers with ATR accessories placed in the FTIR spectrophotometer. The detector used is DTGS (deuterated triglycine sulphate). Measurements are made in the range of 1000-4000 cm- wave numbers. Software OPUS 7.2.139.1.24 (Bruker Optik GmbH, Ettlingen, Germany) is used to display the FTIR spectrum. FTIR spectrum data is stored in xls file.

RESULTS

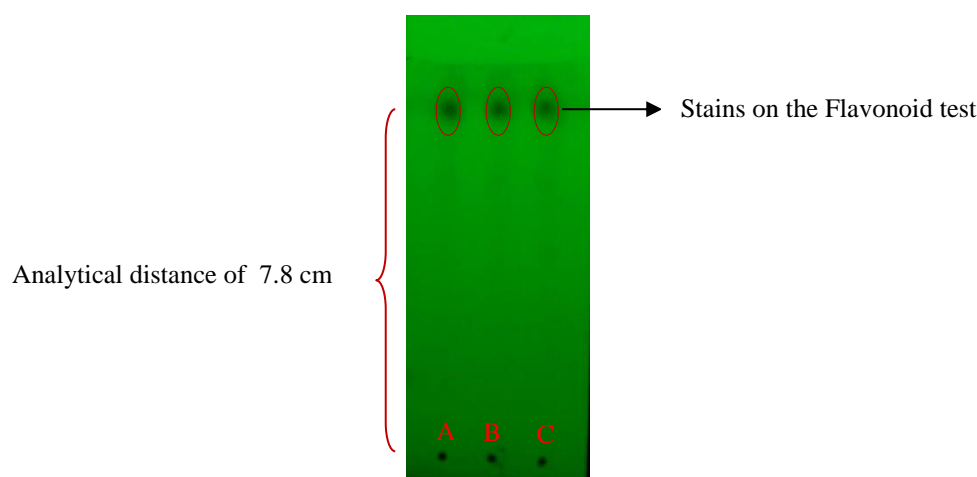


Figure 1. Stain Formed and Analytical Distance on Flavonoid Test. Sampling Place of First Sample Replication (A), Second Replicating Sampling Place (B), Third Replicating Sampling Place (C).

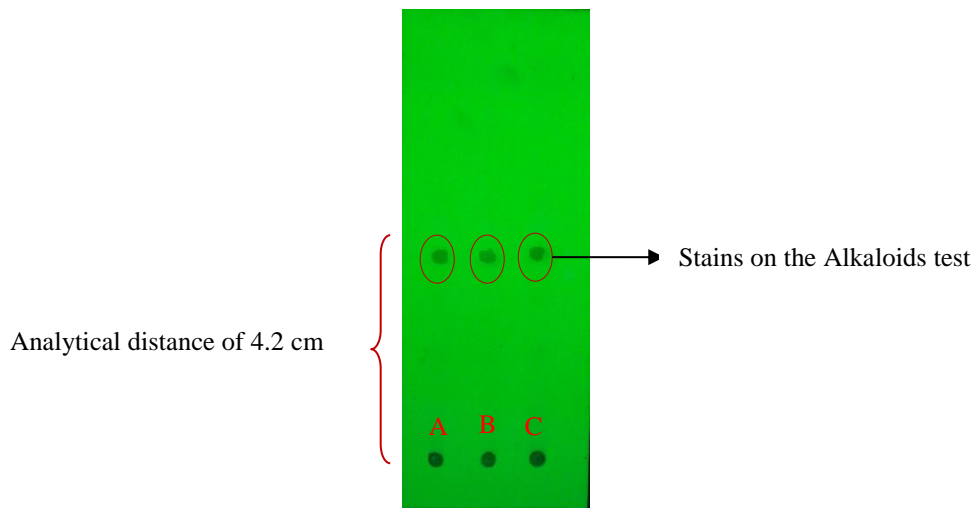


Figure 2. Stain Formed and Analytical Distance on Alkaloid Test. Sampling Place of First Sample Replication (A), Second Replicating Sampling Place (B), Third Replicating Sampling Place (C).

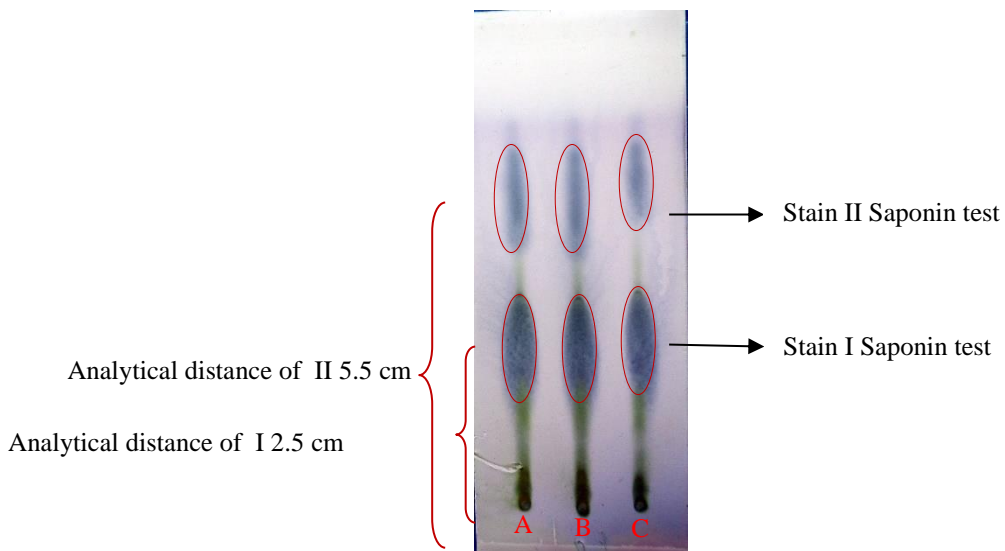


Figure 3. Stain Formed and Analytical Distance on Saponin Test. Sampling Place of First Sample Replication (A), Second Replicating Sampling Place (B), Third Replicating Sampling Place (C).

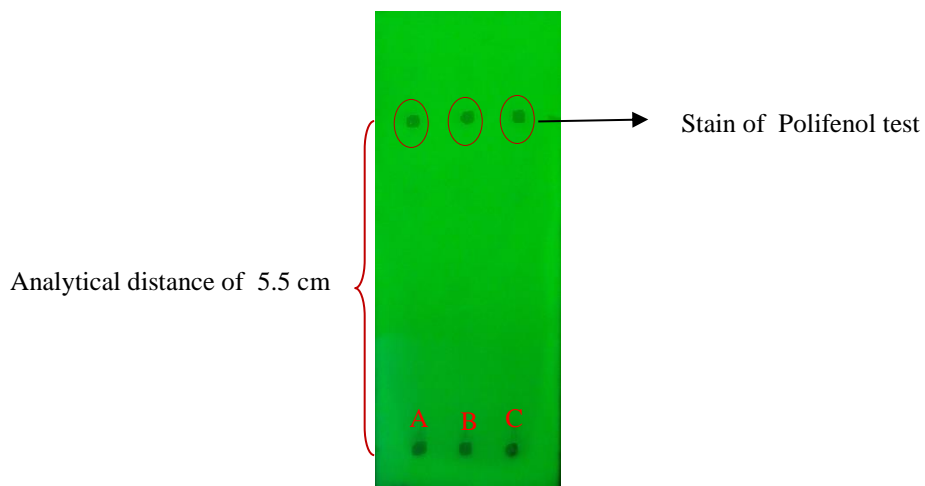


Figure 4. Stain Formed and Analytical Distance on Tanin Test. Sampling Place of First Sample Replication (A), Second Replicating Sampling Place (B), Third Replicating Sampling Place (C).

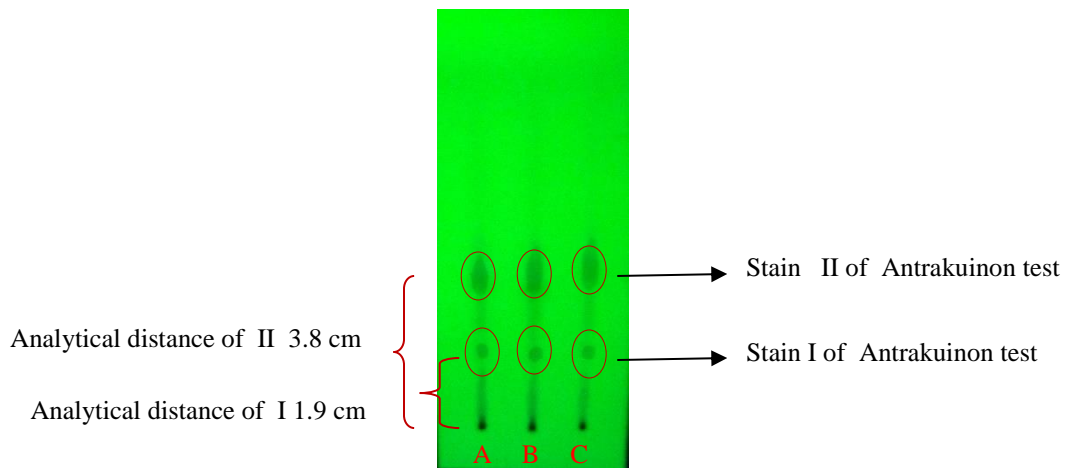


Figure 5. Stain Formed and Analytical Distance on Antrakuinon Test. Sampling Place of First Sample Replication (A), Second Replicating Sampling Place (B), Third Replicating Sampling Place (C).

Table 1. TLC Profile of granule mixture of betel leaf extract and srikaya seed extract

No	Compound	Eluen's Distance (cm)	Analit's distance (cm)	Rf Value
1.	Flavonoid	8.5	7.8	0.91
2.	Alkaloid	8.5	4.2	0.49
3.	Saponin	8.5	2.5 and 5.5	0.29 and 0.65
4.	Polifenol	8.5	7.6	0.89
5.	Antrakinon	8.5	1.9 and 3.8	0.22 and 0.45

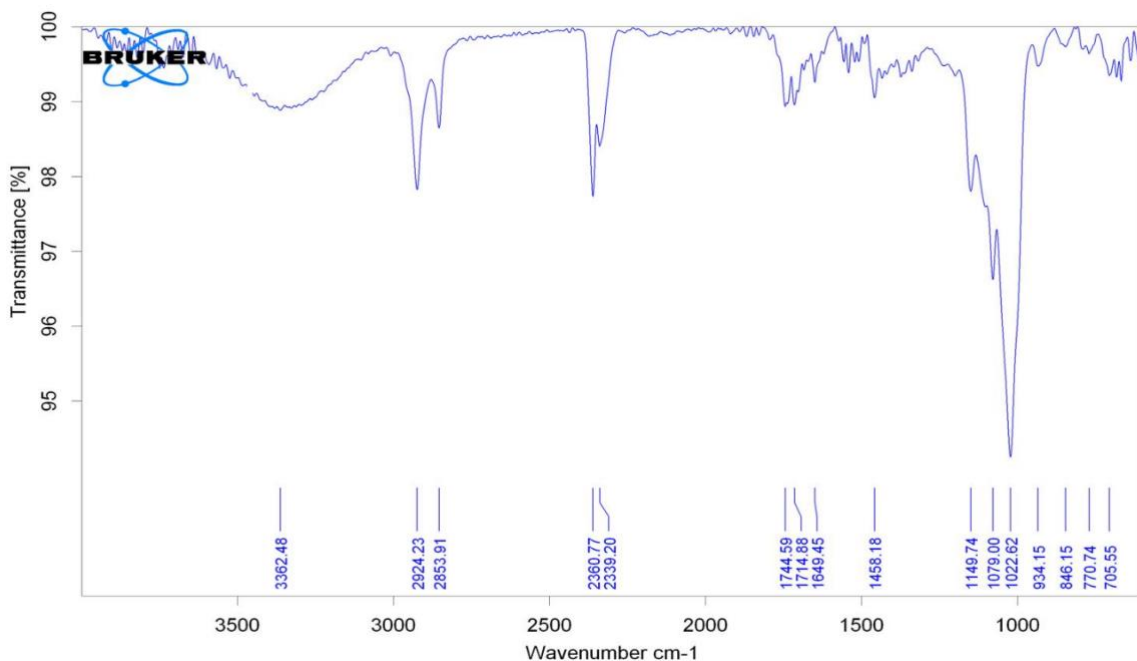


Figure 6. FTIR Profile Granules Mixed Green betel leaf Extract and Srikaya Seed Extract. Interpretation of the results based on the highest absorption peaks so that on the graph above obtained 4 peaks with the highest uptake. Apex Absorption At Wavelength 3362.48 cm⁻¹ (A), Wet-Absorption Absorption At Wavelength 2924.23 cm⁻¹ (B), Wave Absorption At Wavelength 2360.77 cm⁻¹ (C), 1022.62 cm⁻¹ (D).

Table 2. Wavelength Numbers and Functional Groups on Granules

No.	Wavelength (cm ⁻¹)	Bond	Functional Group
1.	3362.48	O-H stretch, H-Bonded	Fenol, alcohol
2.	2924.23	C-H Stretch	Alkana
3.	2360.77	-C≡N	Nitril
4.	1022.62	C-O Stretch	Eter

DISCUSSION

Detection Using Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is a technique of separating the solute by a dynamic differential migration process in a system consisting of two or more phases in which one phase moves continuously in a particular direction. Data obtained from TLC is the value of Retention factor (Rf) which is useful for the identification of compounds. The Rf value can be defined as the distance traveled by the compound from the point of origin divided by the distance traveled by the solvent from the origin. The TLC analysis was performed Rf value for 0.91 flavonoid compound; alkaloids 0.49; saponins 0.29 and 0.65; tannin 0.89; and anthraquinone 0.22 and 0.45.

Detection Using FTIR

Based on the results of IR spectrophotometer analysis, the absorption bands that appear at certain wave numbers are obtained. Weak uptake at wavelength 3362.48 cm⁻¹ OH group of alcohols reinforced with CO group at wavelength 1022.62 cm⁻¹. The absorption at the wavelength of 2924.23 cm⁻¹ indicates the presence of C-H groups from alkanes while the absorption at wavelength 2360.77 cm⁻¹ indicates the presence of a -C≡N group of nitriles. This indicates that the granule mixture of green betel leaf extract and srikaya seed contains saponin. The results of this study should be continued by determining how much the number of C atoms and the H atoms contained using the core magnetic resonance method (NMR) so as to determine the structural formula of the compound. In addition, information about the molecular weight of the compound should also be known using the Liquid Chromatography with Mass Spectrometry (LC-MS) method. A chemical compound in a plant has a structure and a certain molecular weight that can be used as the basis for its identification.

The functional group is a special group of atoms in the molecule that play a role in giving the characteristic chemical reaction to the molecule. The functional group is a reactive member of a molecule so that the functional group determines the properties of many compounds. One of the properties of the compound that acts as a larvacide is its polarity. This biolarvasida naturally will be used in water so it needs polar biolarvasida active compound. In addition to the active compound distribution process, the penetration of the biological membrane is primarily influenced by the lipophilic properties of molecules such as their solubility in fat / water. Polar compounds are compounds formed from atoms that have large electronegativity differences. In polar compounds, the shared electrons are attracted stronger to one of the atoms. As a result one of the atoms will become more negatively charged and the other atom is positively charged.

The OH alcoholic group is polar. In an alcohol, the longer the hydrocarbon chain the lower the solubility. Even if this hydrophobic nature is long enough it defeats the hydrophilic nature of the hydroxyl group. The number of hydroxyl groups can increase solubility in water. Alkanes are saturated aliphatic hydrocarbons, ie hydrocarbons with an open chain and all bonds between carbon atoms are single bonds. All alkanes are difficult to dissolve in water. This is because the alkane molecule is nonpolar, whereas water is a polar solvent. Alkanes also have a chemical nature to react with other substances because it is a saturated hydrocarbon. The ether compounds do not have hydrogen bonds because they have no acidic H atoms attached to their O atoms. Ether has only one type of style between molecules, namely London style. With the increase in the number of C atoms in the carbon chain, the strength of the London style has increased. The C-O group in the ether is polar. However, the solubility of ether in a polar solvent such as water is very small because the ether forms a very weak hydrogen bond with water. This solubility also decreases with the length of the carbon chain because the ether is increasingly non polar. Nitriles are organic compounds that have a functional group -C≡N. Nitriles are a group of toxic compounds because they contain CN groups in their structure. The linear N-C-C geometry in nitriles represents sp hybridization of triple carbon. Nitriles are very polar compounds. Atom N is a very electronegative atom that easily pulls the electrons in a three-dimensional bond toward it. Because it has a pole, the nitrile compound has a Van der Waals force in the form of a very strong permanent dipole. Although nitrile compounds do not form hydrogen bonds with their fellow molecules, but when dissolved into water, the hydrogen bonds will form with water molecules. In addition to forming hydrogen bonds with water molecules, nitrile compounds can also form a permanent dipole - dipole dispersion force with water because these two compounds are polar. The formation of forces between water molecules with this nitrile will release energy. This energy can be used to separate forces between molecules that occur between fellow compounds of nitrile and water, so that they can be mixed perfectly.

As the carbon chain lengthens on the nitrile compound, it reduces the formation of hydrogen bonds with water molecules. this causes the solubility of the compound to decrease.

By studying the functional groups of a compound also serves as a first step in determining the presence of haptoforic and pharmacoforic groups that determine the biological activity of a compound. The Haptoforik group is a group that plays a role in the formation of complex bonds of chemical compounds and receptors whereas the pharmacoforic groups are the groups responsible for the biological response. The biological response results from the interaction of drug molecules with functional groups of receptor molecules. The types of bonds between active molecules and receptors include covalent bonds, hydrogen bonds, ion-dipoles, and van der Waals bonds. Covalent bonds are the strongest bond between receptor and drug compound. Modification of functional groups of a compound can improve the strength of the receptor bond using bioisosterism. Bioisosterism is the replacement of functional groups in an active specific molecule with another group that produces new compounds with better biological activity.

CONCLUSION

The result of analysis using layers chromatography showed that the mix granule of betel leaf and srikaya seed contains flavonoid compound, alkaloid, saponin, tannin, and antrakinon. The result of analysis using FTIR spectrophotometry showed that the granules of srikaya seed mixture and betel leaf contain alcohol group, alkane, nitrile, and eter.

Further research is needed from this granule to obtain secondary metabolite compounds because it is possible there are many other compounds that have not been identified in this study. Analysis by instrumentation method LCMS and C-NMR is needed to obtain more complete and accurate information in identifying molecular structure of isolate compound.

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