

The Physical Properties And Phytochemical Exploration Of Bioinsecticide Granules Mixed Betel Leaf Extract (Piper Betle) And Srikaya Seed Extract (Annonasquamosa)

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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. Granules mixed betel leaf and srikaya seeds contain chemical compounds that can be used as bioinsecticide. When granule formulation is designed, it is necessary to understand the properties of granules to determine the optimal type, grade, and amount of material. In addition, the content of chemical compounds in granules is also an important aspect that must be known. This study aims to determine the physical properties and phytochemical contents of granules mixture of betel leaves extract with srikaya seeds extract. The physical properties to be studied are tap density and fragility of granules. The research was conducted by conducting laboratory test using tap density and sieving machine. The following paper deals with detection of phytochemical in the Granules using Thin Layer Chromatography (TLC) and FTIR. The results obtained the tap density determination on the first, second and third replication, obtained value of 9.33%, 5.28%, and 7.11% and the fragility granules on the first, second and third replication, the value of 0.05%, 0.1%, and 0.2%. 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: granular, tap density, fragility, phytochemical, TLC, FTIR

1. INTRODUCTION

Dengue fever is a challenge that Indonesia must be addressed strategically, nationally, focus and comprehensiveness. The most effective way to overcome dengue fever is to break the life cycle of *Aedes aegypti* mosquito as its vector. Temephos is the only chemical insecticide used to combat the *Aedes aegypti* mosquito larvae, has been known to cause negative effects such as resistance. Indonesia needs to be encouraged to look for new insecticides as control of *Aedes aegypti* mosquito larvae (Wahyuni, 2015). The World Health Organization (WHO) recommends developing bioinsecticides and finding biological control agents as an alternative to dengue vector control in order to target specific, safer, and environmentally friendly ones (WHO 1991 in Wahyuni 2003). Previous research has obtained a granule of green betel leaves (Piper betle) extract with srikaya seeds (*Annonasquamosa*) characteristic of amorphous shape, brownish white color, mesh size 40-60, drying time of 2-6 hours, drying temperature 40-55 degrees Celsius, can kill *Aedes aegypti* mosquito larvae with a dose of 1 gram in 10 liters of water within 105 minutes by 95% with effective duration of 14 days (Wahyuni, 2016).

Betel leaf and srikaya seeds contain chemical compounds that can be used as bioinsecticide (Dwiwahyuni, 2013). 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 (Aulung, 2010). The extracts of srikaya seeds contain chemical compounds of acetogenin group consisting of annonain, squamosin and asimisin (Hermianto et al., 2004). In addition, srikaya seed extract also contains alkaloid compounds, tannins, saponins, flavonoids and fatty oils composed of metal palmitate, metallic stearate and methyl linoleate (Taslimah, 2014). 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.

2. RESEARCH METHOD

2.1 Research Time and Area

The research will be conducted from January to February 2018 and conducted at Pharmacy Laboratory of Jember University for physical properties and chemical compounds of Granulite combination of betel leaves extract (Piper betle) and extract of srikaya seeds (*Annonasquamosa*).

2.2 Research Instruments and Materials

The instruments used in the process of a series of physical properties tests are: Drying bin (memmert), Stainless steel funnel, Stop watch, Digital Scales, tap density, multilevel screen and sieving machine. The instruments used in the analytical phytochemical contains are Bruker's alpha FTIR system with OPUS version 7.0.122. The materials used are compound granules mixture of betel leaves extract and srikaya seeds extract with heating 55°C, glassware,

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butanol, acetic acid, water, ethyl acetat, methanol, n-hexane, chloroform, Plat silica Gel UV-254.

2.3 Research Procedure

2.3.1 The Physical Properties Of The Granules

a. Tap density

A number of granules are inserted into a tilt measuring glass then enforced. Added granules to a volume of 100 ml, recorded as V_0 . The measuring cup is attached to the appliance and then the rotor is turned on. Records of volume changes at 5, 10, 25, 50 and 100 minutes are recorded as V_t . If not already obtained the constant volume is then continued until a constant volume is obtained, recorded as V_k . Calculated value $T\%$ (after volume is obtained constant) and note the weight of granules used. Tap density can be calculated by the formula:

$$\frac{V_0 - V_t}{V_0} \times 100\%$$

b. Fragility

One hundred grams of granules are inserted into a multistage sieve with the top sieve no. 30 Mesh and bottom pan. The sieving is done by sieving machine. The engine runs at 50 amplitudes for 10 minutes. The fragility of the granula is calculated by the formula :

$$\frac{\text{initial weight} - \text{lagging weight}}{\text{initial weight}} \times 100\%$$

2.3.2. Analytical of Phytochemical contents of the granules

a. Detection Using Thin Layer Cromatography (TLC)

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 R_f values were calculated and noted.

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 R_f values were calculated and noted.

TLC Study of Sapogenin

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

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 R_f values were calculated and noted.

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 R_f values were calculated and noted.

b. 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^{-1} 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.

3. RESULT

3.1 The Physical Properties Of The Granules

The tap density determination on the first, second and third replication, obtained value of 9.33%, 5.28%, and 7.11% and the fragility granules on the first, second and third replication, the value of 0.05%, 0.1%, and 0.2%.

3.2 Analytical of Phytochemical contents of the granules

Result I

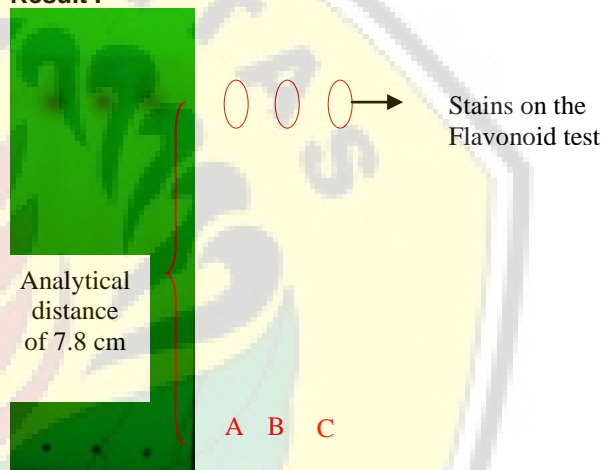


Figure 1. Stain Formed and Analytical Distance on Flavonoid Test. Sampling Place of First Sample Replication (A), Second Replicating Sampling Place (B), Third Replicating Sampling Place (C).

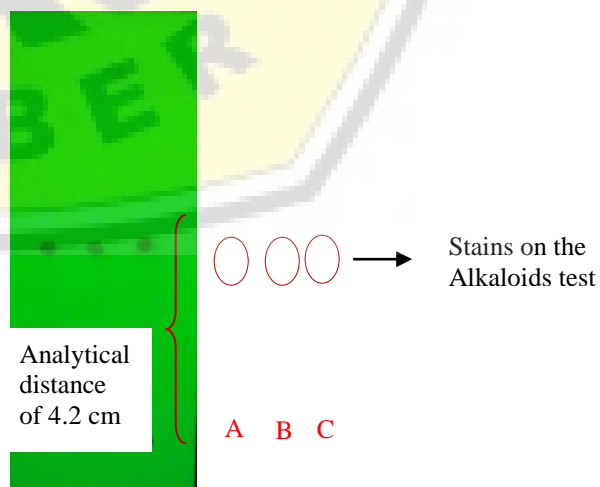


Figure 2. Stain Formed and Analytical Distance on Alkaloid Test. Sampling Place of First Sample Replication (A), Second Replicating Sampling Place (B), Third Replicating Sampling Place (C).

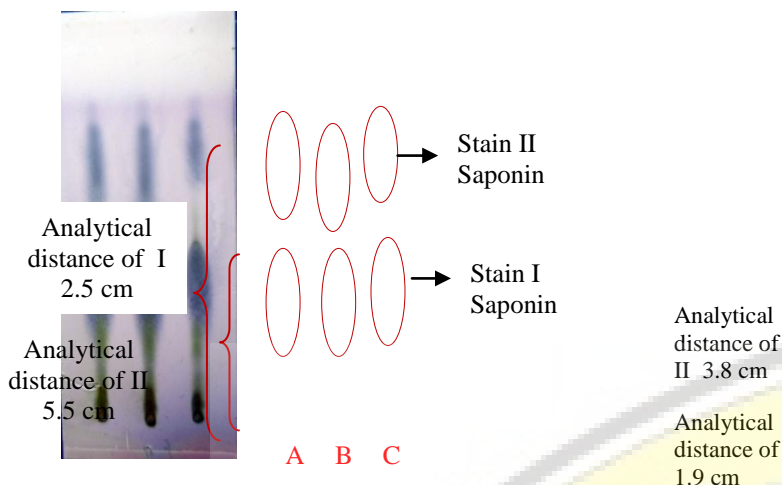


Figure 3. Stain Formed and Analytical Distance on Saponin Test. Sampling Place of First Sample Replication (A), Second Replicating Sampling Place (B), Third Replicating Sampling Place (C).

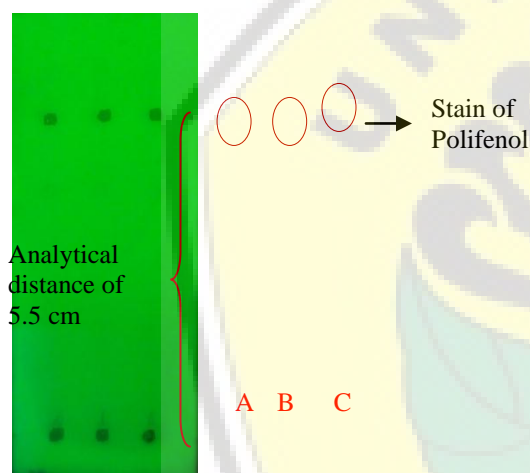


Figure 4. Stain Formed and Analytical Distance on Tanin Test. Sampling Place of First Sample Replication (A), Second Replicating Sampling Place (B), Third Replicating Sampling Place (C).

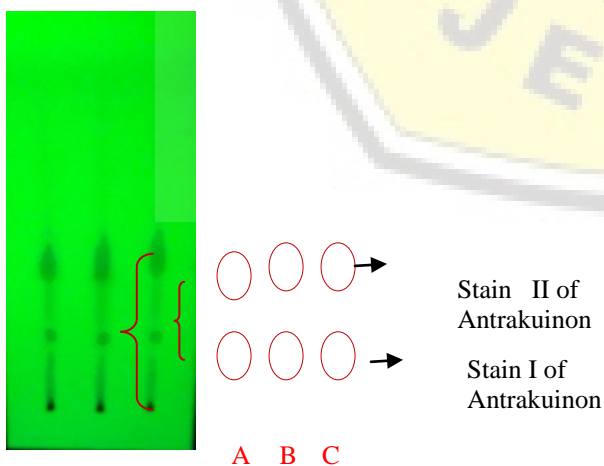


Figure 5. Stain Formed and Analytical Distance on Antrakuinon Test. Sampling Place of First Sample Replication (A), Second Replicating Sampling Place (B), Third Replicating Sampling Place (C).

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 |

Result II

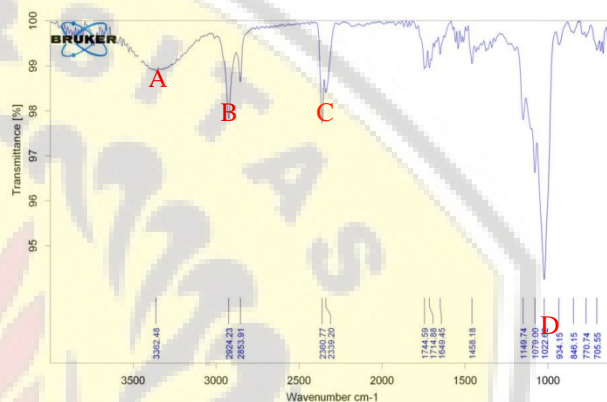


Figure 6. FTIR Profile Granules Mixed Green betel leaf Extract and Srikaya Seed Extract. Interpretation of the results based on the highest absorption peaks so that on the graph above obtained 4 peaks with the highest uptake. Apex Absorption At Wavelength 3362.48 cm^{-1} (A), Wet-Absorption Absorption 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 Strecth | Alkana |
| 3. | 2360.77 | $\text{C}\equiv\text{N}$ | Nitril |
| 4. | 1022.62 | C-O Stretch | Eter |

4. DISCUSSION

4.1 The Physical Properties Of The Granules

a. Tap density

The determination is a change in the volume of the granule when given a pound or to obtain a constant volume. A granule that gets the beat and vibration will fill or occupy in such a way on the empty space between granules, resulting in a compressed volume. The purpose of the determination is to know the compactness of a granule when given vibration or pounding. The density also relates to the flow

properties of a granule. The properties of the granular flow will be good if the value of tap density shows less than 20% (Lachman et al., 2007). The tap density is the ability of granules to remain compact with the presence of pressure. The tap density depends on how much the volume drops when the granule is given vibration or pounding. The criteria for the tap density are: 5-11: special; 12-17: good; 18-22: medium; 23-34: not good; 35-39: very bad; > 40: very bad (Ansel, 2005). Based on the result of examination, it can be seen that granules has exceptional tap density criteria with an average value of 7.24%. The things that can be done to improve the compactness of the granules are 1) Wetting (wetting), the wetting can increase the tap density of granules. This is due to the presence of strong inter-particle bonds with appropriate moisture. Thus, the granules can be compressed well. This affects the resulting granule. Good determination will produce good and compact granules. Granules made with wet granulation are better than granules made by dry granulation method. This is due to the dry granulation of the absence of the wetting process so that the powders to be mixed can not be fused perfectly to be a good granule; 2) The particle shape and texture changes, the shape and texture of the particles can influence the tap density, in the presence of variations in the shape and texture of the particles will produce good resolutions. This reduces the presence of fines in the granular molding process. The shape and size of the particles also affect the porosity (partikel density) which will also affect the determination. If porosity is good it will result in good tap density with low value (<20%). This is because porosity can reduce the presence of cavities at the time of forging the granules; 3) The addition of adhesives and binders, adhesion is an attractive force between molecules of different kinds. This force causes between substances with one another to stick well because the molecule pull each other pull or glue. The addition of an adhesive agent may increase the tap density due to an increase in tensile forces between different molecular types. So that between substances that one with other substances can be binding each other (Nagar et al., 2011).

b. Fragility (%)

Granular fragility is a picture of granular physical stability. It can be observed through its resistance to vibration by placing it on a vibrant stratified screen. The granular fragility indicator can be determined by how much percentage of weight is lost during the test. The fragility of the granules is also influenced by the amount of content in the granules. The fragility of granula is said to be good if its value is less than 1% (Lachman et al, 2007). The fragility value of granules mixture of betel leaves extract and srikayaseeds extract that meet the requirements of good granular fragility is <1%. Exposure to hot temperatures essentially improves the fragility of the granules because during the granule heating process becomes strong by the crystallization of the binder. shows the higher the heating temperature then the granule fragility will be better. But that does not mean that the temperature is too high also improve the fragility of the granule, even can actually make the percentage of granula is higher which means showing granules increasingly fragile or easily destroyed (Ade et al., 2014). The higher the drying the granule fragility will increase. The granule formula that has the smallest granular fragility value makes granular

dust due to fragile treatment tends to stick. Dry granules cause fragile granules to become smaller particles. This is caused by high granular humidity and tend not to much dust or powder so that the resulting granula is still soft and not easily fragile. Granules with high temperature and drying time tend to have a lot of dust or powder, so the granules become easily brittle

4.2 Analytical of Phytochemical contents of the granules

a. 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.

b. 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 $\text{-C}\equiv\text{N}$ 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 $-C\equiv N$. 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 Haptoforic 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 physical properties showed that the mix granule of betel leaf and srikaya seed had met tap density and fragility requirements. This will make the granule will have stable physical properties. 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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