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Green Chemistry: Strategy in Essential Oils Sustainability by Development of Insecticide Using Docking Method

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Abstract. Sustainable agricultural applications in green chemistry was associated with the development of insecticide production based on secondary metabolites, such as essential oils. This research used In Silico modeling for insecticide formulation based on essential oils. The insecticidal formula was made on the basis of the Ki value of multiple docking results between the major components of essential oils as ligand with Spodotera litura receptor (2DJC) studied using Autodock Tools software. Insecticide formula activity test was done by contact method of toxic and leaf contact with essential oils concentration at level 0% - 1%. The results of the in silico study showed that the inhibition constants (Ki) of citronellal and anethol ligands combination were 1.6 mM however of citronellal and eugenol as ligands were 1.75 mM and formulated rasio (v/v), respectively 5 : 1 and 4 : 1. In addition, in vitro activity of insecticide formula with the ratio of 5: 1 possess LC50 value 0.10% (toxic contact) and 0.35% (leaf contact). While the formula with a ratio of 4: 1 possess LC50 value 0.05% (toxic contacts) and 0.31% (leaf contact).

1. Introduction

The green chemistry revolution is providing an number of challenges to those who practice chemistry in industry, education and research. With these challenges however, there are an equal number of opportunities to discover and apply new chemistry, to improve the economics of chemical manufacturing and to enhance the much-tarnished image of chemistry.

The challenge for chemists and others is to develop new products, processes and services that achieve the societal, economic and environmental benefits that are now required, as well as in agriculture to make natural insecticides. This requires a new approach which sets out to reduce the materials and energy intensity of chemical processes and products, minimise or eliminate the dispersion of harmful chemicals in the environment [1].

Many areas of science and engineering have progressed in terms of rapid data generation, data analysis, building mathematical models, and computer simulations. In the biomolecular field, in silico research is a form of computational modeling to study biological aspects as well as interactions at the molecular level [2]. Docking is a computational and modeling method used to determine the interaction between ligands with a receptor or protein [3]. AutoDock is a docking program that has a flexible docking technique and a grid-based quick method for energy evaluation [4]. This computational method uses the Iterated Local Search (ILS) algorithm [5]. The usefulness of the docking program has evolved in areas such as the pharmaceutical field in the development of new drug discovery and design, the industrial field in the manufacture of perfumes and agriculture for example in the manufacture of pesticides. In the biomolecular field by knowing the interaction on the
desired compound we can make a natural pesticide formula with natural base material ie essential oil. Where essential oils also have the opportunity to be developed into other derivative products such as pesticides. The use of docking molecule modeling as an effort to make natural pesticide formulations can reduce the conventional pesticide manufacture conventionally, the preparation of a series of formulation with variations of the bioactive component, making it more time efficient and cost.

Essential oil obtained from citronella oil has the main components of citronellal (36.19%), geraniol (32.82%) and citronellol (11.37%). The main components of citronella have phytotoxic and antimicrobial properties that can be used as pest control [6]. The active components of oil basil are methyl chavicol (estragole), methyl eugenol, linalool, champer. Basilicum oil (Oscimum basilicum L.) contains an insecticidal bioactive element [7]. In Chiou Ling Chang's study, et al (2009) basilicum oil (tested on three species for insecticidal activity showed a steep dose-response relationship. Deadly periods for 90% mortality (LT₉₀) [8]. While the main component of clove oil is eugenol (80-90%) which is as antioxidant and antimicrobial. The antimicrobial mechanisms of eugenol compounds include disrupting cell membrane function, inactivating enzymes, inhibiting chitin synthesis, nucleic acids and proteins and inhibiting energy production [9]. Hummelbrunner and Murray (2001) studies reported that eugenol and citronellal compounds are toxic to grayak caterpillar S. litura with each LD₅₀ being 2.5-157.6 mg / insect and 66.0-111.2 mg / insect [10]. Grayak caterpillars are pests that attack crops such as tomatoes, chilli and tobacco, causing the leaves to become perforated with 20-40% damage to the plants, so that with the manufacture of pesticides from essential oils can help reduce damage to plants.

2. Materials and Methods
2.1 Materials
The materials used in the study in silico are the 3D receptor structure of the grayak caterpillar growth protein (S. litura) downloaded from www.rcsb.org with the 2DJC pdb access code. The ligands used are ligands of the main constituent components of citronella oil (citronellal, geraniol, citronellol), basil oil (anethole) and clove oil (eugenol) with 3D structure downloaded from www.chemspider.com. While the ingredients used in research in vitro are citronella oil (CO), basil oil (BO), clove oil (CLO), acetone, tween 80 and aquades. Insect test used is grayak caterpillar (S. litura) instar 2.

2.2 Methods
2.2.1 Plant Refinery Basil.
The process of distillation begins by inserting water as much as 1- 2 liters into the kettle. Then put chestnut on water and basil leaves weighing 1 kg put on chestnut. Then close the kettle tightly so that no steam will be removed. After all the tools of the distillation process is done by heating. The distillation process is performed 4-5 hours.

2.2.2 Analysis of Essential Oil Components
Essential oil contains volatile compounds, so it can be analyzed by gas chromatography mass spectrometry (GC-MS) method. Gas Chromatography-Mass Spectrometry is a combination of two instruments, namely gas chromatography that serves as a component separator on the sample mixture and mass spectrometry that serves to detect compounds that have been separated on gas chromatography. CO, BO, CLO were characterized using GC-MS with GC-MS type QP2010S / shimadzu. This type of GC-MS has a column length of 30 meters with a column temperature of 60-300 ° C. Temperature injector and temperature detector 280°C which has a gas flow rate of 90 mL/min with a helium gas.

2.2.3 Determination of Ki Value Using In Silico Method
Ligands with 3D structures used are ligands of major constituent components CO (geraniol, citronellal and citronellol), BO (anethole) and CLO (eugenol). Then the ligand is optimized on Hyperchem software. Ligands are stored in (.hin) format. Ligand that has been optimized, changed its extension from (.hin) to (.pdb) in OpenBabel 2.3.2. The macromolecule (receptor) used is Spodoptera litura with
2DJC access code with pdb extension. The protein macromolecules are separated from solvents and ligands using the Discovery Studio Visualizer 3.5. Then do molecular optimization with .pdb format. Macromolecules and ligands were docking using AutoDock Tools software to determine the molecular interactions between ligands and macromolecules to obtain Ki values and known formulas for the manufacture of insecticides. The docking process consists of 4 stages: ligand and protein receptor preparation (file format ".pdb" changed to ".pdbqt"), running Autogrid (change file format to ".gpf"), running AutoDock (file format changed to ".dpf") and analysis of docking results. Docking process is done that is single and multiple ligand docking. Each ligand of the main constituent components CO (geraniol, citronellal and citronellol) and BO (anethole) were docking with 2DJC receptors as well as the main component ligands CO and CLO (eugenol) were docking with 2DJC receptors. Based on Ki value of docking result of each ligand of main component of CO, selected one ligand with lowest Ki value which then docking by multiple ligand docking with anetol ligand and eugenol interchangeably. Multiple ligand docking interaction modeling is done in two stages that produces Ki1 and Ki2 values. First, macromolecular docking with ligand 1 is generated Ki1 value. Then the docking results are stored with the file format ".pdbqt" to redocking with ligand 2 so Ki2 is generated.

2.2.4 Making Natural Insecticide Formulas

The preparation of the formulation solution is based on the results obtained from the in silico test. In silico test, Ki value is generated with unit mM (milli Molar) so that IC50 can be determined. CO and BO formulations as well as CO and CLO can be determined by multiplying the IC50 values by the molecular weight of each ligand with the following equation:

\[ [M] + [L] \rightleftharpoons [M - L] \]  
\[ K_d = \frac{[M][L]}{[M][L]} \]  
\[ Jika \ [M] \gg [L], maka [ML] = [M] \]  
\[ K_d = \frac{[M][L]}{[L]} = [L] \]  
\[ K_d = [L] \quad (Ki=Kd) \]  
\[ Ki = \frac{IC_{50}}{1+L/K_d} \]  
\[ Ki = \frac{IC_{50}}{K_d} \]  
\[ Ki = \frac{IC_{50}}{1+L/K_d} \]  
\[ Ki = \frac{IC_{50}}{1+1} = \frac{IC_{50}}{2} \]  
\[ IC_{50} = 2 Ki \]

According to equation a.6, then are citronellal concentration in CO =IC50 \cdot M_{citronellal} anethole concentration in BO = IC50 \cdot M_{anethole} and eugenol concentration in CLO = IC50 \cdot M_{eugenol}. If the value of IC50 and mass of molecule unrtuk each is included in the equation then obtained the ratio of oil in insecticide formula, that is CO-BO (5: 1) and CO-CLO (4: 1). The volume composition of the formula is assumed in the research of Pinheiro et al (2013), with tween 80 (mL): oil (mL): acetone (mL) ratio is 1: 11600: 20000, so the comparison of natural insecticides is obtained. The natural insecticide formulation was prepared by dissolving CO in the BO (ratio 5: 1), acetone, tween 80. Then put into 100 mL poultry flask and added aquades up to 100 mL volume then shaken until homogeneous. The second formulation is made in the same way but uses CO and CLO (ratio 4: 1). In this study, the concentration interval based on insecticide formula above was used 0%, 0.0625%, 0.125%, 0.25%, 0.5% and 1% concentration.
2.2.5 Test of Natural Insecticide Activity
Activity test of natural pesticidal formula was carried out by contact method of toxic and leaf contact adopted from the research of Pinheiro, et al (2013) [10]. Leaf contact method test is method that done by way of grayak caterpillar instar 2 instilled into container of test (19 x 19 x 30 cm) which contain jatropha leaves ± 15 cm diameter previously sprayed natural insecticide as much as 1.5 mL per leaf side. While the toxic contact method is done by natural insecticides directly sprayed into the grayak caterpillar in a 250 mL glass of glass (diameter 8 cm, height 10 cm). Observation of grayak caterpillar death after 1 hour application for 24 hours.

3. Result and Discussion
3.1 Plant Refinery Basil
Basil oil obtained from the distillation of basil plant (Ocimum basilicum L) result of determination from Biology Department, Faculty of Science Brawijaya University. The distillation process is carried out by the method of steam-water distillation. Basil leaves are used as much as 1221 grams, distillation lasts until all the essential oil runs out of the marked by not dripping oil from the condenser. Essential oils obtained have a yield of 1.16% (w/w) then collected and stored in tightly sealed containers. The oil of basil plant distillation has a clear yellow color with a specific gravity of 0.952 g/mL.

3.2 Analysis of Essential Oils Composition
Based on the analysis using GC-MS it appears that in CO detected 30 peaks. The three main components of CO composition with the largest component of geraniol were 32.30%, citronellal 17.26% and citronellol 10.56% (Table 1). Subsequently on the oil of basil was detected 8 peaks with the dominant peak of anethole compound 83.98% (Table 2). The CLO showed that there were 8 peaks with 1 peak showing the highest content of clove oil, ie eugenol compound as much as 65.90% (Table 3). The peaks described in the chromatogram can be determined by their constituent components based on the available mass spectra. The mass spectra was compared to patterns of fragmentation of similar component patterns in libraries based on WILLEY7.LIB library by ensuring SI values above 94. Simulation interaction of citronellal and anethole molecules with 2DJC receptors as well as citronellal and eugenol molecules with 2DJC receptors (S. litura) in silico.

Table 1. Major components of citronella oil

| Peak | tR (min) | % Area | m/z                  | SI | Compound     |
|------|---------|--------|----------------------|----|--------------|
| 8    | 12.589  | 17.26  | 154,140, 136, 121, 111, 95, 83, 69, 55, 41, 38 | 94 | Citronellal  |
| 11   | 13.800  | 10.56  | 156, 138, 123, 109, 95, 81, 69, 55, 41 | 96 | Citronellol  |
| 13   | 14.304  | 32.30  | 154, 136, 123, 111, 93, 70, 69, 55, 41 | 96 | Geraniol     |
Table 2. Major component of basil oil

| Peak | tR (min) | % Area | m/z          | SI | Compound |
|------|----------|--------|--------------|----|----------|
| 8    | 13.351   | 83.98  | 148,147,133,117,105 | 96 | Anetol    |

Table 3. Major component of clove oil

| Peak | tR (min) | % Area | m/z          | SI | Compound |
|------|----------|--------|--------------|----|----------|
| 3    | 15.938   | 65.90  | 55, 77, 91,103, 131,149,164 | 97 | Eugenol  |

Geometric structure optimization using HyperChem software is done to obtain ligand conformation with the most stable geometry structure. The most stable conformation of the most stable structure of the ligand geometry is represented by several parameters, ie the lowest value of Ki indicating the ligand to interact with the active side of the strong receptor.

Table 4. Result optimized on Hyperchem and docking with Autodock Tools for each ligand

| Essential Oil | Ligand | Log P | Surface Area (Å²) | Hidration Energy (kcal/mol) | Ki Values (mM) |
|---------------|--------|-------|-------------------|---------------------------|----------------|
|               | Geraniol | 2.46  | 444,73            | -3.89                     | 3.29           |
|               | CLO     | Citronellal | 2.26  | 451,41            | 14.12          | 2.18           |
|               |         | Citronellol | 2.75  | 434,70            | -2.78          | 5.13           |
|               | BO      | Anethole | 1.29  | 357,35            | -2.87          | 1.18           |
|               | CO      | Eugenol   | 0.31  | 338,28            | -7.58          | 1.14           |

Docking each ligand with the receptor protein that has been done will result in 10 models of ligant interaction conformation with macromolecules. From 10 kinds of conformation model of docking result obtained one of the most stable conformation model is the lowest price of Ki. By knowing the smallest value of Ki from each component of compilers CO, BO and CLO can be done multiple ligand docking is docking two ligands.

Multiple docking results between pairs of two ligand molecules from citronellal-anetol and citronellal-eugenol with receptors from the S. litura insect macromolecules are presented in Figure 1. Ki's value results from the interaction of these molecules are presented in Table 5.
3.3 Insecticide Activity Test based on Essential Oils
The results of testing of natural insecticide activity showed that CO-BO and CO-CLO based insecticides caused mortality of *Spodoptera litura* larva. The larval mortality of *S. litura* is shown in Figure 2 and Figure 3. Based on the image can be seen that at 0% concentration no mortality occurs in *S. litura*, this indicates that the solvent has no effect on mortality. So this 0% concentration can be used as a control for natural insecticides. In the graph of insecticidal concentration relationship (a) CO-BO (b) CO-CLO with present mortality *Spodotera litura* by toxic contact if we compare percent mortality of natural insecticide formula CO-CLO has a faster percent mortality. The same is true of leaf contact methods. From the mortality percentage of each test, it is known that LC₅₀ values within 24 hours are based on probit analysis on toxic contact method and leaf contact. LC₅₀ with leaf contact method on CO-BO natural insecticide formula that is 0.35% concentration and on natural insecticide formula of CO-CLO 0,308%. While LC₅₀ with poison contact method on CO-BO natural insecticide formula that is 0,101% concentration and on natural insecticide formula CO-CLO 0,052%.

**Table 5.** Ki value of ligands multiple docking

| Ligand                      | Ki₅ Value (mM) |
|-----------------------------|----------------|
| Citronellal dan Anethole    | 1.6            |
| Citronellal dan Eugenol     | 1.75           |

*Figure 1. Multiple docking of two ligands
A. Citronellal-anethole   B. Citronellal-eugenol*
Figure 2. Time correlation after application with % mortality (Toxic Contact Method)
(a) CO-BO       (b) CO-CLO
Figure 3. Time correlation after application with % mortality with% mortality (Leaf Contact Method) (a) CO-BO (b) CO-CLO

4. Results and Discussion
The results of the in silico study showed that the inhibition constants (Ki) of citronellal and anethol ligands combination were 1.6 mM however of citronellal and eugenol as ligands were 1.75 mM formulated ratio (v/v), respectively 5:1 and 4:1. In addition, in vitro activity of insecticide formula with the ratio of 5:1 possess LC_{50} value 0.10% (toxic contact) and 0.35% (leaf contact). While the formula with a ratio of 4:1 possess LC_{50} value 0.05% (toxic contacts) and 0.31% (leaf contact).

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