Synthesis Organonitrogen Compounds from Patchouli Alcohol Through Ritter Reaction with Acetonitrile and Its Toxicity to Artemia salina Leach.

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Received day moth year; Accepted day moth year (will be given)

ABSTRACT

Patchouli oil contains a compound with biological activities to human body called the patchouli alcohol that can be further developed in medical field. This research aimed to synthesize organonitrogen compound from patchouli alcohol through Ritter reaction with acetonitrile and discover its toxicity towards Artemia salina Leach. The isolation of patchouli alcohol from patchouli oil using fractional distillation under reduced pressure method. The synthesis of organonitrogen compound is done at room temperature with the mol ratio of patchouli alcohol: acetonitrile: sulfuric acid is 1:1.5:4 for 24 hours. The result showed that the amount of patchouli alcohol produced from fractional distillation is 65.25%. The main product yielded from the synthesis between patchouli alcohol and acetonitrile through Ritter reaction is 36.93% of N-(4,8a,9,9-tetramethyl decahydro-1,6-metanonaftalen-1-il) acetamide. Starting material used have LC50 of 77.39 ppm. The product of synthesis have higher toxicity level than starting material, which have LC50 value is 10.39 ppm with the potential as medical compound.

Key word: patchouli oil, patchouli alcohol, synthesis organonitrogen compound, Ritter reaction, toxicity

INTRODUCTION

Indonesia is the largest producer of patchouli oil in the world, where 90 % of the world’s patchouli oil needs are supplied from Indonesia [1]. Given the scale abundance of patchouli oil is a challenge for people to explore it further into new products and to increase the resale value. One is the use of patchouli oil in the development of therapeutic areas.

Various studies have shown that the potential of patchouli oil as a base for medicine. Patchouli oil is shown to have pharmacological activity as an agent inhibiting platelet activating factor (PAF) [2], anti microbial, sedative agents, antiseptic [3], antiviral [4], and antifungal [5]. Pharmacological activity is influenced by the content patchouli alcohol is the mayor compound in patchouli oil [3]. Patchouli alcohol can inhibit influenza virus [4]. This encourages the optimisation efforts patchouli alcohol as a pro drug compounds through molecular modification of the structure to be beneficial in the medicine world.

Research have been done to modify the structure of patchouli alcohol during the formation ester compound is carried out using acetic acid and acid catalyst producing patchouli acetat compound [6] and dehydration becomes patchoulene by using a strong acid, H2SO4 [7]. Modify the structure of patchouli alcohol in this study was directed at the
formation of organonitrogen compounds, i.e. hydrocarbons containing N atom in a variety of bonds. Organonitrogen compounds have properties related to biological and physiological potential as a cure for diseases related to disorders of the central nervous system and its ability to interact with receptors of the body [8]. Organonitrogen compounds have biological activity as vasodilator, anti-inflammatory, antiviral, antimicrobial, analgesic, antidepressants, antischistosoma, antitumor, and anticonvulsan [9].

Patchouli alcohol has a rigid structure and including the tertiary alcohol group, which has activity related to the formation of a stable carbocation. Reaction that can be used in the formation organonitrogen compound with alcohol compound as the starting material through formation of carbocations is the Ritter reaction. Ritter reaction is a reaction to the formation N-alkyl carboxamide from aliphatic or aromatic nitrile and carbocations in strong acid media [10]. In the Ritter reaction will be produced carbocations as intermediates. Formation of a stable carbocation is due to the protonation of a strong acid, which carbocations are later attacked by the nucleophilic nitrogen atom. Acetonitrile used in this study as a nitrile reagent that attacks the carbocations.

![Figure 1. Structure of Patchouli Alcohol](image)

Organonitrogen compounds have the ability to interact with receptors of the body because of the hetero group that has a certain affinity and polarity that resulted in the occurrence of molecular interactions through biochemical mechanisms in the body [11]. Further studies to determine the ability of the products synthesized as a drug compound to be done through the toxicity test by brine shrimp lethality test (BSLT). The results of toxicity test the method BSLT reflects its potential as a drug compound. Wijdharti, et al [12] states that BSLT method is a method that is simple, rapid, inexpensive, and reliable and usually done at the preliminary stage of the screening materials that are thought to have anticancer properties before stepping to the test in vitro against tumor cell sustainably.

**EXPERIMENTAL SECTION**

**Materials**

General remarks: Na$_2$SO$_4$ anhydrous, CH$_3$CN, Na$_2$CO$_3$, H$_2$SO$_4$ (95-97%), diethyl ether, and DMSO, was purchased from MERCK, Artemia salina eggs was purchased from UIN Malang, and patchouli alcohol (65,25%) was obtained from fractional distillation under reduced pressure towards patchouli oil that purchased from Blitar, East Java.

**Instrumentation**


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ISSN : 2302 - 4690
Procedure

Isolation Patchouli Alcohol from Patchouli Oil

A number of 75 mL of patchouli oil that has been analyzed compounds with GC-MS, dried with Na$_2$SO$_4$ anhydrous and then inserted into the flask capacity 100 mL and put in a series distillation apparatus with condenser column of 60 cm and vigreux column of 30 cm. The process is then performed by fractional distillation under reduced pressure, the residue was obtained then analyzed with GC-MS and FT-IR.

Synthesis of Patchouli Acetamide Compound Through Ritter Reaction

A number of 28,2 mL of (contain 0,1 mol patchouli alcohol) is inserted into the flask 100 mL three-neck equipped with a thermometer. A number of 7,8 mL of acetonitrile (0,15 mol) is added to the flask. The mixture was cooled to 0 °C, cold condition are maintained with the addition of salt around the flask. A number of 22 mL of 97% sulfuric acid (0,4 mol) was added slowly dropwise to the mixture while stirring with a magnetic stirrer. Having run out of concentrated sulfuric acid, the mixture is left at room temperature while stirring with a magnetic stirrer for 24 hours. Synthesis mixture was poured into the erlenmeyer flask containing 100 mL of cold distilled water. Then added 100 mL of diethyl eter, stirred, and separated by a separating funnel.

Obtained organic phase was neutralized with saturated sodium carbonate solution and saturated sodium chloride solution was added. Neutral organic phase was dried with Na$_2$SO$_4$ anhydrous, then filtered. The solution was concentrated with nitrogen gas. The compounds synthesized were analyzed functional groups with FT-IR and analyzed the content of its components by GC-MS.

Toxicity Test to Artemia salina Leach.

Artemia salina eggs are incubated in brine at pH 7-8 (48 h). Then, series of solutions of test substances at varying concentrations and progressive were prepared in DMSO (dimethyl sulfoxide) and brine. A defined number of larvae introduced into each solution. All solution sand control solutions containing no active substance were left stirring for 24 hours. Counting under a microscope the number of death larvae in each solution used to evaluate the toxicity of the solution. Tests were carried out in triplicate.

Percent of lethality shrimp Artemia salina calculated at each concentration by the formula [13]:

$$\% = \frac{N_t}{N_0} \times 100\%$$

where $N_t$ is the number of shrimp larvae that died after incubation for 24 hours and $N_0$ is the total number of shrimp larvae are included. LC$_{50}$ value is then determined by linear regression on analysis of the similarities between the percent of deaths as the y-axis and the concentration as the x axis.
RESULT AND DISCUSSION

Isolation Patchouli Alcohol from Patchouli Oil

The yield of patchouli alcohol was obtained from fractional distillation under reduced pressure 65.25% at 100 mmHg.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before Distillation</th>
<th>After Distillation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of Oil</td>
<td>Brownish yellow</td>
<td>Brown</td>
</tr>
<tr>
<td>Content of GC-MS analysis</td>
<td>16.91%</td>
<td>65.25%</td>
</tr>
</tbody>
</table>

Table 1. The data content of patchouli alcohol

Figure 2. The chromatogram of patchouli alcohol was obtained from fractional distillation

Synthesis Patchouli Acetamide Through Ritter Reaction

Based on the analysis using GC-MS using a column Rtx-wax, the chromatogram obtained compounds synthesized as shown in Figure 3. Chromatogram compounds synthesized showed a peak that has 14% area of more than 1% and 12 peaks that have M+ = 263 with a retention time sequence, shown in Table 2. Peak with M+ = 263 major expected outcome is a product synthesis, namely N-(4,8a,9,9-tetramethyldecahydro-6,1-1-yl methanonaphtalena) acetamide, or better known as patchouli acetamide.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Retention Time (minute)</th>
<th>% Area</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>22,400</td>
<td>0.55%</td>
<td>30, 41, 43, 60, 70, 91, 107, 121, 135,</td>
</tr>
</tbody>
</table>
Mass spectrum at peak 39 which has 5 areas, 34.31% showed a peak of m/z = 30,41,43, 67, 79, 91, 107, 121, 133, 147, 161, 177, 189, 204, 220, 258, and 263 (shown in Figure 4).

Presence of the carbonyl group in patchouli acetamide compound results cleavage α, peak m/z = 263 release CH₃ so that produce peak m/z = M⁺ - 15 = 248, then the release CO to form peak of m/z = 220. Cleavage α to the C carbonyl produces a peak m/z = M⁺ - 22 = 204. Peak m/z = M⁺ - 59 = 204 is obtained from the release NH₂COCH₃, then the release CH₃ to form the peak m/z = M⁺ - 74 = 189. Peak m/z = 220 of the release of C₂H₂ (M⁺ - 43) then release NH₂COCH₃ thus forming the peak m/z = 161. Peak m/z = 161 have thus generated the release of C₃H₇ peak m/z = 91. Peak m/z = 91 release C₂H₂ respectively so as to produce the peak m/z = 41. Suggested fragmentation pattern for compound patchouli acetamide shown in Figure 6.

Figure 4. The Mass Spectrum Peak 39 with Retention Time 25.8 minutes.
**Figure 5.** Suggested fragmentation for patchouli acetamide

**Figure 6.** Plausible mechanism reaction the Formation patchouli acetamide

**Figure 7.** Structure of patchouli acetamide
Table 3. Data functional group product synthesis

<table>
<thead>
<tr>
<th>Wavenumber (cm⁻¹)</th>
<th>Type of vibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>3299, 98</td>
<td>Stretching N-H</td>
</tr>
<tr>
<td>2947, 03</td>
<td>Stretching C-H</td>
</tr>
<tr>
<td>1647, 10</td>
<td>Stretching C=O in amide</td>
</tr>
<tr>
<td>1546, 80</td>
<td>Bending N-H</td>
</tr>
<tr>
<td>1456, 16</td>
<td>Bending C-H in alkene</td>
</tr>
</tbody>
</table>

Based on FT-IR spectrum of compounds synthesized in Figure 7 and the functional groups of data in Table 3, show a strong absorption at wavenumber 3299 cm⁻¹ which is the N-H stretching vibration of secondary amide. Uptake was supported by the absorption at wavenumber 1647.10 cm⁻¹ which is the vibration of the amide carbonyl stretching and at wavenumber 1546.80 cm⁻¹ which is an N-H bending vibration. This indicates that the presence of secondary amide compounds contained in the compounds synthesized. Based on the percent results between the mass of the synthesis results compared with the theoretical mass, percent yield of product patchouli acetamide through Ritter reaction is 36.93%.

Table 4. The calculation result data LC₅₀ from BSLT test

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>Graph Equation</th>
<th>LC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Starting material</td>
<td>$Y = 0.6462x$</td>
<td>77.38 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$R^2 = 0.9809$</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Product Synthesis</td>
<td>$Y = 4.575x$</td>
<td>10.93 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$R^2 = 0.983$</td>
<td></td>
</tr>
</tbody>
</table>
Product synthesis containing patchouli acetamide 36.93% have a higher toxic properties, where the compounds are patchouli acetamide which has a lone pair of N atom and the carbonyl group has two lone pair on O atom. Presence of three lone pairs on these compounds (patchouli acetamide) its active side to form hydrogen bonds with DNA [...]. This binding causes the protein synthesis process is hampered and toxic effect at low doses.

CONCLUSION

Based on research that has been done, it can be concluded that the main products of the synthesis of patchouli alcohol with acetonitrile trough Ritter reaction is N-(4,8,1,9,9-tetramethyl decahydro-6-1-1-yl methanonaphtalene) acetamide or patchouli acetamide, 36.93%. Synthesized compounds have a toxicity level greater than starting material, which is 10.93 ppm, so the potential for drug compounds.

ACKNOWLEDGMENT

The authors gratefully acknowledge DIKTI for financial support through the Program Kreativitas Mahasiswa 2011 program.

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