Dengan ini ditugaskan kepada:

   Jabatan: Waddek II Fakultas Farmasi Universitas Pancasila
   Jabatan: Dosen Fakultas Farmasi Universitas Pancasila
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   Jabatan: Dosen Fakultas Farmasi Universitas Pancasila

Untuk mengikuti Seminar dengan tema “Penanganan Diabetes Melitus dengan Obat Herbal” dan Membawakan Makalah Berupa Poster, yang diselenggarakan pada:

Hari/tanggal: Selasa - Kamis, 19 - 21 Oktober 2010
Waktu: Pukul 08.00 – 16.00 WIB
Tempat: Auditorium BPPT, Gd. BPPT 2 Lantai 3
        Jl. MH. Thamrin No. 8 Jakarta

Setelah selesai dimohon menyerahkan laporan secara tertulis dalam waktu satu minggu kepada Dekan Fakultas Farmasi Universitas Pancasila.

Demikianlah kami sampaikan, agar dilaksanakan dengan baik.

Jakarta, 12 Oktober 2010


Tembusan:
1. Para Wakil Dekan
2. Para Ka Minat
3. Kasubbag Pulahta
4. Kasubbag Keuangan
5. Kasubbag Kepegawaian
ISOLATION AND IDENTIFICATION OF A CHEMICAL COMPOUNDS IN n-HEXANE PHASE FROM THE METHANOL EXTRACT OF KELADI TIKUS LEAF (Typhonium flagelliforme (Lodd.) Blume, Araceae-

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Abstract: Keladi tikus (Typhonium flagelliforme (Lodd.) Blume) is a plant which empirically has efficacy in treating cancer. In this study, n-hexane phase was fractionated using Vacuum Liquid Chromatography (dichloromethane; isopropanol 98:2 ~ 1:1) and Column Chromatography (SiO2, n-hexane-ethylacetate =3:1) and then each fraction was tested for toxicity with BSLT (brine shrimp lethality test.) method. The most active fraction was purified by TLC preparative and the pure compound was identified by using UV-Vis Spectrophotometer, IR Spectrophotometer, and Gas Chromatography-Mass Spectrometer. The result showed that fraction H.2.5 was the most active fraction with percent mortality 90% and one of the compounds contained in the n-hexane phase of keladi tikus leaves is phytol compound that had 91% similarity with the molecular formula C20H40O and the molecular weight of 296.31.

Keywords: isolation, keladi tikus (Typhonium flagelliforme (Lodd). Blume), BSLT.

1. INTRODUCTION

Keladi tikus (Typhonium flagelliforme (Lodd.) Blume, from familia Araceae, widely known as an alternative therapy to treat various types of cancer (Teo and Ch’ng, 1999). Keladi tikus are native Indonesian plant that grows wild and has not been known by the people of Indonesia. Therefore many studies have been done on keladi tikus to find out its activities as well as to determine the content of their compounds.

Several chemical constituents have been isolated previously which include phenyltridecanoic acid, methyl 13-phenyltridecanoate, several aliphatic compounds (Choo et al., 2001), β-sitosterol, β-daucosterol and 1-O-β-glucopyranosyl-2-(hydroxyloctadecanoyl) amido-4,8-octadecadiene-1,3-diol (Huang et al., 2004).

There is an obvious need for a systematic bioassay guided approach to the study of the bioactive constituents of Typhonium flagelliforme. As such, the present study was undertaken to isolate and identify the active chemical constituents of Typhonium flagelliforme based on a bioactivity-guided fractionation approach. Therefore, the research was aimed to determine the toxicity by using brine shrimp lethality assay and then to isolate and to identify of chemical compounds based on spectroscopic analysis.
2. MATERIALS AND METHODS

2.1. Materials

n-hexane extract from the methanol extract of keladi tikus leaves. *Artemia salina* Leach, Buchi Rotavapor RII, Vacuum Liquid Chromatography, Column Chromatography, UV-Vis Spectrophotometer Shimadzu 1700, IR Spectrophotometer, GC-MS Fisons Instruments 8000 series-MD 800.

2.2. Methods.

**Phytochemicals Screening.** Phytochemical screening to identify compounds such as flavonoids, saponins, tannins, quinones, steroid/triterpenoid, coumarins and volatile oils based on the method of Farnsworth.

**Fractionation.** A total of 15 g of the sample was separated on silica gel 60 using vacuum liquid chromatography with step gradient elution of the following composition dichloromethane; dichloromethane-isopropanol; methanol with a ratio as in Table 1. All the fractions were evaluated for toxicity using Brine Shrimp Lethality assay. From the bioassay results, the most active fraction was further purified by using normal phase flash column chromatography. The stationary phase was made up of a glass column packed with silica gel 60. The mobile phase consisted of combinations of *n*-hexane-ethylacetate (3:1). The chemical composition of fraction was evaluated by using TLC and visualized with UV (254 nm and 366 nm). Anisaldehyde-sulfuric acid reagent was used to identify these compounds.

**Purification and Identification.**

Purification was conducted using preparative TLC (SiO$_2$, *n*-hexane-ethyl acetate = 3:1). Furthermore, the identification used UV-Vis spectrophotometer, IR spectrophotometer and Gas Chromatography-Mass Spectrometry.

**Brine Shrimp Lethality Assay**

The brine shrimps (*Artemia salina*) lethality bioassay was carried out according to McLaughlin *et. al* (1998) and Meyer *et al.* (1982). Artificial sea salt was prepared using
sea salt (38.0 g) in distilled water (1L). The sea water was put in a small tank and a teaspoon of brine shrimp eggs were added to one side of the divided tank, which was covered. The other side was not covered so as to allow light that would attract the hatched shrimps. The tank containing the brine shrimp eggs was left at room temperature for two days to allow the eggs to hatch and mature as nauplii. Sample of three different concentrations 10, 100, 1000 mcg/ml were prepared according to the direction as given in the literature. Ten nauplii were added to each vial. All vials were covered at room temperature for 24 hours under the light and surviving were counted. Observations were made after 24 hours by looking at the number of *Artemia salina* Leach and the dead from each concentration.

Furthermore, calculated mortality rates or mortality (%) by comparing the total number of *Artemia salina* Leach who dies with the total number of *Artemia salina* Leach tested. LC$_{50}$ value was calculated using regression equations with log concentration as X axis and Y axis values as the probit LC$_{50}$ value obtained by calculating the value of X from the equation obtained. A substance is said to have active or toxic if the LC$_{50}$ value $<$1000 µg/ml.

### 3. RESULTS AND DISCUSSION

The phytochemical screening is known that *n*-hexane phase containing the steroid/triterpenoid and saponins which may have been responsible for the observed biological activity.

Vacuum liquid chromatography results obtained 10 fractions, using a mobile phase optimization results are dichloromethane-isopropanol (Table 1) whereas the mobile phase used for the analysis TLC of the fractions using dichloromethane-isopropanol (95:5) which gave the best separation (Figure 1). Brine shrimp lethality test results (LC$_{50}$) for the fractions are given in Table 1.
Figure 1. Chromatogram of the fraction H.1- H.10 by VLC

a) UV 254 nm
b) UV 366 nm
c) Spraying with Anisaldehyde-sulfuric acid
   Mobile phase: dichloromethane-isopropanol (95:5)
   Stationary phase : silica gel plate GF254

Table 1. Results of VLC fractionation of \(n\)-hexane extract and \(LC_{50}\) values

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Solvent for VLC</th>
<th>Weight (g)</th>
<th>(LC_{50}) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H.1</td>
<td>Dichloromethane ; 100</td>
<td>14.52</td>
<td>264.60</td>
</tr>
<tr>
<td>H.2</td>
<td>DCM–Isopropanol; 98:2</td>
<td>15.59</td>
<td>14.06</td>
</tr>
<tr>
<td>H.3</td>
<td>DCM–Isopropanol; 95:5</td>
<td>9.99</td>
<td>27.11</td>
</tr>
<tr>
<td>H.4</td>
<td>DCM–Isopropanol; 92:8</td>
<td>6.8920</td>
<td>26.71</td>
</tr>
<tr>
<td>H.5</td>
<td>DCM–Isopropanol; 90:10</td>
<td>4.3756</td>
<td>18.80</td>
</tr>
<tr>
<td>H.6</td>
<td>DCM–Isopropanol; 85:15</td>
<td>1.9643</td>
<td>60.85</td>
</tr>
<tr>
<td>H.7</td>
<td>DCM–Isopropanol; 75:25</td>
<td>2.0028</td>
<td>39.54</td>
</tr>
<tr>
<td>H.8</td>
<td>DCM–Isopropanol; 60:40</td>
<td>2.6281</td>
<td>19.58</td>
</tr>
<tr>
<td>H.9</td>
<td>DCM–Isopropanol; 50:50</td>
<td>0.9239</td>
<td>18.27</td>
</tr>
<tr>
<td>H.10</td>
<td>Methanol; 100</td>
<td>4.4587</td>
<td>27.67</td>
</tr>
</tbody>
</table>

The fraction H.2 has the highest \(LC_{50}\) value (14.06 ppm). The fraction H.2 was the most cytotoxic fraction among all 10 fractions. This fraction was further purified by using column chromatography.
**Purification by Column Chromatography**

Purification the fraction H.2 by using column chromatography (SiO2, n-hexane-ethyl acetate = 3:1). Eluent used is the result of optimizing both the fraction H.2 and produce the best separation. Performed by TLC analysis of the fractions (Figure 2) and brine shrimp lethality test shown in Table 2. Brine shrimp assay against the fractions calculated per cent of his death in concentration (100 ppm). Based on test results, the fraction H.2.5 have the highest mortality (90%) which was then performed identification.

![Figure 2. Chromatogram of the fraction H.2 by column chromatography](image)

- a) UV 254 nm
- b) UV 366 nm
- c) Spraying with Anisladehide-sulfuric acid
  Mobile phase: hexane-ethylacetate (75:25)
  Stationary phase : silica gel plate GF254

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Weight (g)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H.2.1</td>
<td>0.22</td>
<td>50</td>
</tr>
<tr>
<td>H.2.2</td>
<td>0.14</td>
<td>66.67</td>
</tr>
<tr>
<td>H.2.3</td>
<td>2.95</td>
<td>86.67</td>
</tr>
<tr>
<td>H.2.4</td>
<td>0.42</td>
<td>73.33</td>
</tr>
<tr>
<td>H.2.5</td>
<td>0.19</td>
<td>90</td>
</tr>
<tr>
<td>H.2.6</td>
<td>0.11</td>
<td>83.33</td>
</tr>
<tr>
<td>H.2.7</td>
<td>0.21</td>
<td>70</td>
</tr>
<tr>
<td>H.2.8</td>
<td>0.50</td>
<td>66.67</td>
</tr>
</tbody>
</table>

Table 2. Results column chromatography of the fraction H.2 and percentages of mortality

Presented in “International Conference and Talkshow on Medicinal Plants”. Jakarta 19th – 21st October 2010
Identification the fractions H.2. by using preparative TLC (Silica gel 60 preparative-TLC plates; \( n \)-hexane-ethyl acetate (3:1) produced 3 bands, where both the blue fluorescens at a wavelength of 366 nm were further identified and referred as isolates X (1.2 mg). Then performed a two-dimensional TLC to ensure purity of the isolates. Results of analysis isolates by UV-Vis spectrophotometer provides maximum absorption at a wavelength of 228.2 nm, characteristic for the chromophore group which has a double bond. The measurement results isolate compound X with an IR spectra gave absorption peaks at wave numbers 3421.48 cm\(^{-1}\) (OH), 2927.74 cm\(^{-1}\) (CH stretch), 1663.49 cm\(^{-1}\) - 1532.34 cm\(^{-1}\) (strain C = C), 1445.55 cm\(^{-1}\) -1327.9 cm\(^{-1}\) (CH bending).

The results of analysis by GC-MS of isolates X were obtained spectra (Figure 4). Based on the GC MS spectra can be seen that the peak is detected at a certain retention showed compound X is not pure, this is because many compounds that have the same Rf value (2-dimensional TLC, using methanol). Chromatogram of the fraction H.2.5 by Preparative and 2-dimensional TLC of the fraction H 2.5 (Figure 3). Results of analysis using the database Wiley7n.1, compounds that were detected with a very high quality of similarity 91% is phytol compound. The molecular structure of phytol (Figure 5).

![Figure 3. Chromatogram of the fraction H. 2.5 by Preparative TLC and 2-dimensional TLC](image)

a) UV 366 nm of the fraction H.2.5  
b) UV 366 nm the fraction H.2.5 of the blue fluorescens fraction  
c) 2-dimensional TLC of the blue fluorescens fraction  
  Mobile phase: hexane-ethylacetate (75:25) and hexane-ethylacetate (25:75)  
  Stationary phase: silica gel plate GF\(_{254}\)
4. CONCLUSION

The result of isolation, biological screening and identification showed that the fraction H.2.5 was the most active fraction with percent mortality 90% and one of the compounds contained in the n-hexane phase of keladi tikus leaves is phytol compound that had 91% similarity with the molecular formula C_{20}H_{40}O and the molecular weight of 296.31.
ACKNOWLEDGEMENTS
This research was supported by the Agency for the Assessment and Application of Technology, Indonesia (BPPT). The author are grateful to Prof. Dr. Wahono S.

REFERENCES


Presented in “International Conference and Talkshow on Medicinal Plants”. Jakarta 19th – 21st October 2010