Biological Activity Test of Extracts and Fractions There of Keladi Tikus Leaves (*Typhonium flagelliforme* (Lodd) Blume)

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Abstract: *Typhonium flagelliforme* (keladi tikus), from familia Araceae, is widely used as an alternative therapy to treat various cancer. In this study, the leaves of keladi tikus plant were extracted using methanol and then partitioned using n-hexane, ethylacetate, and n-butanol sequentially. The phytochemical screening, and the biological activity using brine shrimp lethality (BSLT) were carried out in this research. The result of phytochemical screening showed that the leaves contained steroids, flavonoids, and saponins. The results of BSLT method showed that the most active extract was ethylacetate extract (LC$_{50}$ = 55 µg/mL) and the lowest one was the n-butanol extract (LC$_{50}$ = 384 µg/mL). The ethyl acetate extract was tested for cytotoxic activity on T-47D cells using the MTT assay method. The IC$_{50}$ value was 30 µg/mL. The ethylacetate extract was then fractioned by VLC using gradient elution (DCM, isopropanol, and methanol); the fractions were tested using BSLT. The results from the nine fractions showed that the 5th fraction was the most active causing 97% mortality. It can be concluded that the ethyacetate extract is potential therapeutetic bioactive compound.

Keywords: Keladi tikus (*Typhonium flagelliforme* (Lodd), Blume), Brine Shrimp Lethality Test (BSLT), MTT assay.

1. INTRODUCTION

Keladi tikus (*Thyponium flagelliforme*), familia Araceae is one of wild plant species which is reported as traditional medicine for cancer treatment. A few people who have consumed the keladi tikus juice, have reported its “beneficial” effects on combating cancer. Some cancer patients claimed that they had “cured” and felt more healthy after consuming this plant product (Teo and Teo, 1999).

The brine shrimp lethality assay is a method using brine shrimp (*Artemia salina*), to screen medicinal plants for their biological activity. A number of studies have demonstrated the usage of the brine shrimp assay to screen plant extracts, because the brine shrimps eggs is inexpensive, ease of performing the assay, and a very useful bench-top method (McLaughlin et..al, 1991).
There is an obvious need for a systematic bioassay guided approach to the study of the bioactive constituents of *Typhonium flagelliforme*. The aim of the present study was to phytochemical screening of different extracts of keladi tikus *Typhonium flagelliforme* and to test their biological activity.

**MATERIALS AND METHODS**

**Materials**

All plant materials were collected from Balittro Bogor. The plant was identified at the Herbarium Bogoriense, Bogor. *Artemia salina* Leach, sea water, methanol, *n*-hexane, ethylacetate, *n*-butanol, dichloromethane, isopropanol, T-47D cell line, other unlabelled chemicals and reagents were analytical grade.

**Methods.**

**Phytochemicals Screening.**

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

The plant materials were extracted by maseration in methanol. The methanolic extracts were filtered and evaporated using a rotary evaporator at 40°C to give the crude dried extracts. The dried extracts were dissolved in water and partitioned with *n*-hexane, ethyl acetate, *n*-butanol respectively. The extracts were filtered and evaporated using a rotary evaporator at 40°C and used for brine shrimp lethality test (BSLT). From the bioassay results, the most active extract was evaluated for cytotoxic activity on human breast cancer cell line T-47D using MTT assay and fractionation using vacuum liquid chromatography.

**Fractionation.**

A total of 25 g of sample was separated on silica gel 60 using vacuum liquid chromatography (VLC) with step gradient elution of the following composition dichloromethane; dichloromethane-isopropanol; methanol with a ratio as seen in Table 1. All the fractions were evaluated for toxicity using brine shrimp lethality assay.
Brine Shrimp Lethality Bioassay

The brine shrimps (Artemia salina) lethality bioassay was carried out according to Meyer et al. (1982) and McLaughlin et al. (1998). Artificial seasalt was prepared using table salt (3.8% NaCl solution). The seawater was put in a small tank and a teaspoon of the eggs of the brine shrimp, 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, and 1000 µg/mL were prepared by dissolving them in DMSO (not more than 50 µl in 5 mL solution) plus seawater to attain concentrations. 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 shrimp and the dead from each concentration. Furthermore, calculated mortality rates or mortality (%) by comparing the total number of shrimp who dies with the total number of shrimp Leach tested. LC50 value was calculated using regression equations with log concentration as X axis and Y axis values as the probit LC50 value obtained by calculating the value of X from the equation obtained.

Cytotoxic assay

The human breast cancer cell line T-47D was maintained in RPMI-1640 medium with 10% fetal bovine serum (FBS), penicillin-streptomycin. The cells were cultured in 96-well plates containing 100 µL of growth medium per well and were incubated at 37°C in 5% CO2 incubator for 24 hours. Various extract concentrations were added to the cultures. After treatment, the cells were washed with phosphate buffer Saline (PBS), then 20 µL of 3-4,5- dimethylthiazol-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/ mL) per well was added to each cultured medium. After a further 4 hour of incubation, the formazan crystals will be formed. After that, 100 µL of 10% sodium dodecyl sulphate (SDS) was added into each well and were incubated for 24 hour. The plates were read in ELISA microplate reader at 570 nm. The IC50 values were calculated from graphic plot of percentage viable cell versus concentration. The IC50 values express that 50% cell death induced by concentration.
RESULTS AND DISCUSSION
The phytochemicals screening of the leaves extract indicated the presence of major
phytocompound including flavonoids, sterols/triterpenes, saponins, and coumarin, which
may be responsible for the observed biological activity. Table I give a summary of the
results of phytochemical screening of keladi tikus (*Typhonium flagelliforme* (Lodd) Blume)
events.

Brine shrimp lethality is a simple bioassay useful for screening large number of extracts
in the drug discovery process from medicinal plants. The LC$_{50}$ values of the brine
shrimp obtained from the leaves extracts is presented in Figure 1. According to Meyer *et
al.* (1982), an extract was considered toxic to *Artemia salina* if the LC$_{50}$ value resulted
from this test was less than 1000 ppm. The results showed that the ethyl acetate extract
from the leaves has the highest activity (LC$_{50}$ =55 µg/mL) and lower activity was n-
butanol extract (LC$_{50}$=384 µg/mL).

The results of the assessment of the cytotoxic activity of various extract concentration of
ethyl acetate extract of keladi tikus leaf on human breast cancer cell line T-47D determined
by MTT assay, gave IC$_{50}$ value of 30 µg/mL. On the other hand the n-hexane extract and
the chloroform extract from stems and leaves exhibited cytotoxic activity on murine P388

From the bioassay results, the ethyl acetate extract that has the highest toxicity than the
others, was further fractionated using VLC. All fractions were then evaluated for toxicity
using brine shrimp lethality assay. The results are showed in Table 3.

The fraction E1 was the lowest active fraction and the fraction E5 was the most active
fraction. In the future, fraction E5 should be further isolated, purified, and the compounds
should be elucidated.

CONCLUSION
The result of screening and bioassay of various extracts and fractions of *Typhonium
flagelliforme* showed that the most active extract was ethyl acetate with LC$_{50}$ of 55
ug/mL and the fraction which has the highest mortality was E5 with percent mortality of
97%.
ACKNOWLEDGEMENTS
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Table 1. Results of phytochemical screening

<table>
<thead>
<tr>
<th>Chemical group</th>
<th>methanol</th>
<th>n-hexane</th>
<th>ethylacetate</th>
<th>n-butanol</th>
<th>water</th>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sterol/triterpenes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Quinone</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Volatile oil</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coumarine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = presence  - = absence

Figure 1  LC<sub>50</sub> values of different extracts of keladi tikus leaves

Figure 2 Determination of cytotoxic activity of human breast cancer line T-47D of ethyl acetate extract by MTT assay

Table 3 Results of VLC fractionation of ethyl acetate extract and mortality values

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Solvent for VLC</th>
<th>Weight (g)</th>
<th>Mortality (%)</th>
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<tbody>
<tr>
<td>E1</td>
<td>Dichloromethane; 100</td>
<td>0.088</td>
<td>16.66</td>
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<tr>
<td>E2</td>
<td>DCM–Isopropanol; 98:2</td>
<td>1.329</td>
<td>50.00</td>
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<tr>
<td>E3</td>
<td>DCM–Isopropanol; 95:5</td>
<td>6.4353</td>
<td>66.66</td>
</tr>
<tr>
<td>E4</td>
<td>DCM–Isopropanol; 90:10</td>
<td>5.7280</td>
<td>73.33</td>
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<tr>
<td>E5</td>
<td>DCM–Isopropanol; 80:20</td>
<td>7.0302</td>
<td>96.66</td>
</tr>
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<td>E6</td>
<td>DCM–Isopropanol; 70:30</td>
<td>9.9504</td>
<td>46.66</td>
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<td>E7</td>
<td>DCM–Isopropanol; 60:40</td>
<td>7.2726</td>
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<td>E8</td>
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<td>E9</td>
<td>Methanol</td>
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REFERENCES


