TOXICITY OF EXTRACT AND FRACTIONS OF PUSPA BARK 
(Schima wallichii Korth) TO Artemia salina Leach.*

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Field
Natural products-Pharmacology
Oral Presentation

Puspa (Schima wallichii Korth.) is a medicinal plant which potential to be developed as anticancer. Previously research reported that puspa leaves have a cytotoxic activity. In this present works, a toxicity test to extract and fractions of puspa bark was carried out by Brine Shrimp Lethality Test (BSLT). Extraction was done by maceration using ethanol 70%. The concentrated extract was dissolved in water and then fractionated by Liquid-Liquid Extraction (LLE) using n-hexane and ethyl acetate respectively. The result of toxicity test of ethanol extract and fractions of LLE indicated that all sample had a toxic effect to A. salina (LC50 < 1000 ppm). The highest toxic fraction, that was ethyl acetate fraction (LC50 = 24.70 ppm), then was further separated by classical column chromatography (CC). The group of fractions II from CC process, showed the highest toxic activity (LC50 = 11.40 ppm). Preliminary identification of active compound by phytochemical screening and TLC with several spray reagent, predicted that it was terpenoid or polyphenol, however the identity of active compound was still unknown.

Keywords: Schima wallichii Korth., toxicity, Brine Shrimp Lethality Test (BSLT).

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INTRODUCTION

At this time, many traditional healing using plant or herbal drug as the key ingredient. A lot of empirical study and research has scientifically prove the activities, so the using of this natural drug tended to increase. This also supported by public opinion that natural product has minimal risk compare with chemical drug or synthetic drug (Rita et al., 2008).

One of plant that use as traditional drug is puspa. Some research of puspa reported that ethanol extract of puspa leaves has cytotoxic activity to leukemia cancer cells (Nurfaizah, 2008). Ethyl acetate fraction of puspa leaves has cytotoxic and pro-apoptosis activity to leukemia and breast cancer cells leukemia (Kristina, 2009; Ramdan, 2009). These researches showed that puspa leaves has potency to be developed as anticancer.

Secondary metabolite as active ingredient distributed in all parts of the plants i.e bark, rhyzome, leaves, fructus, and seed. Because of this reason, if one part of the plant has been prove any activity so the other parts potentially had the same activity. With that assumption, puspa bark expected have potency as anticancer like puspa leaves (Sajuthi, 2001).

Brine Shrimp Lethality Test (BSLT) is one of common methods in bioactivity test of chemical compounds. This method has developed as general biological test for preliminary study and monitoring the active substance of natural products. This methods also easy in application, cheaper, a little time consuming, and accurate. Toxicity result of this methods has correlation with cytotoxic activity of anticancer compounds (Meyer et al., 1982; Nurhayati et al., 2006; Bawa, 2009).

In this research, toxicity test of extract and its fractions of puspa bark was done by BSLT. In this sense, we want to know the toxicity and potency of puspa bark in development of anticancer from natural producs.

METHODS AND RESULTS

Materials and tools:

Materials: puspa bark, 70% ethanol, aquadest, n-hexane, chloroform, ethyl acetate, ether, amilalcohol, hydrochloric acid, sulfuric acid, nitric acid, acetic acid glacial, formic acid, formaldehyde, ammonia, bismuth-subnitric, mercury(II)chloride, aluminium (III) chloride, dimetil sulfoxide (DMSO), potassium hydroxide, sodium chloride, sodium acetic, vaniline, gelatin, magnesium powder, aluminium foil, precoated silica gel GF254, and silica gel 60 (0.063-0.200 mm) for column.

For toxicity test, A. salina was obtained from Laboratory of organic chemistry, Chemical Department, Faculty of Natural product and Sciences, Universitas Padjadjaran.

Tools: grinder, macerator, rotavapor (Buchi Rotavapor R-3000), separatory funnel, classical column chromatography, UV lamp (Camag UV-betrachter), aerator, electrical lamp, micropipette, volume pipette, and common glassware in laboratory

Methods:

Sample preparation

Bark of puspa plant was collected from mountain range of Kebun Tanaman Obat dan Aromatik Manoko, Lembang, Bandung. The material was sorted, cleaned, coarsely cut, air-dried and finally grinded.

This plants was identified in Herbarium Bandungense School of Science and Biological Technology (SITH) ITB, that belonged to Theaceae, species Schima walichii (DC.) Korth, with sinonim name Schima noronhae Reinw ex. Blume, Schima bancana Miq., and Schima crenata Korth.

Extraction and Fractionation

Powdered puspa bark was extracted by maceration using 70% ethanol (3x24 hour). Filtrat was condensed using vacuum rotavaporator. A 113,18 g of extract was obtained from 1 kg of crude drug, equivalent with 11.32% (w/w) of extractive matters (rendemen).

Fractionation conducted by liquid-liquid extraction (LLE) using
n-hexane, and ethyl acetate respectively, where the extract was dissolved in aquadest previously. About 56.09 g extract fractionated resulted in 3.25 g n-hexane fraction (5.80 %); 5.59 g ethyl acetate fraction (9.97 %) and 35.42 g of water fraction (63.15%).

**Phytochemical screening**

Phytochemical screening were determined from extract and all fractions of LLE using standard procedures [Ditjen POM, 1979; Farnsworth, 1966]. The results showed that in extract, n-hexane fraction, ethyl acetate fraction and water fraction of puspa bark identified flavonoid, polyphenolic compound, mono- and sesquiterpenoid, kuinon and saponin.

**Brine Shrimp Lethality Test (BSLT)**

The extract and all fraction of LLE was routinely evaluated in BSLT with minor modification procedures of Mayer et al. (1982). Toxicities of compound were tested at 1000, 500, 100, 50, 10, 5, 1; 0.5 and 0.1 ppm in medium solution with 1% DMSO (v/v). Ten, one day A.salina were used in each test and survivors counted after 24 h. Three replications were used for each concentration. The blank control were conducted at the same condition without sample. The lethal concentration for 50% mortality after 24 h of exposure were determined using McLaughlin (1990) as the measure of toxicity of the extract or fractions. Any substance was active or tixic if LC<sub>50</sub> values <1000 ppm for extract and LC<sub>50</sub> values ≤ 30 ppm for isolated compound, but LC<sub>50</sub> values greater than 1000 ppm for plant extracts were considered inactive. The result of BSLT showed in Table 1.

Table 1. The result of BSLT to extract and its fraction of LLE

<table>
<thead>
<tr>
<th>Sample /fractions</th>
<th>Linear Regression</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt; (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>y = 26.93 x + 5.489</td>
<td>44.88</td>
</tr>
<tr>
<td>Water</td>
<td>y = 25.41 x + 10.49</td>
<td>35.88</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>y = 22.00 x + 19.36</td>
<td>24.70</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>y = 21.70 x + 5.627</td>
<td>110.66</td>
</tr>
</tbody>
</table>

Determination of targetted compound (TC) was done by combination of preparative-thin layer chromatography (P-TLC) and BSLT. From P-TLC using precoated plate silica gel GF254 and ethyl acetate as mobile phase, there was three bands appeared. Each bands on P-TLC sçaped from the plate was dissove in medium solution then tested by BSLT. Percentage of mortality Band I (Rf=0.64) was 40%, Band II (Rf=0.28) was 60%, and Band III (Rf=0.16) was 50 %. Targettend compound was a compound that showing the highest value in Percentage of mortality.

**Separation and Identification**

Separation was conducted by classical column chromatography (CC) to the highest toxicacl fraction of LLE, that is ethyl acetate fraction. Solvent ethyl acetate used as mobile phase and silica Gel as stationary phase. This process monitored by TLC.

About 1.5 g sample separated by CC obtained 83 sub fractions. Base on similarity pattern of chromatogram TLC then clasified as grouped 16-20 of sub fraction (I), 21-42 sub fraction (II) and 43-45 sub fraction (III). Each group of fraction then tested by BSLT and data showed in Table 2.

Table 2. The BSLT result to Group of Fractions of CC

<table>
<thead>
<tr>
<th>Group of Fractions</th>
<th>Linear Regression</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt; (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>y = 10.13 x + 34.83</td>
<td>31.477</td>
</tr>
<tr>
<td>II</td>
<td>y = 6.498 x + 43.13</td>
<td>11.402</td>
</tr>
<tr>
<td>III</td>
<td>y = 5.731 x + 42.86</td>
<td>17.619</td>
</tr>
</tbody>
</table>

Preliminary identification by TLC with some spray reagent, was done to predict the identity of active compound. Active compound in group of fraction II appeared as yellow-brown (Kuning Kecoklatan=KK) spot visually, purple spot under UV 254 nm, but under UV 366 nm this invisible. After sprayed with 5% sulfuric acid KK turn into brown spot, this also appearence KK after sprayed by 5% potassium hydroxyde. Reaction KK with 5% vanilline-sulfuric acid was appeared as red purplish spot, with 5% Ferri (III) chloride was appeared as
green spot. Chromatogram of TLC showed in Picture 1.

![Chromatogram of TLC with some spray reagent.](image)

**Picture 1 Chromatogram of TLC with some spray reagent.**

**Condition:** Mobile phase: ethyl acetate, Stationary phase: precoated plate silica gel GF254, Observe visually with (1) 5% of potassium hydroxide, (2) 5% of Ferri(III)chloride, (3) 5% of sulfuric acid, and (4) 5% of vanillin- sulfuric acid.

**DISCUSSION**

The extracts and its fractions studied in this work showed significant lethality against brine shrimp (Table 1), which has been successfully used as a simple biological test to guide the separation process of plant extracts in order to detect anticancer compounds. This bioassay has good correlation with cytotoxic activity of anticancer compounds. LC50 values <1000 ppm are considered significant for crude extracts.

Active compound in the high toxical fraction, ethyl acetate fraction, was monitored by P-TLC and BSLT. The result showed that Band II (Rf=0.28) has the highest value in percentage of mortality (60%). This result also supported by CC result, where the group of fractions II showing the highest toxical activity (LC50 = 11,40 ppm). Identification at early stage of active compound by phytochemical screening and TLC with several spray reagent, predicted that it was terpenoid or polyphenol, however the identity of active compound was still unknown.

At this recent works, targeted compound was not an isolate or pure compound, so identification at the early stage only done by phytochemical screening and TLC with some spray reagent. Solution 5% of sulfuric acid used as universal reagent for organic compounds. Solution 5% of vanillin- sulfuric acid used to detect the existence of mono-and sesquiterpenoid.

Solution 5% of ferri(III)chloride use to detect the phenolic compounds, and solution 5% of potassium chloride used to detect kuinon. From chromatogram TLC with some spray reagent, showed that targetted compound or active compound supposed that was terpenoid or polyphenol, however the identity was still unclear.

**CONCLUSION**

Toxocity test of extract and its fraction of LLE indicated high toxical effect to larve Artemia salina Leach. with LC50 values < 1000 ppm. The highest toxical fraction, that was ethyl acetate fraction (LC50 = 24,70 ppm), then was further separated by classical column chromatography (CC). The group of fractions II from CC process, showed the highest toxical activity (LC50 = 11,40 ppm). Identification at early stage of active compound by phytochemical screening and TLC with several spray reagent, predicted that it was terpenoid or polyphenol, however the identity of active compound was still unknown.

This research was preliminary investigation in development anticancer from puspa (Schima wallichii Korth.). So this works are still going on our Laboratory.

**REFERENCES**


Bioassay For Active Plant Constituents. *Plant Medica*. 31-34.


