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Cytotoxicity of the Most Active Fraction of the Seeds of *Swietenia macrophylla* using Human Breast Cancer MCF-7 Cells

Dudi Tohir [a,1,*], Fitriah Sari [a,2], Irma Herawati Suparto [a,b,3]

[a] Department of Chemistry, Faculty of Mathematics and Natural Science, IPB University (Bogor Agricultural University), Bogor 16680, Indonesia
[b] Primate Research Center Animal Studies, IPB University (Bogor Agricultural University), Bogor 16151, Indonesia

* Author's email: (1) dudito@apps.ipb.ac.id; (2) fitriah.sr23@gmail.com; (3) irmasu@apps.ipb.ac.id

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Abstract

Ethyl acetate fraction from *Swietenia macrophylla* was reported to have toxicity against the larvae of *Artemia salina* shrimp larvae. However, there are no reports about *S. macrophylla*, which can inhibit human breast cancer cells MCF-7. Therefore, this study aims to evaluate *S. macrophylla* extract's cytotoxicity using human breast cancer MCF-7 cells assay, followed by confirmation of its toxicity using brine shrimp lethality assay. The most active fraction obtained from the ethyl acetate extract of *S. macrophylla* showed 76.49% inhibition at 50 µg/mL (IC_{50}=34.11 µg/mL). At the same time, the most active fraction may contain a mixture of limonoid compounds after LCMS analysis. The most active fraction obtained from ethyl acetate extract of *S. macrophylla* showed 76.49%

1. Introduction

Swietenia macrophylla belongs to the Meliaceae's family, which has been used as a source of traditional medicine [1]. Almost all parts of the plants possess biological activities such as antimicrobial, anti-inflammatory, antioxidant effects, antimutagenic, antidiabetic, antitumor, and anticancer [2, 3]. Moreover, the natural products of the species have not explored much. Some of them are reported as a folk medicine for the treatment of hypertension, diabetes, malaria [4], antibacterial [5].

There is increasing interest in research on natural products based on antineoplastic activity with low nonspecific toxicity. This can be exemplified by the plant *S. mahogany*. A previous report [5] showed that the $CHCl_3$ and ethyl acetate extracts could kill brine shrimp at the LC_{50} of 13.75 and 11.64 µg/mL. Another report from the ethyl acetate extract of *S. macrophylla* seed, which was evaluated using human colon colorectal HCT116 cells, was able to kill the cancer cells with IC_{50} of 35.35 ± 0.50 µg/mL [6]. Regardless of its biological activity, the mahogany seeds are rich for saponin, alkaloid, steroid, triterpenoid, and tannin [7, 8, 9, 10]. The major compounds that show antineoplastic are triterpenoid and limonoids [11]. Based on the literature search, *S. macrophylla* seeds have never been extensively examined before. Therefore, the need to investigate either biological activities and chemical contents of the plant is urgent. We began with the work of isolating active compounds against human breast cancer MCF-7 cells, which is the subject of this report.

2. Methodology

The research methods consisted of collecting *S. macrophylla* seed sample, sample preparation, determination of water content, extraction, phytochemical analysis, fractionation using vacuum liquid chromatography (VLC), and further purification using open column chromatography. Each fraction obtained from the fractionation was tested for toxicity against *A. salina* larvae. Cytotoxicity tests on MCF-7 cancer cells were carried out in the most active fraction on brine shrimp lethality assay. Finally, the chemical entities of the active fraction were identified using Liquid Chromatography-Mass Spectroscopy (LC-MS) analysis.

2.1. Material and Equipment

The material used in this research was *S. macrophylla* obtained from the Surabaya region. The chemicals used were a variety of organic solvent in analytical grade and several consumable materials for separation. Brine shrimp larvae *A. salina*, seawater, Tween 80, MCF-7 cell

line (ATCC®-HTB[22]), 3-(4,5-dimethylthiazol-2-il) 2,5-difeniltetrazolium bromide (MTT), and doxorubicin (generic) were used for the biological assay. The instruments used were UV light, Vacuum Liquid Chromatography (VLC) equipped with Si- gel 60 G (Merck Art 7731), Column Chromatography equipped with using Si-gel 60 G (Merck Art 7734), Thin Layer Chromatography employing silica gel 60 F254 0.25 mm (Merck Art 5554), a set of anticancer test kits, including enzyme-linked immunosorbent assays (ELISA) readers, and LC-MS instruments.

2.2. Extraction and Isolation

Before extraction, *S. macrophylla* powder samples were measured for their water content (loss of drying) [12]. Some portions of the sample (500 g) were dissolved with *n*-hexane for 6 hours, followed by ethanol three times for 24 hours to obtain the ethanol extract. The ethanol extract was then partitioned by ethyl acetate-water solvent (1:1). The ethyl acetate extract was identified for its phytochemical content (limonoid). The ethyl acetate extract was fractionated by VLC (*n*-hexane-ethyl acetate) to give ten fractions. The most active fraction against the brine shrimp lethality assay (fraction 7) was purified by column chromatography (chloroform-ethyl acetate).

2.3. Determination of cytotoxic characteristics

The cytotoxic characteristic of the most active fractions (fraction 7a, 7b, and 7i), after the second fractionation, were tested for the MCF-7 breast cancer cells by following the MTT method (3-(4.5-dimethyliazo-2-il) 2.5-diphenyltetrazolium bromide) [13]. The principle of the method is a color change of MTT from yellow to blue. The raised color is due to the presence of remaining living MCF-7 cells. In this method, the cytotoxic activity is expressed as a percentage of inhibition. The test was conducted by adding each isolation material to various concentrations in triplicate to the MCF-7 cells. After incubating for 48 hours, the MTT was added into the sample and incubated the plate for 4 hours. The absorption after being treated with MTT was measured using the enzyme-linked immunosorbent assay (ELISA) reader device at λ 595 nm after the addition of a solvent to stop the reaction.

3. Result and Discussion

The moisture content of the sample is 4.34%, which can be kept for long time storage. After extraction, the amount of ethyl acetate extract obtained is 8 g (1.6% w/w of the dried sample). Evaluation of the ethyl acetate extract against brine shrimp lethality assay shows LC_{50} of 156 µg/mL. The extract is positive, containing triterpenoids through a phytochemical test using Liebermann-Burchard reagent. The number of triterpenoid molecules from *S. macrophylla* is still limited and encouraged us to investigate chemically the Indonesian *S. macrophylla*. Purification of the active ethyl acetate using VLC resulted in 10 fractions, which the seventh fraction showed the most active against brine shrimp lethality assay LC_{50} 43.94 µg/mL (3.6 g). Further purification of the seventh fraction using column chromatography with a mixture of chloroform and ethyl acetate gave ten subfraction 7a–7j. The result of the brine shrimp assay for subfraction 7a–7j is depicted in Figure 1.

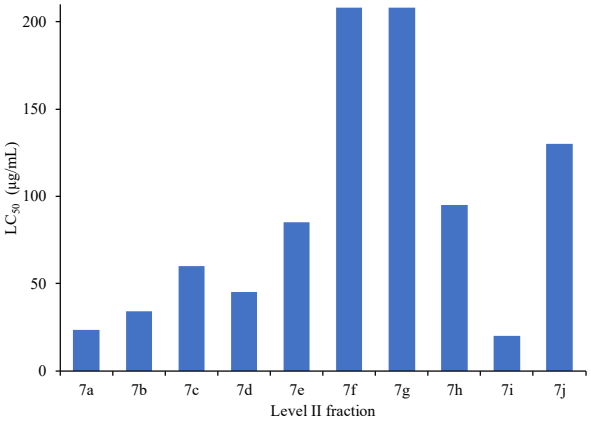

Figure 1. LC_{50} value of subfractions 7a–7j against brine shrimp *Artemia salina.*

From Figure 1, the most active three-active subfractions are 7a, 7b, and 7i, with LC_{50} of 23.55, 34.14, 19.93 µg/mL, respectively. These results are more toxic than the most active fraction reported by Hajianto [14], which was 35.46 µg/mL. Further confirmation of their antitumor activity was carried out using MCF-7 cells. The result of the MCF-7 cytotoxicity assay can be seen in Table 1 and Figure 2.

Table 1. The inhibition of subfractions 7a, 7b, and 7i against MCF-7 cells.

Subfractions	% inhibition of MCF-7 cells at 50 µg/mL
7a	41.59
7b	76.50
7i	30.51

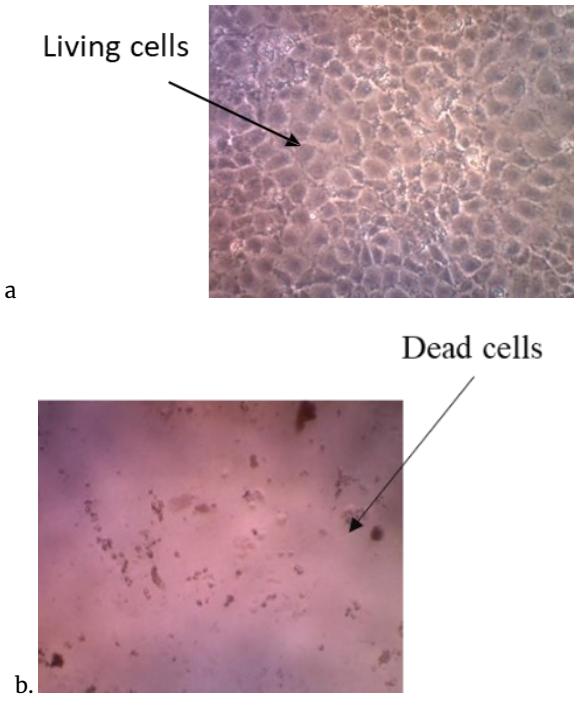

Figure 2. MCF-7 assay of subfraction 7b at 50 µg/mL (b) and blank (a)

Based on the cytotoxicity result, we chose subfraction 7b for further work. Elucidation of subfraction 7b using LCMS (Figure 4) reveals that the subfraction containing $(M+H)^+$ 399.308 mmu and $(M+Na)^+$ 421.340 mmu are consistent with the molecular formula $C_{26}H_{38}O_3$. Searching in the database and combined with chemical information obtained above, the subfraction 7b may contain a type of limonoid compound. Further confirmation is required by NMR spectroscopy.

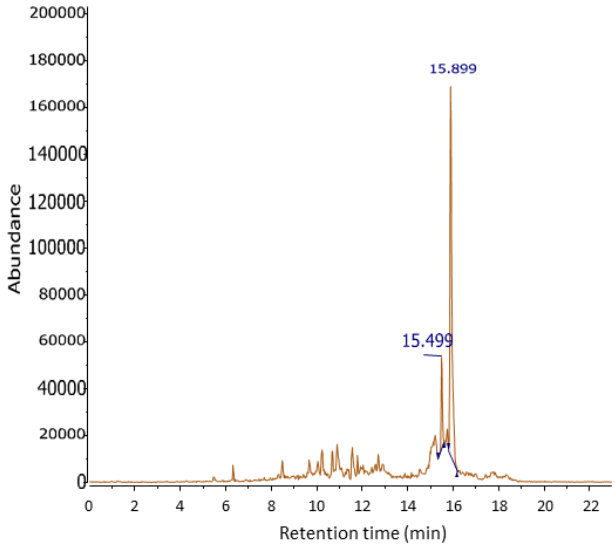

Figure 3. Chromatogram of subfraction 7b

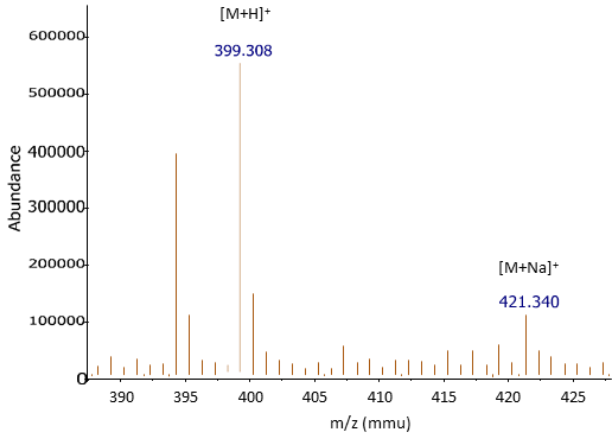

Figure 4. Mass spectrum of subfraction 7b with a retention time of 15.89

4. Conclusion

Subfraction 7b is the most active subfraction from *S. macrophylla* seeds with LC_{50} of 34.14 µg/mL. It inhibits the growth of MCF-7 cells with an inhibition percentage value of 76.49% on the 50 µg/mL of concentration. From LC-MS data and literature comparisons, we may suspect that the class of limonoid compounds may be contained in the subfraction 7b and is responsible for its antitumor activity against brine shrimp and human MCF-7 cells.

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