Extract of Yellow Root (*Arcangelisia Flava* (L.) Merr.) from Several Regions in Kalimantan: Alkaloid Content and Cytotoxicity towards WiDr Colorectal Cancer Cells

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Abstract

Yellow root (*Arcangelisia flava* (L.) Merr.) has been scientifically known to have potential as an antimalarial, antibacterial, antioxidant, and anticancer. The purpose of this study was to determine the profile of alkaloid content and cytotoxicity of yellow root extract from several regions in Kalimantan. The alkaloid content was tested using the thin layer chromatography (TLC) method with dragendorf reagent. Cytotoxic in vitro test was conducted against WiDr colorectal cancer cells using the 3-(4,5-dimethylthiazol-2-il)-2,5-diphenyltetrazolium bromide (MTT) assay. Yellow roots were collected from Samarinda city, Banjarmasin city, Barito Timur regency, Malinau district, and Balikpapan City. The MTT inhibitory concentration 50 (IC₅₀) of yellow root extracts were 573.308 μg/mL; 582.857 μg/mL; 296.326 μg/mL; 114.119 μg/mL; and 320.162 μg/mL respectively. Results of the compound identification indicated that alkaloid was found in *A. flava* from all regions. Alkaloids of *A. flava* extract should be investigated further in order to find possible active agent that could decrease the viability of WiDr colorectal cancer cells.

Keywords: *Arcangelisia flava*, Borneo, colorectal cancer, Kalimantan, WiDr cells

INTRODUCTION

Cancer is a disease caused by abnormal cells, that have high proliferation (Sandra, 2004), anti-apoptosis (Hendarmin, *et al*., 2018), survival ability (Sandra, 2018), and migration (Vallianou, *et al*., 2015) or commonly known as metastasize to other body parts. One of the many cancers suffered is colorectal cancer. Colorectal cancer is one type of cancer that occurs in the colon mucosa where the disease has a high morbidity and mortality rate (Tatuhey, *et al*., 2014).

Colorectal cancer ranks third as a type of cancer that often occurs and ranks fourth as the leading cause of this disease in the world (Bray, *et al*., 2018). Colorectal cancer is the third cause of

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death in men and women in the United States (Kim, et al., 2018). According to the American Cancer Society, this cancer is one of the most malignant diseases in the world and ranks third in a condition that is often diagnosed in the United States and fourth in Asia (Rahmadania, et al., 2016).

Yellow root (*Arcangelisia flava* (L.) Merr.) is a plant from the Menispermaceae family. *A. flava* is a liana plant with a length of up to 20 meters (Widyatmoko, et al., 1998). *A. flava* can grow well on flat, dry, non-muddy soil, and many contain humus, with rainfall of 2000-3000 mm per year (Heyne, 1987). Dayak people in Kalimantan or previously known as Borneo, use *A. flava* to treat hepatitis, fever, infections, digestive disorders, intestinal worms, and cancer sores (Pratama, 2016). Some studies show that *A. flava* is useful as an antimalarial (Lovin, et al., 2012), antibacterial, antioxidant (Maryani, et al., 2013), and anticancer (Keawpradub, et al., 2005). The active compounds of *A. flava* are thought to have anticancer activity, including alkaloids like berberine, palmatine, and jatrorrhizine. (Pratama, 2016).

The content of each plant has different activities. This difference can be caused by internal factors such as hormones, water and genetic balance, as well as external factors such as area height, rainfall, soil type, climate, temperature, reaction soil, gas composition in the ground, and soil nutrient availability (Mpapa, 2016). There present study was conducted to determine the content and cytotoxicity of *A. flava* plants from several regions in Kalimantan.

**MATERIALS AND METHODS**

**Extraction**

*A. flava* stems were obtained from Samarinda city (AfSR), Banjarmasin city (AfBM), Barito Timur regency (AfBT), Malinau district (AfMN), and Balikpapan city (AfBP) (Figure 1). *A. flava* stems were determinated at the Indonesian Institute of Sciences/Lembaga Ilmu Pengetahuan Indonesia (LIPI) Purwodadi (certificate No.0402/IPH.06/HM/IV/2019). Extraction of *A. flava* stems were performed using maceration method with Ultrasound-Assisted Extraction (UAE) and 80% ethanol (Merck, Darmstadt, Germany) as solvent. Five grams of each dry powder sample were dissolved with 100 mL of solvent. Extraction was conducted for 2 minutes with three repetitions. The extract was filtered, and the filtrate was heated at 40°C.

![Figure 1. Samples A. flava from (a) AfSR, (b) AfBM, (c) AfBT, (d) AfMN, (e) AfBP.](image-url)
Phytochemical Test of Alkaloid

Phytochemical tests were conducted using thin layer chromatography (TLC) method. The stationary phase used was silica gel GF254 plate and the mobile phase were chloroform and methanol (2:3). To get stain appearance, the dragendorf reagent was used. Samples that produced orange stains after spraying the dragendorf reagent indicated an alkaloid compound (Minarno, 2015).

Preparation of Sample

Stock solutions made by extracting A. flava as much as 10 mg and dissolved with 1% dimethyl sulfoxide (DMSO) as much as 100 μL. Then dilution was done using Roswell Park Memorial Institute (RPMI) medium (Gibco, Invitrogen cell culture, Carisbad, USA). The concentrations were 500, 250, 125, 62.5 and 31.25 μg/mL. These solutions were used in the WiDr cell cytotoxic test. As the positive control, doxorubicin was used in the concentration of 100, 50, 25, 12.5 and 6.25 μg/mL.

Cytotoxic Test towards WiDr Cells

The cytotoxic tests were conducted in the Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta. WiDr cells were cultured with RPMI medium, then harvested. The cells were transferred into a 96-well plate (Iwaki, Kuala Lumpur, Malaysia) with a total of 30x10^4 cells/well followed by 24 h incubated in an incubator with CO_2. Cells were treated with tested solution as indicated concentrations. The final volume was 100 μL/well. Cells were incubated for 24 h. Positive controls group were treated with doxorubicin (OBG Sanbe 2 mg/mL). Then the cells were added with an MTT reagent (Sigma, St Louis USA) and incubated for 2 h. Then wells were added with 10% sodium dodecyl sulphate (SDS) (Sigma) in 0.1 N HCl (Merck) as much as 100 μL, then wrapped and incubated for 24 h. This stopper solution served to dissolve formazan crystals. Next, the absorbances of solutions were read using an microplate reader (Bio-rad, Hercules, California, USA) with a wavelength of 550-600 nm.

The absorbance values were calculated to obtain the viability percentage of viable cells. Then the percentages were used to find inhibitory concentration 50 (IC₅₀) value. The IC₅₀<50 μg/mL was categorized as having a strong cytotoxic effect, 50 μg/mL<IC₅₀<200 μg/mL was categorized as having a moderate cytotoxic effect, 200 μg/mL<IC₅₀<1000 μg/mL was categorized as having a weak cytotoxic effect, and IC₅₀>1000 μg/mL was categorized as having no cytotoxic effect (Kuete, 2017). The IC₅₀ value was analyzed by using probit in regression of SPSS version 24 (IBM Corp., Armonk, New York, USA) IC₅₀ values were analyzed based on a percent of the viability of viable cells/replication with replication three times.

RESULTS

TLC Results of A. flava Extracts

The results of the phytochemical test were identified by using TLC (Figure 2). Figure 2 shows an orange stain after being given dragendorf reagent. All A. flava samples produced orange stains indicating that all samples contained alkaloid compounds. The Rf values of TLC results were shown in Table 1. All AfSR, AfBM, AfBT, AfMN and AfBP extracts had almost similar Rf values.
The absorbance values of MTT were converted to the percentage of viable cells. (Figure 3 and 4). All AfSR, AfBM, AfBT, AfMN and AfBP extracts decreased the percentage of viable WiDr cells in dose-dependent manners (Figure 3). AfMN extract had the highest capacity in decreasing the percentage of viable WiDr cells. Compared with AfSR, AfBM, AfBT and AfBP extracts, the 125 μg/mL and 250 μg/mL AfMN extracts markedly decreased the percentage of viable WiDr cells. Results of doxorubicin as the positive control in this study showed a clear decrease of the percentage of viable WiDr cells in a dose-dependent manner (Figure 4). Among all investigated A. flava extracts, AfMN extract had the highest cytotoxic effect with the IC$_{50}$ of 114.119±6.617 μg/mL and category of cytotoxic effect was moderate (Table 2).

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### Table 1. TLC Results of A. flava with mobile phase Chloroform : Methanol (2:3), stationary phase Silica gel GF254.

<table>
<thead>
<tr>
<th>No</th>
<th>Sample Name</th>
<th>Rf value 1</th>
<th>Rf value 2</th>
<th>Stain Color</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AfSR</td>
<td>0.36</td>
<td>0.46</td>
<td>Orange</td>
<td>Alkaloid</td>
</tr>
<tr>
<td>2</td>
<td>AfBM</td>
<td>0.34</td>
<td>0.2</td>
<td>Orange</td>
<td>Alkaloid</td>
</tr>
<tr>
<td>3</td>
<td>AfBT</td>
<td>0.32</td>
<td>0.4</td>
<td>Orange</td>
<td>Alkaloid</td>
</tr>
<tr>
<td>4</td>
<td>AfMN</td>
<td>0.32</td>
<td>0.4</td>
<td>Orange</td>
<td>Alkaloid</td>
</tr>
<tr>
<td>5</td>
<td>AfBP</td>
<td>0.32</td>
<td>0.4</td>
<td>Orange</td>
<td>Alkaloid</td>
</tr>
</tbody>
</table>

Figure 3. Effect of A. flava extract from several regions in Kalimantan towards viability of WiDr cells. Blank bar: 31.25 μg/mL, striped bar: 62.5 μg/mL, dotted bar: 125 μg/mL, grey bar: 250 μg/mL, black bar: 500 μg/mL.

**DISCUSSION**

Based on the present results shown in Figure 2 and Table 1, all samples had 2 orange stains with almost similar Rf values and color intensity, marked as alkaloids. Alkaloid compounds have been reported to inhibit cell growth by blocking the G1 phase of the cell cycle (Arung, et al., 2010; Widowati, et al., 2010). Berberine, one of the alkaloid compounds could bind with DNA and RNA and induce DNA damage in cancer cells by regulating the activity of DNA topoisomerase (Wang, et al., 2016).

Among all tested extracts, the AfMN extract whose stem from the Malinau district had the highest cytotoxicity. These results strengthen current knowledge of plant variation. The variation can be caused by internal and external factors.
Table 2. IC\textsubscript{50} values of doxorubicin, AfSR, AfBM, AfBT, AfMN and AfBP extracts towards WiDr cells. Data were obtained from 3 replications.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>IC\textsubscript{50}±SD (mg/mL)</th>
<th>Cytotoxic Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>45.901±15.951</td>
<td>Strong</td>
</tr>
<tr>
<td>AfSR</td>
<td>573.308±33.63</td>
<td>Weak</td>
</tr>
<tr>
<td>AfBM</td>
<td>582.857±12.843</td>
<td>Weak</td>
</tr>
<tr>
<td>AfBT</td>
<td>296.326±14.129</td>
<td>Weak</td>
</tr>
<tr>
<td>AfMN</td>
<td>114.119±6.617</td>
<td>Moderate</td>
</tr>
<tr>
<td>AfBP</td>
<td>320.162±12.188</td>
<td>Weak</td>
</tr>
</tbody>
</table>

SD: Standard deviation

In present study, cytotoxic study with MTT assay was performed. Treated viable WiDr colorectal cancer cells were measured with colorimetry using microplate reader. Lower percentage of viable cells could be considered as inhibition of cell growth/proliferation as well as induction of apoptosis (Sandra, et al., 2017). Therefore, further study should be conducted with different assays to ensure the mechanism of the A. flava stem extract, whether inhibiting cell growth/proliferation, inducing of apoptosis or both at the same time (Sandra, et al., 2017).

CONCLUSION

Alkaloid content of A. flava stem extract could be potential in decreasing viability of WiDr colorectal cancer cells. Further research should be explored on active agent in the alkaloids, especially alkaloids of the AfMN extract.

REFERENCES


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