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Cytotoxic Effect of Water, Ethanol and Ethyl Acetate Extract of Black Cincau (Mesona Palustris BL) against HeLa Cell Culture

Tri Dewanti Widyaningsih*

Department of Food Science and Technology, Faculty of Agricultural Technology, Brawijaya University, Malang Indonesia

Abstract

Black cincau (Mesona palustris BL) or grass black jelly as a traditional Indonesian food that has been used as a folk medicine and as a beverage for many centuries. It was reported that some of pharmaceutical effect of herbs could be related to their possible antioxidant activities. Moreover, some researchers reported that diverse compounds naturally occurring in food could exert an antimutagenic effect due to their antioxidant capacity. The studies to determine the bioactivity of black cincau extract against Hela cell. Cytotoxicity assays was conducted using method the MTT with 10 levels of extract concentration. The results of cytotoxicity assays showed the water extract, ethanol extract and the ethyl acetate extract of black cincau has anticancer activity against Hela cell lines. The third activity of black cincau extracts was significantly different. Water extracts of black cincau at low concentrations (19.53 - 156.25 μg/ml) showed higher anticancer activity than extracts ethanol and ethyl acetate. IC_{50} water extract of black cincau was 132.6 μg/ml, ethanol extract of black cincau was 146.0 μg/ml and ethyl acetate extract of black cincau was 182.96 μg/ml.

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Keywords: Black cincau (Mesona palustris BL), MTT assays, Hela cell, IC_{50}

1. Introduction

The herb of janggelan, or black cincau (Mesona palustris BL), is recognized as material of both a herbal drink and a jelly type dessert in Indonesia. Black cincau as herbal drink is also known in Asian countries, in China and Taiwan similar black cincau is called hsian-tsao (Mesona procumbens Hemsl) (1). It is a traditional

* Corresponding author.
E-mail address: tridewantiw@yahoo.com
herbal drink used as medicine for hypertension, diabetes and liver problem (2), heat-shock, diarrhea, cough, and preventing digestive disorders (3).

There has been much research concerning the properties of bioactive components in hsian-tsao extract. The hsian-tsao extract contains phenolic compounds that significantly contributed to antioxidant activity and free radical scavenging activity (4). The hsian-tsao extract protected tert-butylhydroperoxide-induced hepatic and oxidative damage in rats, and it also reduced the UV-C and/or H2O2-induced DNA damage in human lymphocyte (5) and the water extract of hsian-tsao shown to have antimutagenic activity with *Salmonella typhimurium* bacterial test (Ames test) (6).

Effects of a material is closely associated with the bioactive compounds contained in these materials. The Black cincau extract based on research conducted among bioactive compounds containing phenolic components, tannins, stigmasterol, β-sitosterol, oleanolic acid and ursolic (4) and possibly other compounds. These compounds have a synergistic or antagonistic effects. The water extract of black cincau (*Mesona palustris* BL) have effective as an immunomodulator to increase both the expression of IFN-γ and the immunosurveillance component activity: NK cells, cytotoxic T cells (CD 8 +), and macrophages (7). Whether the compounds contained in extracts of black cincau is an anticancer? It can be tested for cytotoxic to cancer cells in vitro.

This study is aimed to tasted the cytotoxic activity of water extract, ethanol and ethyl acetate black cincau against HeLa cells (cervical cancer) by the method of MTT (3-[4,5-dimetilhiazol-2yl]-2,5-difenil tetrazollum bromida). IC50 values were determined from regression lines of dose-response curves, assuming linearity of response.

2. Materials and Methods

2.1. Materials

The dried plant bulbs of black cincau were collected from the farm in Magetan, East of Java, Indonesia. The herb was ground into a fine powder with a hammermill. The powder was passed through an 80-mesh sieve, collected and sealed by a polyethylene plastic bag, and then stored at 0 - 4 °C for further use.

2.2. Preparation of extracts of black cincau

Three 10 g samples of black cincau powder were soaked for 24 h at room temperature with 200 ml of either ethyl acetate, or ethanol, respectively, followed by filtration through Whatman # 1 filter paper. The filtrates were then evaporated *in vacuo* to dryness and weighed to determine the yields. Water extract of black cincau was prepared with 20-times volume of 100°C boiling water for 2 h. The extraction was evaporated under reduced pressure (34-etha36 kPa) using a rotary vacuum-evaporator at 40 °C and the contents were freeze-dried. The dried extract was pulverized, sieved (100 meshes), sealed in zip plastic bags for future use.

To prepare the stock solutions, 2 mg of each solid residue was dissolved in 140 μl of DMSO and RPMI-1640 was added up to 1 ml. The mixtures were then filtered and sterilized using 0.22 μ microfilters and kept frozen. Serial dilutions (10.000; 5.000; 2.500; 1.250; 625; 312,50; 156,25; 78,125; 39,062; dan 19,531 μg/ml) were freshly prepared from stock solution before use.

2.3. Cell line

Hela cell line was purchased from LPPT Gadjah Mada University Yogyakarta Indonesia. Cells were grown in RPMI-1640 [each 500 ml of RPMI-1640 was supplemented with 10% of fetal calf serum, 5 ml of penicillin/streptomycin (50 IU/ml and 50 μg/ml respectively), 5 ml of sodium pyruvate (1 mM), NaHCO3 (1
and 5 ml of L-glutamine (2 mM)]. The final medium was then sterilized using 0.22 μm microfilters and stored at 4 ºC before use.

2.4. MTT-based cytotoxicity assay

Cytotoxic effect of the extracts against Hela cells was determined by a rapid colorimetric assay, using 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). This assay is based on the metabolic reduction of soluble MTT by mitochondrial enzyme activity of viable cells into an insoluble colored formazan product, which can be measured spectrophotometrically after dissolving in DMSO (8). Hela cells were seeded in 96-well plates (Iwaki) with 2x10^5 cells/well and divided into control and treatment groups. Single serial dilution of black cincau extracts at 10,000; 5,000; 2,500; 1,250; 625; 312.5; 156.25; 78.125; 39.062; dan 19.531 μg/ml. Black cincau extract (5 mg) was dissolved in Dimethyl sulf oxide (DMSO) as stock solution (1mg/ml) then diluted in culture medium until desired concentration. After 24 h incubation, culture medium was removed and cells were washed using PBS (Sigma). 5 mg/ml of MTT (Sigma) was diluted by culture medium (1 ml MTT stock add 10 ml culture medium) and 100 μl of it was added into every well. Then, the plate was incubated for 2-4 h until formazan was produced. MTT reaction was stopped by Sodium Dodecyl Sulfate (SDS) 10% in HCL 0.1 N (Merck). The plate was then covered with paper or aluminum foil and incubated in a dark place over night, followed by incubation, shake for 10 minutes and measured the absorbance using ELISA reader (Bio-Rad) at wave length of 550 nm. Each extract concentration was assayed in 3 wells and repeated 3 times. Standard curve (absorbance against number of cells) for the cell line was also plotted. Determination of the percentage of cell death was calculated by the formula:

\[ \text{Mortality} = \frac{(A - D)}{(B - C)} \times 100\% \]

\[ A = \text{Absorbance control cell} \]
\[ B = \text{Absorbance sample} \]
\[ C = \text{Absorbance control sample} \]
\[ D = \text{Absorbance control media} \]

2.5. Statistical analysis

SPSS was used to perform statistical tests. Analysis of variance was used to distinguish the difference among groups. Significance was assumed at 5% level. For detecting the point of difference the post hoc test was used.

3. Results and Discussion

The results of cytotoxic assays of different extracts of water solution, ethanol and ethyl acetate of black cincau showed that the percentage of cell death is the average has a similar trend, namely the higher the concentration of the extract percentage of cell death is also increasing. From Figure 1 shows that the cytotoxic effects of water extract of black cincau is higher at low concentrations (19.53 to 156.25 μ/ml) compared to ethanol and ethyl acetate extracts. Water extract of black cincau in addition to dissolving the bioactive compounds are also dissolving hydrocolloid compound or gum (gel-forming component), while ethanol and ethyl acetate precipitate hydrocolloid precisely (1). Hydrocolloid compound and bioactive compound are water soluble influence the lysis cell membrane so that causing cell death. Extract of black cincau ware bioactive contains phenolic compounds which were antioxidants (7). Water extract hsian tsao showed antimutagenic activity, and this function was concerned with the content of their phenolic compounds and ascorbic acid (6,9,10).
The cytotoxicity study was carried out for plant extract of black cincau. These extracts were screened for its cytotoxicity against HeLa cell lines at different concentrations to determine the IC\textsubscript{50} (50\% growth inhibition) by MTT assay. The results are tabulated in Table 1 and graphically represented in Fig. 1. It was found that the % (mortality) growth inhibition increasing with increasing concentration steadily up to 19.53 μg/ml on HeLa cell line and IC\textsubscript{50} value of this assay was water extract showed the highest cytotoxic effect among the extracts (IC\textsubscript{50} = 132.6 μg/ml). Ethanol and ethyl acetate extracts cytotoxic against Hela cells (IC\textsubscript{50} = 146.0 and 182.965 μg/ml, respectively).

Table 1. IC\textsubscript{50} value of 3 different extracts of black cincau (\textit{Mesona palustris} BL). Against Hela cells

<table>
<thead>
<tr>
<th>No.</th>
<th>Extraction solvents *</th>
<th>IC\textsubscript{50} (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Water</td>
<td>132.6</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol</td>
<td>146.0</td>
</tr>
<tr>
<td>3.</td>
<td>Ethyl Acetate</td>
<td>182.96</td>
</tr>
</tbody>
</table>

* Extractions were performed as mentioned in experimental section. Extracts of black cincau were tested against Hela cells and IC\textsubscript{50} were determined, using dose-response curves in Fig. 1.

4. Conclusion

The extract of black cincau water, ethanol and ethyl acetate showed potential as anticancer in HeLa cells. The water extract of black cincau at low concentrations (19.53 - 156.25 μg/ml) showed higher anticancer activity than the ethanol extract and ethyl acetate extract. IC\textsubscript{50} water extract of black cincau was 132.6 μg/ml, ethanol extract of black cincau was 146.0 μg/ml and ethyl acetate extract of black cincau was 182.96 μg/ml.
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