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Cytotoxicity study of ethanol extract of the stem bark of asam kandis (*Garcinia cowa* Roxb.) on T47D breast cancer cell line

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ABSTRACT

Objective: To investigate the cytotoxic effect of ethanol extract of the stem bark of asam kandis [*Garcinia cowa* Roxb. (*G. cowa*)] on T47D breast cancer cell line.

Methods: The cytotoxicity of ethanol extract was carried out against human breast cancer cell line (T47D) by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay. The extract was added at various concentrations (0.1, 1, 10 and 100 μg/mL). The level of cytotoxicity was determined by calculating the level of IC_{50} that was based on the percentage of the cell death after 24 h treatment with the extract. Cell morphological changes were observed by using inverted microscope.

Results: The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay showed that ethanol extract of *G. cowa* exhibited significant cytotoxic effect on T47D with IC_{50} value of (5.10±1.68) μg/mL. Morphological alteration of the cell lines after exposure to ethanol extract of *G. cowa* was observed under phase contrast microscope in a dose-dependent manner.

Conclusions: The results suggest the possible use of ethanol extract of asam kandis for preparing herbal medicine for cancer-related ailments.

1. Introduction

Garcinia cowa Roxb. (Guttiferae) (*G. cowa*), known as asam kandis in West Sumatra, is a medium-sized tree with edible fruit which attains a height of about 30 m. It is widely distributed throughout Indonesia and Malay Peninsula[1]. Many parts of *G. cowa* have been used in traditional folk medicine as antipyretic and anti-inflammatory[1].

This specie has been extensively investigated from both biological and phytochemical points of view. Previous phytochemical investigations of *G. cowa* resulted in the isolation of tetraprenyltoluquinone ([2*E*,6*E*,10*E*]-(+)-4β-hydroxy-3-methyl-5β-(3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenyl-2-cyclohexen-1-one)[2], xanthones[2-6], benzophenones[3], dihydrobenzofuran[6], acylphloroglucinol[7] and depsidone[8]. The phenolic constituents have been reported to possess a wide range of biological and pharmacological properties, such as antibacterial[3,5,6], antioxidant, antiinflammatory[9] and cytotoxic

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activities[7].

Hence in the present study, we investigated the cytotoxic effect of ethanol extract of the stem bark of *G. cowa* on T47D breast cancer cell line.

2. Materials and methods

2.1. Plant material and extraction

The stem barks of asam kandis were collected in Padang, Indonesia at January 2014. The plant material was first air-dried in the laboratory for 5 d at room temperature, next oven-dried at 40 ˚C, and then grinded into powder by using an electric mill. The powdered sample was kept in an airtight container until required. About 800 g of the powdered steam bark of *G. cowa* was macerated into 5 L of ethanol (70%) for 3 d. This process was repeated 3 times. Rotary evaporator was used to evaporate and concentrate ethanol extract at 40 ˚C. The resulting extract was kept in the refrigerator.

2.2. Methods

All procedures were carried out in a sterile condition (Class II biohazard cabinet) and aseptic techniques are applied. Good cell culture practice guidance was exercised during the whole process[10].

2.2.1. Cell culturing procedure

T47D cell lines used during the present study were obtained from Tissue Culture Laboratory, Faculty of Medicine, Gadjah Mada University, Jogjakarta, Indonesia. All cell lines were maintained in Roswell Park Memorial Institute 1640 medium with 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin and 100 μg/mL streptomycin. Cells were grown in 25 cm^2 tissue culture flasks in a humidified atmosphere containing 5% CO_2 at 37 ˚C. Once the cells reach 80% confluence, 1 mL of trypsin-ethylenediaminetetraacetic acid solution was added to the flasks for 5-10 min to detach the monolayer cells. The cells were occasionally observed under the inverted microscope until the cell layer was dispersed. Then, 3 mL of complete growth medium was added to the flasks followed by repeated gentle pipetting to split the cell clumps. Approximately 0.5×10^6 to 1×10^6 cells were sub-cultured into a new 25 cm^2 flask which contained 8 mL of fresh medium.

2.2.2. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The ability of the extract to induce growth inhibition on T47D cell line was determined by using MTT assay. This assay is a calorimetric assay that measures the activity of mitochondrial dehydrogenase in living cells, which have the ability to convert pale yellow soluble MTT to insoluble purple formazan product[11]. Cells were attached by incubating suspension cells (180 μL in each well except blank) for over night. Varying concentrations of the extract were prepared from the stock solutions by serial dilution (10-fold dilution start from 100 μg/mL, 10 μg/mL, 1 μg/mL to 0.1 μg/mL) in Roswell Park Memorial Institute 1640 to give a volume of 200 μL in each well of microtiter plates (96-well). The assay for each concentration of extract was performed in quadruplicate and the culture plates were incubated at 37 ˚C with 5% (v/v) CO_2 for 24 h. The well which contains cells and media only were used as a control. After 24 h of incubation, 50 μL of 2 mg/mL MTT solution was added to each well and they were allowed to incubate for an additional 4 h. After 4 h incubation, all supernatant was discarded. Subsequently, 100 μL dimethyl sulfoxide was added to each well and vigorously mixed to dissolve the formazan crystals. Absorbance values at 550 nm was measured with a microplate reader and the percentage cell viability was calculated manually using the formula:

$$\text{Viability (\%)} = \frac{\text{Average absorbance of duplicate extract wells}}{\text{Average absorbance of duplicate control wells}} \times 100$$

A dose-response curve was plotted to enable the calculation of the concentrations that killed 50% of the T47D cells (IC_{50}).

2.3. Statistical analysis

Statistical analysis was carried out with the SPSS 15.0 for Windows software package. The results are expressed as mean±SD. One-way ANOVA and Duncan's multiple range test were used to determine the differences of each variation of the concentration against the percentage of cell viability.

3. Results

3.1. Proliferative effects of T47D

The cytotoxicity effect of *G. cowa* on T47D was evaluated by MTT assay. The ethanol extract of *G. cowa* in multiple concentrations was used. The effective concentration was calculated from concentration-response curve. The cytotoxicity evaluation of the ethanol extract against T47D was shown in Figure 1. Based on the MTT assay, it was found that the ethanol extract of the stem bark of asam kandis had IC_{50} value of (5.10±1.68) μg/mL. The criteria of cytotoxicity for the crude extract, which was established by the U.S. National Cancer Institute, is IC_{50} <20 μg/mL in the preliminary assay[12].

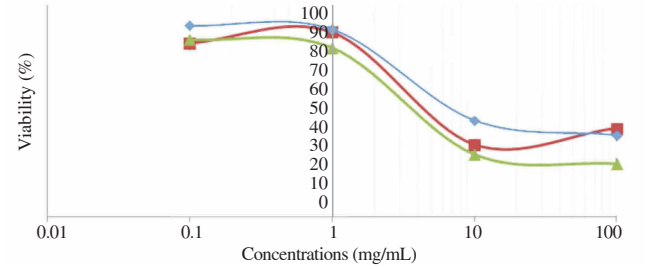

Figure 1. Correlation between concentrations of *G. cowa* extract and viability (%) of cells at concentrations of 0.1, 1, 10 and 100 μg/mL in three repetitions.

3.2. Evaluation on morphological changes upon treatment with extracts

Morphological alteration of T47D cell lines after exposure to *G. cowa* extract was observed under inverted microscope. The cells indicated the most prominent effects. The microscopic observations revealed that the *G. cowa* extract had an outstanding effect on cell death, and the mortality increased correspondingly with concentration increment of the extract treatment. At the highest concentration (100 μg/mL), the cells became shrunken and showed signs of detachment from the surface of the wells, which denoted cell death (Figures 2-6).

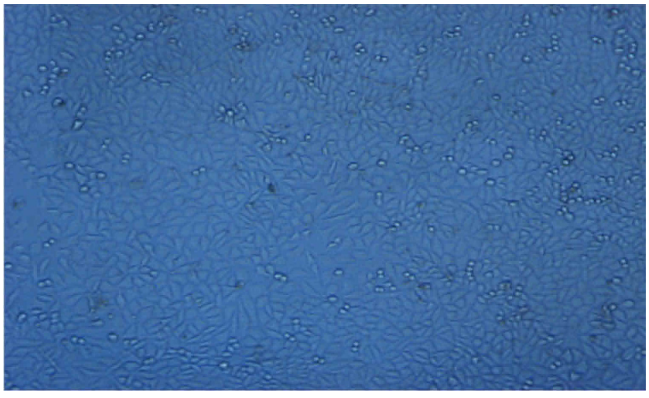

Figure 2. Morphological changes of the T47D cells after 24 h of incubation (control) (100×).

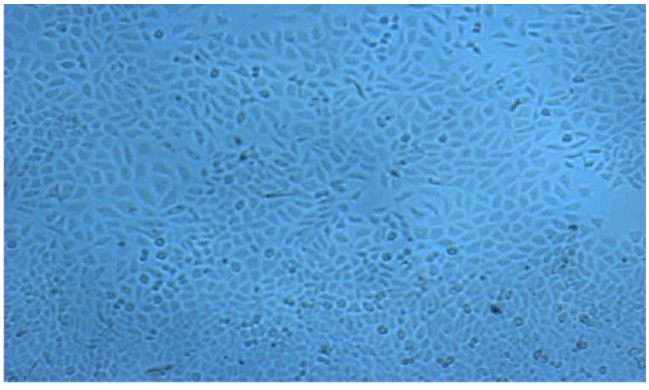

Figure 3. Morphological changes of the T47D cells after treatment with 0.1 μg/mL ethanol extract of *G. cowa* after 24 h of incubation (100×).

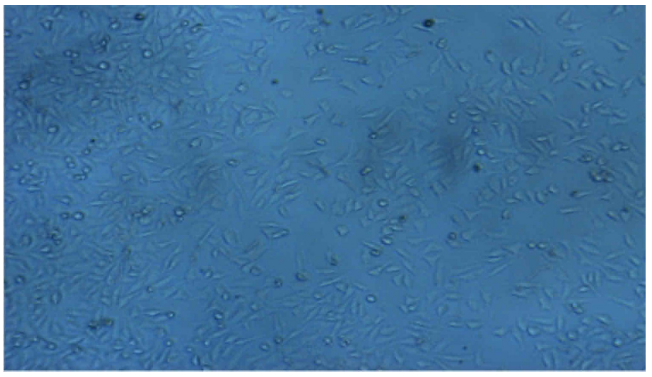

Figure 4. Morphological changes of the T47D cells after treatment with 1 μg/mL ethanol extract of *G. cowa* after 24 h of incubation (100×).

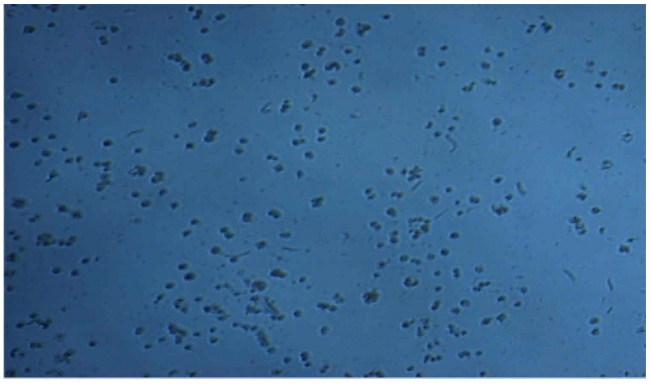

Figure 5. Morphological changes of the T47D cells after treatment with 10 μg/mL ethanol extract of *G. cowa* for 24 h (100×).

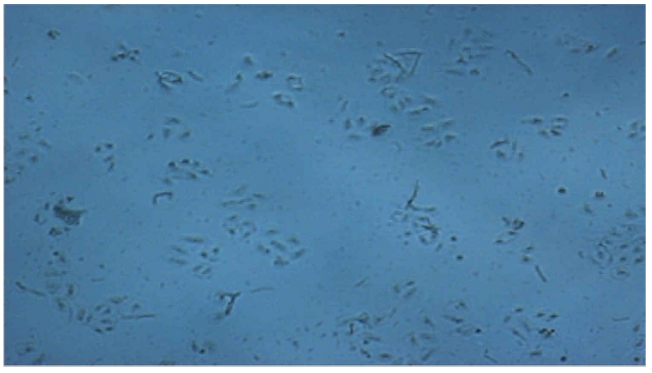

Figure 6. Morphological changes of the T47D cells after treatment with 100 μg/mL ethanol extract of *G. cowa* after 24 h of incubation (100×).

4. Discussion

MTT assay is a colorimetric cytotoxic test to determine the number of living cells based on changes in MTT solution which are colored from yellow to purple formazan crystals by active mitochondria in living cells. MTT was absorbed into living cells and broken through the oxidation reaction by nicotinamide adenine dinucleotide, as enzyme in the mitochondrial respiratory chain was changed into formazan which can not be soluble in water. Purple color intensity was directly proportional to the amount of metabolism active cell. The darker color, the higher the absorbance value, and the more

living cells[11].

Breast cancer cell used in this research was T47D cell. T47D breast cancer cell is sensitive to chemotherapeutic agents and have a fast replication capability that is well-suited for the cytotoxic test. The multiple concentrations of *G. cowa* extract were used and effective concentration was calculated from concentration-response curve. The ethanol extract of the stem bark of *G. cowa* exhibited significant activity against T47D cell lines with a IC_{50} value of (5.10±1.68) μg/mL. One-way ANOVA was followed by Duncan's multiple range test to determine the differences of each variation of the concentration against the percentage of cell viability. The concentrations of 100 μg/mL and 10 μg/mL had similar cytotoxic effect with 34.94% and 36.24% cell viability respectively while the concentrations of 1 μg/mL and 0.1 μg/mL also had similar effect with 85.19% and 85.34% cell viability respectively. A anticancer drugs is expected to be of minimal concentration while giving maximal cytotoxic effects.

T47D cells demonstrated clear structural evidence of apoptosis after treatment with extract. The concentrations of 0.1 μg/mL and 1 μg/mL of *G. cowa* ethanol extract led to the morphological changes of cells including reduction in the size of the cells. Physiologically, cells which detached from the base of the culture plate revealed an interruption of extracellular matrix and inhibition of cell-cell contact. The cells became flat and shrunken with the appearance of small vesicle bodies (apoptotic bodies). Compared to the control, cells shringkage could be seen clearly after being given the extract with concentrations of 100 μg/mL and 10 μg/mL after 24 h of incubation.

In conclusion, the findings of our study suggest the possible use of ethanol extract of asam kandis for preparing herbal medicine for cancer-related ailments.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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