Cytotoxicity Study of Ethanol Extract of the Leaves of Asam Kandis (Garcinia cowa Roxb.) on T47D Breast Cancer Cell line

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ABSTRACT

Objective: To investigate the cytotoxic effect of ethanoic extract of the leaves of asam kandis (Garcinia cowa Roxb.) against T47D breast cancer cells. Methods: The cytotoxicity of ethanol extract was carried out by measuring the activity of mitochondrial dehydrogenase in living cells that have the ability to convert dissolved MTT pale yellow to purple formazan product. The extract was added at various concentrations (0.1, 1, 10 and 100 μg/mL). The level of cytotoxicity was determined by calculating the IC₅₀ value that was based on the percentage of the cell death after 24 hours treatment with the extract. Cell morphological changes were observed by using inverted microscope. Results: The IC₅₀ value showed that ethanol extract of leaves of asam kandis could resist T47D breast cancer cells with IC₅₀ 6.13 ± 3.51 μg/mL. The statistic results proved that ethanol extract of the leaves of asam kandis could inhibit the growth of T47D breast cancer significantly at concentrations of 10 μg/mL and 100 μg/mL. Conclusion: The results suggest that ethanol extract of the leaves of asam kandis was potential source of herbal medicine for cancer-related ailments.

Key words: Asam kandis, Breast cancer, Cytotoxicity, Garcinia cowa Roxb, MTT Assay, T47D.

SUMMARY

- The IC₅₀ value of the ethanolic extract of the leaves of asam kandis (Garcinia cowa Roxb) is 6.13 ± 3.51 μg/ml towards T47D breast cancer cell line. The results suggested that ethanolic extract of the leaves of G. cowa was potential source of herbal medicine for cancer-related ailments.

INTRODUCTION

Plants have beneficial activity in different type of diseases producing in human beings. About 80% of the world’s populations still use the medicinal plants for their primary health care. Many pharmaceutical drug discoveries originated from traditional folk medicine and its associated plant materials and bioactive secondary metabolites. Garcinia (Family Guttiferae) is a large genus of polygamous trees or shrubs distributed in tropical Asia, Africa, and Polynesia. Garcinia cowa Roxb., known asam kandis is a medium-sized tree with edible fruit which attains a height of about 30 m. Traditionally, asam kandis has been used by Minang tribes for many purposes. The dry fruit of kandis is used as seasoning and in traditional folk medicine is used for treating nausea, constipation and ulcers.

Phytochemical investigations of asam kandis resulted in the isolation of xanthones, benzophenones, dyhydrobenzopyran, acylphloroglucinol, depsidone and tetraprenyltoluquinone. Previous investigation on the stem bark of this plant revealed the presence of nine prenylated polyhydroxyxanthones as well as pyrano-xanthones. The organic acid content in fresh leaves, fruits and dried rinds of asam kandis the major constituents. Previous investigation of the stem bark of asam kandis revealed the presence of 6-hydroxy-calabaxanthone, 1,3,6-trihydroxy-7-methoxy-4-(4-acetoxy-3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl) xanthone and cowanin. α-mangostin and cowanin were found to be potent against MCF-7 while 6-hydroxy-calabaxanthone potent against DU-145 cell lines.

In continuing of our studies on asam kandis (G. cowa) we further investigated the cytotoxicity of the leaves of this species on T47D breast cancer cells line.

MATERIALS AND METHODS

Plant materials and extraction

The leaves of G. cowa were collected in Padang. The plant material was air dried in the green house for 3 days at room temperature, followed by oven drying at 40°C for one day. The dry leaves were grinded to powder form using a laboratory blender. The powdered sample was kept in an airtight container until required. About 2 kg of the powdered leaves of G. cowa was macerated in 15 L of ethanol 70% for 3 days. The powdered sample was kept in the refrigerator.

Methods

All procedures were described in our previous study.

PICTORIAL ABSTRACT

Abbreviations used: MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, IC: Inhibition of Concentration.

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DOI: 10.5530/pj.2015.6.9

369
FATMA SRI WAHYUNI et al.: Cytotoxic activity of the leaves of *Garcinia cowa* Roxb.

RESULTS

Cytotoxic effect toward T47D

The cytotoxic effect of ethanol extract of leaves of *asam kandis* on T47D was evaluated by MTT assay. The extract from *asam kandis* in multiple concentrations was used. The effective concentration was calculated from concentration-response curve. The percentage of viability of each plate was shown in Table 1.

Based on the MTT assay (Figure 1), it was found that the ethanol extract of the leaves of *asam kandis* had IC$_{50}$ value of 6.13 ± 3.51 µg/mL. The criteria of cytotoxicity for the crude extract, as established by the U.S. National Cancer Institute (NCI), are an IC$_{50}$<20 µg/mL in the preliminary assay.  

Evaluation on morphological changes upon treatment with extracts

The difference in concentration gives a different picture when viewed with a microscope inverted when compared to control. Figure 2 showed that many cancer cells grow and attached at the base of the flask at a concentration of 0.1 µg/mL and 1 µg/mL. Cells grow very dense and very little distance between one cell to another cell so it almost looks no difference with control. While at a concentration of 10 µg/mL, the cells look less than the concentration of 0.1 µg/mL and 1 µg/mL. Cells are visible only in small groups so that the distance a group of cells with other cells look far. It is also evident from the low absorbance value. Another case in a concentration of 100 µg/mL, the cells look less, there is no longer even visible cell growth. Dead cells will not be visible cell nucleus (white) and opaque. Absorbance also suggests that little living cells.

DISCUSSION

MTT assay is a colorimetric cytotoxic test method to determine the number of living cells based on changes in a solution of 3-(4,5-dimetil-thiazol-2-yl)-2,5-difeniltetrazolium bromide were colored yellow to purple formazan crystals by active mitochondria in living cells. MTT is absorbed into living cells and broken through the oxidation reaction by nicotinamide adenine dinucleotide (NAD$^+$) as enzyme in the mitochondrial respiratory chain into formazan that was not soluble in water.

Purple color intensity is directly proportional to the amount of the active cell metabolism. Formed the darker color, the higher the absorbance value, and the more living cells.

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 ethanol extract from *asam kandis* were used and effective concentration was calculated from concentration-response curve. The results of the cytotoxicity evaluation against T47D cell lines of the *kan-dis* extract were shown in Figure 1. The ethanol extract of the leaves of *asam kandis* exhibited significant activity against T47D cell lines with IC$_{50}$ 6.13 ± 3.51 µg/mL.

Data processing using One Way ANOVA followed by Duncan Advanced Test. Results of One Way ANOVA test (α=0.05) suggesting that the leaves extract asam kandis gave significantly different barriers to the growth of T47D breast cancer cells. The results obtained on a One Way ANOV A test were significantly different, then followed by Duncan test at 5% significance level (Table 1). Duncan further test the data obtained that the concentration of 100 µg/mL exhibited significant activity against T47D cell lines with IC$_{50}$ 6.13 ± 3.51 µg/mL.

Based on the relationship curve between the concentration of the test compound with the % cell viability can be observed that the leaves of *asam*
kandis extract at a concentration of 0.1 µg/mL and 1 µg/mL did not affect the amount of reduction in the number of cells that survive, but at higher concentrations i.e. at a concentration of 10 µg/mL and 100 µg/mL can be observed a decrease in % cell viability, significantly. Based on the statistical results, it can be concluded that the average percentage of cell viability at a concentration test showed a highly significant difference (p<0.01).

CONCLUSION
The results of this research suggest that ethanol extract of the leaves of asam kandis was potential source of herbal medicine for cancer-related ailments.

ACKNOWLEDGEMENT
This work was supported by Faculty of Pharmacy, Andalas University.

CONFLICT OF INTEREST
Authors do not have any conflict of interest.

REFERENCES

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