ACTIVITY OF BENZOPHENONE GLUCOSIDE FROM MAHKOTA DEWA {*Phaleria macrocarpa* (Scheff.) Boerl.} FRUITS ON PROLIFERATION OF HUMAN CERVICAL-CANCER CELLS (HeLa and CasKi) AND HUMAN ESOPHAGEAL CANCER CELLS (TE-2, TE-8, and TE-14)

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ABSTRACT

Phaleria macrocarpa, locally named mahkota dewa, is known as a medicinal plant in Indonesia. Fruits of this plant are traditionally used in treatment of cancer diseases. Several substances were isolated from mahkota dewa fruits. A benzophenone glucoside, 4,6`-dihydroxy-4`-methoxybenzophenone-2'-O-glucoside, has been isolated from an ethyl acetate fraction of mahkota dewa fruits. The structure of this compound was determined based on the analysis of UV, IR, NMR and MS spectral data. Antiproliferative activity was measured on human cervical cancer cells (HeLa and CasKi) and human esophageal cancer cells (TE-2, TE-8, and TE-14) by MTT assay. The result of this study showed that at concentration of 500 µg/mL, benzophenone glucoside derived from mahkota dewa didn't reach CPI_{50} to all tested cells. Those CPI_{50} were 34 µg/mL (HeLa), 32 µg/mL (CasKi), 33.91 µg/mL (TE-2), 35.43 µg/mL (TE-8), 43.04 µg/mL (TE-14). There were no significantly differences (α=0.5) of this activity among HeLa, CasKi, TE-2, TE-8 and TE-14 cells.

Key words : Benzophenone glucoside, *Phaleria macrocarpa*, HeLa, CasKi, TE-2, TE-8, and TE-14 cells, MTT assay

AKTIVITAS ANTIPROLIFERASI BENZOFENON GLUKOSIDA DARI BUAH MAHKOTA DEWA {*Phaleria macrocarpa* (Scheff.) Boerl.} TERHADAP SEL KANKER RAHIM (HeLa dan CasKi) DAN SEL KANKER ESOFAGUS MANUSIA (TE-2, TE-8, and TE-14)

ABSTRAK

Phaleria macrocarpa, dikenal sebagai mahkota dewa merupakan salah satu tanaman obat di Indonesia. Buah dari tanaman tersebut digunakan secara tradisional untuk mengobati berbagai penyakit kanker. Beberapa senyawa telah diisolasi dari buah mahkota dewa. Suatu benzofenon glukosida, 4,6`-dihidroksi-4`-metoksibenzofenon-2'-O-glukosida telah diisolasi dari fraksi etil asetat buah mahkota dewa. Struktur senyawa tersebut ditentukan berdasarkan analisis spektrofotometri *UV*, *IR*, *NMR* dan *MS*. Aktivitas antiproliferasi diukur terhadap sel kaker rahim (HeLa dan CasKi) dan sel kanker esofagus (TE-2, TE-8, and TE-14) dengan metode MTT. Hasil penelitian menunjukkan, bahwa pada konsentrasi 500 µg/mL, senyawa uji tidak mencapai CPI_{50} pada semua sel yang diuji. Nilai CPI_{50} untuk masing-masing sel adalah 34 µg/mL (HeLa), 32 µg/mL (CasKi), 33,91 µg/mL (TE-2), 35,43 µg/mL (TE-8), 43,04 µg/mL (TE-14) dan tidak terdapat perbedaan bermakna antara aktivitas senyawa ini (α=0.5) terhadap semua sel yang diuji.

Kata kunci : Benzofenon glukosida, *Phaleria macrocarpa,* sel HeLa, CasKi, TE-2, TE-8, and TE-14, metode MTT

INTRODUCTION

Plants have a long history of use in the treatment of cancer. They have provided some of the currently used effective anticancer agents such as vinblastine, vincristine, etoposide, teniposide, and paclitaxel (Alexandrova *et al*., 2000). The difficulties in the treatment of cancer, have led to many researches done to find out effective anticancer compounds. Beside act to cancer cells, many cancer therapies affect normal cells to cause serious adverse effects, such as bone marrow function inhibition, nausea, vomiting and alopecia. More effective anticancer drugs with high selectivity against only malignant cells and with ability to repress tumor metastasis are desired (Ueda *et al*., 2002). Indonesia has many medicinal plants and mahkota dewa (*Phaleria macrocarpa*) is one widely used and distributed in Indonesia. The stems, leaves and fruits of mahkota dewa are used for medicinal treatment. Traditionally, this plant is used in the treatment of cancer disease. The ethanol extract of *P. macrocarpa* is known to have antiproliferative activity on Leukemia cells L 1210 and antioxidant activity (Lisdawati, 2002; [a]Diantini, *et al*., 2006;). Several benzophenone substances had been isolated and identified from fruits and leaves of *P. macrocarpa* ([b]Diantini, *et al*., 2006; Hakim *et al*., 2004; Hartati *et al*., 2005; Kusmardiyani *et al*., 2004; Nawawi, 2004). In

this study, we investigated antiproliferative activity of benzophenone glucoside isolated from the fruits of *P. macrocarpa* on several cancer cells (HeLa, CasKi, TE-2, TE-8, and TE-14).

MATERIALS AND METHODS

Plant Materials

P. macrocarpa fruits were collected from Purworejo, Central Java, Indonesia. The plant was determined in School of Biological Science and Technology, Bandung Institute of Technology, Bandung, Indonesia.

Extraction and Isolation

The dried powder of fruits (2000 g) was extracted with 70% ethanol (3 x, each 24 hr) at room temperature, and the solvent was then evaporated under reduced pressure at 55-60ºC to yield a concentrated extract (475 g). The extract was partitioned with a mixture of *n*-hexane-water (3 : 1) to afford an *n*-hexane fraction (11.69 g) and a water layer. The water layer was further extracted with ethyl acetate and *n*-butanol successively to give ethyl acetate, *n*-butanol, and water fractions (39 g, 118.37 g, and 278 g, respectively). The ethyl acetate fraction was chromatographed over silica gel G 60 using an *n*-hexane-ethyl acetate-methanol mixture of increasing polarity to give five fractions (A, B, C, D and E). Repeated chromatography of the fraction C (23.17 g) over silica gel G 60 with the eluent of the *n*-hexane-aceton-methanol mixture led to the isolation of a pale yellow compound (3.24 g).

Structure Elucidation

UV spectra were measured with a Varian Conc. 100 instrument. IR spectra were determined with a Perkin Elmer FTIR Spectrum One spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded with a JEOL AS400 operating at 400 (^{1}H) and 100 (^{13}C) MHz using residual and deuterated solvent peaks as reference standards. Mass spectrum was obtained with VG Autospec mass spectrometer (EI mode).

Cell Lines

Human cervical cancel cells (HeLa and CasKi) and human esphageal cancer cells (TE-2, TE-8 AND TE-14) were maintained in RPMI-1640 medium (Invitrogen) supplemented with 100 U/ml penicillin, 100 ug/ml streptomycin and 10% fetal bovine serum (FBS). The cells were maintained at 37^{o}C/5% CO2 in a humid environment.

Stock Solution of Benzophenone Glucoside

A stock solution of 1000 µg/ml were made in PBS, filtered and stored at 4º C, for use in tissue culture. The further dilutions were made in RPMI-1640 medium (Invitrogen).

Antiproliferative Assay

Cell proliferation analysis was performed on cells in the presesnce of increasing concentrations of benzophenone by tetrazolium assay using WST as describe previously. Briefly. Cells ($1x10^4$/well) were plated in 96-well plates, 50 µl for each well. After the initial cell seeding, various concentrations of benzophenone (1,25 – 500 µg ml) were incubated for 24 h. WST-8 assay (Dojindo Laboratories, Tokyo, Japan), 10 µl of the cell counting solution was added to each well and incubated in a 100 µl/well in 1 N HCl, and the absorbance of the solution was read at 450-650 nm using a microtiter plate reader (Becton Dickinson, Franklin Lakes, N.J, USA). After 24 h exposure, the 50% inhibitory concentration (CPI_{50}) was calculated as percentage of control by interpolate logarithmic concentration curve. Results were derived from duplicate experiments.

Result and Discussion

Cell culture techniques play a key role in the development of new anticancer drugs by imposing additional constraints on those of receptor interaction alone, such as drug uptake and efflux, interaction with other cellular receptors, and cellular metabolism. Microcultures combined with colorimetric and other methods for measuring antiproliferative effects, have provided the basis for large-scale screening of cytotoxic and cytostatic drugs (Baguley *et al*., 2002).

Some cytotoxicity assays are based on alterations of plasma membrane permeability, uptake dyes that normally excluded by viable cells, but these assays has a disadvantage because the initial sites of damage of many cytotoxic agents are intracellular. Cells may be irreversibly damaged and commited to die, but the plasma membrane is still intact. Thus, these assays tend to underestimate cellular damage when compared to other methods.

On this study we measured the activity of benzophenone glucoside on proliferation of several cell lines by WST-assay. WST-assay measures cell survival based on incapability of dead cells to metabolize various tetrazolium salt (MTT, XTT, or WST).

The isolation and structure determination of benzophenone glucoside had been described before ([b]Diantini, *et al*., 2006).

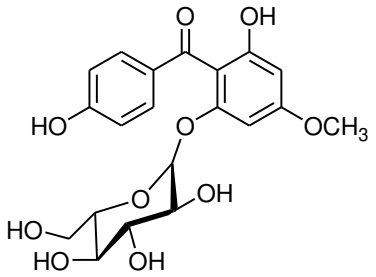

Figure 1. Structure of benzophenone glucoside

In this study, we report activity of benzophenone glucoside on proliferation of cervical cancer cells (HeLa and CasKi) and human esophageal cancer cells (TE-2, TE-8, TE-14).

Activity of benzophenone glucoside on HeLa, CasKi, TE-2, TE-8, TE-14 cells proliferation can be seen in Table or Figures below:

Table 1. Cell Proliferation Inhibition (CPI) of Benzophenone Glucoside on HeLa, CasKi, TE-2, TE-8 and TE-14 Cancer Cells

Treated Cells	Cell Proliferation Inhibition (CPI) of Benzophenone glucoside at different concentrations (µg/mL)									
	1	2	4	7.8	15.6	31.3	62.5	125	250	500
HeLa	0.261	3.041	5.003	7.619	11.216	16.857	21.517	26.095	30.592	34.189
CasKi	0.169	2.603	4.757	6.630	8.971	10.845	14.029	16.839	21.521	32.478
TE-2	0.870	2.899	2.899	4.348	6.667	9.662	13.043	15.845	19.903	33.913
TE-8	1.634	6.879	11.866	12.898	14.703	15.563	18.143	21.324	29.837	35.426
TE-14	0.654	1.214	1.867	2.428	2.894	4.669	7.190	13.725	22.129	43.044

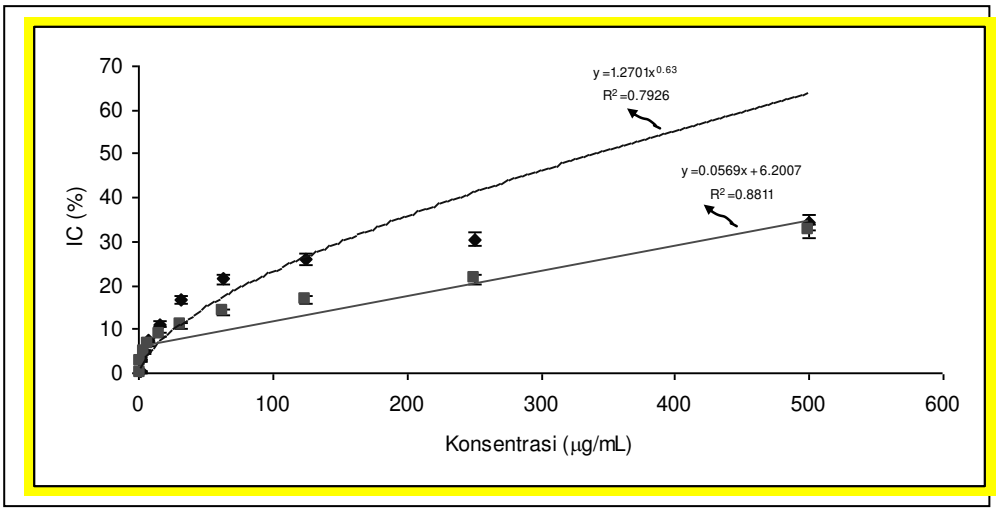

Figure 2. Activity of benzophenone glucoside on proliferation of human cervical cancer cells (HeLa and CasKi).

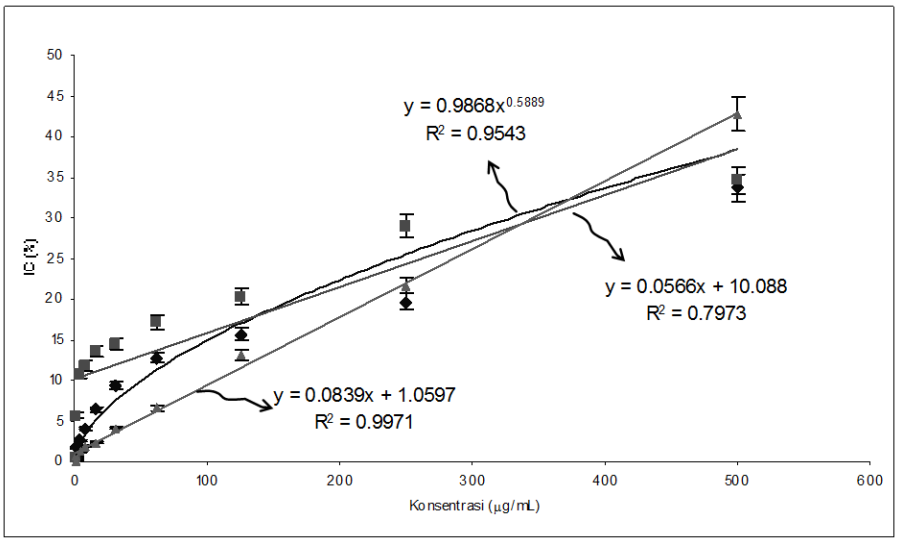

Figure 3. Activity of benzophenone glucoside on proliferation of human esophageal cancer cells (TE-2, TE-8, TE-14)

As shown in Table 1 or Figures 1 and 2, benzophenone glucoside isolated from the fruits of mahkota dewa had low activity on proliferation of HeLa, CasKi, TE-2, TE-8, TE-14 cells. At a concentration of 500 µg/mL, this substance didn't reach CPI_{50} on all tested cells. CPI_{50} values were 34 µg/mL (HeLa), 32 µg/mL (CasKi), 33.91 µg/mL (TE-2) , 35.43 µg/mL (TE-8), 43.04 µg/mL (TE-14). These data showed that benzophenone glucoside was inactive on the tested cells and there were no significantly difference (α=0,05) on activity of the substance on proliferation of human cervical cancer cells (HeLa, CasKi) and human esophageal cancer cells (TE-2, TE-8, TE-14 cells) as well. The activity of benzophenone glucoside on proliferation of the attached cells is lower as compared to floating cancer cells (leukemia P 388 cells) with IC_{50}=72.5 µg/mL ([c]Diantini *et al.*, 2006). Benzophenone glucoside isolated from mahkota dewa fruits was considered to have low activity on proliferation of all cells tested because the IC_{50} > 5 µg/mL. The capability of the fruits of mahkota dewa to cure cancer empirically might be due to another mechanism, eg antiangiogenesis or improvement of immune system.

CONCLUSION

Benzophenone glucoside isolated from mahkota dewa fruits has low activity on proliferation of human cervical cancer cells (HeLa, CasKi) and human esophageal cancer cells (TE-2, TE-8, TE-14 cells).

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