CYTOTOXICITY EFFECT OF RESVERATROL OLIGOMERS AND THEIR DERIVATIVE AGAINST HUMAN CANCER CELL LINES

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Abstract: Eleven **resveratrol oligomers isolated from *Hopea* (Dipterocarpaceae) and their derivative, were evaluated for in vitro cytotoxicity against a panel of human tumor cell lines. Among them, two compounds ampelopsin H (6) and vaticanol B (4) showed as cytotoxicity againt cell Hela-S3, Raji, and Myeloma**

Introduction

In our continuing phytochemical study of the Dipterocarpaceae family occuring in Indonesia, we have examined the resveratrol derivatives constituents of *Hopea mengarawan, H. odorata,* and *H. nigra*. *Hopea* is a relatively large genus belonging to the family Dipterocarpaceae and is distributed mainly in Southeast Asia [1;2;3]. This family has proven to be a rich source of oligostilbene compounds derived from the resveratrol (4, 3', 5'-trihydroxystilbene) [6-12].

Materials and Methods

Samples of the stem bark of *H. Mengarawan, H. odorata,* and *H. nigra* were collected in December 2003 from the Experimental Garden in Carita, Banten, Indonesia. The plant was identified by the staff at the Herbarium Bogoriense, Kebun Raya Bogor, Bogor, and a voucher specimen had been deposited at the Herbarium.

The milled dried stem bark of *H. mengarawan* (5 kg) was extracted exhaustively with acetone. The acetone extract on removal of the solvent under reduced pressure gave a brown residue (400 g). A portion (40 g) of the total acetone extract was fractionated by vacuum liquid chromatography (VLC) and purified by repeated column chromatography on silica gel eluted with various solvent systems. From this method we obtained four oligostilbenes, namely balanocarpol (1) (300 mg), heimiol A (2) (200 mg), vaticanol G (3) (70 mg), and vaticanol B (4) (200 mg). The structures of these compounds (1 – 4) were established on the basis of their spectral data, including UV, IR, and NMR spectra in comparison with the previously reported data [6-12] and by direct comparison with the authentic samples. From the dried and milled stem bark of *H. odorata* (3.8 kg) was isolated four componds, namely balanocarpol (1) (300 mg), hopeaphenol (5) (1500 mg), ampelopsin H (6) (250 mg), and hemilesyanol C (7) (120 mg), whereas from the dried and milled stem bark of *H. nigra* (4.6 kg) to give vaticanol G (1) (200 mg) (Fig. 1). The isolation procedure and spectroscopic data of all compounds were described in previous papers [11]. Derivates of the compounds we synthesis by methylation and acethylation of balanocarpol and hopeaphenol. Methylation of these compounds was allowed to react with K_2CO_3 and Me_2SO_4 in dry acetone under reflux for 6 h. The crude product as purified by cromatographyc method. Acethylation of these compounds was allowed to react with anh. Acetic acid in piridin under reflux for 24 h. The crude product as purified by chromatographyc method and to identify by spectrocophy UV, IR, NMR From this reaction, we found deca-methyl-O-hopeaphenol (8), and deca-acethyl-hopeaphenol (9), penta-methyl-O-balanocarpol (10), hexa-acethyl-balanocarpol (11).

All of compounds we evaluated for in vitro cytotoxicity against a panel of human tumor cell lines Hela S3, Raji, and Myeloma. The invitro cytotoxicity test was investigated using plate with 96 wells, with cell density $2x10^4$ cells per ml. Into each well was added 100 µl cells in culture medium (87,5% RPMI 10,4 g/L; 2% penstrep; and 10% FBS) which was then incubated in CO_2 incubator for 12-24 hours at 37°C. Each sample was dissolved in culture medium containing 0,05% DMSO, and 100 µl of each sample in different concentrations was added into each well in triplicate and was then incubated in CO_2 incubator for 12-24 hours at 37°C. MTT solution (10 µl per 100 µl medium) was added to all wells of an assay, and plates were incubated for 4 hours at 37°C in CO_2 incubator. As much as 100µl formazon (10% SDS and 0, 01 N hydrochloric acid) was added into each well and mixed on a shaker for 5 minutes. The wells were incubated in the dark room for 12-24 hours at room temperature. The absorbance was measured using multiwell scanning spectrophotometers (ELISA reader) at wavelength 595 nm. The absorbance is directly proportional to the number of living cells. So the dead cell could be calculated to determine LC_{50}. Doxorubicin, a medicine for lymphoma, leukaemia and acute tumor, was also measured its cytotoxic activity as positif control comparison. The cytotoxic activity of the samples against Hela-S3 cell measured as LC_{50} were provided in Table 1. Hela-S3, a *continuous cell line* that lived as adherent cell, is a cell derivate of ephythell cell of human cervix cancer. Further investigation of cytotoxic activity of the samples was held against Raji cell (Table 1). The cell that resembles lymphoblast cell found by R.J.V Pulvertaft (1963) from *Burkitt's lymphoma* at the left of the upper jaw of a 11 year old negro boy. *Myeloma cell, the first from* Merwin Plasma Sel Tumor-11 (MPC-11) which isolated from mice Balb/c and collected by J. Fahey on 1967.