

Crude ethyl acetate extract of marine microalga, *Chaetoceros calcitrans*, induces Apoptosis in MDA-MB-231 breast cancer cells

ABSTRACT

Background: Marine brown diatom *Chaetoceros calcitrans* and green microalga *Nannochloropsis oculata* are beneficial materials for various applications in the food, nutraceutical, pharmaceutical and cosmeceutical industries. **Objective:** This study investigated cytotoxicity of different crude solvent extracts from *C. calcitrans* and *N. oculata* against various cancer cell lines. **Materials and Methods:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was carried out to screen the cytotoxic effects of hexane (Hex), dichloromethane (DCM), ethyl acetate, and methanol extract from *C. calcitrans* and *N. oculata* toward various cancer cell lines. Flow cytometry cell cycle was used to determine the cell cycle arrest while the mode of cell death was investigated through acridine orange/propidium iodide (AOPI) staining, Annexin V-Fluorescein Isothiocyanate (FITC) and Terminal deoxynucleotidyl transferase-mediated d-UTP Nick End Labeling (TUNEL) assays. Expression profile of apoptotic and proliferative-related genes was then determined using the multiplex gene expression profiler (GeXP). **Results:** Crude ethyl acetate (CEA) extract of *C. calcitrans* inhibited growth of MDA-MB-231 cells, with IC₅₀ of 60 µg/mL after 72 h of treatment. Further studies were conducted to determine the mode of cell death at various concentrations of this extract: 30, 60 and 120 µg/mL. The mode of cell death was mainly apoptosis as shown through apoptosis determination test. The expression data from GeXP showed that caspase-4 was upregulated while B-cell leukemia/lymphoma 2 (Bcl-2) was down regulated. Thus, caspase-4 induction endoplasmic reticulum death pathway is believed to be one of the mechanisms underlying the induction of apoptosis while Bcl-2 induced S and G2/M cell cycle phase arrest in MDA-MB-231 cells. **Conclusion:** CEA extract of *C. calcitrans* showed the highest cytotoxicity on MDA-MB-231 via apoptosis.

Keyword: Apoptosis; *Chaetoceros calcitrans*; Crude ethyl acetate extract; Gene expression profiler; MDA-MB-231