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IN-VITRO CYTOTOXIC ACTIVITY OF CRUDE METHANOL EXTRACT AND ITS FRACTIONS OF SRI LANKAN MARINE BROWN ALGAE Choonospora minima AGAINST HUMAN RHABDOMYOSARCOMA (RD) CELL LINE

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Abstract

Seaweeds are an important source of bioactive metabolites in drug development and nutraceuticals. The aim of this study was to investigate the *in vitro* cytotoxic activity of de-polysaccharide methanol extract and partition fractions of hexane, chloroform, ethyl acetate (EtOAc) and aqueous fractions of brown algae Choonospora minima against human rhabdomyosarcoma (RD) cell line. The selected fractions and crude methanol extract were subjected for MTT and neutral red uptake assay to determine the cell viability. Apoptotic morphological features were observed using crystal violet and fluorescence dye Hoechst staining methods. The results of both MTT and neutral red assay showed that the decrease of the percentage of cell viability in dose dependent manner as signified by cell death. The hexane and chloroform fractions of C.minima showed potential cytotoxic activity with IC₅₀ of 93.98±1.33 µg/ml and 106.94±1.68 µg/ml against human rhabdomyosarcoma (RD) cell line compared to the standard cycloheximide (IC₅₀: 36.17 ± 1.78 µg/ml). In addition, a significant cytotoxic activity was observed between total crude extract and fractions of C.minima against RD cell lines (P>0.05). Further, potential cytotoxic activity of hexane $(133.13\pm4.50 \,\mu\text{g/ml})$ and chloroform $(142.09\pm0.64 \,\mu\text{g/ml})$ fractions of C.minima was confirmed by neutral red assay compared to the standard cycloheximide (32.78±0.91µg/ml). Gas chromatography-mass spectrometry analysis of the hexane fraction revealed the presence of several anti-cancer compounds. In addition, apoptotic morphological features such as cell membrane blebbing, formation of membrane bound vesicle, nuclear fragmentation and micro nuclei formation, cellular shrinkage, cellular aggregation and formation of cell clumps and chromatin condensation were observed both in hexane and chloroform fractions of C.minima when stained with crystal violet and florescence dye Hoechst stain. The results suggest that the hexane fraction of C. minima is a potential source of natural compounds to combat as anti-cancer therapy. The research grant (ASP/01/RE/SCI/2017/50) by University of Sri Javewardenepura is acknowledged.

Keywords: Choonospora minima, Anti-cancer, Rhabdomyosarcoma, MTT, Neutral red, Morphology