ABSTRACT

Santalum album L., the East Indian Sandalwood, is a well-known tree in traditional medicine. Its medicinal importance has been extensively reported for small molecules. In this study the bioactive peptides from sandalwood was explored. A bioactive peptide was purified from somatic seedlings of *Santalum album* L. (sandalwood) extracts using gel filtration and RP-HPLC separation process. The molecular mass of purified peptide was found to be 858 Da and the sequence was determined by MALDI-ToF-PSD-MS as 'RLGDGCTR' (cyclic), named cyclosaplin. The chemical synthesis of cyclosaplin was outsourced and the chemistry was confirmed by HPLC, and ESI-MS. The structure of cyclosaplin was elucidated by molecular modeling associated with dynamics using GROMACS that was further used in docking studies. It showed strong binding affinities towards all cancer-related proteins (EGFR Kinase, VEGFR2 Kinase, Protein Kinase B, p38, MMP-2, MMP-9, Procaspase 3, Procaspase 7, Caspase 9, TRAIL, and SURVIVIN) except PTEN. A few amino acid residues of cyclosaplin actively interacted forming H-bonds and hydrophobic contacts. The cytotoxic activity of cyclosaplin was tested against human breast cancer (MDA-MB-231) cell line in a dose and time-dependent manner. The purified peptide exhibited significant antiproliferative activity with an IC_{50} 2.06±0.08 µg/mL. In a mechanistic approach, apoptosis was observed in differential microscopic studies (SEM, Live/Dead staining, and DAPI staining) for cyclosaplin treated MDA-MB-231 cells that were further confirmed by mitochondrial membrane potential, DNA fragmentation assay, cell cycle analysis, and caspase 3 activities. In addition, apoptosis related genes were measured at the mRNA level using reverse transcription-PCR. Based on *in silico* docking, the co-localization studies revealed that the cyclosaplin sensitizes MDA-MB-231 cells by possibly binding to EGFR and induces apoptosis. Silk fibroin based 3D *in vitro* tumor model was used for evaluation of anticancer drug activity of cyclosaplin. The proliferation rate, glucose consumed, LDH and MMP-9 activity of breast cancer cells was higher in 3D constructs compared to 2D. Higher concentrations of drug were required to achieve 50% cell death in 3D culture than 2D cultures. The cyclosaplin treated MDA-MB-231 cells showed significant decrease in MMP-9 activity in 3D constructs. Microscopic analysis revealed the formation of cell clusters evenly distributed in the scaffolds. The drug treated cells were less in number, smaller and showed unusual morphology. The findings indicate the promising role of cyclosaplin in cancer therapeutics.

Keywords: Apoptosis, Caspase 3, Cyclosaplin, EGFR, Sandalwood, and Silk fibroin tumor model.