

MTT Assay-Guided Isolation of a Cytotoxic Lead from *Hedyotis umbellata* and Its Mechanism of Action against Non-Small Cell Lung Cancer A549 Cells

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Abstract : Introduction: Cancer is one of the leading causes of death worldwide. Although existing therapy effectively kills cancer cells, they do affect normal growing cells leading to many undesirable side effects. Hence there is need to develop effective as well as safe drug molecules to combat cancer, which is possible through phyto-research. The currently available plant-derived blockbuster drugs are the example for this. In view of this, an investigation was done to identify cytotoxic lead molecules from *Hedyotis umbellata* (Family Rubiaceae), a widely distributed weed in India. Materials and Methods: The methanolic extract of the whole plant of *H. umbellata* (MHU), prepared through Soxhlet extraction method was further fractionated with diethyl ether and n-butanol, successively. MHU, ether fraction (EMHU) and butanol fraction (BMHU) were lyophilized and were tested for the cytotoxic effect using 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay against non-small cell lung cancer (NSCLC) A549 cell lines. The potentially active EMHU was subjected to chromatographic purification using normal-phase silica columns, in order to isolate the responsible bioactive compounds. The isolated pure compounds were tested for their cytotoxic effect by MTT assay against A549 cells. Compound-3, which was found to be most active, was characterized using IR, ¹H- and ¹³C-NMR and MS analysis. The study was further extended to decipher the mechanism of action of cytotoxicity of compound-3 against A549 cells through various in vitro cellular models. Cell cycle analysis was done using flow cytometry following PI (Propidium Iodide) staining. Protein analysis was done using Western blot technique. Results: Among MHU, EMHU, and BMHU, the non-polar fraction EMHU demonstrated a significant dose-dependent cytotoxic effect with IC₅₀ of 67.7µg/ml. Chromatography of EMHU yielded seven compounds. MTT assay of isolated compounds explored compound-3 as potentially active one, which inhibited the growth of A549 cells with IC₅₀ value of 14.2µM. Further, compound-3 was identified as cedrelopsin, a coumarin derivative having molecular weight of 260. Results of in vitro mechanistic studies explained that cedrelopsin induced cell cycle arrest at G2/M phase and down-regulated the expression of G2/M regulatory proteins such as cyclin B1, cdc2, and cdc25C, dose dependently. This is the first report that explores the cytotoxic mechanism of cedrelopsin. Conclusion: Thus a potential small lead molecule, cedrelopsin isolated from *H. umbellata*, showing antiproliferative effect mediated by G2/M arrest in A549 cells was discovered. The effect of cedrelopsin against other cancer cell lines followed by in vivo studies can be performed in future to develop a new drug candidate.

Keywords : A549, cedrelopsin, G2/M phase, *Hedyotis umbellata*

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