

Investigation of Cytotoxic Compounds in Ethyl Acetate and Chloroform Extracts of *Nigella sativa* Seeds by Sulforhodamine-B Assay-Guided Fractionation

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Abstract : A Sulforhodamine-B assay-guided fractionation on *Nigella sativa* seeds was conducted to determine the presence of cytotoxic compounds against human hepatoma (HepG2) cells. Initially, a freeze-dried sample of *Nigella sativa* seeds was sequentially extracted into solvents of increasing polarities. Crude extracts from the sequential extraction of *Nigella sativa* seeds in chloroform and ethyl acetate showed the highest cytotoxicity. The combined mixture of these two extracts was subjected to bioassay guided fractionation using a modified Kupchan method of partitioning, followed by Sephadex® LH-20 chromatography. This chromatographic separation process resulted in a column fraction with a convincing IC₅₀ (half-maximal inhibitory concentration) value of 13.07µg/ml, which is considerable for developing therapeutic drug leads against human hepatoma. Reversed phase High-Performance Liquid Chromatography (HPLC) was finally conducted for the same column fraction, and the result indicates the presence of one or several main cytotoxic compounds against human HepG2 cells.

Keywords : cytotoxic compounds, half-maximal inhibitory concentration, high-performance liquid chromatography, human HepG2 cells, *nigella sativa* seeds, Sulforhodamine-B assay

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