

## **Synopsis**

Cancer has become the leading disease-related cause of death in the human population. For example, in the United States, cancer is the second leading cause of death behind cardiovascular disease, and it is projected that cancer will become the leading cause of death in the coming years. The medical treatment of cancer still has many unmet needs. The main curative therapies for cancer, surgery and radiation, are generally only successful if the cancer is found at an early localized stage. Once cancer has progressed to metastatic stage, these therapies are less successful. Hence, chemotherapeutic drugs are used for the treatment of these advanced tumors, particularly in the case of the common epithelial tumors such as lung, colorectal, breast, prostate, and pancreatic cancers. New chemotherapeutic drugs are necessary since most of the cancers acquire resistance towards existing anticancer drugs.

Nature has always been an attractive source of new chemotherapeutic agents, as a tremendous chemical diversity is found in millions of species of plants, animals, and microorganisms. Microorganisms (bacteria, fungi, actinomycetes) serve as readily renewable and inexhaustible source of novel bioactive metabolites. Endophyte, a microorganism that reside in the internal tissues of living plants

without causing any immediate overt negative effects, are potential sources of novel natural products for exploitation in medicine.

*Mappia foetida* is distributed in western part of peninsular coastal India from *Konkan ghats* to northern parts of the Kanara, Nilgiris, Anamalis, and Pullneys hills of India. It is a rich source of alkaloids such as camptothecin, 9-methoxy camptothecin, mappicin, sitosterol and lupeol and other natural products having anticancer and antimicrobial properties. Since, the secondary metabolite production in fungal endophytes is greatly influenced by their competitive ecological niche and interaction with host metabolism, *Mappia foetida* was chosen for endophytic fungal isolation. Sirsi, (North Karnataka, India) because of its rich biodiversity was chosen as a location for collection of the plant samples.

The organic solvent extracts obtained from mycelia and culture filtrates of all the endophytic fungal isolates were screened for their cytotoxic activity against HeLa (human cervical carcinoma) cell line. Organic extracts of 8 fungal isolates exhibited significant cytotoxic activity, among which *Phoma tropica* (S1/3) culture filtrate extract exhibited most significant cytotoxic activity against HeLa cell line with  $IC_{50}$  of 25 $\mu$ g/ml.

Dichloromethane extract of the *Phoma tropica* culture filtrate was subjected to bioassay-guided column chromatographic fractionation which resulted in the isolation of purified cytotoxic secondary metabolite. Based on the analysis of various

spectroscopic techniques such as NMR, FTIR, LC-MS/MS, HRMS, CHNOS elemental analysis and X-ray diffraction studies, the purified metabolite was identified as 2-Hydroxy-2,4-dimethyl-5-[-1-propen-1-yl]-3(2H)furanone(Phomafuranone).

Phomafuranone exhibited significant cytotoxic activity against various cancer cell lines (HeLa, Jurkat, COLO 205, HT-29, HCT-15, HCT 116, A549, A-431 and OVCAR-3)

Further studies were undertaken to elucidate the mechanism of cytotoxicity of the purified metabolite on human cancer cell lines. Phomafuranone contains a conjugated unsaturated  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  carbonyl pharmacophore moiety. Polyunsaturated carbonyl compounds are referred to as "Michael acceptors" and they behave as soft electrophiles. Michael acceptors react with strong biological nucleophiles such as thiols. The reactivity of electrophilic Phomafuranone with thiol containing biomolecules like glutathione, cysteine and N-acetyl cysteine was investigated by spectrophotometric methods.

Cell cycle progression analyses of treated HCT 116 (colorectal carcinoma) cell line by flow cytometry revealed that Phomafuranone arrested significant proportion of cells in G2/M phase. HCT 116 cells were arrested specifically in early mitotic phase of cell cycle as indicated by time dependent increase in phospho-histone3 (Ser10) levels and nuclear import of Cyclin B1. On treatment cancer cells with Phomafuranone, time dependent depletion of intracellular reduced glutathione levels was observed. Depletion of reduced glutathione in turn led to elevated intracellular ROS levels. Pre-treatment of HCT 116 cell lines with thiol containing antioxidants like N- acetyl

cysteine (NAC) and reduced glutathione (GSH) completely abrogated its cytotoxic effect, suggesting Phomafuranone induced thiol-mediated cytotoxicity. The elevated ROS levels led to mitochondrial membrane depolarization as indicated by cytochrome c release in cytosol from mitochondria in a time dependent manner. Cytochrome c release was followed caspase 9 mediated apoptotic cell death. Thus, our results suggest that Phomafuranone induced redox imbalance mediated apoptosis in HCT 116 cell line by intrinsic pathway.