Anti-cancer potential of a supercritical CO_2 extract of Vernolac nutraceutical, a viable polyherbal formulation

P. C. Rathnayake, S. R. Samarakoon, K. S. Senathilake

Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Sri Lanka

Therapeutic resistance and adverse side effects limit the efficacy of conventional cancer treatments, highlighting the need for safer, more potent alternatives. Natural bioactive compounds have gained attention due to their synergistic mechanisms, low toxicity, and reduced potential for resistance. However, conventional extraction methods often fail to extract the spectrum of phytochemicals, which can lead to a loss of bioactive compounds. Supercritical CO_2 extraction provides a safer and more efficient alternative, yielding higher concentrations of active compounds. This study evaluated the anti-proliferative potential of an optimized supercritical CO_2 extract of 'Vernolac', a commercially available polyherbal nutraceutical containing aerial parts of *Vernonia zeylanica* and *Leucas zeylanica*, seeds of *Nigella sativa*, roots of *Hemidesmus indica*, and rhizomes of *Smilax glabra*. Cytotoxicity was assessed by using Sulforhodamine B (SRB) assay on Caco-2, MCF-7, NTERA-2 cl.D1, and normal MCF-10A cells. Induction of apoptosis was evaluated by acridine orange/ethidium bromide staining using fluorescence microscopy and caspase-3/7 activity assays. The scratch assay measured effects on cell migration, expression of apoptosis-related genes (*TP53*, *Survivin*), and autophagy-related gene *mTOR* was quantified and analysed using reverse transcriptase-quantitative PCR. Antioxidant capacity was measured using the DPPH assay, and intracellular ROS levels were determined using the nitroblue tetrazolium assay. The supercritical CO_2 extract showed potent cytotoxicity against NTERA-2 cl.D1 cells (IC_{50} at 41.12 µg/mL, 48 h) with negligible toxicity toward MCF-10A cells (IC_{50} > 1000 µg/mL). Based on these results, NTERA-2 cl.D1 cells were selected for further assays. The extract induced early apoptosis, as evidenced by fluorescence microscopy and elevated levels of caspase-3/7 activities. In Vernolac extract-treated cells, up-regulation of *TP53* expression and downregulation of *survivin* and *mTOR* expressions were evidenced, which confirms apoptosis regulation after Vernolac exposure. Additionally, the extract significantly inhibited cell migration and increased intracellular ROS levels. These findings demonstrate that the Vernolac extract possesses strong anticancer activity against cancer stem-like NTERA-2 cl.D1 cells, which supports its potential as a therapeutic candidate for targeting cancer stem cells.

Keywords: *Vernolac, Supercritical CO_2 extraction, Anti-proliferative, Nutraceutical, Cancer stem-like cell*