# Therapeutic potential of a nutraceutical formula (Vernolac) in cancer therapy through integrated network pharmacology and computational simulations approach

S. D. V. Gunawardane, M. M. Milhan, P. C. Rathnayaka, P. S. Gurusinghe, K. S. Gunaratne, A. Kanagasundaram, T. M. D. Darshanamala, D. C. Senevirathne, K. S. Senathilake, S. R. Samarakoon

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

Vernolac is a commercially available polyherbal nutraceutical formulation comprised *Vernonia zeylanica* aerial parts, *Nigella sativa* seeds, *Hemidesmus indicus* roots, *Leucas zeylanica* aerial parts, and *Smilax glabra* rhizome. The present study employed an integrative network pharmacology-based approach with *in vitro* validation to explore the anticancer potential of Vernolac. Phytochemicals present in Vernolac were retrieved from public databases and filtered based on drug-likeness using SwissADME. Protein targets of phytochemicals were predicted using SwissTargetPrediction and intersected with cancer-related targets retrieved from GeneCards to identify common targets. Common targets were used to construct a protein-protein interaction network using the STRING database and analyzed in Cytoscape to identify core targets based on degree. Clustering, topology, hub node analysis, and formula-herb-compound-target-disease network construction were performed on core targets using Cytoscape. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were performed to identify key pathways regulated by phytochemicals in Vernolac. Molecular docking and dynamics simulations were conducted to evaluate the binding affinities of key ligand-target interactions. The *in vitro* antiproliferative activity of Vernolac was assessed in MCF-7, Caco-2, NTERA-2 cl.D1, and MCF-10A, using the Sulforhodamine B assay. A total of 155 drug-like phytochemicals and 137 core overlapping targets were identified. Key phytochemicals, including vernolactone, thymoquinone, quercetin, nigellidine, and carvacrol, were found to interact with key hub nodes, including AKT1, BCL2, CASP3, CTNNB1, EGFR, JUN, SRC, TNF, and STAT3. Pathway enrichment analyses indicated a significant involvement of targets in multiple cancers, such as prostate, endometrial, bladder, pancreatic cancer, and leukemia. The molecular docking and dynamics simulation studies identified novel target-ligand interactions. *In vitro* experiments demonstrated substantial antiproliferative activity against Caco-2 ($IC_{50}$ = 173.2 µg/mL), MCF-7 ($IC_{50}$ = 124.5 µg/mL), and NTERA-2 cl.D1 ($IC_{50}$ = 61.5 µg/mL), while sparing normal MCF-10A cells ($IC_{50}$ = 1075 µg/mL). Vernolac may exert anticancer effects through apoptosis induction, immune modulation, antioxidant, anti-inflammation, antiproliferative, and chemoradiosensitizing mechanisms. This study suggests that Vernolac may exhibit chemoradioprotective potential by alleviating therapy-induced toxicity, supporting its promise as a potential adjunct to conventional cancer treatments.

**Keywords:** *Nutraceuticals, Cancer, Network pharmacology, Molecular docking, Molecular dynamics simulation*