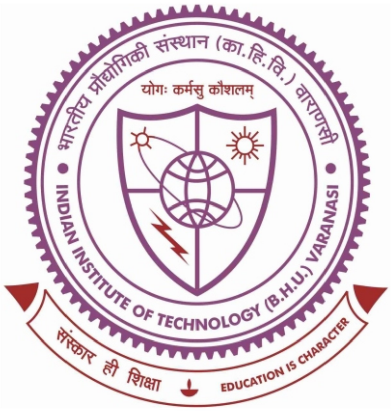
Exploring the Cytotoxic Potential of Secondary Metabolites of *Araucaria cunninghamii* Mudie: LCMS-Based Approach



Thesis submitted in the partial fulfillment for the Award of Degree

Doctor of Philosophy

By

Bharat Sahu

DEPARTMENT OF PHARMACEUTICAL ENGINEERING & TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY
(BANARAS HINDU UNIVERSITY)
VARANASI - 221005
INDIA

Roll No. 18161509

Year 2024

Conclusions and Recommendation

Conclusions

In my thesis, the primary objective was to identify the NP-based anticancer lead molecules for drug discovery. Work was focused on LC-MS-based dereplicationm metabolomics and network pharmacological approaches. *A. cunninghamii* plant was selected for the tis work. Stretgy one was focused on the dereplication to target the new metabolites from the A. *cunninghamii* gum-resins. And second strategy was focused to LC-MS-based metabolomis and network pharmacology to understand the cytotoxic mechanism of secondary metabolites of *A. cunninghamii* leaves.

In the gum resin of *A. cunninghamii,* a new molecule agatheol methylether **(4.17)** and eight **(4.9-4.16)** known labdane diterpenoid and five **(4.18-4.22)** abietane diterpenoids were isolated. All these compounds were characterized by 1D and 2D NMR (^{1}H, ^{13}C-DEPT-135, ^{1}H-^{1}H COSY, HSQC, and HMBC experiments) and HRMS analysis. Thereafter, cytotoxicity activity of compounds has been screened against human cancer cell lines (A549, SCC09, MDAMB231, HS578T, FaDU, Molt4, and MCF7) and breast epithelial *cell line(fr2)* as well by employing MTT assay. Compound **4.17** seemed to be most active with IC_{50} values of 9 µg/mL against Hs 578T and MOLT-4 cells, showing that it can be a potential anticancer lead molecule. Molecular docking interaction of active compounds with anticancer target α, β-tubulin (PDB ID: 1JFF) and EGFR (PDB ID: 6DUK) has been performed. The binding energy of compounds ranged from -6.06 kcal mol^{-1} to -7.22 kcal mol^{-1} against α, β-tubulin dimer protein, and from -8.8 Kcal mol^{-1} to -7.11 Kcal mol^{-1} against EGFR protein, respectively. The binding energy,

ligand efficiency, and interactions of ligands with α, β-tubulin protein (PDF: 1JFF) and EGFR (PDB ID: 6DUK) are presented in the **Table 4.4** and T**able 4.5** respectively.

In the leaves of *A. cunninghamii*, the extract exhibited significant cytotoxic potential, with an IC50 value of 25 µg/mL. The extract was planned for phytochemical investigation for the isolation of bioactive secondary metabolites via bioassay-guided fractionation, combined with LC-HRMS analysis and DNP strategic database mining. Further, eight (AC1-AC8) compounds were characterized by using 1D and 2D NMR (^{1}H, - ^{13}C, DEPT-135, ^{1}H-^{1}H COSY, HSQC, and HMBC experiments) spectrum and HRMS. Additionally, Compounds were evaluated for *in-vitro* cytotoxicity studies against human gastric adenocarcinoma (AGS cell line), *in-silico*, eight compounds (AC1-AC8) were selected for network pharmacology analysis and AC8 was rejected based on Lipinski's rule violations in the virtual ADME analysis. Molecular docking studies of compounds with EGFR, PIK3R1 AND GSK3B were evaluated with minimum binding affinity -7 kcal/mol to -9.4 kcal/mol. Molecular simulations of compound AC2 and AC5 against GSK3β and EGFR shows avg RMSD deviation were found 0.55-0.66 nm at around 25ns. All these results suggest that the ligand was finding its most favorable binding state as the simulation run progressed, all these results suggest that at the initial stage, the ligand was finding its most favorable binding state as the simulation run progressed, the ligand was found quite stabilized in the binding pocket.

In future these secondary metabolites may be used for drug discovery, detailed pharmacological and toxicological studies may be planned on the identified leads.

Recommendations for Future Research

Based on the findings of this study, several recommendations can be made to further explore the potential of ***A. cunninghamii*** metabolites in anticancer drug discovery

Comprehensive Structural Characterization

Further spectroscopic and crystallographic studies are needed to confirm the absolute stereochemistry of the newly isolated agatheol methylether and other diterpenoids. Advanced techniques such as **NMR-based quantitative metabolomics** can be applied to better understand metabolite variations in different plant parts and extraction conditions.

Expanded Cytotoxicity and Mechanistic Studies

Extensive *in-vitro* cytotoxicity screening on additional cancer cell lines, including multi-drug-resistant strains, should be conducted. Detailed **mechanistic studies**, including apoptosis assays, cell cycle analysis, and proteomics-based approaches, can help elucidate the precise molecular targets.

In-Vivo **Validation**

Preclinical studies using animal models should be performed to evaluate the pharmacokinetics, bioavailability, and efficacy of promising compounds. The toxicity profile and therapeutic index should be established to assess the safety and feasibility of these compounds for further development.

Network Pharmacology and AI-Driven Drug Discovery

Integrating artificial intelligence (AI) and machine learning in network pharmacology can enhance predictions of potential molecular targets and synergistic drug interactions.

Multi-omics approaches combining genomics, transcriptomics, and proteomics data can provide deeper insights into the pathways influenced by the secondary metabolites.

Structure-Activity Relationship (SAR) and Synthetic Modifications

A detailed SAR study should be undertaken to optimize the cytotoxic activity and selectivity of the diterpenoids. Semi-synthetic modifications and nanoparticle-based delivery systems can be explored to improve the solubility and bioavailability of these molecules.

Ethnopharmacological and Sustainable Utilization Studies

Investigating traditional medicinal uses of ***A. cunninghamii*** in indigenous communities may provide valuable insights for future drug development. Sustainable harvesting and cultivation strategies should be developed to ensure the conservation of this plant species while supporting pharmaceutical applications. By implementing these recommendations, future research can build upon the findings of this study, further advancing the discovery of novel anticancer therapeutics from ***A. cunninghamii.***