MOLECULAR INSIGHTS AND THERAPEUTIC POTENTIAL OF NATURAL COMPOUNDS

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CERTIFICATE

This is to certify that the thesis entitled **'Molecular insights and therapeutic potential of natural compounds'** being submitted by Mr. Vipul Kumar to the Indian Institute of Technology Delhi for the award of the degree of **'Doctor of Philosophy'**, is a record of the bonafide research work carried out by him, which has been prepared under my supervision in conformity with the rules and regulations of Indian Institute of Technology Delhi. The research reports and the results presented in the thesis have not been submitted for any degree or diploma in any other University or Institute.

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ABSTRACT

The high cost of drugs and the speed of growing drug resistance has made modern medicine unaffordable to people in economically impoverished and developing countries. However, traditional home remedies are often cheaper and have fewer side effects but are not always accepted because of a lack of scientific understanding. Therefore, the goal of this thesis was to investigate the molecular mechanisms of compounds found in natural sources using computational simulations supplemented with experimental evidence. Withanolides derived from the Ashwagandha plant and compounds from the honeybee propolis are the main focus of this thesis. Firstly these natural compounds were investigated against cancer-related targets. Withaferin-A (Wi-A) and Withanone (Wi-N) from Ashwagandha and Caffeic Acid Phenethyl Ester (CAPE) from honeybee propolis were found to be potent against DNA methyltransferases. The studies suggested that Withanolides might also be potent against Phosphodiesterase-4D (PDE4D) related cancers. Furthermore, the antiviral properties of the natural compounds were studied. Withanolides such as Withanoise-IV, Withanoside-V and Withanone and honeybee propolis-related compounds such as CAPE, individually and in combination were effective against Severe Acute Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) *in silico* and *in vitro* experiments. Additionally, natural products were explored to investigate their ability to overcome cancer resistance by inhibiting efflux proteins such as ATP-binding cassette subfamily G member 2 (ABCG2). The study suggested that Stock1n-87939 (3-(5,11-dioxoisoindolo[2,1-a]quinazolin-6(5H,6aH,11H)-yl)-N-(1H-indol-4yl)propenamide) could be a potent ABCG2 inhibitor. Ashwagandha and honeybee propolis compounds have been shown to have neuroprotective effects in the literature. However, no experimental evidence was present on whether they could penetrate the Blood Brain Barrier (BBB). Hence, steered molecular dynamics simulation and the umbrella sampling approach were used to study the passive diffusion of the natural compounds through BBB. It was found that only CAPE, but not the Withanolides (Wi-A and Wi-N), was found to be permeable through BBB and hence could be used as potential neurotherapeutics.

Overall, the thesis presents compelling evidence supporting the potential of natural products as a valuable source for drug discovery. The implications of these findings could pave the way for the development of safer and more effective drugs.

सार

दवाओं की उच्च लागत और बढ़ती दवा प्रतिरोध की गति ने आर्थिक रूप से गरीब और विकासशील देशों में आधुनिक चिकित्सा को लोगों के लिए अवहनीय बना दिया है। हालाँकि, पारंपरिक घरेलू उपचार अक्सर सस्ते होते हैं और इनके कम दुष्प्रभाव होते हैं लेकिन वैज्ञानिक समझ की कमी के कारण इन्हें हमेशा स्वीकार नहीं किया जाता है। इसलिए, इस शोध प्रबंध का लक्ष्य प्रायोगिक साक्ष्य के साथ पूरक कम्प्यूटेशनल सिमुलेशन का उपयोग करके प्राकृतिक स्रोतों में पाए जाने वाले यौगिकों के आणविक तंत्र की जांच करना है। अश्वगंधा पौधे से प्राप्त विथेनोलाइड्स और हनीबी प्रोपोलिस से यौगिक इस शोध प्रबंध के मुख्य केंद्र हैं। सबसे पहले इन प्राकृतिक यौगिकों की जांच कैंसर संबंधी लक्ष्यों के विरुद्ध की गई। अश्वगंधा से विथाफेरिन-ए (Wi-A) और विथेनोन (Wi-N) और हनीबी प्रोपोलिस से कैफिक एसिड फेनिथाइल एस्टर (CAPE) डीएनए मिथाइलट्रांसफेरेज़ के खिलाफ शक्तिशाली पाए गए। हमारे अध्ययनों ने सुझाव दिया कि विथेनोलाइड्स फॉस्फोडिएस्टरेज़-4डी (पीडीई4डी) से संबंधित कैंसर के खिलाफ भी शक्तिशाली हो सकते हैं। इसके अलावा, हमने प्राकृतिक यौगिकों के एंटीवायरल गुणों पर ध्यान दिया। Withanoise-IV, Withanoside-V और Withanone जैसे Withanolides और CAPE जैसे हनीबी प्रोपोलिस-संबंधित यौगिक, व्यक्तिगत रूप से और संयोजन में, सिलिको और इन विट्रो प्रयोगों में गंभीर एक्यूट एक्यूट रेस्पिरेटरी सिंड्रोम कोरोनावायरस 2 (SARS-CoV-2) के खिलाफ प्रभावी हैं। इसके अतिरिक्त, एटीपी-बाइंडिंग कैसेट सबफैमिली जी मेंबर 2 (एबीसीजी2) जैसे एफ्लक्स प्रोटीन को बाधित करके कैंसर प्रतिरोध को दूर करने की उनकी क्षमता की जांच करने के लिए प्राकृतिक उत्पादों का पता लगाया गया है। अध्ययन ने सुझाव दिया कि Stock1n-87939 (3-(5,11-डाइऑक्सोइसोइंडोलो[2,1-a]quinazolin-6(5H,6aH,11H)-yl)-N-(1H-indol-4yl)propenamide) हो सकता है एक शक्तिशाली ABCG2 अवरोधक। अश्वगंधा और हनीबी प्रोपोलिस यौगिकों को साहित्य में न्यूरोप्रोटेक्टिव प्रभाव दिखाया गया है। हालांकि, इस बात का कोई प्रायोगिक साक्ष्य मौजूद नहीं था कि वे ब्लड ब्रेन बैरियर (बीबीबी) में प्रवेश कर सकते हैं या नहीं। इसलिए, बीबीबी के माध्यम से प्राकृतिक यौगिकों के निष्क्रिय प्रसार का अध्ययन करने के लिए स्टीयर आणविक गतिशीलता सिमुलेशन और छाता नमूनाकरण दृष्टिकोण का उपयोग किया गया था। यह पाया गया कि केवल CAPE, लेकिन Withanolides (Wi-A और Wi-N) नहीं, BBB के माध्यम से पारगम्य पाया गया और इसलिए इसे संभावित न्यूरोथेरेप्यूटिक्स के रूप में इस्तेमाल किया जा सकता है।

कुल मिलाकर, शोध प्रबंध दवा की खोज के लिए एक मूल्यवान स्रोत के रूप में प्राकृतिक उत्पादों की क्षमता का समर्थन करने वाले सम्मोहक साक्ष्य प्रस्तुत करती है। इन निष्कर्षों के निहितार्थ सुरक्षित और अधिक प्रभावी दवाओं के विकास का मार्ग प्रशस्त कर सकते हैं।

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LIST OF ABBREVIATIONS

ACE2	Angiotensin-converting enzyme 2
ABCG2	ATP-binding cassette subfamily G member 2
ARC	Artepillin C
ADME	Absorption Distribution Metabolism Excretion
BBB	Blood Brain Barrier
CADD	Computer-Aided Drug Discovery
CAPE	Caffeic Acid Phenethyl Ester
COVID-19	CoronaVirus Disease
DNMTs	DNA Methyl Transferases
HIF	Hypoxia Inducible Factor
HTS	HighThroughput Screening
HTVS	HighThroughput Virtual Screening
LBDD	Ligand-Based Drug Discovery
MM	Molecular Mechanics
MMGBSA	Molecular Mechanics Generalised Born and Surface Area
M^{pro}	Main Protease
POPC	Phosphatidylcholine
POPE	Phosphatidylethanolamine
PDE4D	Phoshphodiestrase 4D
QSAR	Quantitative Structure-Activity Relationship
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
SARS-CoV-2	Severe Acute Respiratory Syndrome-CoronaVirus 2
SBDD	Structure-Based Drug Discovery
SMD	Steered Molecular Dynamics Simulation
TCM	Traditional Chinese Medicine
TEG	Triethylene Glycol
TMPRSS2	Transmembrane Serine Protease 2
Wi-A	Withaferin-A
Wi-N	Withanone