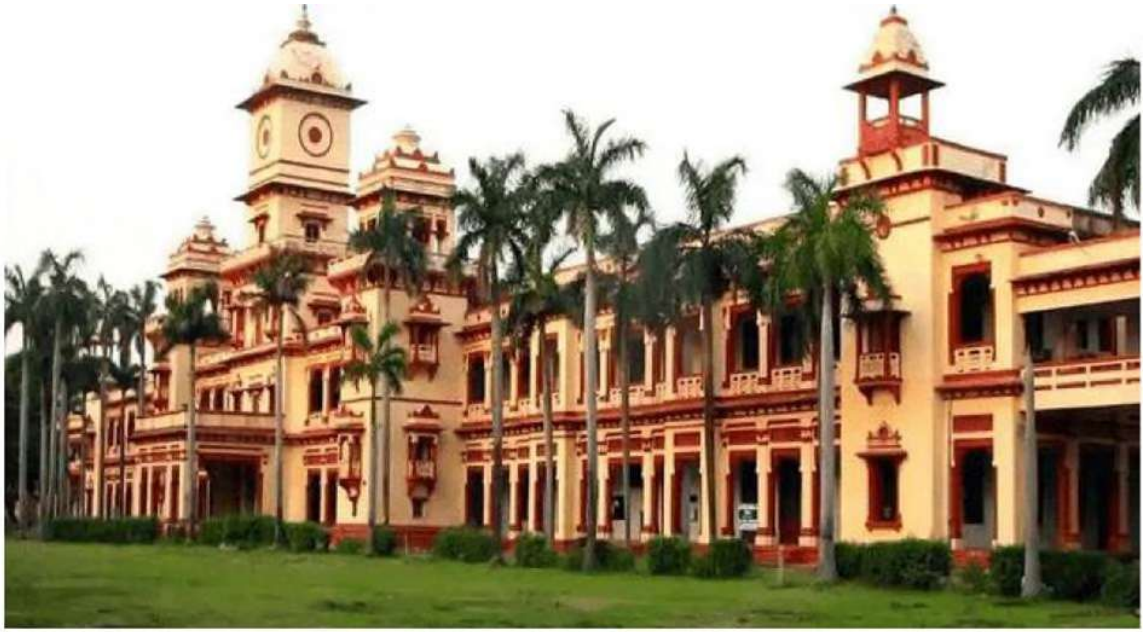

THERAPEUTIC EFFECTS OF GEDUNIN AND DERIVATIVE

Thesis submitted in partial fulfillment for the Award of Degree

Doctor of Philosophy

By

Priya Dagar

SCHOOL OF BIOCHEMICAL ENGINEERING INDIAN INSTITUTE OF TECHNOLOGY (BANARAS HINDU UNIVERSITY) VARANASI - 221005 INDIA.

ROLL NO.: 18011008 October 2022

DEDICATED TO MY BELOVED HUSBAND, MY PARENTS
FOR THEIR LOVE, SUPPORT, AND ENCOURAGEMENT

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It is certified that the work contained in the thesis entitled ***" Therapeutic Effects of Gedunin and Derivative"*** by ***"Priya Dagar"*** has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of Comprehensive, Candidacy, and SOTA.

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I, "**Priya Dagar**", certify that the work embodied in this thesis is my own bonafide work and carried out by me under the supervision of "Dr. Abha Mishra" from" July 2018 to October 2022 ", at the "School of Biochemical Engineering", Indian Institute of Technology (BHU), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma. I declare that I have faithfully acknowledged and given credits to the research workers wherever their works have been cited in my work in this thesis. I further declare that I have not wilfully copied any other's work, paragraphs, text, data, results, *etc*., reported in journals, books, magazines, reports, dissertations, theses, *etc*., or available at websites and have not included them in this thesis and have not cited as my own work.

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ACKNOWLEDGEMENTS

Through this page, I offer my salutation to ***Mahamana Pt. Madan Mohan MalviyaJi****, the creator of this pious seat of learning.*

It is indeed my proud privilege to express my deep sense of gratitude, respect, indebtedness, and sincere regard to ***my Supervisor, Dr. Abha Mishra****, for her excellent supervision, skilled and valuable guidance, stimulating discussion, unfailing support, immense help, and constant encouragement over the entire period of my association with him. I am grateful to her for her sincere concern for academics, personal welfare, and parental care throughout the research period she has extended to me to complete my research work effectively. I can never forget her affectionate, caring nature and moral support, which always provides the feeling of being at home. I have no words in my dictionary to explain her. I am proud to have a teacher like her who is always motivating and supportive, even in the most adverse situations. She has been a source of inspiration for me to have an optimistic approach to life and do my best.*

I express my heartfelt thanks to ***Dr. Vikash Kumar Dubey, the Coordinator,*** *and* ***DPGC Convener,*** *the* ***School of Biochemical Engineering, Indian Institute of Technology (Banaras Hindu University), Varanasi****, School of Biochemical Engineering, IIT (BHU) for their constant support and blessings.*

It is a profound privilege to be a student of the School of Biochemical Engineering. I want to express my thanks to the faculty members of the school, ***Dr. Vikas Kumar Dubey, Dr.Sanjay Kumar,*** *and* ***Dr. Vishal Mishra,*** *who have helped me a lot in over coming the bottlenecks I encountered during my studies*

with their valuable advice.

I am thankful to ***Dr. S. Hemalatha,*** *my external RPEC member, Department of Pharmaceutical Engineering and Technology, IIT(BHU), for giving me valuable suggestions throughout my research period.*

I am highly obliged to express my appreciation to wards my seniors ***Mr. Deepanker, and Ms. Aditi Bhatnagar****, for their affection and support during the whole journey.*

I have been highly blessed with a friendly and cheerful group of fellow research scholars. I want to express my heartfelt gratitude to mainly ***Ms. Sonali, Mr. Ravi, and Mr. Abhay,*** *who directly or indirectly supported my research work. Their companionship and lively discussions in and outside the laboratory were great sources of inspiration.*

I am also grateful to the non-teaching staff members ***Mr. Ramashankar Singh****,* ***Mr. Dinesh, Mr. Subhash****,* ***Mr. Amit , and Mr. Arun*** *for their support and cooperation during my research.*

Words plunge insufficient to express my regards and deep emotions to my ***beloved husband*** *and my* ***parents*** *for being the source of unconditional love and inspiration to move on the way to my goal of achieving higher education. Their everlasting encouragement, patience, sacrifice, and blessings have brought me to this stage. Parents being earthly Goddeserve much more than I can express in words.*

I want to express my gratitude towards the departments of IIT (BHU), Varanasi, for providing the necessary facilities for smoothly conducting my research work. School of Biochemical Engineering for providing the lab facilities like FTIR,

HPLC, and Central Instrumentation Facility Centre.

(CIFC), ICAR New Delhi for seed material, SAIFIIT Bombay for HR-LCMS. I take this occasion to acknowledge the financial assistance the Ministry of Human Resource and Development provides in the form of a Teaching Assistantship.

Again, I wish to express a word of thanks to all those hands that helped me in some way or the other in pursuing my research work and completing the thesis. I apologize unreservedly for any mistakes, omissions, or failure to acknowledge fully. Finally, I bow my head humbly before the almighty ***God,*** *without whose consent and blessings this work would have been impossible.*

Date:

Place:IIT(BHU),Varanasi ***(PRIYA DAGAR)***

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LIST OF ABBREVIATIONS AND SYMBOLS

MAPC	Multipotent adult progenitor cells
JAK/SAT	Janus kinase signal transducers/activators of transcription
HSP90	Heat shock protein 90
PARP	Poly adenosine diphosphate ribose polymerase
PAMPs	Pathogen-associated molecular patterns
QSAR	Quantitative structure-activity relationships
L-AAO	L- amino acid oxidase
ASV	Anti-snake venom
PLA	Phospholipase A
CROFAB	Crotalidae polyvalent immune fab
DDT	Dichloro diphenyl trichloroethane
BRCA	Breast cancer gene
TMPRSS	Transmembrane serine protease
PTEN	Phosphate and tensin homolog
TP53	Tumor protein 53
MCF-7	Michigan cancer foundation -7
BRs	Brassino steroids
NADPH	Nicotinamide adenine dinucleotide phosphate
NADH	Nicotinamide adenine dinucleotide
NADK	Nicotinamide adenine dinucleotide kinase
MNADK	Mitochondria localized Nicotinamide adenine dinucleotide kinase
KRAS	Kirsten rat sarcoma virus
GLT-1	Glutamate transporter
ROS	Reactive oxygen species
TEM	Transmission electron microscope
DM	Diabetes mellitus
HPLC	High-performance liquid chromatography
HRLCMS-Q-TOF	High-resolution liquid chromatograph mass spectrometer

	quadruple time of flight
AJS-ESI	Agilent Jet Stream -Electrospray ionization mass spectrometry
CCL_4	Carbon tetrachloride
LC	Liquid chromatography
PDB	Protein data bank
ADMET	Absorption, Distribution, Metabolism, Excretion, and toxicity
TPSA	Topological polar surface area
BBB	Blood-brain barrier
LD50	Lethal dose 50
MD	Molecular dynamics
OPLS	Optimized Parameters for Liquid Simulations All Atom
DLID	Drugs like density
CNS	Central nervous system
CYP	Cytochrome P
GI	Gastrointestinal tract
HIA	Human intestinal absorbtion
HBD	Hydrogen bond donar
HBA	Hydrogen bond acceptor
HERG	Human ether-a-gogorelated gene
5'NT	5'Nucleotidase
CSL	Composite site lability
WHO	World health organization
DG	Free energy change
ACHE	Acetylcholinesterase
PMF	Potential mean scores
RMSD	Root mean square density
RMSF	Root mean square fluctuations
SASA	Solvent accessible surface area
PGP	P-glycoprotein
IC_{50}	Inhibitory concentration
OCHEM	Organic chemistry
DNN	Deep neural network
CDK2	Cyclin-dependent kinase

MTT3-	[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
R^2	Coefficient of determination
Q^2	goodness of prediction
RMSE	Root mean square error
MAE	Mean absolute error
CNN	Convolutional neural network
AI	Artificial intelligence
INCHI	International chemical identifier
RNN	Recurrent neural network
NLP	Natural language processing
LSTM	Long Short-Term Memory networks
VGGNET	Visual Geometry Group network
RELU	Rectified Linear Unit activation function
DNS	3,5-Dinitrosalicylic acid
GBSA	Generalized Born and surface area continuum solvation
PNPG	4-Nitrophenyl-β-D- glucopyranoside
DMSO	Dimethyl Sulfoxide
HEPG2	Human liver cancer cell line
DPBS	Dulbecco's phosphate buffer saline
FBS	Fetal bovine serum
PBS	phosphate buffer saline
H2DCFDA	Dichlorodihydrofluorescein diacetate
GLN	Glutamine
ASP	Aspartic acid
TYR	Tyrosine
HIS	Histidine
LEU	Leucine
TRP	Tryptophan
ILE	Isoleucine
PHE	Phenylalanine
KDA	Kilo dalton
OC	Ovarian cancer
CEA	Carcino embryogenic antigen

PA1	Ovarian teratocarcinoma cell line
PC3	prostate cancer cell line
FITC	Fluorescein isothiocyanate
FL1	fluorescence parameter 1

Preface

The work in the thesis has been published, and most of the chapters are reworking of these published work. The entire Thesis work has been divided into 11 chapters: Chapter 1 deals with a general introduction about Gedunin($C_{28}H_{34}O_7$) and its relation with cancer, Diabetes mellitus, and snake anti-venom. It also highlights the motivations and significance of the research work. Chapter 2 presents the literature survey covering the uses and applications of gedunin against snake venom, diabetes, liver, prostate, and ovarian cancer. Chapter 3 describes the ripen fruit materials, various techniques (polarity-based extraction using soxhlet, HPLC, HRLCMS, and Column chromatography) involved in the Extraction, purification, and characterization of gedunin from *Azadirachta indica* and their results and discussion. Chapter 4 presents the methods of computational analysis including docking, simulation, and pharmacokinetics analysis of gedunin against snake venom enzymes and their results and discussion. Chapter 5 deals with *insilico* analysis of modified gedunin derivative $C_{26}H_{31}N_2O_6F$ with their methods, results, and discussion. Chapter 6 deals with methods, results, and discussion of QSAR and Retrosynthesis of $C_{26}H_{31}N_2O_6F$. Chapter 7 deals with methods, results, and discussion of molecular property prediction of $C_{26}H_{31}N_2O_6F$(modified gedunin derivative) using LSTM, CNN, VGGNET, and k-means clustering. Chapter 8 deals with the method, results, and discussion of the antidiabetic potential of gedunin using alpha-glucosidase and alpha-amylase enzymes. Chapter 9 covers the materials, methods, results, and discussion of the anti-cancer activity of gedunin using liver cancer cell HepG2, PA1, and PC3 where ROS activity, Apoptosis activity, scratch assay/migration assay and colony

formation assay/proliferation assay has been shown. Chapter 10 deals with Transcriptomics studies of gedunin using the Hela cell line with methods, results, and discussion. Chapter 11 presents a brief summary and conclusions of experimental findings.

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CELL LINE, LIVER CANCER CELL LINE HEPG2, PA1 OVARIAN AND PC3PROSTATE CANCER CELLS