Molecular modelling and Invitro studies of the therapeutic potential of *Ichnocarpus frutescens* and *Hemidesmus indicus*

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DOCTOR OF PHILOSOPHY

By

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List of Abbreviations

3T3-L1 - Mouse Embryonic Fibroblast Cell Line

Abs - Absorbance of sample

Ac - Absorbance of Control

ACTB - Beta-Actin (Housekeeping Gene)

AKT - Protein Kinase B

AL - Aluminum

ALL - Acute Lymphocytic Leukemia

AML - Acute Myeloid Leukemia

ATP - Adenosine Triphosphate

AXL - AXL Receptor Tyrosine Kinase

AXL - AXL Receptor Tyrosine Kinase

B.C. - Before Christ

BBB - Blood-Brain Barrier

Bcl-2 - B-Cell Lymphoma 2 (Anti-apoptotic Protein)

BE - Binding Energy

Ca - Calcium

Caspase-3 (CASP3) - Cysteine-Aspartic Acid Protease 3 (Apoptotic Gene)

CD3OD - Deuterated Methanol

CLL - Chronic Lymphocytic Leukemia

CML - Chronic Myeloid Leukemia

CNS - Central Nervous System

Ct - Cycle Threshold

Cu - Copper

CVD - Cardiovascular Disease

DKA - Diabetic Ketoacidosis

DLS - Dynamic Light Scattering

DMEM - Dulbecco's Modified Eagle Medium

DNA - Deoxyribonucleic Acid

DNase - Deoxyribonuclease

DPP-4 - Dipeptidyl Peptidase-4

DPPH - 2,2-Diphenyl-1-Picrylhydrazyl

EDS - Energy Dispersive Spectroscopy

EGFR - Epidermal Growth Factor Receptor

FBS - Fetal Bovine Serum

Fe - Iron

FITC - Fluorescein Isothiocyanate

FRAP - Ferric Reducing Antioxidant Power

FT-IR - Fourier-Transform Infrared Spectroscopy

g/mol - Grams per Mole

GA Runs - Genetic Algorithm Runs

GDM - Gestational Diabetes Mellitus

GLP-1 - Glucagon-like Peptide-1

GLUT-4 - Glucose Transporter Type 4

HER2 - Human Epidermal Growth Factor Receptor 2

HI ZnOxNP - *Hemidesmus Indicus* Zinc Oxide Nanoparticles

HPLC - High-Performance Liquid Chromatography

HR-LCMS - High-Resolution Liquid Chromatography-Mass Spectrometry

HR-MS / HRMS - High-Resolution Mass Spectrometry

IC_{50} - Half-Maximal Inhibitory Concentration

IDF - International Diabetes Federation

IF ZnOxNP - *Ichnocarpus Frutescens* Zinc Oxide Nanoparticles

IR - Insulin Receptor

IV – Intravenous

kcal/mol - Kilocalories per mole (energy unit).

Km - Michaelis-Menten Constant

LogP - Partition Coefficient

m/z - Mass-to-Charge Ratio

MAPK - Mitogen-Activated Protein Kinase

MD - Molecular Dynamics

MFI - Mean Fluorescence Intensity

Mg - Magnesium

mg/mL - Milligrams per Milliliter

mL - Milliliter

mM - Millimolar

MM/GBSA - Molecular Mechanics/Generalized Born Surface Area

mRNA - Messenger RNA

MS/MS - Tandem Mass Spectrometry

MTT - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

Na - Sodium

NaCl - Sodium Chloride

nm - Nanometer

NMR - Nuclear Magnetic Resonance

NPT - Constant Number of Particles, Pressure, and Temperature

ns - Nanoseconds

NVT - Constant Number of Particles, Volume, and Temperature

OD - Optical Density

P - Phosphorus

p21 (CDKN1A) - Cyclin-Dependent Kinase Inhibitor 1A

p53 - Tumor Suppressor Gene

PBS - Phosphate-Buffered Saline

PDB - Protein Data Bank

PDBQT - Protein Data Bank Quaternion Translation Format

PDGFR - Platelet-Derived Growth Factor Receptor

PDI - Polydispersity Index

PI - Propidium Iodide

PSA - Polar Surface Area

qRT-PCR - Quantitative Real-Time Polymerase Chain Reaction

RMSD - Root Mean Square Deviation

RMSF - Root Mean Square Fluctuation

RNA - Ribonucleic Acid

RNAse - Ribonuclease

RoG - Radius of Gyration

ROS - Reactive Oxygen Species

rpm - Revolutions per Minute

rRNA - Ribosomal RNA

RTK - Receptor Tyrosine Kinase

SAED - Selected Area Electron Diffraction

SASA - Solvent Accessible Surface Area

SDF - Structure Data File

SEM - Scanning Electron Microscopy

SGLT2 - Sodium-Glucose Cotransporter-2

Si - Silicon

SP - Standard Precision

SYBR - SYBR Green (Fluorescent Dye)

T1DM - Type 1 Diabetes Mellitus

T2DM - Type 2 Diabetes Mellitus

TEM - Transmission Electron Microscopy

TIP3P - Transferable Intermolecular Potential with 3 Points (Water Model)

TLC - Thin Layer Chromatography

TMS – Tetramethylsilane

U/mL - Units per milliliter (enzyme activity).

UV-VIS - Ultraviolet-Visible Spectroscopy

VEGFR - Vascular Endothelial Growth Factor Receptor

Vmax - Maximum Reaction Rate

WBC - White Blood Cells

WHO - World Health Organization

XP - Extra Precision

Zn – Zinc

List of Symbols

Å - Angstrom (Unit of Length)

α-A - α-Amylase

α-G - α-Glucosidase

μg/mL (μg) - Micrograms per milliliter (concentration unit).

% - Percentage (used for proportions, concentrations, and statistical data).

> - Greater than (used in comparisons).

+ - Addition or increase (used in reactions or combinations).

→ - Process or transition indicator.

/ - Denotes a ratio.

= - Equality or equivalence.

Fe^{3+} - Ferric ion (oxidized iron).

Fe^{2+} - Ferrous ion (reduced iron).

°C - Degrees Celsius (temperature).

pH - Measure of acidity or alkalinity.

× 100 - Multiplication factor for percentage calculation.

ΔG - Change in free energy (binding or reaction energy).

≥ - Greater than or equal to.

≤ - Less than or equal to.

$Å^2$ - Square Ångström.

ΔGbind - Binding free energy.

ΔEcoulomb - Coulomb energy (electrostatic interaction energy).

ΔElipo - Lipophilic energy (from hydrophobic interactions).

ΔEele - Electrostatic solvation energy (polar solvation effects).

ΔEvdW - Van der Waals energy (non-covalent interaction energy).

2-NBDG - A fluorescent glucose analog.

μM - Micromolar (concentration unit).

μL - Microliter (volume unit).

V: Reaction velocity (rate of enzymatic reaction)

Vmax: Maximum reaction velocity (enzyme saturation point)

Km: Michaelis-Menten constant (substrate concentration at half Vmax)

ΔGtotal - Total free energy

ΔGprotein - protein free energy

ΔGligand - ligand free energy

TΔS - Entropic contribution to free energy.

m/z - Mass-to-charge ratio (mass spectrometry).

± - Plus-minus (standard deviation).

$p < 0.05$ - Statistical significance indicator.

× g - Gravitational force (centrifuge speed).

M - Molar concentration.

PREFACE

India is renowned for its rich heritage of herbal medicinal knowledge. In India, approximately 80% of the rural population uses medicinal herbs or indigenous medical systems. Among the numerous medicinal plants that originate from India, *Ichnocarpus frutescens* and *Hemidesmus indicus* are notable for their various traditional uses in treating a range of ailments. This research focuses on the extraction, purification, and characterization of bioactive compounds from root of *Hemidesmus indicus* and *Ichnocarpus frutescens*. The antidiabetic potential of these compounds was evaluated using *invitro* and *insilico* studies, targeting α-Amylase (α-A) and α-Glucosidase (α-G) inhibition. Additionally, *invitro* glucose uptake and wound healing assays were conducted to assess their therapeutic efficacy. The antioxidant potential of the plant extracts and isolated compounds was also investigated. The anticancer properties of the phytoconstituents were explored through biological and computational studies. Additionally, zinc oxide nanoparticles were also synthesized from the methanolic root extract of *Hemidesmus indicus* and *Ichnocarpus frutescens*, and their biological evaluation, including antioxidant, antidiabetic, and anticancer potentials, was conducted. Bioactive compounds silychristin was isolated from the roots of *Hemidesmus indicus* and hyperoside was isolated from *Ichnocarpus frutescens* using Column chromatography and were characterized using TLC, HPLC, 1H and 13C NMR, and HR-MS techniques. Hyperoside and silychristin exhibited significantly inhibitory effects against α-A and α-G than the standard drug, acarbose. The NBDG glucose uptake assay indicated that both compounds significantly enhanced glucose uptake compared to the control, with silychristin showing a more pronounced effect. DPPH assay, revealed that hyperoside exhibited the strongest antioxidant activity, while Silychristin and ascorbic acid showed similar antioxidant activities.

Biological evaluation and computational studies were conducted to assess the anticancer potential of phytoconstituents in the A549 lung cancer cell line. The studies included apoptosis, cell cycle analysis, and gene expression analysis. Silychristin and hyperoside exhibited 50% cell growth inhibition at concentrations of 96.46 µg/mL and 74.61µg/mL, respectively. The Annexin V apoptosis assay revealed that Hyperoside marked increase in late apoptotic and necrotic cell populations. Silychristin, while less potent, still induced apoptosis. Cell cycle analysis revealed distinct mechanisms of action for the two compounds. Hyperoside caused G2-M phase arrest, indicating interference with mitotic progression, while silychristin induced a strong G0-G1 phase arrest, effectively blocking DNA synthesis. Gene expression analysis indicated that the Bcl-2 gene was downregulated, whereas p21, p53, and Caspase-3 genes were upregulated compared to the housekeeping gene. Hyperoside treatment increased p21 expression 9-fold and silychristin 7-fold. For p53 expression, hyperoside caused a 2.4-fold increase, while silychristin led to a 3.7-fold increase. Caspase-3 expression was enhanced 4-fold by hyperoside and 6.7-fold by silychristin.

The zinc oxide nanoparticles using root extracts of *Hemidesmus indicus* (HI ZnOxNP) and *Ichnocarpus frutescens* (IF ZnOxNP) were also explored for their therapeutic applications, including antioxidant antidiabetic and anticancer potential. The synthesized nanoparticles were characterized through UV-VIS Spectroscopy, Particle Size Distribution and Zeta Potential Analysis, FT-IR Spectroscopy, Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM). HI ZnOxNP exhibited superior free radical scavenging and ferric ion-reducing power compared to IF ZnOxNP. HI ZnOxNP demonstrated stronger inhibition of α-A, while IF ZnOxNP exhibited more potent inhibition of α-G.The anticancer studies on the A549 lung cancer cell line revealed that both HI ZnOxNP and IF ZnOxNP exhibit significantly higher

anticancer potential compared to their parent root extracts. Flow cytometry analysis demonstrated that HI ZnOxNP induced apoptosis more effectively than IF ZnOxNP, with a predominant late apoptotic response and minimal necrosis, highlighting its targeted cytotoxic mechanism. Both ZnOxNP also caused cell cycle arrest, with HI ZnOxNP inducing G0/G1 phase arrest and IF ZnOxNP exhibiting dual-phase inhibition, disrupting both DNA synthesis (S phase) and mitosis (G2/M phase). The combination of ZnOxNP with natural phytochemicals offers a novel strategy to improve the bioavailability, cellular uptake, and therapeutic efficacy of plant-derived compounds. These findings contribute to a deeper understanding of the therapeutic applications of *Hemidesmus indicus* and *Ichnocarpus frutescens*, suggesting their potential in treating type 2 diabetes, oxidative stress-related conditions, and cancer.

Keywords: *Hemidesmus indicus, Ichnocarpus frutescens, Bioactive Compounds, Cancer, Type 2 diabetes.*