DECLARATION

I declare that the thesis entitled "**Screening of phytochemicals in selected plants from Sikkim and Darjeeling Himalayan region for potential drug development against metabolic disorder and inflammation**" has been prepared by me under the guidance of Dr. Ranabir Sahu, Assistant Professor, Department of Pharmaceutical Technology, University of North Bengal, West Bengal.

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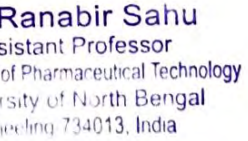
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CERTIFICATE FROM THE SUPERVISOR

It is certified that the work contained in the thesis titled **"Screening of phytochemicals in selected plants from Sikkim and Darjeeling Himalayan region for potential drug development against metabolic disorder and inflammation"** by Priya Das, has been carried out under the supervision of **Dr. Ranabir Sahu**, Assistant Professor, Department of Pharmaceutical Technology, University of North Bengal, West Bengal. The work has not been submitted elsewhere for the requirements for the degree of Doctor of Philosophy (PhD) in Pharmacy.



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Date: 21/10/2024

Place: NBU

Priya Das

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DEDICATED

TO MY RESPECTED PARENTS

AND

NEPHEW

PREFACE

The continuous search for bioactive compounds from natural sources has led to the exploration of medicinal plants with potential therapeutic applications. Among these, *Asparagus racemosus* willd (*A. racemosus*) and *Duabanga grandiflora (D. grandiflora*) are recognized for their traditional medicinal uses. This thesis focuses on the comprehensive screening of phytochemicals from these two plant's different parts (Aerial part of *A.racemosus* and Leaves and flowers of *D.grandiflora*) and investigates their biological activities.

The study begins with the extraction of phytochemicals from aerial part of *A.racemosus* and leaves and flowers of *D.grandiflora*, followed by detailed chemical profiling using advanced analytical techniques such as High-Performance Thin-Layer Chromatography (HPTLC), Liquid Chromatography-Mass Spectrometry (LC-MS), and Gas Chromatography-Mass Spectrometry (GC-MS). These methods allow for the identification and characterization of bioactive compounds, providing valuable insights into their chemical compositions. GC-MS and LC-MS identified bioactive compounds were found to have antioxidant, antidiabetic, anti-inflammatory, antimicrobial etc. activities which supported our findings. Various phenolic acids (Caffiec acid, myricetin, para-coumaric acid and gallic acid), two flavonoids (quercetin and naringenin) and steroid (stigmasterol) were identified and quantified by HPTLC analysis.

Subsequent sections of this thesis are dedicated to in vitro studies, where the antioxidant, anti-inflammatory, antimicrobial, and cytotoxic properties of the plant extracts were evaluated. These bioassays form the foundation for understanding the therapeutic potential of the isolated phytochemicals. The methanolic extracts from both plant parts demonstrated significant antioxidant, anti-inflammatory, antimicrobial, and cytotoxic properties, suggesting their potential for future drug development.

The research also extended to in vivo studies, with a focus on evaluating the anti-diabetic and anti-inflammatory activities of the extracts. These studies provided critical data on the efficacy of the extracts in biological systems. Notably, the research offers new insights into the liver-protective properties of the methanolic extracts prepared from the aerial parts of *A.*

racemosus and the leaves of *D. grandiflora* in STZ-Nicotinamide-induced diabetic rats. Both short- and long-term tests showed a significant rise in blood glucose levels in the diabetic control groups compared to normal, extract-treated, and conventional drug-treated groups. The methanolic extracts (ARME and DGME) exhibited beneficial effects by reducing oxidative stress, as evidenced by lower catalase (CAT), glutathione (GSH), and malondialdehyde (MDA) activities in the liver of diabetic rats. These extracts also protected the liver from damage caused by free radicals and restored antioxidant levels to normal. In addition, the extracts significantly reduced paw inflammation within the stipulated time frame.

Furthermore, this research explores the green synthesis of nanoparticles using these plant extracts, showcasing an eco-friendly approach to nanotechnology with potential applications in medicine and other fields.

The findings of this research contribute to the growing body of knowledge on medicinal plants and their applications in modern therapeutic practices. By integrating traditional medicine with modern scientific methodologies, this study opens new possibilities for the development of novel drugs with minimal side effects. It is my hope that this work will inspire further research in the fields of natural product chemistry and pharmacology.

This thesis is the result of dedicated efforts, and I extend my deepest gratitude to all those who have supported and guided me throughout this academic journey.

ABBREVIATIONS

AA – Ascorbic Acid

ACHN: Human kidney adenocarcinoma cell line

ADT: Adipose tissue

AE: Acarbose

AgNPs : Silver Nanoparticles

AgNPs-AR : *A.racemosus* mediated Silver Nanoparticles

AGEs: Advanced glycation end products

Alp: Alkaline phosphatase

ANOVA: Analysis of variance

ARME : *A.racemosus* methanolic extract

ATR: Attenuated total reflectance

AuNPs: Gold nanoparticles

BF- Bundle of fibers

BSI: Botanical Survey of India

C: Crystal

CA: Caffiec acid

CAT: Catalase

CDGF: *D.grandiflora* flower chloroform extract

CDGL: *D.grandiflora* leaves chloroform extract

CdS : Cadmium sulphide

CdSNPs : Cadmium sulphide Nanoparticles

CeO2 : Cerium oxide

CeO2NPs : Cerium oxide Nanoparticles

c-REL: Transcriptional repressor of inflammatory genes

CC: Column chromatography

CH: Chloroform extract

COX – Cyclooxygenase

CRT: Cortex

CuO : Cupper Oxide

CuONPs : Cupper Oxide Nanoparticles

CV: Central vein

CVDs: Cardiovascular diseases

DGME: *D. gramdiflora* leave methanolic extract

DLS – Dynamic Light Scattering

DM: Diabetes mellitus

DMEM: Dulbecco's Modified Eagle Medium

DMSO – Dimethyl Sulfoxide

DNSA: 3,5-dinitrosalicylic acid

DPP-4: Dipeptidyl peptidase-4

DPPH: 2,2 -di (4-tetra-octyl phenyl)-1-picrylhydrazyl

EA: Ethyl acetate extract

ED_{50} : Effective Dose 50 (dose that produces 50% of the maximal response)

EDGL: *D. grandiflora* ethyl acetate extract of leaves

EDTA: Ethylenediamine tetra acetic acid

EDX – Energy Dispersive X-ray

ELISA: Enzyme-linked immunosorbent assay

EPC: Epidermal cell

EP: Epidermis

F- Fibres

FBS: Fasting blood sugar

FR: Free radicals

FRAP: Ferric reducing power assay

FTIR – Fourier Transform Infrared Spectroscopy

GA – Gallic Acid

GC: Gas chromatography

GC-MS : Gas chromatography and mass spectroscopy

GD: Gestational diabetes

GAE: Gallic acid equivalents

GLP-1: Glucagon-like peptide-1

GSH: Glutathione

GPX: Glutathione peroxidases

GMP: Good Manufacturing Practices

GRX: Glutathione Reductase

GLUT-4: Glucose transporter -4

GS – Green Synthesis

GSSG: Oxidized form of GSH

HD: Herbal drug

HDGF: *D. grandiflora* flower hydroalcoholic extract

HDGL: *D. grandiflora* leaves hydroalcoholic extract

HPLC: High Performance Liquid Chromatography

HPTLC: High performance thin layer chromatography

HM: Herbal medicine

HbA1C: Glycated haemoglobin A1c H2O2: Hydrogen peroxide

IC_{50}: 50% inhibitory concentration

IDDM: Insulin dependent diabetes mellitus

IKKα :IκB kinase or inhibitor of nuclear factor kappa B kinase α

IKBα : Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha

INM: Inflammatory mediators

IP – Intraperitoneal (injection into the peritoneal cavity)

IR: Insulin resistance

LD_{50} – Lethal Dose 50 (dose that causes death in 50% of the population)

LCMS : Liquid chromatography and mass spectroscopy

LF: lignified fibre

LOD: Limit of detection

LOQ: Limit of quantification

MBC: Minimum bactericidal concentration

MDA: Malondialdehyde

MDGF: *D. grandiflora* flower methanol extract

MDGL: *D. grandiflora* leaves methanol extract

ME : Methanolic extract

MEM : Mueller Hinton broth

MER: Medullary rays

MHB: Mueller Hinton broth

MIC : Minimum inhibitory Concentration

MNPs – Metal Nanoparticles

MP: Medicinal plants

MR: Myricetin

MS: Metabolic syndrome

MTT : 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide

NA: Nicotinamide

NCCS : National Centre for Cell Science

NDGF: *D. grandiflora* flowers n-hexane extract

NDGL: *D. grandiflora* leaves n-hexane extract

NIDDM: Non-Insulin dependent diabetes mellitus

n-H: n-hexane extract

NMR : Nuclear magnetic resonance

NP: Natural products

NPs – Nanoparticles

NSAIDs: Nonsteroidal anti-inflammatory medications

OD: Oxidative damage

ODT: Optical density of test sample

OG: Oil granules

OS: Oxidative stress

OGTT: Oral Glucose tolerance test

PBS: Phosphate buffer solution

PCS: Parenchymatous cell with starch

PEC: Pericycle

PH- Phloem fragments

PM: Powder microscopy

PPH: Postprandial Hyperglycemia

PVE- Pitted vessels element

PYT: Phytotherapeutic

OG: Oil granules

OGTT: Oral Glucose tolerance test

OS: Oxidative stress

p-CA : Para coumaric acid

PKC: Protein kinase C

PO – Per Os (oral administration)

PPAR-γ: Peroxisomes proliferators-activated receptor gamma

PUFA: Polyunsaturated fatty acids

QR: Quercetin

QRE: Quercetin equivalents in milligrams per gram

R- Raphide

R2: Correlation coefficient

RELA or RELB: Rel/NF-kappa B family of transcription factors

Rf : Retention factor

ROS: Reactive oxygen species

RSD: Relative standard deviation

RT – Room Temperature

RV-Reticulate vessels

SEM – Scanning Electron Microscopy

SGOT: Serumglutamic oxaloacetic transaminase

SGPT: Serum glutamic pyruvic transaminase

SOD: Superoxide dismutase

SG: Starch granules

SRL :Sisco Research Laboratory

STZ: Streptozotocin

STAT: Signal transduction and activation of transcription

TCA: Trichloroacetic acid

TEM – Transmission Electron Microscopy

TF- Trachaides fragment

T1DM: Type 1diabetes mellitus

T2D: Type 2diabetes

T2DM: Type 2diabetes mellitus

TAK1: TGF-β-activated kinase 1

TBARS: Thiobarbituric acid reactive substance

TPM: Traditional Phytomedicine

TC: Trichome

TCA: Tri-chloro acetic acid

TLC: Thin layer chromatography

TS: Transverse section

TLRs: Toll-like receptors

TNF-α : Tumor necrosis factor-α

TNFR : Tumor Necrosis Factor Receptors

TZDs: Thiazolidinediones

UPLC : Ultra performance liquid chromatography

UV-Vis – Ultraviolet-Visible Spectroscopy

US FDA: United States Food and Drugs Administration

VE- Vessel's element

VEGF: Vascular endothelial growth factor

VIT-E: Tocopherol or Vitamin-E

WHO: World Health Organization XANOX: Xanthine oxidase

XLV: xylem vessel

XRD – X-ray Diffraction

ZnO: Zinc Oxide

ZnONPs: Zinc Oxide Nanoparticles