PREPARATION, PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF ANTI-DIABETIC ACTIVITY OF POLYHERBAL EXTRACT

Thesis submitted in partial fulfilment for the Award of Degree

Doctor of Philosophy

By

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It is certified that the work contained in the thesis titled ***"Preparation, phytochemical investigation and evaluation of anti-diabetic activity of polyherbal extract"*** by ***Mr. Amit Kumar Singh*** 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 Examination, Candidacy and SOTA for the award of Ph.D. degree.

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I, ***"Amit Kumar Singh"***, certify that the work embodied in this thesis is my own bonafide work and carried out by me under the supervision of ***"Dr. Sunil Kumar Mishra"*** from ***"January 2019" to "March 2024"***, at the ***"Department of Pharmaceutical Engineering & Technology", 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 thatI have not willfully 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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LIST OF ABBREVIATIONS

Abbreviation: Full name	Abbreviation: Full name
AGEs: Advanced glycation end products	**ICP-MS**: Inductively Coupled Plasma Mass Spectrometry
ALP: Alkaline phosphatase	**IHC**: Immuno Histochemistry
ALT: Alanine transaminase	**KEGG**: Kyoto Encyclopedia of Genes and Genomes
AP: *Andrographispaniculata* Nees.	**KM**: Kaempferol
As: Arsenic	**LC-MS**: Liquid chromatography-mass spectroscopy
AST: Aspartate aminotransferase	**LDL**: Low-density lipoprotein
ATA:Alpha Aocospiro A	**MDA**: Malondialdehyde
BA: *Berberisaristata* DC.	**MDT**: Molecular Docking Test
Bcl-2: B-Cell Lymphoma 2	**META**: Metabolite
BF: Biological Function	**MF**: Molecular Function
BMI: Body mass index	**MODY**: Maturity onset diabetes of the young
BP:Biological process	**NA**: *Nyctanthesarbortristis* L.
BQL: Below the quantification limit	**Ni**: Nickel
BUN: Blood urea nitrogen	**OGTT**: Oral Glucose Tolerance Test
CAT: Catalase	**OUT**: Operational taxonomic unit
CBC: Complete blood count	**Pb**: Lead
CC: Cellular Component	**PHE**: Poly Herbal Extract
CCA: Canonical correspondence analysis	**PI**: *Premnaintegrifolia* L.
Cd: Cadmium	**PPI**: Protein-Protein Interaction
CRE: Creatinine	**QCT**: Quercetin
DM: Diabetes mellitus	**RE**: Rutin equivalent
DMR: Dabar Madhu Rakshak	**ROS**: Reactive oxygen species
DMR: Dabur Madhu Rakshak	**RT**: Retention time, Rutin
DMSO: Dimethylsulfoxide	**SCFA**: Short Chain Fatty Acid
DNSA: 3,5 Dinitro Salicylic Acid	**SEA**: Similarity Ensemble Approach
DPP-IV: Dipeptidyl peptidase IV	**SGLT2**: Sodium-glucose transport protein 2
FBG: Fasting Blood Glucose	**SOD**: Superoxide Dismutase
GAE: Gallic acid equivalent	**STP**: Swiss Target Prediction
GC-MS: Gas chromatography-mass spectroscopy	**STP**:Swiss Target Prediction
GLP-1: Glucagon-like peptide 1	**STZ**: Streptozotocin
GLUT: Glucose transporter	**STZ**: Streptozotocin
GM: Gut Microbiota	**T1DM**: Type 1 Diabetes mellitus
GO: Gene Ontology	**T2DM**: Type 2 Diabetes mellitus
GSH: Glutathione peroxidase (reduced glutathione)	**TB**: *Terminaliabellerica* Roxb.
GSK3: Glycogen synthase kinase 3	**TC**: *Terminaliachebula* Retz., Total cholesterol
HbA1c: Glycated hemoglobin	**TCMSP**: Traditional Chinese Medicine Systems Pharmacology
HbA1c: Hemoglobin A1C	**TFC**: Total flavonoid content

HDL: High density lipoprotein
Hg: Mercury
HNF1α: Hepatocyte nuclear factor 1 α
HOMA-IR: Homeostasis Model Assessment of Insulin Resistance
HOMA-β: Homeostasis Model Assessment of β-Cell Function
HPLC: High-performance liquid chromatography
HPTLC: High Performance Thin Layer Chromatography
HRMS: High-resolution mass spectroscopy
TG: Target Gene, Triglyceride
TLC: Thin Layer Chromatography
TNF-α: Tumor Necrosis Factor-α
TP: Total protein
TPC: Total phenolic content
UA: Uric acid
UDCA: Ursodeoxycholic acid
VLDL: Very low density lipoprotein

PREFACE

This thesis on "Preparation, phytochemical investigation and evaluation of anti-diabetic activity of polyherbal extract" aims at giving an insight at various aspects of the chronic disease named as diabetes mellitus and the risk factors that lead to it; the various ways it develops in the human body; the old and new approaches to treatment, both from a pharmacological and a computational (bioinformatics approach) point of view; ways to prevent and to manage the diabetes complications; how to improve the lives of the diabetic patients who are faced with not only physical but also psychological problems; statistical data from around the world which focuses on epidemiology and outlines the issue of the cost of diabetes.The entire thesis work has been divided into nine chapters,which fulfill four objectives as follows:

Chapter 1 deals with the general introduction to diabetes mellitus and its relation to oxidative stress and antidiabetic activity of medicinal plants, the phytochemical composition of polyherbal extracts (polyphenols, flavonoids, and others), gut microbiota dysbiosis, and the enrichment of beneficial short-chain fatty acids through the gut microbiome. It also covers the general strategy adopted for antidiabetic activity and highlights the motivations and significance of research work. The objectives of the thesis work are also part of Chapter 1. **Chapter 2** presents the literature survey covering strategies for selection of medicinal plants with antidiabetic activity, extraction, purification, and characterization of phytochemicals. It also covers the classification of polyphenols and flavonoids related to diabetic management and therapeutic applications. **Chapter 3** describes the research objectives and plan of work, which include step-by-step differentiation of the work.

Chapter 4 presents materials and methods adapted to perform this work. This includes various techniques involved in extraction, purification, and characterization. *In vitro* and *in silico* evaluation of phytochemicals from polyherbal extract (PHE) as an antidiabetic agent and antidiabetic activity evaluation through gut microbiota eubiosis by phytochemical gut microbiota metabolite brain axis. **Chapter 5** deals with all the results of in vitro, in silico, and in vivo studies of PHE with the involvement of short-chain fatty acids through beneficial gut microbiota enrichment. **Chapter 6** presents the discussion of the whole studies with phytochemicals like polyphenols and flavonoids and the evaluation of the antioxidant potential of these through antidiabetic activity in traditional ways as well as in a in a newer way with the eubiosis of gut microbiota. **Chapter 7** deals with the conclusion of the whole study, which focuses mainly on the antidiabetic evaluation of PHE through various approaches. **Chapter 8** deals with future aspects of the study involving clinical trial studies on human volunteers. **Chapter 9** presents a brief overview of publications during the experimental work and findings.

Finally, our study strengthened the possibility that PHE and possibly gut microbiota metabolites from this investigation could one day be novel medications and approaches in the field of pharmacological intervention for diabetes.When taken as a whole, the encouraging PHE reported in this study may prove to be a lead medication option in the future that helps with diabetes management.

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