Dedicated

To

My Beloved Parents

Department of Pharmaceutical Engineering and Technology
Indian Institute of Technology
(Banaras Hindu University)
Varanasi – 221 005

CERTIFICATE

This is to certify that the revised thesis entitled "COMPARISON OF THERAPEUTICALLY INTERESTING BIOACTIVITY PROFILES OF *WITHANIA SOMNIFERA* EXTRACTS IN RODENT MODELS" is being submitted by Mr. Amitabha Dey in partial fulfilment for the award of Ph.D. in Department of Pharmaceutical Engineering and Technology, IIT (BHU), Varanasi is a record of bonafide work carried out by him.

Date of Submission: 17th January, 2018

(Prof. Sushil Kumar Singh)
(Supervisor)

Forwarded by

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

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DECLARATION BY THE CANDIDATE

I, AMITABHA DEY, certify that the work embodied in this thesis is my own bonafide work and carried out by me under the supervision of Dr. Vikas Kumar from July, 2013 to August, 2016 at the Department of Pharmaceutics, Indian Institute of Technology (Banaras Hindu University), 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 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.

Date: August 19th, 2016

Place: Varanasi **(AMITABHA DEY)**

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It is certified that the above statement made by the student is correct to the best of my knowledge.

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Supervisor

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I, Amitabha Dey, declare that the work embodied in this Ph.D. thesis is my own bonafide work carried out by me under the supervision of Dr. Vikas Kumar for a period of about 3 years and 2 months from July, 2013 to August, 2016 at Indian Institute of Technology (Banaras Hindu University), Varanasi. The matter embodied in this Ph. D. thesis has not been submitted for the award of any other degree/diploma.

Date: August 19th, 2016

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This is to certify that Mr. Amitabha Dey, a bonafide research scholar of this Department, has satisfactorily completed the course work/comprehensive examination/candidacy/SOTA/pre-submission seminar requirement, which is a part of his Ph.D. programme

Date: August 19th, 2016

Place: Varanasi

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***Date:** August 19th, 2016*

Place:** Varanasi* ***(Amitabha Dey)

CONTENTS

LIST OF ABBREVIATIONS

Abbreviations	Full forms
AChE	Acetylecholinesterase
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
ANOVA	Analysis of variance
AVP	Arginine vasopressin
BChE	Butyrylcholinesterase
CMC	Carboxymethyl cellulose
CAECU	Central Animal Ethical Committee of the University
CNS	Central Nervous System
COX	Cyclooxygenase
CRH	Corticotrophin releasing hormone
DA	Dopamine
IC_{50}	Half Maximal Inhibitory Concentration
ELISA	Enzyme Linked Immunosorbant Assay
GABA	Gamma Amino Butyric Acid
GOT	Glutamic oxaloacetic transaminase (Aspartate transaminase)
GPT	Glutamic pyruvic transaminase (Alanine transaminase)
HPA axis	Hypothalamic–Pituitary–Adrenal axis
i.p.	Intraperitoneal injection
MAO	Monoamine oxidase
NAD	Nicotinamide adenine dinucleotide

NMDA	N-methyl-D-aspartate
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
NE	Norepinephrine
NO	Nitric oxide
iNOS	Inducible nitric oxide synthase
NF-κβ	Nuclear factor kappa beta
p.o.	Per oral
SNRIs	Serotonin–Norepinephrine Reuptake Inhibitors
SSRIs	Selective Serotonin Reuptake Inhibitors
5-HT	5-Hydroxytryptamine (Serotonin)
SEM	Standard Error of Mean
TCA	Tricyclic antidepressant
TEG	Triethylene glycol
TNF-α	Tumor Necrosis Factor-alpha
WHO	World Health Organization

LIST OF SYMBOLS

Symbols	Denotes
α	Alpha
β	Beta
dl	Decilitre
°C	Degree Celsius
g	Gram
Hz	Hertz
hr	Hour
kg	Kilogram
IU	International Units
µg	Microgram
mA	Milliampere
mg	Milligram
ml	Millilitre
µl	Microlitre
min	Minute
ms	Millisecond
ng	Nanogram
%	Percentage
±	Plus minus
sec	Second
v/v	Volume/volume
w/v	Weight/volume
w/w	Weight/weight
W	Watt
×g	Times gravity

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PREFACE

Ayurveda is the oldest known system of medicine still widely practiced in India and popularity of diverse therapeutic modalities mentioned in ancient Ayurvedic literature has continued to increase during recent decades. In Ayurveda, Rasayana herbs are always recommended for treating metabolic and neurologic conditions. Diabetes is a slowly and silently progressing metabolic disorder, which ultimately leads to structural and functional deteriorations of almost all bodily organs including those of circulating fluids and central and peripheral nervous systems. Diabesity has been associated with various co-morbidities including central nervous system (CNS) disorders such as depression, anxiety, cognitive dysfunctions, and neuropathic pain and inflammation. Treating mental health problems in diabetic patients however remains a challenging field in medical science. Many tonics or rejuvenators oftenly used in Ayurvedic system of medicine for prevention of psychiatric or neurologic comorbidities contain Ashwagandha concoctions as one of their major active ingredients and currently Ashwagandha is recommended by most of Ayurvedic practitioners for treating all such conditions. Ashwagandha (*Withania somnifera*) is a traditionally known rejuvenative herb of solanaceae family, and withanolides are quantitatively the major bioactive secondary metabolites of the plant identified and most currently commercialised *Withania somnifera* extracts or products are now often analytically characterised by their contents of total withanolides. Extensive efforts made during past few decades have identified not only broad spectrums of therapeutically interesting pharmacological properties of diverse types of *Withania somnifera* extracts obtained from different parts, but also of withanolides and other structurally unique bioactive constituents of such extracts. Amongst them the ones dealing with anti-stress, anticancer and anti-inflammatory activities of extracts

rich in withanolides, have attracted the most attention of modern researchers. Preclinical and clinical information now available on diverse types of *Withania somnifera* extracts and numerous of their known bioactive constituents strongly suggest that appropriate combinations of phytochemicals and other bioactive substances encountered in its extracts could as well be used for prevention of metabolic or environmental stress triggered diseases and their syndromes. Keeping in mind, the encouraging withanolides and the data available regarding the use of *Withania somnifera* in treating stress triggered diseases, present study was conducted to fully exploit the potential of *Withania somnifera* in this promising area. The present research work includes the pharmacologically validated rodent bioassays to verify whether parts of the plant other than roots could also be used for the prevention of metabolic or environmental stress triggered diseases and to identify plant metabolites potentially useful for prevention and cure of mental health problems accompanying, or caused by, metabolic diseases. Metabolic effects during stressful conditions using validated behavioural rodent models followed by elucidation of mechanism(s) of observed action(s) through biochemical estimations were evaluated. Observations made during such efforts strongly suggest that, *Withania somnifera* is an adaptogenic Rasayana herb with a uniquely broad psychopharmacological activity profile and could be herbal lead for prevention and treatments of stress triggered diseases commonly associated with metabolic disorders. Further efforts to identify the roles of diverse other known bioactive secondary metabolites of the plant are necessary for better understanding of Ayurvedic pharmacology of this plant.