DECLARATION

I hereby declare that the thesis entitled "**SCREENING AYURVEDA BASED THERAPEUTIC PREPARATIONS AGAINST MULTIPLE ANTIBIOTIC-RESISTANT GASTRO-ENTERIC DISEASE CAUSING BACTERIA AND TO REVEAL THE MODE OF ACTION OF THE POTENTIAL PREPARATION(S)**" is an authentic research work carried out by me in the Department of Biotechnology, University of North Bengal, Darjeeling – 734013, West Bengal, India, under the supervision of Prof. (Dr.) Ranadhir Chakraborty, Department of Biotechnology, University of North Bengal, Darjeeling – 734013, West Bengal, India. I also affirm that the thesis is an original work and has not been submitted in part or full for any degree, diploma or any other academic award to this or any other University or Institution.

Place: SILIGURI

Date: 27.12.2023

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CERTIFICATE

The research work presented in this thesis entitled "**SCREENING AYURVEDA BASED THERAPEUTIC PREPARATIONS AGAINST MULTIPLE ANTIBIOTIC-RESISTANT GASTRO-ENTERIC DISEASE CAUSING BACTERIA AND TO REVEAL THE MODE OF ACTION OF THE POTENTIAL PREPARATION(S)**" has been carried out under my direct supervision by **Mr. Subhanil Chakraborty**. This work is original and has not been submitted for any degree or diploma to this or any other University or Institution.

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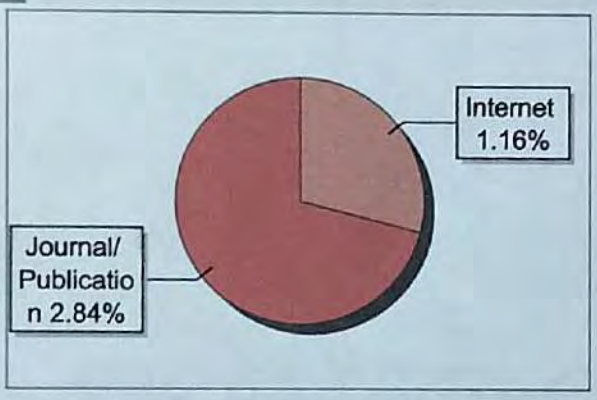
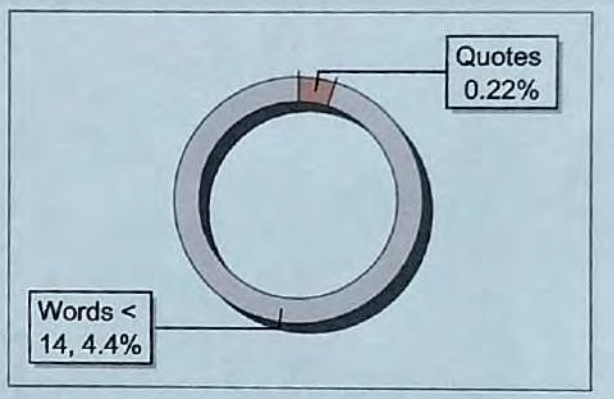
Author Name	Subhanil Chakraborty
Title	SCREENING AYURVEDA BASED THERAPEUTIC PREPARATIONS AGAINST MULTIPLE ANTIBIOTIC-RESISTANT GASTRO-ENTERIC DISEASE-CAUSING BACTERIA AND TO REVEAL THE MODE OF ACTION OF THE POTENTIAL PREPARATION(S)
Paper/Submission ID	1214605
Submitted by	nbuplg@nbu.ac.in
Submission Date	2023-12-14 14:14:39
Total Pages	101
Document type	Thesis

Result Information

Similarity **4 %**





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Student Papers	Yes
Journals & publishers	Yes
Internet or Web	Yes
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Subhanil Chakraborty 27/12/2023

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Dedicated

To

My Family

My Father

Late Sri Murari Mohan Chakraborty

And

Honorable

(Late)

Prof. Ashim K. Chakravarty

Acknowledgment

This thesis entitled
"SCREENING AYURVEDA BASED THERAPEUTIC PREPARATIONS AGAINST MULTIPLE ANTIBIOTIC-RESISTANT GASTRO-ENTERIC DISEASE-CAUSING BACTERIA AND TO REVEAL THE MODE OF ACTION OF THE POTENTIAL PREPARATION(S)" is the most significant scientific adventure in my life and I am truly honored to acknowledge the persons who supported me all through with their co-operation, guidance and empathy for the successful conception of the research work.

I first arrived in University of North Bengal ten years back to meet **Professor (Dr.) Ranadhir Chakraborty** at the Department to Biotechnology, University of North Bengal. Little did I know then, this person will become the pole star of my life while supervising and guiding my doctoral research in most unique and eloquent way. He put me like "The charge of the light brigade" into my scientific endeavor with a childlike enthusiasm, unending inspiration and contagious curiosity.

It is my humble duty and great pleasure to express my indebtedness and respect to my prudent mentor Prof. Dr. Ranadhir Chakraborty, Department of Biotechnology, NBU, for his active guidance, painstaking supervision, valuable advice and constant encouragement that he rendered for the present work. His constant vigilance and care have been lavishly bestowed upon me in every stage of this work. He is the key person to steer my academic career in right direction. Any words of gratitude would not be enough to express my thanks for his tremendous support and advice. I would also like to take this opportunity to sincerely thank the better half of my guide, Dr. Ratna Sarkar for her kind words of inspiration and mother like care.

I take immense pleasure to show great appreciation to all respected teachers of my department. Any thanksgiving in response would feel microscopic to the support and

learnings I received from them. I am indebted to Dr. Dipanwita Saha for introducing me to the world of "fantastic fungi" and Dr. Shilpi Ghosh for leading me through the "paradigm of proteins". Any word of acknowledgement would fall short for the support I gratefully accepted from Dr. Anoop Kumar. He treated me like a younger brother and always showered me with his teaching and mentoring, I would like to congratulate and thank Dr. Manab Deb Adhikary for being inspirational in joining this galaxy of stars.

I feel pride to have acquainted with the most dynamic duo I have ever come across, Dr. Ashish Kumar Nanda and Late Dr. Ashim Kumar Chakravarty. I learned a lot from them and in return, they expected me to challenge them with logical contradictions. All I received from them is "the gift of the magi". I am indebted to Dr. Min Bahadur from Department of Zoology, NBU for all his guidance related to animal studies and related permissions and Late Dr. Palash Mandal from Department of Botany, NBU for identification of my plants.

I am honored to always being on the receiving end of love, care and immense support rendered by all non-teaching staffs like Dipak da, Raja Da, Biswajit Da, late Sujay da and most importantly my brother Sri Arup Ghosh.

I suppose, any meagre word would not fit the gravity to express how indispensable, my lab mate friends' presence was for my research. My special thanks to my ever-enthusiastic lab mates, Anup bhai, Amit, Biprans da, Rudra, Tilak Da, Sreeparna di, Suparna di, Bhaskar da, Arvind bhaiya, Subhajit, Vivek Ranjan, Prayaas, Partha, Sriradha, Chandana, Shilpa, Anusuya, Soumya, Anukul, Deep and my childhood friend Sudipta for their immense co-operation and co-ordination for the research work.

I am also thankful to all other scholars especially Somnath bhai, Gargee di, Moushree, Deepika, Shanta, Nithya, Upasna, Swarnab, Aditi, Ayan and my sister Khushboo Lepcha for their continuous co-operation and support throughout my time at NBU.

I offer cordial thanks to Dr. Anindya Sundar Ghosh of IIT, Kharagpur, Dr. Biswanath Chakraborty of Kalyani University, Dr. Ranjan Nandi of NICED and Dr. Shanti Mandal, who were an important advisor during my project work and provided me with

their valuable support through their scholars and themselves to facilitate characterization of my samples.

Words of thanks would be too meager for profound support and blessings rendered to me by my parents Mrs. Saraswati Chakraborty and late Mr. Murari Mohan Chakraborty, who remain deprived of my time and attention during the preparation of this thesis. My entire family including my in-laws were also very supportive.

I would like to convey my sincere thanks to Dr. A. Mukherjee, Scientist and Head of INSPIRE Division, Department of Science and Technology, New Delhi, for awarding me INSPIRE Fellowship vide sanction order DST/INSPIRE Fellowship/2016/IF 160786 and University of North Bengal for providing the infrastructural facilities.

Finally, yet most importantly, I would like to express my heartfelt thanks to my beloved wife **Dr. Babli Roy** for her enduring love and unending enthusiasm to inspire me at every stage of the research and helping me silently and invisibly to navigate through every obstacle I faced. Without her 360° support, I would have been long lost.

Before I finish, I want to express my gratitude to all the biologists, the scientists and research scholars in this field of work whose studies have guided me to complete this work. However, the words might not reach, but I feel it is important to thank my holy abode, the nature and the mountains of the Himalayas. I have possibly left out the names of many other people who helped me in a way or other during this research work and I express my sincere thanks and heartfelt sorry to all of them.

Place: SILIGURI

Date: 27/12/2023

(Subhanil Chakraborty)

"Do not go gentle into that good night, Old age should burn and rave at close of day; Rage, rage against the dying of the light."

Preface

Gastro-enteric diseases, particularly Diarrhea effortlessly overwhelm the public health system in India by easy means of spreading through either food or drinking water and leading to the scale of the epidemic; causing enormous loss of life even today. Even after decades of research, the problem of efficiently combating multiple antibiotics resistant pathogens by the existing arsenal remains to be resolved. There are several references in the literature, printed age-old books and internet, describing remedies from enteric diseases using traditional knowledge and practice.

Different ayurveda preparations, elixirs and mixtures of natural products and minerals were used in the control of gastro-enteric diseases. The present research project is to screen most suitable preparations/candidates and explore the efficacy of those age old Indian natural remedial preparations in the control of gastro-enteric pathogens with main emphasis on the genus *Salmonella, Escherichia, and Shigella*. The major problem of treating gastro-enteric diseases today is the emergence of multiple antibiotics resistant pathogen and growing complexity of resistance mechanism and perplexing mixed etiology.

The activity of the screened preparations was checked against multiple drug resistant pathogens along with testing the MIC values, MBC values and other regular safety, efficacy and antimicrobial assays. The mode of action of the potential preparation(s) were ascertained using combination of methods of electron microscopy and high through-put technologies including chemical analysis and fluorescence based cytological profiling. Physico-chemical characteristics of the effective preparation(s) were determined using different analytical methods for identification of active components of the preparation with a view of developing the Ayurveda based potential preparation AP-01 into more patient-compliant dosage forms for future uses.

List of Tables

List of Tables

AGORA	Antimicrobials: A Global Alliance for Optimizing their Rational Use in Intra-Abdominal Infections
AK	Amikacin
AMC	Amoxycillin
AMP	Ampicillin
AT	Aztreonam
BCP	Bacterial Cytological Profiling
CAZ	Cephazolin
CDC	Centre for Disease Control and Prevention
CIP	Ciprofloxacin
CLSI	Clinical Laboratory Standards Institute
CPM	Cefepime
CRP	C-reactive protein
CTX	Ceftriaxone
D	Doxycycline
DAPI	4′,6-diamidino-2-phenylindole
DEC	*diarrheagenic Escherichia coli*
DMSO	Di methyl sulphoxide
DNA	Deoxy-ribo Nucleic Acid
DOR	Doripenem
eCDC	European Centre for Disease Control and Prevention
E	Erythromycin
EAEC	*enteroaggregative Escherichia coli*
EHEC	*enterohemorrhagic Escherichia coli*
EIEC	enteroinvasive *Escherichia coli*
EPEC	enteropathogenic *Escherichia coli*
ETEC	enterotoxigenic *Escherichia coli*
EUCAST	European Committee on Antimicrobial Susceptibility Testing

FDA	Food and Drug Administration
FTIR	Fourier Transform Infra-Red
GFP	Green Fluorescent Protein
GI	Gastro Intestinal
GTPR	Gastro-intestinal Tract Pathogens Repository
HCS	High-Content Screening
ICMR	Indian Council of Medical Research
IEC	intestinal epithelial cell line
K	Kanamycin
Kg	Kilo gram
LB	Luria Bertani broth
LBDD	Ligand-based drug design
LCMS	Liquid Chromatography Mass Spectrometry
LE	Levofloxacin
µg	microgram
µl	microliter
ml	milliliter
MAR	Multiple Antibiotics Resistant
MBC	minimum bactericidal concentration
MHA	Muellar-Hinton agar
MIC	minimum inhibitory concentrations
MOA	Mode of Action
MRP	Meropenem
MS	Mass Spectrometry
MTCC	Microbial Type Culture Collection Center
MTT	(3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide)
nm	nano meter

NA	Nalidixic acid
NCBI	National Center for Biotechnology Information
NDA	New Drug Application
NICED	National Institute of Cholera and Enteric Diseases
NMR	Nuclear Magnetic resonance
NOR	Norfloxacin
OFX	Ofloxacin
ORT	oral rehydration therapy
PBS	Phosphate Buffer Saline
PCA	Principal component analysis
PCR	Polymerase Chain Reaction
PHF	Poly Herbal Formulations
PIT	Piperacillin/Tazobactam
PLC	Public Limited Company
PRWA	Percent residual wound area
R & D	Research and Development
RCPA	Retail chemist prescription audit
RIE	rat intestinal epithelial
RNA	Ribo Nucleic Acid
S	Streptomycin
SBDD	Structural-based drug design
SD	Standard deviation
S.E.M	Standard Error of Mean
SEM	Scanning Electron Microscopy
SXT	Sulphamethoxazole Trimethoprim
Usaid	United States Agency for International Development
v/v	volume per volume
WHO	World Health Organization