

**LICHEN DIVERSITY OF ULTAPANI FOREST RANGE,
MANAS BIOSPHERE RESERVE, ASSAM AND BIOLOGICAL
ACTIVITY OF SELECTED SPECIES**

**A THESIS SUBMITTED TO
THE DEPARTMENT OF BOTANY, BODOLAND UNIVERSITY**

**FOR THE AWARD OF
DEGREE OF DOCTOR OF PHILOSOPHY IN BOTANY**

**UNDER
FACULTY OF SCIENCE AND TECHNOLOGY**



**SUBMITTED BY
PUNGBILI ISLARY**

**ENROLLMENT NO.: BOTPHD-02
PH.D. REGISTRATION NO.: FINAL.BOT00235 of 2019-2020
MAY, 2024**



BODOLAND UNIVERSITY
DEPARTMENT OF BOTANY

Debargaon, P.O.: Rangalikhata
Kokrajhar-783370, BTR, Assam, India

Dr. Rebecca Daimari
Assistant Professor

Contact: 9859164753

Email: rebeccadmr@gmail.com

Dated: May, 2024

CERTIFICATE

I am pleased to forward this Ph.D. thesis entitled “**LICHEN DIVERSITY OF ULTAPANI FOREST RANGE, MANAS BIOSPHERE RESERVE, ASSAM AND BIOLOGICAL ACTIVITY OF SELECTED SPECIES**” by Ms. Pungbili Islary, submitted for the award of the Degree of Doctor of Philosophy (Ph.D.) in Botany in the Faculty of Science and Technology.

Ms. Pungbili Islary has carried out this work under my supervision and fulfils all the requirements under the Ph.D. Regulations of Bodoland University. She has been registered under Bodoland University with Provisional Registration No. PROV.BOT00235 of 2019-2020 and Ph.D. Final Registration No. FINAL.BOT00235 of 2019-2020.

I further declare that the thesis is entirely original and her own investigation and no part of the thesis have been submitted to any other university or anywhere for the award of any degree.

Daimari
27/05/2024
Signature

Dr. Rebecca Daimari
Assistant Professor
Dept. of Botany
Bodoland University
Kokrajhar-783370, Assam

DECLARATION

I, Pungbili Islary, declare that the thesis entitled “**LICHEN DIVERSITY OF ULTAPANI FOREST RANGE, MANAS BIOSPHERE RESERVE, ASSAM AND BIOLOGICAL ACTIVITY OF SELECTED SPECIES**” is a bonafide Ph.D. work done by me under the supervision of Dr. Rebecca Daimari, Assistant Professor, Department of Botany, Bodoland University. This thesis is submitted to Bodoland University for the award of degree, Doctor of Philosophy (Ph.D.) at Bodoland University bearing Enrollment no. BOTPHD-02 and final Registration no. FINAL.BOT00235 of 2019-2020. The thesis is the result of my own investigation and is original, and has not been previously submitted for the award of any degree or other research works.

Place: Kokrajhar

Dated: May, 2024

Pungbili Islary

(Pungbili Islary)

Department of Botany

Bodoland University

ACKNOWLEDGEMENT

This study on **“Lichen diversity of Ultapani Forest Range, Manas Biosphere Reserve, Assam and Biological Activity of Selected Species”** was undertaken for the award of degree, Doctor of Philosophy (Ph.D.) in Botany under the Faculty of Science. The findings of the present study are the outcome of my extensive effort for my Ph.D. programme conducted during the period of 2019-2023. This work would not have been possible without the supportive advice, motivation, suggestions, and assistance of several individuals in other ways.

First and foremost, I want to express my deep sense of gratitude to my supervisor, Dr. Rebecca Daimari, Department of Botany, Bodoland University, Kokrajhar, Assam, for her unwavering guidance, support, kindness, and supervision. I express my sincere gratitude to her for her patience, insightful suggestions, encouragement, and company for whom I conducted my research.

I must express my sincere gratitude to the Member Secretary, Assam State Biodiversity Board (ASBB) and the official team for allowing me to explore the study area. I extend my appreciation to the Divisional Forest Officer (DFO), Haltugaon Forest Division of the Forest Department, Bodoland Territorial Region, for providing necessary information and granting me permission to conduct in study area.

I express my whole hearted thanks to Dr. Manjil Basumatary, Academic Registrar; Dr. Prahlad Basumatary, Deputy Academic Registrar; Prof. Hilloljyoti Singha, former Head of the Department, Zoology; Prof. Sandeep Das, former Dean, Faculty of Science and Teachnology; Prof. Sujit Deka, Dean, Faculty of Science and Teachnology; Prof. Sanjay Basumatary, Head of the Department, Chemistry; Dr. Hemen Sarma, Head of the Department, Botany; Dr. Sanjib Baruah, Assistant Professor, Botany; Dr. Yutika Narzary, Assistant Professor, Botany; Dr. Arvind Kumar Goyal, Assistant Professor, Biotechnology; Dr. Rajeeb Brahma, Assistant Professor, Physics; Dr. Kaylan Dey, Assistant Professor, Physics of Bodoland University, for suggesting numerous ideas and remarks during my entire progress seminar that helped me strengthen my Ph.D. work.

I want to express my heartfelt gratitude to Mr. Durga Brahma, Mr. Keshab Brahma, Mr. Bana Kr. Brahma, Mr. Giren Brahma, staffs of Ultapani Forest Range and NGO and Mr. Jaisar Basumatary and his brother (inhabitants of Ultapani) for their kind assistance during field visit.

I express my sincere gratitude and profuse thanks to Dr. Dalip Kumar Upreti, Emeritus Scientist, CSIR-NBRI Lucknow; Dr. Sanjeeva Nayaka, Principal Scientist, CSIR-NBRI Lucknow; Dr. Gaurav Kumar Mishra, Scientist, CSIR-NBRI Lucknow; Dr. Komal Kumar Ingle, Technical Assistant, CSIR-NBRI Lucknow and Dr. Siljo Joseph, Scientist, Kerala Forest Research Institute for their monumental guidance on identification of lichen taxa. They encouraged me with immense source of inspiration, valuable guidance, constructive criticism, and always exhorted me in keeping myself intact with my studies in high spirit, which really gave impetus to my research work.

It is my privilege to express my heartfelt gratefulness and sincere thanks to Dr. Debasmita Dubey, Assistant Professor, Medical Research Laboratory, IMS and SUM hospital, Bhubaneshwar, India for assisting in my antimicrobial work.

I am really appreciative to Dr. Rajesh Kumar Meher, Postdoctoral fellow, ACTREC, Tata Memorial Center, Mumbai, India for helping me in my cytotoxic work.

I am highly obliged to Dr. Ananta Swargiary, Assistant Professor, Department of Zoology and Dr. Anjalu Basumatary, Assistant Professor, Department of Physics, Bodoland University for their help to use Spectrophotometer for my antioxidant work.

I owe the Ministry of Tribal affairs (MoTA), Government of India for the financial assistance under National Fellowship and Scholarship for Higher Studies of ST (Scheduled Tribe) students (NFST) (Award No: 202021-NFST-ASS-01574) for the Ph.D. programme.

Lastly, I feel extremely proud to express my deep favors, amazing gratitude beyond accountability to my father, Mr. Balaram Islary and mother, Mrs. Chaimuni Islary for their eternal love, rock-solid support and incessant sacrifices to figure my career and

personality as I can never ever think of achieving this innovative and difficult task without the affection, adoration and blessings bequeathed by them. I also offer my thanks to my younger brother, Mr. Biringat Islary and sister, Ms. Bhagabati Islary for their love and care which has been my greatest strength always with me in this tough journey.

Furthermore, I would like to express my profound gratitude to all of my Scholar mates for their helpful cooperation in upholding the excellent spirit of the research environment.

I apologise profusely to everyone whose name could not be mentioned specifically on this page.

I bowed down to the All-Mighty God, whose favours made it possible for me to finish my research work.

Pungbili Islary

Pungbili Islary

List of Figures

Fig. 3.1.	Map showing the location of Ultapani Forest Range (UFR)	15
Fig. 4.1.	Representation of different growth forms of lichen taxa	32
Fig. 4.2.	Family wise representation of the lichen taxa	33
Fig. 4.3.	Representation of the lichen genera	34
Fig. 4.4.	Lichen diversity of UFR	187
Fig. 4.5.	Number of endemic species found in different states of India	215
Fig. 5.1.	GC-MS chromatograph of the methanol extract of <i>A. ornatoides</i> volatile compounds with putative chemical structures of most abundant molecule	227
Fig. 5.2.	A - Standard graph of Gallic acid curve B - Total phenolic contents (mg GAE/g DW) of different extracts	229
Fig. 5.3.	A - Standard graph of Quercetin curve B - Total flavonoid contents (mg QE/g DW) of different extracts	229
Fig. 5.4.	A - Standard graph of Ascorbic acid B - Total antioxidant compounds by phosphomolybdenum method (mg AAE/g DW) of different extracts	230
Fig. 5.5.	A - Standard graph of Ascorbic acid B - Ferric reducing antioxidant power (mg AAE/g DW) of different extracts	230
Fig. 5.6.	A - Standard graph of Ascorbic acid B - IC50, scavenging DPPH radical between extracts	231
Fig. 5.7.	A - Standard graph of Trolox B - IC50, ABTS assay between extracts	233
Fig. 5.8.	A - Standard graph of Ascorbic acid B - IC50, Inhibitory activity towards lipid peroxidation between extracts	234

Fig. 6.1.	Inhibition of proliferation of cancer cells against all the extracts	239
Fig. 6.2.	IC50, Cancer cell lines of methanol extract	240
Fig. 6.3.	A - Treatment of OVCAR-3 cancer cells with methanolic extract of <i>A. ornatoides</i> at a specific IC50 concentration	240
	B - Viability and apoptotic percentage of the OVCAR-3 cancer cell	
Fig. 6.4.	A comprehensive visual representation of the impact of methanolic extract on OVCAR-3 cancer cells at the IC50 concentration	241
Fig. 6.5.	Demonstrating the extract influences viability and apoptotic cell death of healthy cells across a range of concentrations	242

List of Photo plates

Plate 1	A - Ultapani river (flows in reverse direction)	16
	B - Photograph inside Ultapani forest	
Plate 2	A - Naa Bhandhar (Mach Bhandhar)	17
	B - Saralbhanga river	
Plate 3	A - Laopani river	18
	B - Semi-evergreen forest	
Plate 4	A - Photograph from outside forest	19
	B - Golden langur on tree	
Plate 5	A - Remains of the tree felling within forest	223
	B - Human settlement after deforestation	
Plate 6	A - Clearance of the forest	224
	B - Remains of the burn down trees	
Plate 7–18	Disc diffusion assay against bacteria	278-289
Plate 19–21	Disc diffusion assay against yeast	290-292
Plate 22–33	Agar-well diffusion assay against bacteria	293-304
Plate 34–36	Agar-well diffusion assay against yeast	305-307
Plate 37–38	MIC	308-309
Plate 39	MBC and MFC	310
Plate 40–50	Lichen species	311-321
Plate 51–53	TLC plate developed lichen substances	322-324

List of tables

Table 4.1.	Growth forms, families, genera and lichen taxa from UFR	32
Table 4.2.	List of lichen taxa, their growth forms and families along with their frequency and relative abundance of UFR	35-42
Table 4.3.	New distributional records of lichen to India discovered in UFR	188-190
Table 4.4.	New distributional records of lichen to Assam discovered in UFR	190-198
Table 4.5.	New distributional records of lichen taxa to the BTR region discovered in UFR	198-209
Table 4.6.	Endemic species to India with substratum and their distribution discovered in UFR	209-213
Table 4.7.	Comparison of lichen families of UFR with lichen biota of Assam	215-217
Table 4.8.	List of lichen species used by ethnic groups and their usages discovered in UFR	218
Table 4.9.	Commercial lichen species used as spice in different states of India found in UFR	218
Table 4.10.	Encroached area, number of encroached villages, forest cover reduction and number of increasing populations of UFR (Source: Haltugaon Forest Division, Kokrajhar)	220
Table 5.1.	Temperature values for refluxing at Soxhlet, extraction yield and colours of <i>A. ornatoides</i> dry extracts using different solvents	225
Table 5.2.	Preliminary phytochemical screening of <i>A. ornatoides</i> with various solvents	225-226
Table 5.3.	Compounds detected and identified in the methanol extract by GC-MS	227-228
Table 5.4.	Results of four assays among five different extracts by	231

	Kruskal-Wallis test	
Table 5.5.	Percentage scavenging rate of DPPH free radicals by extracts	232
Table 5.6A.	DPPH assay among the extracts in each concentration by Kruskal-Wallis test	232
Table 5.6B.	DPPH assay among the concentration in each extract by Kruskal-Wallis test	232
Table 5.7.	Percentage scavenging rate of ABTS radicals by extracts	233
Table 5.8A.	ABTS assay among the extracts in each concentration by Kruskal-Wallis test	233-234
Table 5.8B.	ABTS assay among the concentration in each extract by Kruskal-Wallis test	234
Table 5.9.	Percentage scavenging rate of lipid peroxidation radicals by extracts	235
Table 5.10A.	Lipid peroxidation assay among the extracts in each concentration by Kruskal-Wallis test	235
Table 5.10B.	Lipid peroxidation assay among the concentration in each extract by Kruskal-Wallis test	235
Table 7.1.	ZOI of bacterial strains and gentamicin against the lichen extracts (20 µl of 15 mg/ml) by disc-diffusion assay	245-246
Table 7.2.	ZOI of bacterial strains and gentamicin against the lichen extracts (20 µl of 20 mg/ml) by disc-diffusion assay	246
Table 7.3.	ZOI of bacterial strains and gentamicin against the lichen extracts (20 µl of 25 mg/ml) by disc-diffusion assay	246
Table 7.4.	ZOI of <i>C. albicans</i> and fresocan against varied concentration of the lichen extracts (20 µl) by disc-diffusion assay	246-247
Table 7.5.	ZOI of bacterial strains and gentamicin against the lichen extracts (50 µl of 15 mg/ml) by agar-well diffusion assay	247
Table 7.6.	ZOI of bacterial strains and gentamicin against the lichen extracts (50 µl of 20 mg/ml) by agar-well diffusion	247-248

	assay	
Table 7.7.	ZOI of bacterial strains and gentamicin against the lichen extracts (50 μ l of 25 mg/ml) by agar-well diffusion assay	248
Table 7.8.	ZOI of <i>C. albicans</i> and fresocan against varied concentration of the lichen extracts (30 μ l) by agar-well diffusion assay	248
Table 7.9.	MIC and MBC values of lichen extracts against isolated MDR bacterial strains	249-250
Table 7.10.	MIC and MFC values of lichen extracts against isolated MDR yeast strain	250
Table 7.11.	MBC/MIC and MFC/MIC values of extracts against tested organisms	250

Abbreviation used

ABTS = 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)

AO = Acridine Orange

BUBH = Bodoland University Botanical Herbarium

CFU = Colony forming unit

Coll. = Collector

CRF = Chirang Reserve Forest

CSIR = Council of Scientific and Industrial Research

Diam. = Diameter

DMSO = Dimethyl sulphoxide

DPPH = 1-diphenyl-2,2-picrylhydrazil

DW = Dry weight

Elev. = Elevation

EtBr = Ethidium bromide

FBS = Fetal Bovine Serum

FRAP = Ferric-reducing antioxidant power assay

G = Gram

GA = Gyrophoric acid

GAE = Gallic acid equivalent

GAW = Glycerine-alcohol-water

GE = Glycerine-acetic acid

GPS = Global positioning system

H = Hour

IC50 = Concentration of sample providing 50% inhibition

M = Meter

MBC = Minimum bactericidal concentration

MDR = Multidrug resistance

MFC = Minimum fungicidal concentration

MHA = Muller-Hinton agar

MIC = Minimum inhibitory concentration

Min = Minutes

ml = Milliliter

mm = Millimeter

NB = Nutrient broth

NCCLS = National Committee for Clinical Laboratory Standards

NIST = National Institute Standard and Technology

PBS = Phosphate buffered saline

Rf = Retention factor

SDA = Sabourard dextrose agar

SIRS = Systemic Inflammatory Response Syndrome

TLC = Thin Layer Chromatography

TTC = 2,3,5-triphenyl tetrazolium chloride

UFR = Ultapani Forest Range

UNESCO = The United Nations Educational, Scientific and Cultural Organization

US = United State

UV = Ultra violet

WHO = World health organization

YPD = Yeast Peptone Dextrose

ZOI = Zone of inhibition

μ l = Microlitre

μ m = Micrometer