

Document Information

Analyzed document	Sourav Chakraborty_Tea Science.pdf (D140826276)
Submitted	6/20/2022 12:44:00 PM
Submitted by	University of North Bengal
Submitter email	nbuplg@nbu.ac.in
Similarity	0%
Analysis address	nbuplg.nbu@analysis.arkund.com

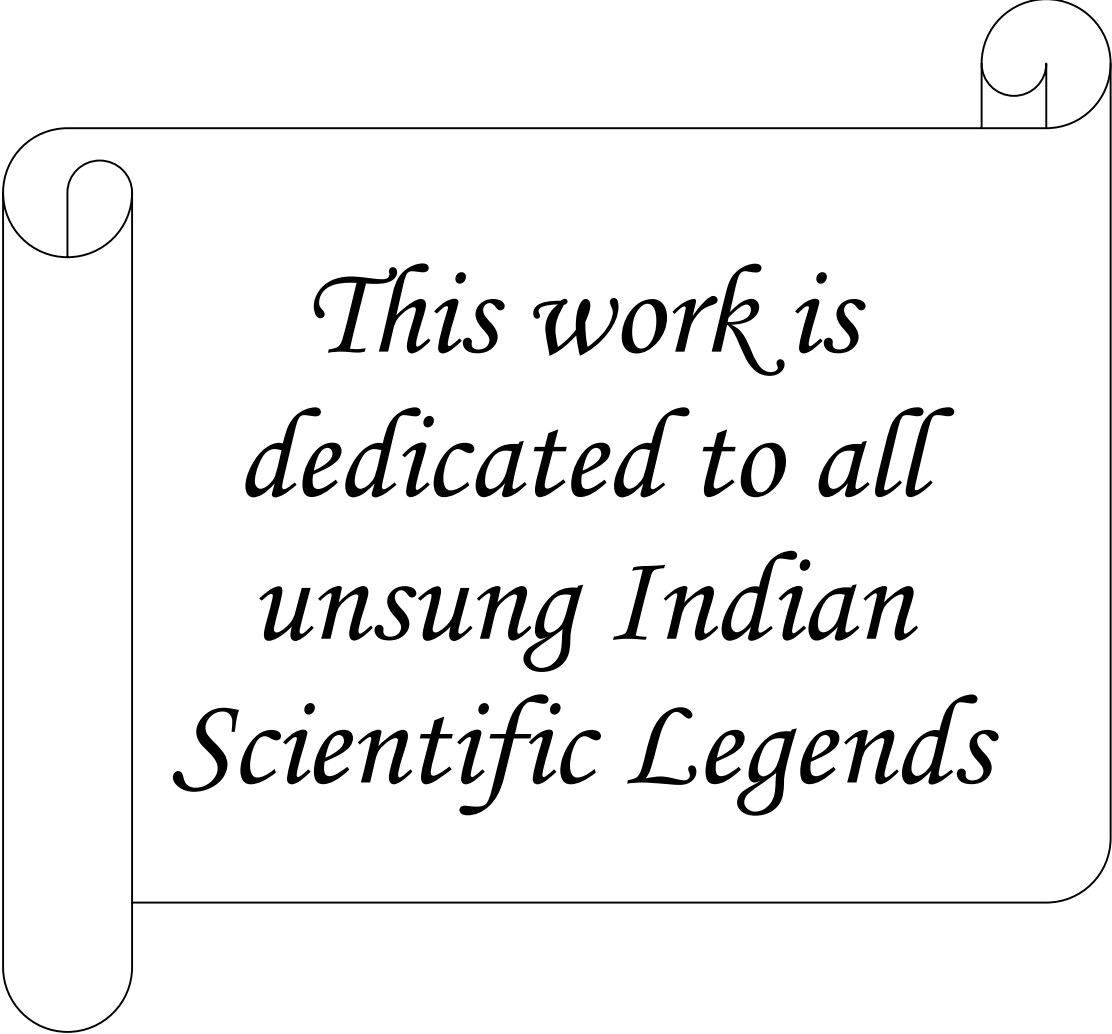
Sources included in the report

Entire Document

1. Introduction India has been referred to as a mega-diversity nation on account of its enriched faunal and floral variations. It encloses a range of biodiversity hotspots among which the Himalaya Hotspot harbours several indigenous and endemic species. The Eastern Himalayan region comprises of the Darjeeling hills (average 6700ft) sharing borders with Bhutan and Sikkim in the North East and Eastern Nepal in the west being therefore deemed to be a characteristic geographic terrain that unifies the diversified flora of Bhutan, India and Nepal. Climbing species play a critical role in intensifying the floristic richness of this naturally scenic landscape amidst the magnificent Tea gardens. The Gourd family Cucurbitaceae holds the second position after Papilionaceae in respect of the climber dominating angiospermic families of Darjeeling hills with around twenty seven reported species. Among the collective 11.61% endemic climbers persisting in Darjeeling region of Eastern Himalaya (Samanta, 1998); an under explored ethnomedicinally relevant indigenous climbing Cucurbit is *Herpetospermum darjeelingense* (C.B.Clarke) H.Schaefer & S.S.Renner [*Edgaria darjeelingensis* C.B.Clarke]. The plant has been stated to be endemic along with being previously reported as an endangered floral entity (Samanta, 1998; Chakraborty et al., 2021a). The Darjeeling hills are located between 27.0410°N and 88.2663°E geographical coordinates with the indigenous Cucurbit being sheltered at altitudinal range of 1450-3000m. *Herpetospermum darjeelingense* is a typical shrubby climber characterized by axillary shoot tendrils employed for climbing purposes. Morphologically, the observable tendrils in the plant are modified axillary buds specialized in providing support and anchorage to the vining stem. Traditionally *Herpetospermum darjeelingense* is known as 'Cathil' in the Chitwan district of Nepal with its fruits being economically valued as a source of vegetable by the local people comprising representative members of the ancient tribal ethnic group 'Tharu' (Mueller-Boeker, 1993). The 'Tamang' community in centralized locations of Nepal and Darjeeling Eastern Himalaya also has been reported to use the climbing cucurbit plant in herbal veterinary formulations being commonly referred to as 'Tangsarkato' and 'Jangali Karela' by the Tamang's and local Nepali inhabitants respectively. The seeds of *Herpetospermum darjeelingense* have been stated to be used in bovine treatment as per the ethno-medicinal based traditional knowledge of the 'Tamang' community. The treatment methodology involves the mixing of the pulverized plant seeds with corn flour being subsequently fed to the cattle's in order to relieve them from bovine pyrexia (Shrestha and Khadgi, 2019). A solitary report by Chakraborty et al (2021a) too highlighted the potential

Dr. Pradyumn Chakraborty
Dr. Pradyumn Chakraborty
Assistant Professor
Department of Tea Science
University of North Bengal

Sourav Chakraborty
29.06.22



*This work is
dedicated to all
unsung Indian
Scientific Legends*

DECLARATION

I declare that the thesis entitled “Exploring bioactivity, isolation of potential biomolecules and *in vitro* regeneration of *Herpetospermum darjeelingense* (C.B.Clarke) H.Schaef. & S.S.Renner- an endemic and endangered Cucurbit collected from Tea growing regions of Darjeeling hills” has been prepared by me under the supervision of Dr. Malay Bhattacharya, Assistant Professor of Department of Tea Science, University of North Bengal. No part of this thesis has formed the basis for the award of any degree or fellowship

Sourav Chakraborty
[Sourav Chakraborty]
Department of Tea Science,
University of North Bengal
Raja Rammohunpur, Siliguri-734013
Date: *29.06.22*



UNIVERSITY OF NORTH BENGAL

DEPARTMENT OF TEA SCIENCE

P.O. NORTH BENGAL UNIVERSITY, Raja Rammohunpur, Dist. Darjeeling,
West Bengal, INDIA, PIN 734013,

Ref. No.

Dated

Certificate

I certify that Mr. Sourav Chakraborty has prepared the thesis entitled “Exploring bioactivity, isolation of potential biomolecules and *in vitro* regeneration of *Herpetospermum darjeelingense* (C.B.Clarke) H.Schaefer. & S.S.Renner- an endemic and endangered Cucurbit collected from Tea growing regions of Darjeeling hills”, for the award of Ph.D. degree of the University of North Bengal, under my supervision. He has carried out the work at the Department of Tea Science, University of North Bengal. I may further declare all the results incorporated in this thesis have not been submitted for any other degree elsewhere.

Place: Raja Rammohunpur
Date: 29.06.2022

[Dr. Malay Bhattacharya]
Supervisor
University of North Bengal
Raja Rammohunpur, Darjeeling,
West Bengal, India, PIN 734013

Dr. Malay Bhattacharya
Assistant Professor
Department of Tea Science
University of North Bengal

Preface

Research is an interesting domain of gaining insights on fascinating subjects. The Doctor of Philosophy programme is a journey and certainly not a destination, since it serves as an inception to prepare students in thinking independently; discover interesting problems; design and conduct experiments; analyze the deciphered results besides reaching a plausible conclusion. The Ph.D. journey cannot be sailed alone and requires a captain who can guide the ship in reaching a safe shore amidst the turbulent waves analogous to a novice scholar initiating research under an experienced mentor. My Ph.D. journey has been an excellent voyage owing to the perfect guidance of my supervisor Dr. Malay Bhattacharya, Assistant Professor in the Department of Tea Science, University of North Bengal. He has been a friend, philosopher and guide throughout the process starting from my selection of research topic to conducting and analyzing experiments; besides providing valuable inputs in

publishing articles to delivering seminar presentation. I would take the opportunity to express my sincere gratitude towards him on account of his extremely helpful advices, active cooperativeness including providing noble and sympathetic guidance to accomplish this work. I express my heartfelt respect and gratefulness to the other teachers of the Department of Tea Science, namely Dr. Chandra Ghosh and Dr. Sonali Ray for their invaluable priceless suggestions which served in fruitful compilation of the Ph.D. thesis.

Special mention of Prof. (Dr.) Pranab Ghosh of the Department of Chemistry, University of North Bengal is a must on him being a guiding light in meeting my objectives related to natural product chemistry; without whose mentorship the experimental work related to that theme could not be completed. Further, I would also like to acknowledge the inspirational outlook received from Prof. (Dr.) Arnab Sen of the

Department of Botany, University of North Bengal during my studentship during M.Sc. special paper and Post-graduate dissertation.

I was blessed with a fantastic group of lab-mates of Molecular Biology and Tissue Culture Laboratory who worked in unison to help me traverse through this dream namely Mr. Sahadeb Sarkar, Ms. Sumedha Saha, Mr. Soumya Majumder, Mr. Arindam Ghosh, Ms. Sukanya Acharyya, Ms. Baishakhi Ghosh, Ms. Preeti Subba, Ms. Chayanika Rabha, Ms. Sudeshna Nandi, Ms. Reha Labar and Dr. Gargi Sen to whom I express my cordial heartfelt thanks.

I will remiss if I don't acknowledge Mr. Mainak Mukherjee, Soil Analyst, Soil Testing Facility, Department of Tea Science and other office staff of the Tea Science Department for their rendered assistance and cooperation besides affirmative outlook received from Dr. Tanmayee Mishra, another faculty member of the Department of Tea Science for timely completion of this work.

I would like to express my gratitude to my colleagues of Government Model School, Old

Malda and Darjeeling Government College, Darjeeling for their motivational attitude throughout this academic journey.

I am extremely grateful to my parents, family members, friends and well wishers for helping me to have this opportunity, guiding me and providing me constant moral support. Special thanks to my brother Mr. Shantoshubhro Bhattacharya, who helped in photo editing and other vital software issues related to the work.

I must also convey my sincere regards and deep gratitude to all those persons whose names are not stated however have rendered their sympathetic and valuable advice at all stages throughout my life which ultimately paved the way to complete this work.

Last but not the least I bestow my thanks to the Almighty whose blessings were the ultimate requirement to accomplish this herculean task.

Sourav Chakraborty 23.06.22
[Sourav Chakraborty]

LIST OF TABLES

Table	Title	Page
2.1	Major forest types of Darjeeling district of West Bengal	15
2.2	Tea plantation and cultivation area statistics	28
2.3	Location wise distribution of tea estates in Darjeeling district	28
2.4	A statistical account of tea gardens and production outputs	29
2.5	Rank based comparative classification system in Cucurbitaceae	35
2.6	List of some <i>in vitro</i> propagated cucurbit crop plants	60
4.1	GenScan study of nucleotide sequences	98
4.2	ProtParam analysis of peptide sequences	100
4.3	Portrayal of GOR4 and Phyre ² result of amino acid sequences	102
4.4	Prosite analysis of peptide sequences	103
4.5	Domain architecture of amino acid sequences	104
4.6	Physico-chemical parameters of soil expressed in average of the collection sites	112
4.7	Representative overview of qualitative biochemical analysis of sample extracts	114
4.8	Antimicrobial activity of Silver nanoparticle formulation	128
4.9	Detected metabolites in methanolic extract of <i>H. darjeelingense</i> through GC-MS probe	130a
4.10	Identification and study of GC-MS detected bioactive molecules	130 b, c, d
4.11	GC-MS detected metabolites in Petroleum-benzene extracts of <i>H. darjeelingense</i> from Tea and non-Tea growing regions of Darjeeling hills	138
4.12	Biological activity and source of unique GC-MS detected metabolites in Petroleum-benzene extracts of <i>H. darjeelingense</i> from Tea and non-Tea growing regions of Darjeeling hills	140
4.13	GC-MS area % of detected metabolites in manifold locations	143 a
4.14	Correlation analysis based heat map of <i>H. darjeelingense</i> plants growing in manifold ecological spots	145
4.15	Cardinal genes responsible for the synthesis of enzymes controlling the metabologenesi of bioactive molecules	151
4.16	Metabolomic bioenergetics of signature compounds in <i>H. darjeelingense</i>	159
4.17	Binding affinity (Kcal/mol) of protein with ligand targets in respect of hepatoprotective molecule based <i>in silico</i> study	160
4.18	Binding affinity (Kcal/mol) of protein with ligand targets in respect of antidiabetic molecule based <i>in silico</i> study	163
4.19	Physicochemical characterization of test drug molecules	170
4.20	ADMET analysis of test drug compounds	173
4.21	Binding affinity (Kcal/mol) of protein with ligand targets in respect of isolated anti-breast cancer molecule based <i>in silico</i> study	174
4.22	Binding affinity (Kcal/mol) of protein with ligand targets in respect of isolated anti-lung cancer molecule based <i>in silico</i> study	177
4.23	Average growth control % of human breast cancer cell line MDA-MB-231 against isolated pure compounds	180
4.24	Average growth control % of human lung cancer cell line HOP-62 against isolated pure compounds	181
4.25	Effect of plant growth regulators on regeneration of <i>H. darjeelingense</i>	185
4.26	GC-MS detected metabolites of natural plant versus tissue cultured plant	186 a
4.27	Bioactivity and sources of unique metabolites investigated in methanolic extract of tissue cultured plant	189
4.28	Representation of <i>in vitro</i> endophytic fungal growth	198
4.29	GC-MS detected metabolites of EC-HD and EF-HD plant extract	203

LIST OF FIGURES

Figure	Title	Page
2.1	Geological map of the Darjeeling hill territory	30
2.2	Biochemical structure of different Cucurbitacins	41
4.1	rbcL gene based DNA barcode of <i>H. darjeelingense</i>	95
4.2	Nucleotide BLAST analysis of rbcL gene sequence of <i>Herpetospermum darjeelingense</i>	96
4.3	rbcL gene sequence based maximum likelihood type phylogenetic tree	105
4.4	rbcL gene sequence based neighbor joining type phylogenetic tree	106
4.5	(a)- Graphical representation of mean DPPH induced free radical scavenging activity by non polar to polar solvent extracts; interaction and DPPH inhibition kinetics of <i>H. darjeelingense</i> extracts in (b) Acetone, (c) Ethanol and (d) Methanol	118-119
4.6a	Graphical depiction of detection of IC ₅₀ value of Methanol extract	120
4.6b	Graphical depiction of detection of IC ₅₀ value of Acetone extract	120
4.7	Graphical representation of mean ferric reducing power assay of Acetone and Methanol extracts	121
4.8	Graphical representation of solvent wise mean flavonoid content expressed as quercetin equivalent (mg QE/g).	121
4.9a	Graphical representation of mean DPPH induced free radical scavenging activity of non-polar to polar solvent fractions	122
4.9b	Graphical representation of mean percentage of DPPH induced free radical scavenging activity of Ethanol sub-fractions	123
4.10	Estimation of total phenol in various solvent extracts of <i>H. darjeelingense</i>	123
4.11	Graphical portrayal of H ₂ O ₂ scavenging activity	124
4.12	Graphical depiction of NO scavenging activity	124
4.13	Anti-microbial assay against <i>Bacillus subtilis</i> , <i>Escherichia coli</i> and <i>Klebsiella pneumoniae</i>	125
4.14	UV-Visible spectral analysis of Silver nano formulation	126
4.15	Scanning electron micrograph of synthesized biogenic nanoparticles	127
4.16	DPPH scavenging activity of Silver nano formulation	129
4.17	Anti-lipid peroxidation activity of Silver nano formulation	129
4.18 a & b	Representative GC-MS chromatogram of methanolic extracts of <i>H. darjeelingense</i>	131
4.19	Molecular structure of GC-MS detected signature metabolites in methanolic extract of <i>H. darjeelingense</i>	132
4.20	GC-MS chromatogram of Petroleum benzene extracts of <i>H. darjeelingense</i> specimens of tea garden area	137
4.21	GC-MS chromatogram of Petroleum benzene extracts of <i>H. darjeelingense</i> specimens of non-tea garden area	137
4.22	Chemical classes of investigated metabolites in <i>H. darjeelingense</i> samples of tea growing area	139
4.23	Chemical classes of explored metabolites in <i>H. darjeelingense</i> samples of non-tea growing area	139
4.24	Chemical structure of bioactive metabolites in <i>H. darjeelingense</i> samples of tea and non-tea growing area	139
4.25	NTSYS generated chemo-taxonomy based dendrogram	146
4.26	Metabologensis scheme of major bioactive metabolites in <i>H. darjeelingense</i>	148
4.27	Molecular docking study of investigated hepatoprotective compounds	160
4.28	Graphical depiction of anti-lipid peroxidation potential of methanolic sample extracts	162
4.29	Molecular docking study of investigated antidiabetic metabolites	163
4.30	Graphical depiction of antidiabetic potential of methanolic sample extracts	165
4.31	Compound 1	166
4.32	Compound 2	166
4.33	Compound 3	167
4.34	Compound 4	168

4.35	Graphical representation of drug likeness model score of Cerin	172
4.36	Graphical representation of drug likeness model score of Betulinic acid	172
4.37	Graphical representation of drug likeness model score of β -Sitosterol	172
4.38	Graphical representation of drug likeness model score of Lupeol	172
4.39	Graphical representation of drug likeness model score of Adriamycin	172
4.40	Molecular docking study of isolated compounds against breast cancer associated proteins	174 a-e
4.41	Molecular docking study of isolated compounds against lung cancer associated proteins	178 a-e
4.42	Cellular growth curve of human breast cancer cell line MDA-MB-231 against test compounds	181
4.43	Inhibition of growth of MDA-MB-231 cell lines on effect of test compounds	182
4.44	Cellular growth curve of human lung cancer cell line HOP-62 against test compounds	183
4.45	Inhibition of growth of HOP-62 cell lines on effect of test compounds	184
4.46	Different stages of <i>in vitro</i> regeneration	186
4.47	GC-MS chromatogram of methanolic extract of tissue cultured plant	186
4.48	Class wise representation of bioactive metabolites in tissue cultured plant	187
4.49	Signature compounds in methanolic extracts of tissue cultured plant of <i>H. darjeelingense</i>	187
4.50	Operational Cholesterol versus Phytosterol biosynthetic pathway in <i>H. darjeelingense</i>	193
4.51	Comparative metabolomics of terpenoid molecules in naturally growing and tissue cultured plant	194
4.52	Carotenoid biosynthesis pathway operating in tissue cultured plants of <i>H. darjeelingense</i>	194
4.53	Comparative schematic representation of fatty acid derivatives in naturally growing and tissue cultured plant	195
4.54	Culture exhibiting endophytic fungus	198
4.55	GC-MS chromatogram of EC-HD plant extract	199
4.56	Biochemical structures of secondary metabolites detected through GC-MS in EC-HD plant extract	200
4.57	GC-MS chromatogram of EF-HD plant extract	201
4.58	Graph of detected biomolecules in EC-HD and EF-HD sample extract based on GC-MS area percentage	202
4.59	Histopathology of endophyte under compound microscope	217
4.60	Contrasting metabologensis of Terpene derivatives in EC-HD and EF-HD plant extract	219
4.61	Divergent biogenesis of Fatty acid derivatives in EC-HD and EF-HD plant extract	222

LIST OF APPENDICES

	Title	Page #
Appendix A	Selected publications	A1
Appendix B	Datasheet used for collection of germplasm and recording field data	A2
Appendix C	Preparation of Phosphate buffer	A3
Appendix D	List of abbreviations	A4-5