“R.S”

Dedicated to my spiritual Guru

Parama Prememamaya Sri Sri Thakur Anukulchandra

DECLARATION BY THE CANDIDATE

I hereby declare that the thesis entitled **"Studies on *Bungarus fasciatus* venom from Eastern and North East India and characterization of poorly immunodepleted PLA_2 enzymes**" submitted to School of Sciences, Tezpur University, in part fulfilment for the award of the degree of **Doctor of Philosophy** in the Department of Molecular Biology and Biotechnology, is a record of original research work carried out by me. Further, I declare that no part of this thesis has been reproduced elsewhere for award of any other degree.

Amit Talukdar

Place: Tezpur (**Amit Talukdar)**

Date: 08-07-2024 **Registration No.: TZ201043 of 2019**

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CERTIFICATE OF THE PRINCIPAL SUPERVISOR

This is to certify that the thesis entitled "**Studies on *Bungarus fasciatus* venom from Eastern and North East India and characterization of poorly immunodepleted PLA_2 enzymes**" submitted to the School of Sciences, Tezpur University in part fulfilment for the award of the degree of Doctor of Philosophy in Molecular Biology and Biotechnology is a record of original research work carried out by **Mr. Amit Talukdar** under my supervision and guidance.

All help received by him from various sources have been duly acknowledged. No part of this thesis has been submitted elsewhere for award of any other degree.

Place: Tezpur **(Dr. Robin Doley)**

Date: 08-07-2024 **Principal Supervisor**

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- Amit Talukdar

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List of Symbols and Abbreviations

%	Percent
<	Lesser Than
>	Greater Than
=	Equals to
&	And
±	Plus or Minus
Σ	Summation
Å	Angstrom
^{o}C	Degree Celsius
µ	Micron
µg	Microgram
µl	Microlitre
µm	Micrometer
µmol	Micromolar
2G	Second-generation
3D	Three Dimensional
3G	Third-generation
3FTx	Three-Finger Toxin
5′-NUC	5′-Nucleotidase
AChE	Acetylcholinesterase
AMD	Age-Related Macular Degeneration
ANOVA	Analysis of Variance
APS	Ammonium Persulphate
APTT	Activated Partial Thromboplastin Time
BCAV	*Bungarus candidus* Antivenom
BCIP	5-bromo-4-chloro-3-indolyl-phosphate
BF-CT1	*Bungarus fasciatus* Cytotoxin-1
BFMAV	*Bungarus fasciatus* Monovalent Antivenom
BME	2-mercaptoethanol
bnAb	Broadly Neutralizing Antibody
BLASTp	Basic Local Alignment Search Tool-protein

BSA	Bovine Serum Albumin
BSPAV	Bharat Serums Polyvalent Antivenom
$CaCl_2$	Calcium Chloride
CASTp	Computed Atlas of Surface Topography of proteins
CE	Collision Energy
CNBr	Cyanogen Bromide
Co.	Company
CRiSP	Cysteine-Rich Secretory Proteins
CTL	Snake C-Type Lectins
CTX	Cytotoxin
CV	Column Volume
DCE	Directed Chemical Evolution
DLF	Direct Lytic Factor
DTNB	5,5′-Dithiobis(2-nitrobenzoic acid)
E	East
ED_{50}	Median Effective Dose
ELISA	Enzyme-Linked Immunosorbant Assay
ESI	Electrospray Ionization
eV	Electron volt
FHAV	Freeze-Dried Hemorrhagic Antivenom
Fc	Fragment constant
FNAV	Freeze-Dried Neurotoxic Antivenom
FWHM	Full Width at Half Maximum
g	Gram
GMQE	Global Model Quality Estimate
h	Hour
HBS	Habu Serum Factor
HIV	Human Immunodeficiency Virus
HR	Hemorrhagin
i.e.	That is
Ig	Immunoglobulin
i.v	Intravenous
INR	Indian Rupee

ISCICS	Irula Snake Catchers' Industrial Cooperative Society
KCl	Potassium Chloride
kDa	kiloDalton
km	kilometer
KSPI	Kunitz-type Serine Protease Inhibitor
kV	kiloVolt
LAAO	L-Amino Acid Oxidases
LC-MS/MS	Liquid Chromatography-Tandem Mass spectrometry
LD_{50}	Median Lethal Dose
Ltd.	Limited
M	Molar
m	Meter
mA	Milliampere
MALDI-TOF	Matrix Assisted Laser Desorption Ionization-Time of Flight
MeCN	Acetonitrile
mg	Milligram
min	Minute
ml	Millilitre
mm	Millimeter
mM	Millimolar
MS	Mass spectrometry
MW	Molecular weight
MWCO	Molecular weight Cut-off
m/z	Mass-by-Charge ratio
N	North
NaCl	Sodium Chloride
$NaHCO_3$	Sodium Bicarbonate
NAMAV	*Naja atra* Monovalent Antivenom
NBAV	Neuro Bivalent Antivenom
NBT	Nitro Blue Tetrazolium
NCBI	National Center for Biotechnology Information
NCT	Normal Clotting Time
NH_4CO_3	Ammonium Carbonate

nm	Nanometer
No.	Number
n-P	Neutralization Potency
NPAV	Neuro Polyvalent Antivenom
NRi	Non-retained fraction for peak i
O.D	Optical Density
OHMAV	*Ophiophagus hannah* Monovalent antivenom
OHVA-PLA_2	*Ophiophagus hannah* venom acidic Phospholipase A_2
PAGE	Poly-Acrylamide Gel Electrophoresis
PBS	Phosphate-Buffered Saline
PLA_2	Phospholipase A_2
PPP	Platelet-Poor Plasma
PSPAV	Premium Serums Polyvalent Antivenom
PT	Prothrombin Time
PVDF	Polyvinylidene Difluoride
Pvt.	Private
RBC	Red Blood Cell
Ri	Retained fraction for peak i
ROC	Republic of China
RP-HPLC	Reverse-Phase High Performance Liquid Chromatography
rpm	Revolutions per minute
RT	Recalcification Time
s	Second
SABU	Serum Anti Bisa Ular
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus-2
scFv	Single Chain Variable fragment
sdAb	Single-Domain Antibody fragment
SDS	Sodium Dodecyl Sulphate
S.E	Standard Error
SELEX	Systematic Evolution of Ligands by Exponential enrichment
SOP	Standard Operating Procedure
$sPLA_2$	Secreted Phospholipase A_2
Snaclecs	Snake c-type lectin-like proteins

SSI	Small Synthetic Inhibitor
SVHY	Snake Venom Hyaluronidase
SVMP	Snake Venom Metalloproteinase
SVSP	Snake Venom Serine Protease
TBS	Tris-Buffered Saline
TBST	Tris-Buffered Saline with Tween 20
TCA	Trichloroacetic Acid
TEMED	N,N,N',N'-tetramethylethylenediamine
TFA	Trifluoroacetic Acid
USA	United States of America
US FDA	United States Food and Drug Administration
V	Volt
VEGF	Vascular Endothelial Growth Factor
V_HH	Heavy-Chain Variable Domain
Vi	Chromatographic area of peak i
VICC	Venom Induced Consumption Coagulopathy
VNGF	Vascular Nerve Growth Factor
VPAV	VINS Polyvalent Antivenom
v/v	Volume per volume
WHO	World Health Organization