

## Contents

---

	<b>Page No.</b>
<b>Abstract</b>	<b>i-iii</b>
<b>Declaration by Candidate</b>	<b>Iv</b>
<b>Certificate of Supervisor</b>	<b>v</b>
<b>Acknowledgement</b>	<b>vi-vii</b>
<b>Table of Contents</b>	<b>viii-xi</b>
<b>List of Figures</b>	<b>xii-xiv</b>
<b>List of Tables</b>	<b>xv</b>
<b>List of Abbreviations &amp; Symbols</b>	<b>xvi-xviii</b>
<b>Chapter 1: General Introduction and Review of Literature</b>	<b>1-18</b>
1.1. Snakebite: a global perspective	1
1.2. Snakebite in India	2
1.3. Antivenom therapy and its limitations	3-5
1.4. Venomics and Proteomics	5-6
1.5. Asian pit vipers	7
1.6. Green pit vipers	7-11
1.7. Green pit vipers of India	11-14
1.8. Haemostatic system	14-15
1.9. Snake venom protein targeting haemostatic system	15
1.10. Snake venom thrombin-like enzymes (SVTLEs)	15-17
1.11. Aim of the study	17
1.12. Objectives	18
<b>Chapter 2: Materials and Methodology</b>	<b>19-35</b>
2.1. Materials	19-22
2.1.1. Venom collection	19
2.1.2. Antivenoms	20-21
2.1.3. Chemicals and Reagents	21-22

2.1.4. Columns	22
2.2. Methodology	23-35
2.2.1. Profiling and fractionation of venom	22-24
2.2.1.1. SDS-PAGE	22
2.2.1.2. Rp-HPLC	23-24
2.2.2. In solution trypsin digestion and LC-MS/MS	24-25
2.2.3. <i>In vitro</i> enzyme activity	25-31
2.2.3.1. Phospholipase A <sub>2</sub> activity	25
2.2.3.2. Coagulation activity	26-27
2.2.3.3. Thrombin-like activity assay	27
2.2.3.4. Plasmin-like activity	27
2.2.3.5. Caseinolytic activity	28
2.2.3.6. Haemolytic activity	28-29
2.2.3.7. Platelet aggregation activity	29-30
2.2.3.8. Plasminogen activation activity	30
2.2.3.9. Fibrinogenolytic activity	30-31
2.2.3.10. Fibrinolytic activity	31
2.2.3.11. Plasma clot formation/dissolution activity	31
2.2.4. Immuno-reactivity assays	31-34
2.2.4.1. Immuno-blotting study	32
2.2.4.2. Neutralization of biochemical activities	32-33
2.2.4.3. Immuno-depletion study	33-34
2.2.5. Clinical case study	34
2.2.6. Computational studies	34-35
2.2.6.1. Multiple sequence alignment	34
2.2.6.2. Homology modelling and Structure validation	35
<b>Chapter 3: Comparative proteomic and biochemical profiling of Indian green pit vipers</b>	<b>36-50</b>
3.1. Introduction	36-37
3.2. Results	37-45

3.2.1. Comparative analysis of SDS-PAGE profiles	37-38
3.2.2. Comparative analysis of chromatographic profiles	38-40
3.2.3. Phospholipase A <sub>2</sub> activity	40-41
3.2.4. Coagulation activity	41-42
3.2.5. Thrombin-like activity	42-43
3.2.6. Proteolytic activity	43-44
3.2.7. Haemolytic activity	44-45
3.3. Discussion	46-50
<b>Chapter 4: Immunological cross-reactivity of Indian green pit viper venoms with Indian Polyvalent Antivenom and Thai Green pit viper monovalent antivenom</b>	51-64
4.1. Introduction	51-52
4.2. Results	52-60
4.2.1. Immuno-blot analysis	52-53
4.2.2. Neutralization of biochemical activities by antivenoms	53-56
4.2.3. Immuno-depletion study	56-59
4.2.4. Clinical data on green pit viper bites	59-60
4.3. Discussion	60-64
<b>Chapter 5: Proteomics of <i>Trimeresurus erythrurus</i> venom from Mizoram, India</b>	65-99
5.1. Introduction	65-66
5.2. Results	66-99
5.2.1. Fractionation of crude venom by Rp-HPLC	66-67
5.2.2. SDS-PAGE analysis of Rp-HPLC fractions	67-68
5.2.3. Protein decomplexation by ESI-LC-MS/MS	68-89
5.2.4. Platelet aggregation effect of crude venom	90
5.3. Discussion	91-99
<b>Chapter 6: Characterization of Erythrofibrase: a haemostatically active protein from <i>Trimeresurus erythrurus</i> venom</b>	100-119
6.1. Introduction	100-101
6.2. Results	101-113

6.2.1. Purification of erythrofibrase from <i>Trimeresurus erythrurus</i> venom	101-104
6.2.2. Functional characterization of erythrofibrase	104-105
6.2.3. Effect of erythrofibrase on fibrinogenolytic and fibrinolytic system	105-109
6.2.4. Sequence alignment of erythrofibrase and structure prediction	110-112
6.2.5. Immuno-reactivity of erythrofibrase with Antivenom:	112-113
6.3. Discussion	113-119
<b>Chapter 7: Conclusion and Future prospects</b>	120-122
7.1. Conclusion	120-121
7.2. Future prospects	122
<b>Bibliography</b>	123-145
<b>List of publications</b>	146
<b>Appendix I:</b> Alignment of peptide fragments obtained from LC-MS/MS of <i>Trimeresurus erythrurus</i> venom	xix
<b>Appendix II:</b> Permissions and Approvals from Ethical committee	xx
<b>Appendix III:</b> Reprint of publication	xxi

---