

## TABLE OF CONTENTS

DECLARATION	ii
CERTIFICATE	iii
ABSTRACT	v-ix
ACKNOWLEDGEMENT	x-xi
TABLE OF CONTENTS	xii-xvii
LIST OF TABLES	xviii-xix
LIST OF FIGURES	xx-xxix
LIST OF APPENDIX	xxx

### Chapter I

<b>1</b>	<b>INTRODUCTION</b>	1-8
----------	---------------------	-----

### Chapter II

<b>2</b>	<b>LITERATURE REVIEW</b>	9-32
2.1	Importance of ethnomedicinal survey	9-10
2.2	Ethnomedicine in Sikkim with emphasis on west district	10-11
2.3	Necessity of pharmacognostic studies	12-13
2.4	Free radicals and oxidative stress	13-14
2.5	Plants as natural antioxidants	14-15
2.6	Phytochemical studies in plants	16-17
2.7	<b>Pharmacological activities of herbal formulations</b>	17-28
2.7.1	Anti-inflammatory activity	17-19
2.7.2	Antimicrobial activity	19-21
2.7.3	Hepatoprotective activity	21-22
2.7.4.	Antidiabetic activity	23-25
2.7.5	Cytotoxic activity	25-26
2.7.6	Anti-hypertensive activity	26-28
2.8	Influence of extraction methods on extraction of bioactive compounds from plants	28-30
2.9	Isolation of bioactive phytoconstituents from plants and bioassay guided purification	30-31
2.10	Identification of bioactive compound of interest	32

<b>Chapter III</b>		
<b>3</b>	<b>MATERIALS AND METHODS</b>	37-60
3.1	<b>Study area</b>	37
3.1.1	Temperature	37
3.1.2	Rainfall	37
3.1.3	Sunshine Hours	37
3.2	Survey	38
3.3	<b>Pharmacognostic study</b>	38-41
3.3.1	Organoleptic Tests and Powder Microscopy	39
3.3.2	Physicochemical study	39
3.3.2a	Determination of total ash	39
3.3.2b	Determination of acid insoluble ash	40
3.3.2c	Determination of water soluble ash	40
3.3.2d	Determination of solvent extractive values	40
3.3.2d (i)	Alcohol soluble extractive value	40
3.3.2d (ii)	Water soluble extractive value	41
3.3.2e	Loss on drying	41
3.3.2f	Determination of ph values	41
3.3.3	Fluorescence analysis	41
3.4	Thin layer chromatography (tlc)	42
3.5	<b>Qualitative phytochemical estimation</b>	42-45
3.5.1	Test for amino acids	43
3.5.2	Test for anthraquinones	43
3.5.3	Test for phytosterol	43
3.5.4	Test for tannin	43
3.5.5	Test for triterpenoids	44
3.5.6	Test for cardiac glycosides	44
3.5.7	Test for alkaloids	44
3.5.8	Test for glycosides	44
3.5.9	Reducing sugar	45

3.5.10	Test for flavonoids	45
3.5.11	Test for resin	45
3.6	<b>Quantitative estimation of phytochemicals</b>	45-47
3.6.1	Total alkaloid content (TAC)	45
3.6.2	Total tannin content (TC)	46
3.6.3	Total steroid content (TSC)	46
3.6.4	Total phenol content (TPC)	47
3.6.5	Total flavonoid content (TFC)	47
3.6.6	Total orthodihydric phenol (TOPC)	47
3.7	Antimicrobial activity	48
3.8	<i>In vitro</i> cytotoxic activity	48-49
3.9	<i>In vitro</i> antidiabetic activity	49
3.10	<b>Antioxidant activity</b>	50-51
3.10.1	1,1-diphenyl-2-picrylhydrazyl scavenging assay (DPPH)	50
3.10.2	2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt scavenging activity (ABTS <sup>+</sup> )	50
3.10.3	Superoxide scavenging activity (SO)	50
3.10.4	Nitric oxide scavenging activity (NO)	51
3.10.5	Metal chelating activity (MC)	51
3.10.6	Ferric reducing antioxidant power (FRAP)	51
3.11	Anti-hypertensive activity (AHA)	52
3.12	Influence of extraction methods on the bioactivity of <i>fraxinus floribunda</i>	52-53
3.13	<b><i>In vivo</i> pharmacological activity</b>	53-56
3.13.1	Antidiabetic activity in streptozotocin-induced diabetic rats	53-56
3.13.1a	Acute toxicity study	54
3.13.1b	Oral glucose tolerance test (OGTT)	54
3.13.1c	Induction of non-insulin dependent Diabetes mellitus (NIDDM)	54
3.13.1d	Bodyweight	54
3.13.1e	Estimation of biochemical parameters	55

3.13.1f	Histopathology	55
3.13.2	Anti-inflammatory activity	55
3.13.3	Hepatoprotective activity	56
3.14	<b>Bioassay-guided partial purification</b>	56-60
3.14.1	Sample preparation	56-57
3.14.2	Preliminary screening	57
3.14.3	Column chromatography	57-58
3.14.4	Thin layer chromatography (TLC) and merging of fractions	58
3.14.5	Gas Chromatography - Mass Spectrometry analysis	58-59
3.14.6	Nuclear magnetic resonance spectroscopy (NMR)	59-60
3.14.6a	Sample preparation	59-60
3.14.6b	NMR analysis	60
<b>Chapter IV</b>		
<b>4</b>	<b>RESULTS</b>	62-254
4.1	Survey and documentation of ethnomedicine used by the traditional herbal practitioners	62-63
4.2	<b>Pharmacognostic study</b>	90-150
4.2.1	Organoleptic study	90
4.2.2	Powder microscopy	90
4.2.3	Physicochemical analysis	104
4.2.4	Fluorescence study	104
4.2.5	Thin layer chromatography	150
4.3	<b>Estimation of phytochemicals content</b>	158-166
4.3.1	Qualitative estimation of phytochemicals	158-166
4.3.2.	Quantitative estimation of phytochemicals	166
4.4	<b>Antimicrobial activity</b>	166-173
4.5	<b><i>In vitro</i> cytotoxic activity</b>	173-193
4.6	<b><i>In vitro</i> antidiabetic activity</b>	193-199
4.7	<b>Free radical scavenging activity</b>	199-204
4.8	<b>Anti-hypertensive activity</b>	204
4.9	<b>Correlation</b>	204-210

4.9.1	Pearson correlation	204-209
4.9.2	Principal Component Analysis	209-210
4.10	<b>Influence of extraction methods on the bioactivity of</b>	213-218
	<i>Fraxinus floribunda</i>	
4.10.1	Antioxidant activity	213-214
4.10.2	Phytochemical content	214
4.10.3	<i>In vitro</i> antidiabetic activity	214-218
4.11	<b>Pharmacological assays</b>	218-229
4.11.1	Anti-inflammatory activity	218
4.11.2	Hepatoprotective activity	218-223
4.11.3	<i>In vivo</i> antidiabetic activity	223--229
4.11.3.a	Acute toxicity study	223
4.11.3.b	Oral glucose tolerance test (OGTT)	223
4.11.3.c	Effect on fasting blood glucose	224
4.11.3.d	Body weight	224
4.11.3.e	Effect of extracts on lipid profile	224
4.11.3.f	Histopathology	224-229
4.12	<b>Bioassay guided partial purification of bark of</b>	229-247
	<i>fraxinus floribunda</i>	
4.12.1	Column and Thin layer chromatography	229-230
4.12.2	GC-MS analysis	230
4.12.3	NMR analysis	246-247
 <b>Chapter V</b>		
<b>5</b>	<b>DISCUSSION</b>	255-293
5.1	Survey of ethnomedicine in west Sikkim	255-257
5.2	<b>Pharmacognostic study</b>	257-264
5.2.1	Organoleptic tests	258
5.2.2	Powder microscopy	259
5.2.3	Physicochemical analysis	259-261
5.2.4	Fluorescence analysis	261
5.2.5	Thin layer chromatography	261-264

5.3	Phytochemical content	265-267
5.4	Antimicrobial activity	268-269
5.5	<i>In vitro</i> cytotoxic activity	269-270
5.6	<i>In vitro</i> antidiabetic activity	271-272
5.7	Antioxidant or free radical scavenging activity	272-277
5.8	Anti-hypertensive activity	277-278
5.9	<b>Correlation</b>	278-279
5.9.1	Pearson correlation	278-279
5.9.2	Principal component analysis	279
5.10	<b>Influence of extraction methods on the bioactivity of <i>Fraxinus floribunda</i></b>	280-83
5.10.1	Antioxidant activity	281-282
5.10.2	<i>In vitro</i> antidiabetic activity	282-283
5.11	<b>Pharmacological assays</b>	283-287
5.11.1	Anti-inflammatory activity	284-285
5.11.2	Hepatoprotective activity	285-286
5.11.3	<i>In vivo</i> antidiabetic activity	286-287
5.12	<b>Bioassay guided partial purification of bark of <i>Fraxinus floribunda</i></b>	288-294
5.12.1	GC-MS analysis and identification of phytochemicals	288-292
5.12.2	NMR analysis	292-293
<b>Chapter VI</b>		
<b>6</b>	<b>CONCLUSION</b>	295
	<b>SUMMARY</b>	296-298
	<b>BIBLIOGRAPHY</b>	299-344
	<b>INDEX</b>	345-347
	<b>APPENDIX-A</b>	
	Chemicals used	348-349
	<b>APPENDIX-B</b>	
	Abbreviation used	350-354
	<b>APPENDIX-C</b>	
	List of publications	355
	<b>APPENDIX-D</b>	
	Animal ethical committee certificate	360
	<b>APPENDIX-E</b>	
	Published articles [Based on the Thesis works]	361