ACKNOWLEDGEMENT

“In the name of God, the most gracious, most merciful”

It is my cherish privilege to express my deep sense of gratitude to Uttarakhand Technical University, Dehradun and I consider myself lucky to be a part of this University.

I bow to the feet of my guide and supervisor Prof. (Dr.) Avijit Mazumder, Director, Pharmacy Institute of Noida Institute of Engineering & Technology, Greater Noida, UP, whose inspiring, priceless guidance and enthusiasm with never ending drive for work, was a constant source of inspiration for me to accomplish this thesis work with zest and zeal. He was my mentor and research guide at M. Pharm level too.

I convey my gratitude to my co-supervisor Prof. (Dr.) Shamim Ahmad, Director, Translam Institute of Pharmaceutical Education and Research, Meerut, UP, who always had a lending ear to all kind of problems. His valuable suggestions were very fruitful for shaping up my ideas and research, without which my effort would have not been rewarded.

I am also grateful to the Prof. (Dr.) Umesh Kumar Singh, Principal, Kharvel Subharti College of Pharmacy, S.V. Subharti University, Meerut for providing me the necessary facilities and infrastructure to pursue my research work.

I owe my sincere respect to Dr. Ranjit Singh, Dr Rupa Mazumder, Dr. Anurag, Dr. Manddeep K Arora, Dr. Manish Sinha and Mr. Ankit Kumar for their valuable, inspiring suggestions with constant encouragement and support in analytical works, which provided impetus and paved the way for the successful completion of this research work.

I express my gratitude to all the faculty members of the Department of Pharmacy, LLRM Medical College, Meerut for believing in me and constantly cheering me to do the best.

I am also thankful to the Central Drug Research Institute, Lucknow, UP, for providing me the analytical facilities to pursue my research work.

On a personal note, I would like to express deep gratitude to my family who provided me constant encouragement and support to pursue my research work.

My generous gratitude to all laboratory animals sacrificed for finding the solutions of the aimed questions in this research work. I would always look out for ways to put in to practice, the ‘3R’ principles to make it easier, for animal welfare.

At last but not least, I acknowledge all those who knowingly and unknowingly contributed in making my work easier and a real success.

Date: Amrendra Kumar Chaudhary
DEDICATED
TO MY
FAMILY
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Certificate</td>
<td>ii</td>
</tr>
<tr>
<td>Abstract</td>
<td>iii</td>
</tr>
<tr>
<td>Acknowledgement</td>
<td>vii</td>
</tr>
<tr>
<td>Dedication</td>
<td>viii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xiv</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xv</td>
</tr>
<tr>
<td>List of Symbols and Abbreviations</td>
<td>xviii</td>
</tr>
</tbody>
</table>

## CHAPTER 1: INTRODUCTION 1-42

1.1 Neuropharmacology
   - 1.1.1 Learning and Memory 1
   - 1.1.2 Dementia 10
   - 1.1.3 Nootropics 12
   - 1.1.4 Nutrition and Memory 12
   - 1.1.5 Mechanistic approach of phytoconstituents for antiamnesic activity 13

1.2 Wound
   - 1.2.1 Burn wound 14
   - 1.2.2 Wound healing 19

1.3 Peptic ulcer
   - 1.3.1 Epidemiology 25
   - 1.3.2 Gastric anatomy 25
   - 1.3.3 Classification of peptic ulcer 26
   - 1.3.4 Symptom 26
   - 1.3.5 Etiology 26
   - 1.3.6 Pathophysiology 27
   - 1.3.7 Diagnosis 29
1.3.8 Treatment of peptic ulcer disease 30
1.3.9 Mechanistic approach of phytoconstituents for antiulcer activity 32
1.4 Infections 33
1.4.1 Mechanistic approach of phytoconstituents for antimicrobials activity 33
1.5 Rationality of herbal medication instead of synthetic 34
1.6 Mechanistic approach of herbs at cellular levels 35
1.7 Plants profile 37
1.7.1 Cedrus deodara Loud. 37
1.7.2 Pinus roxburghii Sarg. 39

CHAPTER 2: LITERATURE REVIEW 43-60

2.1 Review of pharmacological and phytochemical studies of Cedrus deodara Loud. 43
2.2 Review of pharmacological and phytochemical studies of Pinus roxburghii Sarg. 55
2.3 Aim and Objective 60

CHAPTER 3: MATERIALS AND METHODS 61-81

3.1 Chemicals and drugs 61
3.2 Animals 61
3.3 Ethical consideration 61
3.4 Methodology 62
3.4.1 Collection and authentication of plant material 62
3.4.2 Drying and comminution of plant material 62
3.4.3 Isolation of volatile oils 62
3.4.4 Preparation of plant extracts 62
3.4.5 Preliminary phytochemical testing 63
3.4.6 Thin layer chromatography profiling 67
3.4.7 Dose selection 68
3.4.8 Antimicrobial screening: Agar dilution method 68
3.4.9 Memory enhancing activity: Morris water maze test 70
3.4.9.1 Behavioral testing
3.4.9.2 Biochemical estimation of markers of oxidative stress
3.4.10 Wound healing activity
  3.4.10.1 Acute dermal toxicity
  3.4.10.2 In-vivo healing evaluation: “Burn wound model”
3.4.11 Determination of in-vitro antioxidant activity of oils and extracts
  3.4.11.1 Total antioxidant capacity
  3.4.11.2 Reducing power assay
3.4.12 Antiulcer activity
  3.4.12.1 Pylorus ligation induced gastric ulceration
  3.4.12.2 Ethanol induced gastric ulcers
  3.4.12.3 Histopathological evaluation
3.4.13 Isolation and characterization of phytoconstituents from most bioactive extract
  3.4.13.1 Column chromatography of chloroform extract of Cedrus deodara Loud.
  3.4.13.2 Processing of the isolated fractions
  3.4.13.3 Characterization of isolated compounds
3.4.14 Determination of in-vitro antioxidant activity of isolated compounds
  3.4.14.1 Total antioxidant capacity
  3.4.14.2 Reducing power assay
3.5 Statistical analysis

CHAPTER 4: RESULTS
4.1 Collection and authentication of plant materials
4.2 Isolation of volatile oils
4.3 Preparation of plant extracts
4.4 Preliminary phytochemical testing
4.5 TLC profiling of volatile oils and extracts
4.6 Antimicrobial screening of volatile oils and extracts  
4.7 Standardization of different biochemical parameters used in study  
4.8 Effect of Cedrus deodara Loud. and Pinus roxburghii Sarg. on memory  
  4.8.1 Effect of volatile oils and chloroform extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg. on escape latency of mice using MWM Paradigm  
  4.8.2 Effect of volatile oils and chloroform extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg. on probe trial of mice  
  4.8.3 Effect of volatile oils and chloroform extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg. on MDA and GSH in frontal cortex and hippocampus of MWM behavior experienced mice  
4.9 Effect of Cedrus deodara Loud. and Pinus roxburghii Sarg. on wound  
  4.9.1 Effect of volatile oils and chloroform extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg. on wound contraction  
  4.9.2 Effect of volatile oils and chloroform extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg. on epithelialization time  
  4.9.3 Effect of volatile oils and chloroform extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg. on hydroxyproline content  
4.10 In-vitro antioxidant activity of volatile oils and extracts  
  4.10.1 Total antioxidant capacity  
  4.10.2 Reducing power ability  
4.11 Effect of Cedrus deodara Loud. and Pinus roxburghii Sarg. on ulcer  
  4.11.1.1 Effect of chloroform extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg. on gastric content, pH, total and free acidity in pylorus ligation induced ulceration  
  4.11.1.2 Effect of Cedrus deodara Loud. and Pinus roxburghii Sarg. on number of ulcer, ulcer score and ulcer index in pylorus ligation induced ulceration  
  4.11.2 Effect of Cedrus deodara Loud. and Pinus roxburghii Sarg. on number of ulcer, ulcer score and ulcer index in ethanol induced gastric ulcers  
  4.11.3 Histopathological evaluation
4.12 Isolation and structural elucidation of compounds from chloroform extract of

_Cedrus deodara_ Loud.

4.12.1 TLC Profiling

4.12.2 Spectral identification of compounds

4.13 _In-vitro_ antioxidant activity of isolated compounds from chloroform extract of

_Cedrus deodara_ Loud.

4.13.1 Total antioxidant capacity

4.13.2 Reducing power ability

CHAPTER 5: DISCUSSION

CHAPTER 6: CONCLUSION

REFERENCES

APPENDICES

Appendix I: Animal approval certificate

Appendix II: Authentication certificate of _Cedrus deodara_ Loud.

Appendix III: Authentication certificate of _Pinus roxburghii_ Sarg

LIST OF PUBLICATIONS

CURRICULUM VITAE
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Different dilution of extracts, volatile oils and standard drugs in agar dilution susceptibility tests</td>
<td>70</td>
</tr>
<tr>
<td>3.2</td>
<td>The experimental protocol for memory enhancing activity</td>
<td>71</td>
</tr>
<tr>
<td>4.1</td>
<td>Percentage yield of volatile oils of Cedrus deodara Loud. and Pinus roxburghii Sarg.</td>
<td>84</td>
</tr>
<tr>
<td>4.2</td>
<td>Phytoconstituents present in volatile oils of the Cedrus deodara Loud. and Pinus roxburghii Sarg.</td>
<td>85</td>
</tr>
<tr>
<td>4.3</td>
<td>Phytoconstituents present in chloroform and methanolic extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg.</td>
<td>85</td>
</tr>
<tr>
<td>4.4</td>
<td>Antibacterial activity of volatile oils, chloroform extract and methanolic extract of Cedrus deodara Loud. by dilution method</td>
<td>89</td>
</tr>
<tr>
<td>4.5</td>
<td>Antifungal activity of volatile oils, chloroform extract and methanolic extract of Cedrus deodara Loud. by dilution method</td>
<td>90</td>
</tr>
<tr>
<td>4.6</td>
<td>Antibacterial activity of volatile oils, chloroform extract and methanolic extract of Pinus roxburghii Sarg. by dilution method</td>
<td>90</td>
</tr>
<tr>
<td>4.7</td>
<td>Antifungal activity of volatile oils, chloroform extract and methanolic extract of Pinus roxburghii Sarg. by dilution method</td>
<td>90</td>
</tr>
<tr>
<td>4.8</td>
<td>Effect of volatile oils and chloroform extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg. on reference memory during trial 1 on MWM paradigm</td>
<td>94</td>
</tr>
<tr>
<td>4.9</td>
<td>Effect of volatile oils and chloroform extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg. on MDA and GSH level in frontal cortex and hippocampus of MWM behavioral tested mice</td>
<td>99</td>
</tr>
<tr>
<td>4.10</td>
<td>Effect of volatile oils and chloroform extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg. on epithelialization time and hydroxyproline content</td>
<td>101</td>
</tr>
<tr>
<td>4.11</td>
<td>Effect of chloroform extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg. on gastric content, pH, total acidity and free acidity in pylorus ligation induced ulceration in rats</td>
<td>104</td>
</tr>
<tr>
<td>4.12</td>
<td>Pooled fraction of chloroform extract of Cedrus deodara Loud.</td>
<td>109</td>
</tr>
<tr>
<td>4.13</td>
<td>$^1$H-NMR (300 MHz) spectral data of CdC-B in CDCl$_3$</td>
<td>112</td>
</tr>
<tr>
<td>4.14</td>
<td>$^1$H-NMR (300 MHz) spectral data of CdC-C in CDCl$_3$</td>
<td>115</td>
</tr>
<tr>
<td>4.15</td>
<td>$^1$H-NMR (300 MHz) spectral data of CdC-F in CDCl$_3$</td>
<td>117</td>
</tr>
<tr>
<td>4.16</td>
<td>$^1$H-NMR (300 MHz) spectral data of CdC-G in CDCl$_3$</td>
<td>120</td>
</tr>
<tr>
<td>4.17</td>
<td>$^1$H-NMR (300 MHz) spectral data of CdC-H in CDCl$_3$</td>
<td>122</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Fig. No.</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Taxonomy of long term memory system together with specific brain structures involved in each system</td>
<td>3</td>
</tr>
<tr>
<td>1.2</td>
<td>Memory model for the waking brain</td>
<td>4</td>
</tr>
<tr>
<td>1.3</td>
<td>Memory model for the sleeping brain</td>
<td>5</td>
</tr>
<tr>
<td>1.4</td>
<td>Schematic diagram of memory process</td>
<td>7</td>
</tr>
<tr>
<td>1.5</td>
<td>Schematic diagram of Morris water maze</td>
<td>8</td>
</tr>
<tr>
<td>1.6</td>
<td>Etiopathogenesis of wound</td>
<td>16</td>
</tr>
<tr>
<td>1.7</td>
<td>The process of normal wound healing</td>
<td>20</td>
</tr>
<tr>
<td>1.8</td>
<td>The development of gastric and duodenal ulcers</td>
<td>28</td>
</tr>
<tr>
<td>1.9</td>
<td>The plant <em>Cedrus deodara</em> Loud.: (A) Tree, (B) Needles &amp; cone and (C) Stem wood</td>
<td>38</td>
</tr>
<tr>
<td>1.10</td>
<td>The plant <em>Pinus roxburghii</em> Sarg.: (A) Tree, (B) Needles &amp; cone and (C) Stem wood</td>
<td>42</td>
</tr>
<tr>
<td>4.1</td>
<td>TLC of volatile oils of <em>Cedrus deodara</em> Loud.</td>
<td>86</td>
</tr>
<tr>
<td>4.2</td>
<td>TLC of volatile oils of <em>Pinus roxburghii</em> Sarg.</td>
<td>86</td>
</tr>
<tr>
<td>4.3</td>
<td>TLC of chloroform extract of <em>Cedrus deodara</em> Loud.</td>
<td>87</td>
</tr>
<tr>
<td>4.4</td>
<td>TLC of chloroform extract of <em>Pinus roxburghii</em> Sarg.</td>
<td>87</td>
</tr>
<tr>
<td>4.5</td>
<td>TLC of methanolic extract of <em>Cedrus deodara</em> Loud.</td>
<td>88</td>
</tr>
<tr>
<td>4.6</td>
<td>TLC of methanolic extract of <em>Pinus roxburghii</em> Sarg.</td>
<td>88</td>
</tr>
<tr>
<td>4.7</td>
<td>Standard curve for estimation of protein</td>
<td>91</td>
</tr>
<tr>
<td>4.8</td>
<td>Standard curve for estimation of MDA</td>
<td>92</td>
</tr>
<tr>
<td>4.9</td>
<td>Standard curve for estimation of GSH</td>
<td>92</td>
</tr>
<tr>
<td>4.10</td>
<td>Effect of volatile oils and chloroform extracts of <em>Cedrus deodara</em> Loud. and <em>Pinus roxburghii</em> Sarg. on reference memory during trial 1 as compared to control group on MWM paradigm</td>
<td>95</td>
</tr>
<tr>
<td>4.11</td>
<td>Effect of volatile oils and chloroform extracts of <em>Cedrus deodara</em> Loud. and <em>Pinus roxburghii</em> Sarg. on working memory during trial 1 and trial 2 on MWM paradigm</td>
<td>96</td>
</tr>
<tr>
<td>4.12</td>
<td>Representation of swimming paths during the MWM in the last day of evaluation (day 7): (A) Control, (B) Piracetam, (C) Cd O 50, (D) Cd O100, (E) Pr O 50, (F) Pr O 100, (G) Cd C 50, (H) Cd C 100, (I) Pr C 50 and (J) Pr C 100</td>
<td>97</td>
</tr>
<tr>
<td>4.13</td>
<td>Effect of volatile oils and chloroform extracts of <em>Cedrus deodara</em> Loud. and <em>Pinus roxburghii</em> Sarg. on probe trial of mice using MWM paradigm</td>
<td>98</td>
</tr>
<tr>
<td>4.14</td>
<td>Effect of volatile oils and chloroform extracts of <em>Cedrus deodara</em> Loud. and <em>Pinus roxburghii</em> Sarg. on percentage wound contraction</td>
<td>100</td>
</tr>
<tr>
<td>4.15</td>
<td>Total antioxidant capacity of volatile oils and chloroform extracts of <em>Cedrus deodara</em> Loud. and <em>Pinus roxburghii</em> Sarg.</td>
<td>102</td>
</tr>
<tr>
<td>4.16</td>
<td>Reducing power activity of the volatile oils and chloroform extracts of <em>Cedrus deodara</em> Loud. and <em>Pinus roxburghii</em> Sarg.</td>
<td>103</td>
</tr>
</tbody>
</table>
4.17 Effect of chloroform extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg. on number of ulcers (A), ulcer score (B), ulcer index (C) and percentage ulcer inhibition (D) in the ulcerated region of pylorus ligation induced ulcers in rats  
105

4.18 Effect of chloroform extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg. on number of ulcers (A), ulcer score (B), ulcer index (C) and percentage ulcer inhibition (D) in the ulcerated region of ethanol induced gastric ulcers in rats  
106

4.19 Effect of chloroform extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg. on pylorus ligation induced gastric ulcer in rats. The section of stomach tissue was stained with hematoxylin and eosin to assess the pathological changes using light microscopy (x100): (A) received vehicle, (B) received standard, (C) received chloroform extract of Cedrus deodara Loud. at 50 mg/kg, (D) received chloroform extract of Cedrus deodara Loud. at 100 mg/kg, (E) received chloroform extract of Pinus roxburghii Sarg. at 50 mg/kg and (F) received chloroform extract of Pinus roxburghii Sarg. at 100 mg/kg  
107

4.20 Effect of chloroform extracts of Cedrus deodara Loud. and Pinus roxburghii Sarg. on ethanol induced gastric ulcer in rats. The section of stomach tissue was stained with hematoxylin and eosin to assess the pathological changes using light microscopy (x100): (A) received vehicle, (B) received standard, (C) received chloroform extract of Cedrus deodara Loud. at 50 mg/kg, (D) received chloroform extract of Cedrus deodara Loud. at 100 mg/kg, (E) received chloroform extract of Cedrus deodara Loud. at 100 mg/kg, (E) received chloroform extract of Pinus roxburghii Sarg. at 50 mg/kg and (F) received chloroform extract of Pinus roxburghii Sarg. at 100 mg/kg  
108

4.21 TLC of isolated compounds form chloroform extract of Cedrus deodara Loud.  
109

4.22 HRESI-Mass spectra of compound CdC-B  
110

4.23 FT-IR spectra of CdC-B  
111

4.24 $^1$H-NMR spectrum of compound CdC-B  
111

4.25 Structure of compounds isolated from chloroform extract of Cedrus deodara Loud.  
112

4.26 HRESI-Mass spectra of compound CdC-C  
113

4.27 FT-IR spectra of CdC-C  
114

4.28 $^1$H-NMR spectrum of compound CdC-C  
114

4.29 HRESI-Mass spectra of compound CdC-F  
116

4.30 FT-IR spectra of CdC-F  
116

4.31 $^1$H-NMR spectrum of compound CdC-F  
117

4.32 HRESI-Mass spectra of compound CdC-G  
118

4.33 FT-IR spectra of CdC-G  
119

4.34 $^1$H-NMR spectrum of compound CdC-G  
119
<table>
<thead>
<tr>
<th>4.35</th>
<th>HRESI-Mass spectra of CdC-H</th>
<th>121</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.36</td>
<td>FT-IR spectra of CdC-H</td>
<td>121</td>
</tr>
<tr>
<td>4.37</td>
<td>$^1$H-NMR spectrum of compound CdC-H</td>
<td>122</td>
</tr>
<tr>
<td>4.38</td>
<td>Total antioxidant capacity of chloroform extract of <em>Cedrus deodara</em> Loud. and isolated compounds</td>
<td>123</td>
</tr>
<tr>
<td>4.39</td>
<td>Reducing power activity of chloroform extract of <em>Cedrus deodara</em> Loud. and isolated compounds.</td>
<td>124</td>
</tr>
</tbody>
</table>
LIST OF SYMBOLS AND ABBREVIATIONS

- % v/v  Percent volume by volume
- % w/w  Percent weight by weight
- -/+  Not detected/detected
- <  Less than
- >  Greater than
- °C  Degree centigrade
- µg  Micro gram
- µg/ml  Microgram per milliliter
- µg/µl  Microgram per micro liter
- µl  Micro liter
- ¹H-NMR  Proton nuclear magnetic resonance spectroscopy
- 5-HT  5-Hydroxytryptamine
- AChE  Acetyl cholinesterase
- ACP/ALP  acid/alkaline phosphatase
- AD  Alzheimer’s disease
- AMPA  α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
- ANOVA  Analysis of variance
- AR  Analytical reagent
- CNS  Central nervous system
- CdC  Chloroform extract of Cedrus deodara Loud.
- CdM  Methanolic extract of Cedrus deodara Loud.
- CdO  Volatile oil of Cedrus deodara Loud.
- CdC-B  Chloroform extract of Cedrus deodara Loud.- fraction B
- CdC-C  Chloroform extract of Cedrus deodara Loud.- fraction C
- CdC-F  Chloroform extract of Cedrus deodara Loud.- fraction F
- CdC-G  Chloroform extract of Cedrus deodara Loud.- fraction G
- CdC-H  Chloroform extract of Cedrus deodara Loud.- fraction H
- CDCl₃  Deuterated chloroform
- CFU/ml  Colony forming unit per milliliter
- cm  Centimeter
- COX  Cyclooxygenase
- CPCSEA  Committee for the purpose of control and supervision of experiments on animals
- DART-MS  Direct analysis in real time mass spectrometry
- DU  Duodenal ulcers
- ECM  Extracellular matrix
- ESI-MS  Electro spray ionization mass spectrometry
- EtOAc/EA  Ethyl acetate
- FC  Frontal cortex
- FT-IR  Fourier transform infrared spectroscopy
- NO  Nitric oxide
- NSAIDs  Non steroidal antiinflammatory drugs
- OECD  Organisation for economic co-operation & development
- PDGF  Platelet derived growth factor
- PG  Prostaglandins
- PKC  Protein kinase C
- PMNs  Polymorpho nuclear neutrophils
- Pr C  Chloroform extract of *Pinus roxburghii* Sarg.
- Pr M  Methanolic extract of *Pinus roxburghii* Sarg.
- Pr O  Volatile oils of *Pinus roxburghii* Sarg.
- psi  Pound force per square inch
- PTZ  Pentylenetetrazole
- PUFA  Polyunsaturated fatty acids
- rpm  Revolutions per minute
- s  Second
- SD  Standard deviation
- SOD  Superoxide dismutase
- SRM  Spatial reference memory
- SSD  Silver sulphadiazine
- STM  Short term memory
- TAC  Total antioxidant capacity
- TBARS  Thiobarbituric acid reacting substances
- TBSA  Total body surface area
- Temp.  Temperature
- TGF-β  Transforming growth factor beta
- TLC  Thin layer chromatography
- TNF  Tumor necrosis factor
- VIP  Vasoactive intestinal peptide
- w/v  Weight by volume