

## CONTENTS

|   | <b>Page no.</b> |
|---|-----------------|
| Abstract                                | i-iv            |
| Declaration                             | v               |
| Certificate by the Principal Supervisor | vi              |
| Certificate of the External Examiner    | vii             |
| Content                                 | viii-xv         |
| List of Tables                          | xvi-xvii        |
| List of Figures                         | xviii-xx        |
| List of abbreviation                    | xxi-xxii        |
| Acknowledgements                        | xxiii-xxiv      |

## CHAPTER I

### INTRODUCTION

|  |       |
|--|-------|
| 1.1. General   | 1-4   |
| 1.2. Hair growth cycle                               | 5-8   |
| 1.3. Factors responsible for hair growth             | 9-10  |
| 1.4. Hair ailments                                   | 10-14 |
| 1.5. Alopecia areata                                 | 14-15 |
| 1.6. Medical status for Alopecia treatments          | 15-16 |
| 1.7. Phytopharmaceuticals used for treating Alopecia | 16-17 |
| 1.8. Objectives                                      | 18    |

## CHAPTER II

### REVIEW OF LITERATURE

|   |       |
|---|-------|
| 2.1. Hair ailments  | 19-22 |
| 2.2. Alopecia areata  | 22-24 |
| 2.3. Synthetic drugs for treating Alopecia                        | 24-30 |
| 2.4. Natural Products and their activity against Alopecia areata  | 30-31 |
| 2.5. Applications of various plant extracts on hair regenerations | 31-41 |
| 2.6. Animal models used for the study of hair regenerations       | 42-44 |

## CHAPTER III

### MATERIALS AND METHODS

|  |       |
|--|-------|
| 3. Overall Methodology of the Study          | 45    |
| 3.1. Collection of plants                    | 46    |
| 3.2. Morpho-phenological study of the plants | 46    |
| 3.3. Genomic study of the selected plants    |       |
| 3.3.1. DNA isolation                         | 47    |
| 3.3.1.1. Equipments used                     | 47-48 |
| 3.3.1.2. Reagents and chemicals              | 48-49 |
| 3.3.1.3. Buffers                             | 49    |
| 3.3.1.3.1. Extraction buffer                 | 49    |
| 3.3.1.3.2. High salt TE buffer               | 50    |
| 3.3.1.3.3. TAE buffer                        | 50    |
| 3.3.1.4. Loading dye and fluorochrome        | 50    |

|   |       |
|---|-------|
| 3.3.1.5. Bromophenol blue   | 50    |
| 3.3.1.6. Ethidium bromide   | 50-51 |
| 3.3.1.7. DNA isolation protocol   | 51-52 |
| 3.3.1.8. Modifications of the protocol  | 53    |
| 3.3.1.9. Purity and yields of isolated DNA from selected plants                   | 53    |
| 3.3.2. Genome size determination of selected plants                               | 53    |
| 3.3.2.1. Preparation of Otto I buffer   | 53-54 |
| 3.3.2.2. Preparation of Otto II buffer  | 54    |
| 3.3.2.3. Preparation of stain or flurochrome                                      | 54    |
| 3.3.2.4. Procedure of genome size determination                                   | 54-55 |
| 3.4. Isolation of phyto-compounds from the selected plants                        |       |
| 3.4.1. Isolation of phyto-compounds from <i>Eclipta alba</i>                      |       |
| 3.4.1.1. Preparations of crude extracts from <i>Eclipta alba</i>                  | 55-56 |
| 3.4.1.2. Fractionation of crude extracts from <i>Eclipta alba</i>                 | 56    |
| 3.4.1.3. Thin layer chromatography of crude extracts from <i>Eclipta alba</i>     | 56-57 |
| 3.4.2. Isolation of phyto-compounds from <i>Aloe barbadensis</i>                  |       |
| 3.4.2.1. Preparations of crude extracts from <i>Aloe barbadensis</i>              | 57    |
| 3.4.2.2. Fractionation of crude extracts from <i>Aloe barbadensis</i>             | 57    |
| 3.4.2.3. Thin layer chromatography of crude extracts from <i>Aloe barbadensis</i> | 57-58 |
| 3.5. Chemical characterization and structure elucidation of isolated compounds    | 58    |

|   |       |
|---|-------|
| 3.5.1. High performance liquid chromatography (HPLC)                  | 58-59 |
| 3.5.2. Fourier Transformation Infrared Spectroscopy (FTIR)            | 60    |
| 3.5.3. Mass spectroscopy  | 60    |
| 3.5.4. Nuclear Magnetic Resonance (NMR) Spectroscopy                  | 60    |
| 3.6. Biological characterization of the isolated compounds            |       |
| 3.6.1. Antibacterial assay of the isolated compounds                  | 60    |
| 3.6.1.1. Test organisms   | 61    |
| 3.6.1.2. Media  | 61    |
| 3.6.1.2.1. Nutrient Agar medium                                       | 61    |
| 3.6.1.2.2. Muller Hinton agar medium                                  | 61-62 |
| 3.6.1.3. Determination of antibacterial activity                      | 62-63 |
| 3.6.2. Antifungal assay of the isolated compounds                     | 63    |
| 3.6.2.1. Test organisms   | 63    |
| 3.6.2.2. Media used   | 64    |
| 3.6.2.3. Determination of antifungal activity                         | 64-65 |
| 3.6.3. Antioxidant assay  | 65    |
| 3.6.3.1. Chemicals and Reagents used                                  | 65    |
| 3.6.3.2. Determination of free radical scavenging activity            | 65-66 |
| 3.6.4. Assessment of cell cytotoxicity on murine macrophage cell line | 66-67 |
| 3.7. Inducing Alopecia areata on animal model using warfarin          | 67    |
| 3.7.1. Animal Husbandry and Maintenance                               | 67    |
| 3.7.2. Inducing Alopecia areata                                       | 68    |
| 3.8. Application of isolated phyto-compounds                          | 68    |

|                                      |       |
|--------------------------------------|-------|
| 3.8.1. Acute dermal irritation study | 69    |
| 3.8.2. Qualitative study             | 69    |
| 3.8.3. Haematological study          | 69-70 |
| 3.8.4. Study of serum                | 70    |
| 3.8.5. Histological study            |       |
| 3.8.5.1. Chemicals                   | 71    |
| 3.8.5.2. Preparations of reagents    |       |
| 3.8.5.2.1. Acid-alcohol solution     | 71    |
| 3.8.5.2.2. Mayer's albumin           | 71    |
| 3.8.5.3. Staining Protocol           | 71-74 |

## CHAPTER IV

### RESULTS

|  |       |
|--|-------|
| 4.1. Morpho-phenological characters of the selected plants | 75    |
| 4.1.1. <i>Eclipta alba</i>                                 | 76-78 |
| 4.1.2. <i>Aloe barbadensis</i>                             | 78-80 |
| 4.2. Genomic study of the plants                           |       |
| 4.2.1. DNA isolation                                       | 80-81 |
| 4.2.2. Genome size determination of the plants             | 81-83 |
| 4.3. Isolation and purification of Medicinal compounds     |       |
| 4.3.1. Isolation from <i>Eclipta alba</i>                  | 83-85 |
| 4.3.2. Isolation from <i>Aloe barbadensis</i>              | 86-87 |

|  |         |
|--|---------|
| 4.4. Chemical characterization and Structure elucidation of the purified compounds |         |
| 4.4.1. <i>Eclipta alba</i>   | 88-93   |
| 4.4.2. <i>Aloe barbadensis</i>   | 94-98   |
| 4.5. Biochemical characterizations   |         |
| 4.5.1. Microbial assay   |         |
| 4.5.1.1. Antibacterial assay   | 99-101  |
| 4.5.1.2. Antifungal assay  | 102-103 |
| 4.5.2. Antioxidant assay   | 103-105 |
| 4.5.3. Cytotoxicity assay  | 105-106 |
| 4.6. Acute dermal irritation study of the purified compounds                       | 106-107 |
| 4.7. Induction Alopecia in Wistar albino rats                                      | 108-109 |
| 4.8. Qualitative study of hair regeneration  | 110-113 |
| 4.9. Haematology and serum biochemical study                                       | 114-115 |
| 4.10. Histological study of the treated and control skin                           | 116-118 |

## CHAPTER V

### DISCUSSIONS

|  |         |
|--|---------|
| Discussions  | 119-120 |
| 5.1. Morpho-phenological characterization of the plants selected |         |
| 5.1.1. <i>Eclipta alba</i>                                       | 120     |
| 5.1.2. <i>Aloe barbadensis</i>                                   | 120-121 |

|   |         |
|---|---------|
| 5.2. Genomic study of the plants  |         |
| 5.2.1. DNA isolation of plants  | 121-122 |
| 5.2.2. Genome size determination  | 122-123 |
| 5.3. Isolation of the phyto-compounds                                       |         |
| 5.3.1. Isolation from <i>Eclipta alba</i>                                   | 124-125 |
| 5.3.2. Isolation from <i>Aloe barbadensis</i>                               | 125     |
| 5.4. Chemical characterization and Structure determination of the compounds |         |
| 5.4.1. <i>Eclipta alba</i>  | 125-127 |
| 5.4.2. <i>Aloe barbadensis</i>  | 127-128 |
| 5.4.3. Structural similarities of Minoxidil with Ea 1 and Av 4              | 128-129 |
| 5.5. Biochemical characterization   | 125     |
| 5.5.1. Microbial assay of the phyto-compounds                               |         |
| 5.5.1.1. Antibacterial activity   | 130-133 |
| 5.5.2. Antifungal activity  | 133-134 |
| 5.5.3. Antioxidant assay of the isolated compounds                          | 134-136 |
| 5.5.4. Cytotoxicity assay   | 136-137 |
| 5.6. Acute dermal irritation study of the phyto-compounds                   | 137-138 |
| 5.7. Inducing alopecia in wistar albino rats                                | 138-140 |
| 5.8. Qualitative study of hair growth in wistar albino rats                 | 140-141 |
| 5.9. Haematology and biochemical analysis of serum                          | 141-142 |
| 5.10. Histological analysis   | 142-143 |

**CHAPTER VI**  
**CONCLUSIONS & FUTURE WORK**

|                     |         |
|---------------------|---------|
| 6.1. Conclusions    | 144-146 |
| 6.2. Future work    | 147     |
| <br>                |         |
| <b>REFERENCES</b>   | 148-165 |
| <b>PUBLICATIONS</b> | 166     |