

# INTRODUCTION

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### 1.1. General

Hair is one of the important parts of body, derived from ectoderm of the skin and it is one of the most protective appendages on the body<sup>1</sup>. Human hair is an outgrowth of protein, an amorphous matrix high in sulphur proteins in which the keratin fibres are embedded (Fig.1.1). Keratin is an insoluble cystine-containing helicoidal protein complex and is the main component of hair fibre. It composes of 50% carbon, 20% oxygen, 17% nitrogen, 6% hydrogen, 5% sulphur and trace amounts of magnesium, arsenic, iron, chromium and minerals<sup>2</sup>. Hair follicles extend from the surface of the skin through the epidermis into the dermis. The hair fibre is a cylindrical, keratinized, often pigmented filament that can be seen above the skin.

Morphologically, fully formed hair fibres composed of three parts which are cuticle, cortex and medulla<sup>3</sup>. The cuticle is a chemically resistant region surrounding the cortex which is 10% of the hair fibre by weight. Each cuticle cell contains a thin outer membrane, the epicuticle. The cuticle cell membranes consist of three major layers: A layer is the resistant layer with a high cystine content (> 30%); the exocuticle,

sometimes called the B layer, also rich in cysteine (~15%) and the third layer C is called as endocuticle, which is low in cysteine content (~3%)<sup>4-6</sup>. The cysteine-rich proteins of the cuticle belong to the group of proteins called keratin-associated proteins. The hair cuticle is the first line of defence against all forms of damage and it acts as a protective barrier for the softer inner structure including the medulla and cortex. The cuticle is responsible for much of the mechanical strength of the hair fibre. Although the cuticle is the outermost layer of the hair, it does not contain melanin. Cortex is the intervening layer of the hair i.e. between the cuticle and the medulla. It is made of spindle shaped cell and pigmented granules. The cortex constitutes the major part of the fibre mass of human hair and consists of cells and intercellular binding material<sup>7</sup>. The cortical cells of the cortex do not show the presence of trichohyalin. Trichohyalin is a structural protein that is produced and retained in the cells of the inner root sheath and medulla of the hair follicle<sup>8</sup>. Matrix comprises the largest structural subunit of the cortex of human hair fibre which contains highest concentration of disulfide bonds. Although structurally different, keratin associated proteins are also found in the matrix of the cortex. Medulla is the inner most layer of the hair. It is made of cells that form a shaft through the middle of the hair. Different amounts of medulla may be present in the hair. Fine hairs consist of cuticle and cortex only<sup>9</sup>, but with increasing fibre diameter, the medulla can be present. Medullary cells are loosely packed and during dehydration, they leave a series of vacuoles along the fibre axis. These cells are spherical and hollow inside and are bound together by a cell membrane complex. The medulla contributed

negligibly to the chemical and mechanical properties of human hair fibres<sup>10-11</sup>.

The complete hair follicle from where the hair fibre grows, consists of nine distinctive epidermal layers (Fig.1.2), hair matrix, medulla, hair cortex, hair cuticle, cuticle of the inner root sheath, Huxley's layer, Henley's layer, companion layer and outer root sheath arranged concentrically from core to periphery with dermal papilla and connective tissue follicle<sup>12</sup>. In the hair follicle, hair cortex and hair cuticle constitute the major part of the hair shaft that penetrates the skin. Both the tissues undergo heavy keratinization to form the hair shaft, although their components or protein are different. The three concentric layers around the shaft constitute the inner root sheath (IRS), which supports the growth and differentiation of the shaft consist of thin overlapping scales facing the hair cuticle<sup>13</sup>. The cells of the inner root sheath cuticle partially overlap with the cuticle cells of the hair shaft, anchoring the hair shaft tightly to the follicle. Inner root sheath cells produce keratins and trichohyalin that serve as an intracellular "cement" giving strength to the inner root sheath to support and mould the growing hair shaft, as well as guide its upward movement. The inner root sheath separates the hair shaft from the outer root sheath, which forms the external concentric layer of epithelial cells in the hair follicle<sup>14</sup>. The intermediate Huxley's layer is the last of the keratinizing tissues in the IRS and hair shaft. When the IRS is absent, the hair cuticle may become entangled in the dermis or epidermis creating rough surface. Henle's layer keratinizes at a very early phase of hair growth and consequently

keratinized Henle's cells are observable at the initial stages of the hair follicle<sup>15</sup>.

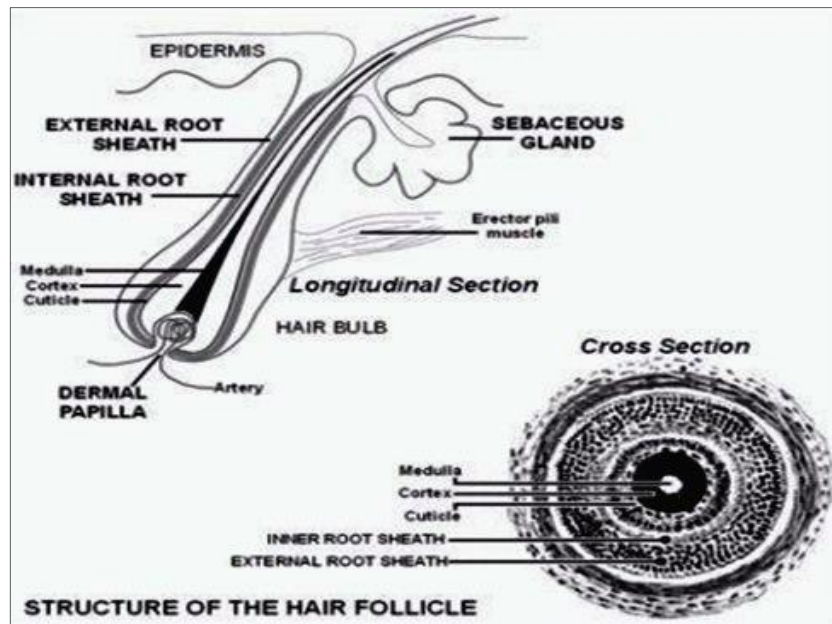


Fig.1.1. Structure of Hair<sup>16</sup>

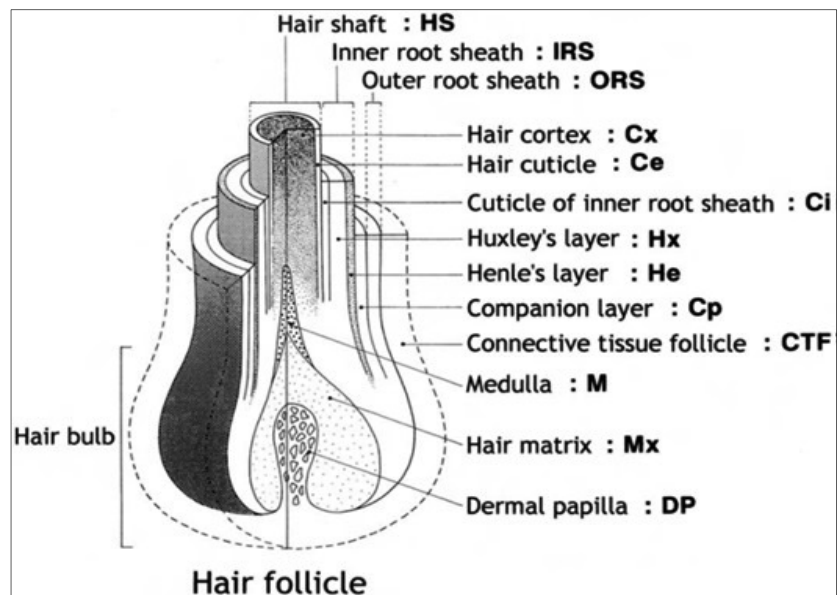


Fig.1.2. Structure of Hair follicle<sup>15</sup>

## **1.2. Hair growth cycle**

Hair follicle is an integral component of the skin and each hair is a keratinized product of the follicle. The dermal papilla (DP) is located at the deepest end of the hair follicle and is thought to play an essential role in the induction of new hair follicles and the maintenance of hair growth. Studies have shown that DP size is well correlated with hair growth cycle and the cell number of DP is increased in the anagen phase<sup>17-19</sup>. Each and every hair follicle undergoes a cycle of activity - the hair grows to a maximum length, then growth ceases and subsequently the hair is shed and replaced (Fig.1.3). This characteristic three-part cyclical growth pattern of hair is the result of follicular activity<sup>20</sup>. The phases of the hair growth cycle have been described as anagen, catagen and telogen.

### **Anagen phase**

The anagen stage is the growing phase, characterized by intense metabolic activity in the hair bulb. For scalp hair, this activity generally lasts for two to six years. Metabolically active and dividing cells around the dermal papilla of the follicle grow upward during this phase to form the hair shaft. This phase includes not only the growth of the hair, but also a rapid proliferative rate of all hair follicle cells, with the highest activity observed in matrix cells<sup>21</sup>. Anagen involves the complete re-growth or regeneration of the follicle i.e., the hair shaft. Duration of anagen phase is genetically predetermined and depends on size and body location of the hair follicle<sup>22</sup>.

Anagen phase is consists of six stages: Anagen I is the period when the cells of the dermal papilla increase in size and show increased RNA synthesis. At the same time, germ cells at the base of the hair sac undergo vigorous mitotic activity. During Anagen II, the lower part of the follicle grows down into the dermis and partially encloses with the dermal papilla, which is surrounded by matrix ring. This stage is marked by continued mitotic activity in the 'germ' region and proliferation of the hair follicular melanocytes. During Anagen III, the follicle attains its maximum length, which is about three times the length that it has in the resting condition. The bulb is now completely formed and the papilla cavity is constricted at its base. The melanocytes become aligned along the papilla cavity and each develops melanin granules. The internal sheath is now an elongated cone, which extends up to the capsule and club of the old hair<sup>23</sup>. In Anagen IV, melanocytes in the papilla develop dendrites and begin to form melanin. Although the hair has formed, it is still within the cone of the internal root sheath, which now extends upward to about the level of the sebaceous gland. The cuticles of the hair and the internal sheath are clearly visible. In the upper part of the bulb, a cone of cells, which will become cortex and medulla of the hair, contains pigment granules<sup>24</sup>. In Anagen V, the tip of the hair has broken through the tip of the internal sheath, through the intersection of the capsule with the external sheath, and has grown to about the level of the epidermis. The bulb attains its final characteristic shape, which in some hairs is laterally compressed, in others rounded and symmetrical. Anagen VI begins as soon as the hair emerges at the skin surface and continues until the onset of catagen. In this stage, the hair

emerges from the cone of the external root sheath and forces its way to the surface along the original hair shaft, which gets pushed aside, and eventually the clubbed hair is discharged<sup>25</sup>.

### **Catagen phase**

The anagen phase is followed by a short resting phase catagen, which can be divided into eight subphases starting with late anagen and ending in early telogen. The catagen stage is the transition stage, lasts for only a few weeks. During this phase, metabolic activity slows down and the base of the hair bulb migrates upward in the skin toward the epidermal surface. The regression of the hair follicle in catagen is characterized by termination of protein and pigment production in the hair follicle and reformation of the extracellular matrix<sup>23</sup>. Catagen is the first component of the initial hair cycle occurring after morphogenesis. During catagen, there are chemical and structural changes take place in the hair follicle which leads to progressive decline or degeneration of hair follicle. Follicular melanogenesis (formation of melanin) also ceases during this stage, and some follicular melanocytes undergo apoptosis as well. Towards the end of the stage, the dermal papilla condenses and moves upward, coming to rest beneath the hair-follicle<sup>26-27</sup>. The purpose of catagen is to delete the old hair shaft factory and to initiate the stem cells of the bulge and the papilla to set the stage for the formation of a new follicle<sup>28</sup>.

## Telogen phase

In telogen, the hair follicle has decrease about half of its previous size and does not extend beyond the upper dermis. This phase is characterised by a lack of pigment-producing melanocytes and inner root sheath. The telogen phase is the resting stage, also lasts only a few weeks. At this stage, growth has stopped completely, and the base of the bulb has shrunken to the point at which it approaches the level of the sebaceous gland. A new hair begins to grow beneath the telogen follicle, pushing the old telogen fibre out. The telogen fibre is eventually shed<sup>29</sup>. All three stages of the hair growth are showed in Fig.1.3.

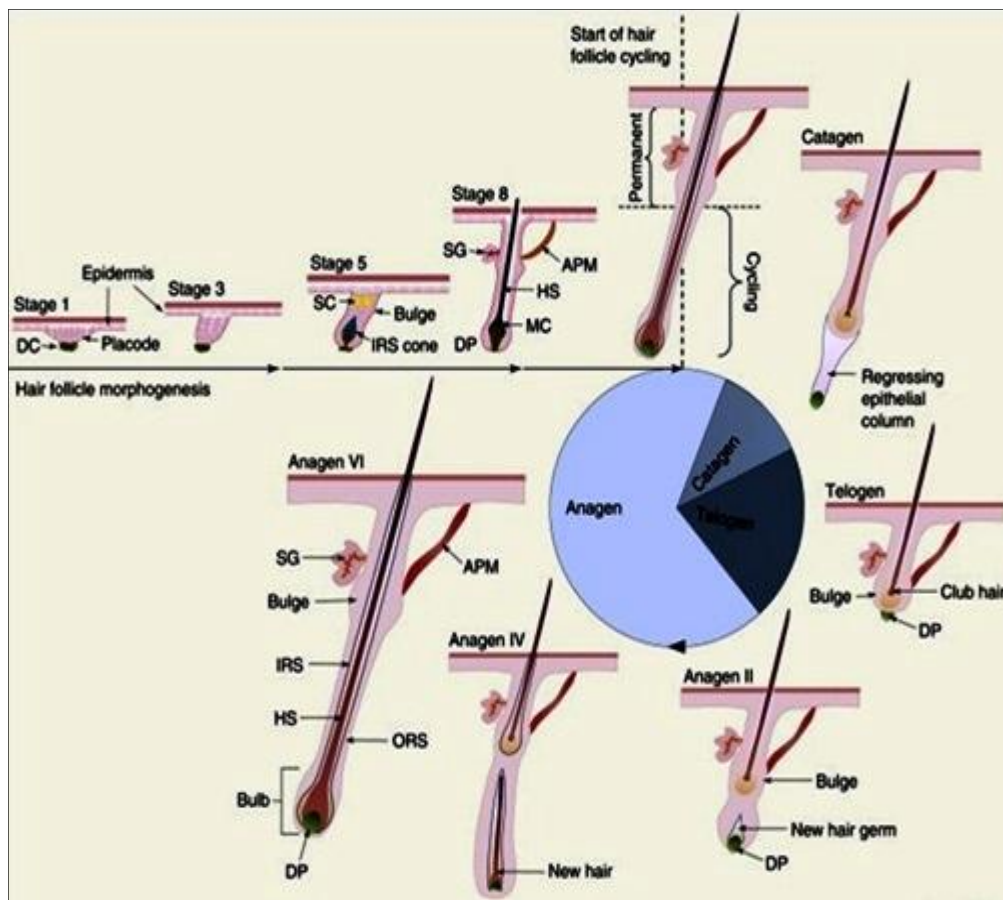


Fig.1.3. Different stages of hair growth cycle<sup>30</sup>



### **1.3. Factors responsible for hair Growth**

There are a number of factors that affect hair growth. Among them, the key factors that affect hair growth are growth hormones and cytokines which are produced by the body<sup>20, 31</sup>. These include epidermal growth factor (EGF), transforming growth factor-a (TGF-a) and transforming growth factor-b (TGF-b), keratinocyte growth factor (KGF), insulin-like growth factor-1 (IGF-1), interleukin-1 (IL-1), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF)<sup>32</sup>. Hair follicles have receptors for androgens and estrogens that can affect hair follicle activity.

The most important and well-known factors involved in hair loss are androgens. Androgens are known to cause hair regression and balding in genetically predisposed individuals. Testosterone and its active metabolite dihydrotestosterone (DHT) are two major androgens and DHT is considered to be more potent in triggering hair loss<sup>33-34</sup>. There are other hormones including those derived from the thyroid and the pituitary glands can also have significant effect on hair growth. In addition, there are cytokines that act on the cells in a hormone-like manner<sup>20</sup>. Intra-follicular activity may be the result of local or systemic factors. Possible local factors include an increased number of androgen receptors, functional polymorphisms of the androgen receptor, increased local production of dihydrotestosterone or reduced local degradation of dihydrotestosterone. Possible systemic factors are increased circulating androgens providing

increased substrate for the conversion to dihydrotestosterone or increased systemic production of dihydrotestosterone at distant sites such as the prostate gland<sup>35</sup>.

Experiments have demonstrated that during synchronized cycling in rats, there is a distinct change in the number, location and activation of mast cells, macrophages, langerhans cells and T cells. This establishes a relation between the immune system and hair growth cycle<sup>36</sup>. ICAM-1, an adhesion molecule, which is expressed by some follicle compartments leads to the accumulation of perifollicular macrophages. This also suggests that changes in the number, location or activity of these macrophages affect hair cycle control<sup>37</sup>. McDonagh and Tazi-Ahnini<sup>38</sup> suggested several factors which showed association with Alopecia areata. HLA alleles were the first to show a strong association with Alopecia areata. Interleukin-1 cluster genes, mainly the IL-1 receptor antagonist, show a strong association with disease severity in Alopecia areata and a number of other autoimmune and inflammatory diseases. When the hair follicle bulb or the bulge isthmus region is attacked by cell infiltration, it leads to severe forms of hair loss or transformation from terminal hair to vellus hair. These components of the skin immune system can therefore be said to control hair growth<sup>25</sup>.

#### **1.4. Hair ailments**

Hair ailments are common dermatologic disorders throughout the world recognized for more than 2,000 years<sup>1</sup> and have been estimated to affect more than 50% of the world population irrespective of gender, races,

region or culture<sup>1, 39</sup>. Although hair loss is not a life-threatening disorder, but it has a great impact on a person's self-respect, mental health, and overall quality of life<sup>40</sup>. Treatment of hair problems such as seborrheic dermatitis, telogen effluvium, anagen effluvium, male and female pattern hair loss, alopecia, head lice and dandruff have been a subject of major concern for cosmetologists and dermatologists throughout the globe. Complaints about continuing loss of scalp hairs are becoming increasingly common as patients become more and more conscious of their appearance due to physiological alopecia (normal hair loss)<sup>41-42</sup>. In physiologic alopecia, a decrease in the length of the anagen stage is observed as an increase in the number of hairs going into their telogen phase. After these hairs go into their telogen phase and fall out, the new hair grown is lighter colored, finer and has a still shorter anagen phase<sup>42</sup>.

Telogen effluvium is directly related to the cycles of hair growth<sup>43</sup>. If a significant number of hair follicles simultaneously enter into dormant phase (telogen), clinically it is likely to be development of telogen effluvium condition. Usually, averages of 100 hairs are lost each day, but this becomes more significant in telogen effluvium where more than 100 hairs are lost along with 30-50% of body hair can be lost. telogen effluvium may be precipitated by severe illness, injury, infection, surgery, crash diets, psychological stress, giving birth, thyroid disorders, iron deficiency, anemia or drugs<sup>44</sup>. Drugs that cause telogen effluvium include antithyroid agents, hormones, anticonvulsants, anticoagulants, beta blockers, angiotensin-converting enzyme inhibitors and lithium<sup>45</sup>.

Anagen effluvium is a diffuse hair loss condition associated with hair follicles in the anagen growth phase. It most commonly occurs when metabolic and mitotic activity of the follicle is rapidly suppressed by any drugs, chemotherapy or radiation<sup>46</sup>. Loose anagen hair disorder is another form of anagen effluvium, which is mostly seen in childrens but may occasionally be seen in adults. This condition occurs due to defective anchorage of the hair shaft to the follicle resulting in easily pluckable hair. In this condition, hair growth is slow and diffuse alopecia is observed<sup>47-48</sup>.

Male pattern baldness can occur at any age when blood androgen levels rise. The first change seen is a reduction of hair in the region of the temples. Men with androgenic alopecia typically have lower levels of total testosterone, higher levels of unbound/free testosterone, and higher levels of total free androgens including DHT<sup>49-51</sup>.  $5\alpha$ -reductase is responsible for converting free testosterone into DHT. The enzymes are present predominantly in the scalp and prostate<sup>52</sup>. The anagen growth phase is influenced and shortened by the oversensitivity to DHT of hair sacs. Moreover, the afflicted hair roots will mortify during the balding process and slowly, the healthy hairs will be replaced<sup>44, 53</sup>. Female pattern hair loss also called “female androgenic alopecia” is the commonest hair loss problem observed in women and prevalence increases with advancing age. There is an increase in vellus follicle numbers with increasing severity of hair loss in women with female alopecia, suggesting but not demonstrating terminal follicle miniaturization<sup>54-55</sup>. Affected women may experience psychological distress and impaired social functioning<sup>56</sup>. The histology of

female pattern hair loss is identical to that of male androgenic alopecia. While the clinical pattern of the hair loss differs between men, the response to oral anti-androgens suggests that female pattern hair loss is an androgen dependant condition, at least in the majority of cases<sup>57</sup>.

Other than alopecia related problems, dandruff is a common scalp disorder affecting almost half of the population of any ethnicity and both genders. *Malassezia spp.* flora plays a key role in combination with the unusual capacity of some corneocytes to be coated by these yeasts<sup>58</sup>. Substantial evidence indicates that keratinocytes play an active role in the generation and expression of immune-pathological reactions<sup>59</sup>. Upon stimulation of a critical colonization of corneocytes by *Malassezia* yeasts, the release of pro-inflammatory mediators is increased. This could lead to the subclinical micro-inflammation present in dandruff. Dandruff can precipitate Telogen effluvium and exacerbate androgenic alopecia<sup>60-61</sup>. Human head lice (*Pediculus humanus capitis*) are cosmopolitan parasites found in all areas of the world and in all socio-economic classes. As many as 6–12 million people worldwide are newly infested with head lice each year<sup>62-64</sup>. Head lice infestation represents an important problem from the standpoint of social health. Their presence may cause itching and loss of sleep. The louse's saliva and feces may ensitise people to their bites, thereby, exacerbating the irritation (mild fever, muscular aches and, occasionally, swelling of the cervical glands). Excessive scratching increases the chance of secondary infections<sup>65-66</sup>.

Hairs are largely resistant to the action of acids, but the disulfide bonds in hair can be broken apart by alkali solutions because alkali used to break the disulfide bonds in the keratins and make the hair weak. Such property is exploited in perms and chemical hair straightening processes which damages hair and increase its breakage and brittleness<sup>67</sup>.

### **1.5. Alopecia**

Among the various hair disorders, Alopecia is of major concern. It is most common type of hair ailment, which affects a large population of both men and women<sup>35</sup>. This disease can develop at any age of life and the most common effected population is the children and young adults. About 30-48% of patients are reported to be affected before the age of 20 and around 70% of cases occur between ages 10 and 25<sup>50, 68</sup>. Alopecia is characterized by areas of non-scarring hair loss that range from single patch to multiple patches that become confluent. Alopecia totalis is loss of all scalp hair and Alopecia universalis refers to loss of all scalp and body hair. In case series, Alopecia totalis and Alopecia universalis are less common than Alopecia and account for 4.5-30% of all alopecia cases<sup>69-70</sup>. Apart from metabolic and hereditary causes, alopecia has also been observed as a major side effect of anticancer, immune-suppressant, and many others drug treatments<sup>71-72</sup>. There are various factors that affect hair growth. It has been reported that hormones and cytokines are the key factors that affect hair growth. Hair follicles known to have receptors for hormones like androgens and estrogens that individually affect the activity hair follicles<sup>20</sup>. Other hormonal factors including those derived from

thyroid and the pituitary glands and natural compounds (hormone analogous structure) can also have significant effects on hair growth. It has been reported that the skin immune system also plays a role in hair growth and development. The hair follicle bulb or the bulge isthmus region is attacked by the cell infiltration which leads to severe hair loss or transformation from terminal hair to vellus hair. Immuno-suppressive drugs like cyclosporine, FK506 and glucocortico-steroids have been proved for control hair growth in the body<sup>34</sup>.

### **1.6. Medical status for Alopecia treatment**

Currently minoxidil and finasteride are two synthetic drugs finding concomitant use for treatment of alopecia, but their side effects have reduced their usage<sup>71, 73</sup>. The mechanisms of both the drugs involved in alopecia treatment are still unclear. It seems to open potassium channels and increase the proliferation and differentiation of epithelial cells in the hair shaft<sup>74</sup>. However, local irritation, itching, dryness and erythema may occur when minoxidil is topically used, as well as systemic side effects such as dizziness, tachycardia, angina pectoris and fluid retention. In case of Finasteride, impotence (erectile dysfunction), abnormal ejaculation, decreased ejaculatory volume, abnormal sexual function, gynecomastia, testicular pain, impairment of muscle growth and severe myopathy were observed in patients as side effect<sup>75</sup>.

At present, there are many allopathic as well as ayurvedic medicines/ formulations available in the market for different hair ailments. Minoxidil, finasteroids, cyclosporine, spironolactone, cimetidine etc are synthetic

drugs commonly used for hair problems like alopecia, telogen effluvium, male and female pattern baldness<sup>35, 76-78</sup>. Pyrithione zinc and selenium disulfide are commonly used chemicals in anti-dandruff shampoos and cosmetic products. All these synthetic compounds are having different minor and major side effects and also they are not confirmatory for complete treating hair ailments<sup>79-80</sup>. Currently available treatment options in the management of dandruff include therapeutic use of zinc pyrithione, salicylic acid, imidazole derivatives, glycolic acid, steroids, sulphur and tar derivatives. However, these agents have certain limitations, either due to poor efficacies or due to compliance issues. Furthermore, these drugs are unable to prevent recurrence, which is a common troublesome clinical problem<sup>81</sup>. For Head lice, topical treatments that are available are pyrethrins (extracted from chrysanthemums), pyrethroids (synthetic derivatives of pyrethrins) and lindane, all of which have a neurotoxic mode of action<sup>82-85</sup>.

### **1.7. Phyto-pharmaceuticals used for treating Alopecia**

Plants and herbs have been the source of valuable medicines since ancient times. With the development of science and technology, considerable progress has been made in the synthetic drugs for use in modern system of medicine<sup>86</sup>. Due to various harmful side effects of synthetic drugs, plants and plant products are still considered to be the major sources of treatments and have extensive use in ethno-medicine, traditional medicines as well as in modern system<sup>72</sup>.



Drugs of the modern system of medicine are mostly of synthetic origin and utilize fossil resources like petrochemicals which are basically non-renewable. Synthetic drugs are also known to possess toxic and side effects in consumption. Other than these, population rise, inadequate supply of drugs, severe side effects of allopathic medicines, prohibitive cost of treatments and development of resistance to currently used drugs has led to increase use of plant materials as a source of medicines<sup>87-88</sup>. However, the renewable resources like plant constitute the major ingredients of traditional system of medicine including folklore. Global history indicates that 80% of about 4 billion population cannot afford the products of the Western Pharmaceutical companies and have to rely on traditional medicines<sup>89</sup>. Long history of use and better patient tolerance as well as easy availability and public acceptance etc. are the factors associated with the plants drugs.

Despite the various advantages, ayurveda does have some drawbacks to it. The first disadvantage is no scientific evidence that many of the medications and healing techniques actually work for patients who are using ayurveda. In today's world we also have many complex medical problems that need proper medical care to treat. This type of holistic healing practice might not work for complex medical problems where medication and advanced therapies or surgeries are required to keep the individual alive. Ayurveda might not work for these individuals and could even make the condition they suffer from worse<sup>88</sup>.

## 1.8. Objectives

The work embodied in the thesis proceeded with the following objectives which are identified against the broad perspectives of the urgent need to forward new phyto-compounds as potential drugs to cure hair ailments like alopecia described in the preceding introduction.

The objectives of the investigation are as follows:

1. Selection of *Eclipta alba* Hassk and *Aloe barbadensis* Miller, their morpho-phenology and genomic studies.
2. Isolation and purification of medicinal compounds from both the selected medicinal plants.
3. Chemical characterization and structure elucidation of the purified phyto-compounds.
4. Biochemical characterization of purified phyto-compounds.
5. Study the effect of purified compounds on Alopecia areata induced animal model at follicular and dermal level.