Chapter II

Literature Review

Contents

2.1 Introduction

Hair is a skin appendage which covers only 0.1% of the total skin surface area. It plays a vital role in thermal insulation, protecting against environmental trauma, collecting sensory information and social and sexual communication [1, 12, 22]. Hair is made up of hair follicles which are formed at the end of the third gestational month. It is developed through the series of 'messages' between the epidermal keratinocytes and mescenchymal dermal fibroblast as shown in **Figure 1** [20]. The first message is from the dermal fibroblast which initiates the thickening of the epidermis to form the placode. The second message is from the epidermal placode which directs the clustering of the dermal fibroblast to form the dermal papilla (DP). The third message is from the DP which initiates the downgrowth of the epidermal placode, forming a peg. The keratinocytes and DP interacts through a unique set of growth factors to form the structure of the hair follicle [1, 22-23].

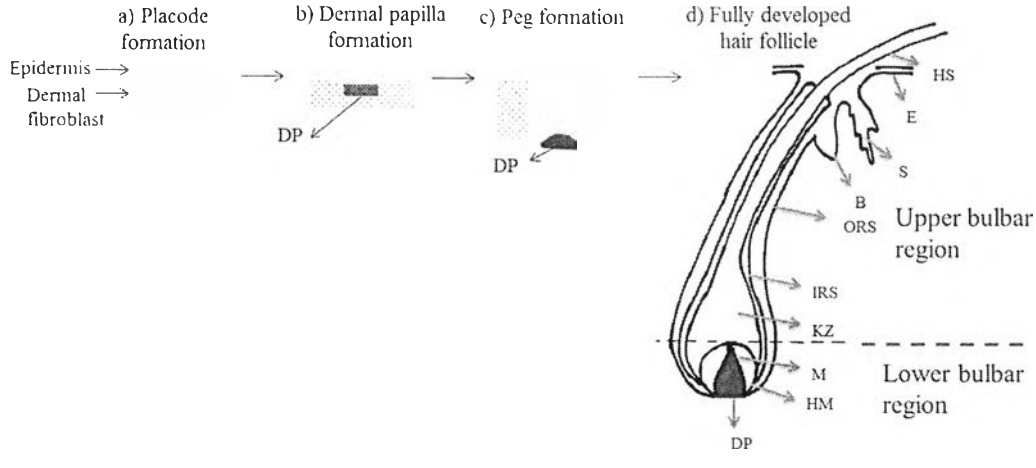


Figure 1. Hair follicle embryogenesis. DP (dermal papilla), HM (hair matrix cell), M (melanocytes), KZ (keratinocytes), IRS (inner root sheath), ORS (outer root sheath), B (bulge region), S (sebaceous glands), E (epidermis), HS (hair shaft)

2.2 The biology of hair

2.2.1 The structure of hair follicle

The structure of the fully developed hair follicle is shown in **Figure 1d).** Fully developed hair follicle consists of hair bulb, located within the skin and a hair shaft, located above the skin.

The hair bulb can be divided into two parts, upper and lower bulbar region. The upper bulbar region contains differentiated keratinocytes and is located at the dermis while the lower bulbar region contains undifferentiated keratinocytes and is located at the subcutaneous layer of the skin. The lower bulbar region is made up the outer root sheath cells surrounding the hair matrix cells which itself surrounds the DP. The DP is composed of dermal papilla cells which are clusters of specialized dermal fibroblast cells, and the extracellular matrix. It has been known that the keratinocytes will only form the hair structure in presence of DP [23]. Therefore, it has been concluded that DP plays essential role in the induction of new hair follicles and the maintenance of hair growth [24]. It also consists of melanocytes which are present at the tip of the DP. The upper region is made up of the three lines of inner root sheath namely: Henley; Huxley and cuticle layers, outer root sheath and the bulge region which contains the bulge stem cells.

The hair shaft is made up of the medulla, cortex and cuticle layers which are formed through the keratinization of the hair matrix cells [1, 9, 20, 22, 25].

2.2.2 The hair cycle

The hair cycle, shown in **Figure 2**, consist of the three phase namely: anagen, growth phase; catagen, involution phase; telogen, resting phase [5, 9, 23, 25-29].

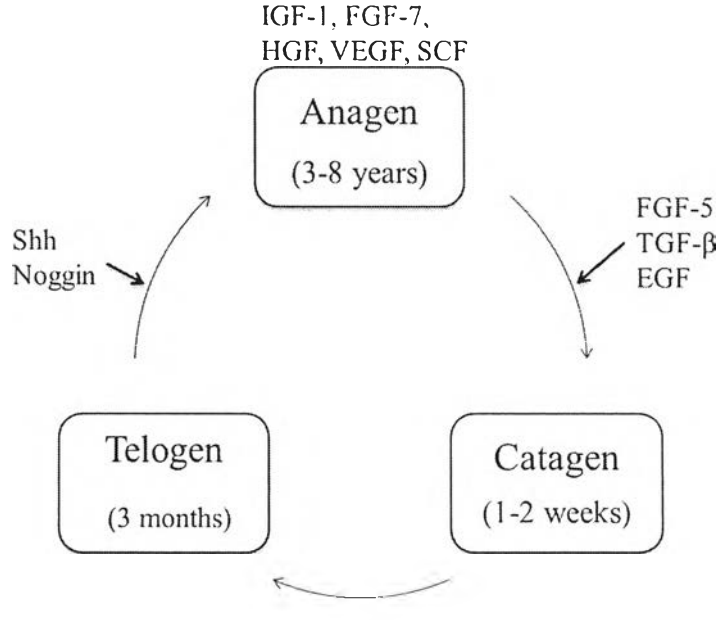


Figure 2. The hair cycle

The fully developed hair follicle is in the anagen phase of the hair cycle. The anagen phase can be divided into three stages. In the early anagen, the hair matrix cells proliferate and differentiate to form the inner root sheath and hair shaft. In mid anagen phase, the melanocytes present at the tip of the DP transfers the melanin produced to the cortex layer of the hair shaft. In the late anagen phase, the full developed hair follicle is formed and is characterized by the formation of the hair bulb surrounding the DP, located in the subcutaneous layer of the skin and the new hair shaft emerges from the skin surface. At this stage, an exogen phase might occur where the old hair shaft sheds from the skin [13, 20, 27, 30]. The anagen phase last for 3-8 years before the hair enters the catagen phase.

The catagen phase is where the keratinocytes and melanocytes of the hair follicle undergo apoptosis while the dermal papilla cells are known to be resistant to apoptosis. During this phase, length of the hair follicle is reduced by 70%. As the growth size is reduced, the DP will condense and move upwards near the bulge region in the dermis but is still in contact with the epithelial cells. In the catagen phase, the hair becomes a club hair [1-2, 9, 27, 29]. The catagen phase last for 1-2 weeks before the hair enters the telogen phase.

The telogen phase is a resting phase where no molecular and morphological changes occur. The hair follicles are short in length, lacks melanin and an inner root sheath. The hair stays in the telogen phase for 3 months before it enters the anagen phase through interaction between the DP and the bulge stem cells. The DP signals the stem cells to proliferate down towards the subcutaneous layer forming the hair matrix cells which then differentiates to form the other layers of the hair follicles.

The length of the hair depends on how long the hair stays in the anagen phase while the size of the hair shaft depends on the size of the DP's extracellular matrix [9, 29].

On average, 90% of the hair is in its anagen phase, 1% in the catagen phase and 9% in the telogen phase. This refers to a mosaic pattern of hair growth in humans unlike in other mammals where the hair grows in synchronized form [1].

2.2.3 The mechanism of hair cycle

At each stage of the hair cycle, there are certain regulators which are responsible for the changes in the size and length of the hair follicles. The regulatory molecules include growth factors and/or cytokines which are either up-regulated or

down-regulated in the dermal papilla cells or keratinocytes. During the anagen phase; IGF-1, HGF, FGF-7, VEGF and stem cell growth factors (SCF) are up-regulated within the dermal papilla cells. They function as paracrine growth factors on the follicular keratinocytes and melanocytes [20-21].

During the transition from the anagen to the catagen phase; fibroblast growth factor-5 (FGF-5), transforming growth factor–β (TGF-β) and epidermal growth factor (EGF) are up-regulated within the follicular keratinocytes. These growth factors act in autocrine/paracrine manner within the hair follicle. In addition, anti-apoptotic genes such as Bcl-2 are down-regulated in the outer root sheath cells of the hair follicle. Growth factors responsible for maintaining the hair follicle in the anagen phase are also down-regulated during the catagen phase [5, 28-29, 31].

No growth factors or cytokines have been identified during the transition from the catagen phase to the telogen phase [28]. However, in order to complete the cyclic process, sonic hedgehog (Shh) and noggin are up-regulated within the hair follicle resulting in the transition from the telogen phase to anagen phase.

2.3 Hair loss

2.3.1 Alopecia and the types of alopecia

Alopecia is a generic term of abnormal hair loss. It is one of the dermatological disorders which is common throughout the world and is of great concern [12]. The most common forms of scalp hair loss are alopecia areata, telogen effluvium, chemotherapy- induced alopecia and AGA. Alopecia areata is an autoimmune disorder where the lymphocytes attack the anagen hair bulb. Telogen effluvium is a condition in which excessive shedding of the hair follicle takes place. It is caused due to the premature entry of the hair follicle into the telogen phase. Chemotherapy-induced alopecia is caused due to the chemotherapy drugs given to cancer patients. The drug disrupts the proliferation of the hair matrix cells causing a premature entry into the catagen phase which leads to the loss of hair follicle. AGA is caused by two androgens namely, T and 5α-DHT [28].

2.3.2 Androgenic alopecia

AGA is the major type of scalp hair loss that affects 60-70% of the population [1, 4]. It affects 50% of male by the age of 50 years and up to 70% of all males in

the later life while it affects only 25% of women by the age of 49 years and 41% at the age of 69 years [13]. It is characterized by the miniaturization of the large, thick pigmented terminal hair with a diameter of greater than 0.03mm to small, fine, non-pigmented vellus hair with a diameter of less than or equal to 0.03mm [1, 2]. This characteristic of AGA is shown in **Figure 3**. The miniaturization is due to the over-production of 5α-DHT which results in the premature entry of the hair follicle into the catagen phase and the delay in the transition from the telogen phase to anagen phase, resulting in the shortening of the anagen phase [32].

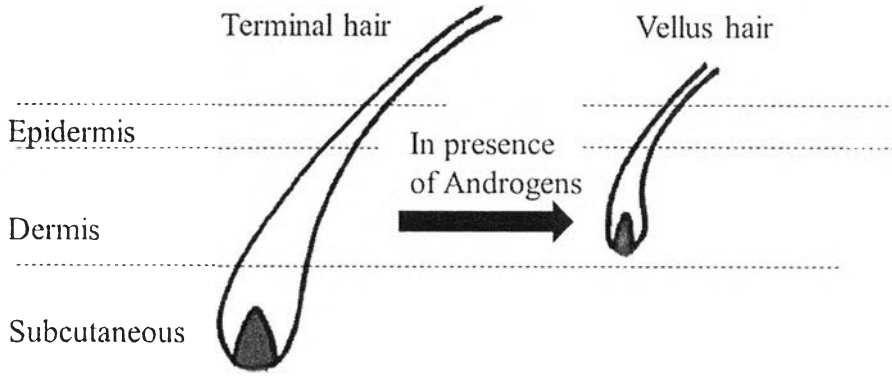


Figure 3. Characteristic of AGA

The difference in AGA between male and female is observed through the difference in the hair loss pattern [5]. Hamilton-Norwood scale consists of 8 stages and is used to describe male pattern baldness. It starts with the bitemporal recession of the frontal hairline followed by the diffuse thinning over the vertex, which eventually lead to a complete hair loss in this region. The bald patch enlarges and will eventually join the receding frontal hairline. At the later stage, only the parietal and occipital hair is left which might get thinner and will eventually lead to complete baldness [3]. Sinclair and Ludwig scales are the two scales used to describe the pattern of hair loss in female. Sinclair scale uses midline parting and consists of 5 grades: grade 1 refers to normal hair, grade 2 refers to widening of the central part, grade 3 refers to widening of the central part and thinning of the hair on either side of the central part, grade 4 refers to the emergence of a diffuse hair loss over the top of the scalp and grade 5 indicates advanced hair loss [13]. Ludwig scale has been used traditionally to describe female pattern hair loss using sideline parting. It consists of 3 grades: grade 1 refers to small amount of thinning of the hair on the crown with no problems observed in the frontal hair line, grade 2 refers to pronounced thinning of the hair on the crown, grade 3 refers to total baldness in the

same area as in grade 1 and 2 [13, 33]. However in both sex, AGA is caused by T and 5α-DHT, the two potent androgens [28].

2.3.3 Androgens

Androgen is the generic term for any natural or synthetic compound, usually a steroid hormone, which stimulates or controls the development of male and female characteristics. T is the primary and the major circulating androgen within human body. It is formed through the steroidogenesis pathway, shown in **Figure 4**, starting from cholesterol, converted into pregnenolone which then undergoes α-hydroxylation forming dehydroepiandrosterone (DHEA). DHEA is enzymatically converted to T by 3β-hydroxysteroid dehydrogenase (3β-HSD) in the prostate. In the circulating system, only a small fraction of T is present in the free form while the rest is in equilibrium with either sex-hormone binding globulin (70%) or albumin (19%). Within the prostate and target organ, T is enzymatically converted to 5α-DHT by the 5α-R [26, 34]. T can also be converted to estradiol and androstenedione by aromatase and 17β-hydroxysteroid dehydrogenase type 2 (HSD17β2), respectively [35].

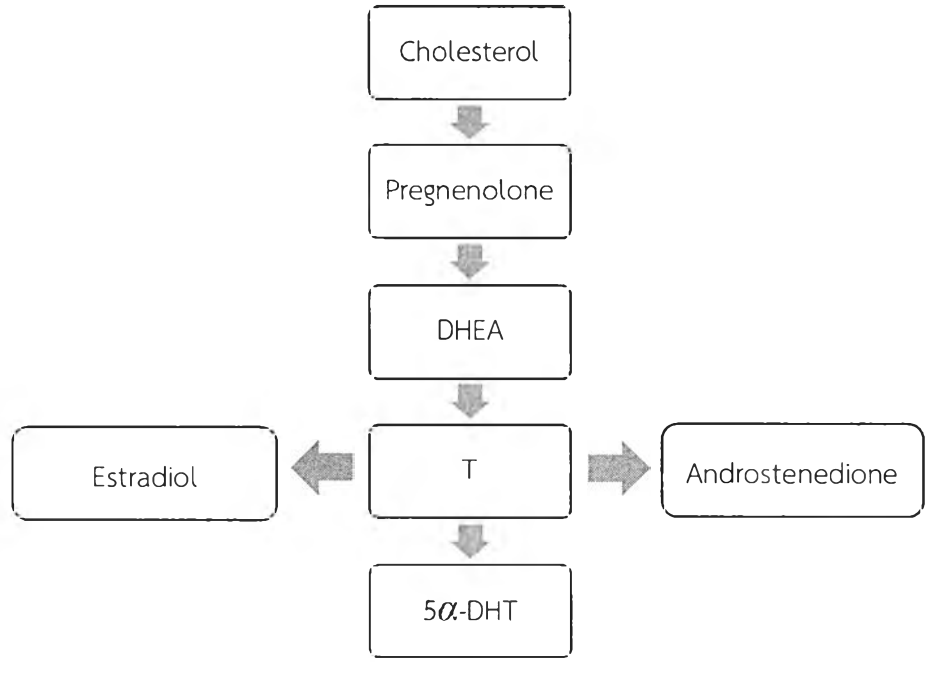


Figure 4. Steroidogenesis pathway. DHEA (dehydroepiandrosterone), T (testosterone), 5α-DHT (5α-dihydrotestosterone)

2.3.4 Mechanism of androgens action

T and 5α-DHT are the two major types of androgens that cause AGA. T is the major circulating androgen and is converted to 5α-DHT within the dermal papilla cells, which are the only site of androgens action within the hair follicle, by the 5α-R. Both T and 5α-DHT can bind to the AR, a member of steroid-thyroid hormone nuclear receptor superfamily, to form a receptor-ligand complex. The complex is then translocated to the nucleus where it acts as a transcriptional factor, regulating the expression of androgen-sensitive genes. The androgen-sensitive genes are the growth factors genes produced by the dermal papilla cells during the hair cycle [3, 8, 22, 36]. However, 5α-DHT has 5 times higher binding affinity and 10-fold higher potency than T does in inducing androgen-sensitive genes and is over-produced during AGA [1, 6]. The mechanism of androgens action within the dermal papilla cell is shown in **Figure 5.**

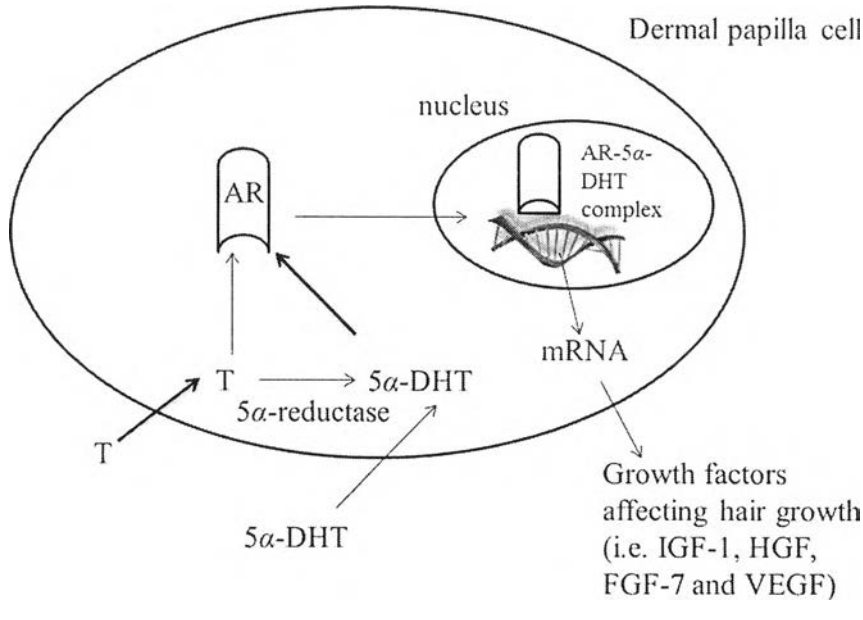


Figure 5. Mechanism of androgens action within the dermal papilla cell. T (testosterone), 5α-DHT (5α-dihydrotestosterone), AR (androgen receptor), AR-5α-DHT (receptor-ligand complex)

2.3.5 Current treatments for androgenic alopecia

Minoxidil and finasteride are the only two FDA-approved synthetic drugs used for the treatment of AGA. Minoxidil is used in the form of topical application while finasteride is taken orally.

Minoxidil, a vasodilator and potassium channel opener is known to prolong the anagen phase and convert vellus hair to terminal hair. This effect is seen through the up-regulation of VEGF and FGF-7 within the dermal papilla cells and due to the growth-promoting activity on the hair epithelial cells [10-11]. In addition, it is the only drug used to treat AGA in females. However, it is only effective on 30-35% of the patient and the treatment must be continued for life time [9]. In addition, the side effects of 2% and 5% minoxidil solutions include scalp irritation, pruritis, dryness, scaling, itchness, redness, contact dermatitis and hypertrichosis [12-13].

Finasteride, a synthetic azo-steriod, is a 5α-R2 inhibitor. It is known to bind irreversibly with the enzyme and inhibits the conversion of T to 5α-DHT. This inhibition reduces the concentration of 5α-DHT in serum by 68% [3], with the observed side effects of impotence, abnormal ejaculation, abnormal sexual function, myalgia, testicular pain and gynecomastia [14]. It might also cause malformation of the external genitalia in male fetuses if taken by women who are or may become pregnant [5]. In addition, it has been known to be effective to only 48% of the patient [12].

Other possible treatment for AGA is to block the interaction of T and/or 5α-DHT with the AR. Cyproterone acetate, spironolactone and flutamide blocks this interaction but all of them have side effects such as it affects the prostate and seminal vesicle testosterone levels and causes libido in males while causing menstrual disturbances, depression, breast tenderness and feminization of fetuses in females [13, 37].

2.4 Potential targets for the discovery of new hair-growth promoters in treating androgenic alopecia

As AGA is caused due to the over-production of 5α-DHT which results in the shortening of the anagen phase, therefore one potential target for finding new hair growth promoters is to inhibit the formation of 5α-DHT by inhibiting the 5α-R within dermal papilla cells. An additional increase in the expression of the growth factors

produced by dermal papilla cells in presence of androgens would prolong the anagen/growth phase of the hair cycle, resulting in hair growth.

2.4.1 5α-reductase enzyme

5α-R [E.C.1.3.99.5] are NADPH-dependent, membrane associated hydrophobic enzymes. It irreversibly reduces the $\Delta^{4,5}$ double bonds of steriods substrates such as T into a much more potent androgen, 5α-DHT.

2.4.1.1 Types

5α-R1 and 5α-R2 are the two isoform of this enzyme.

2.4.1.2 Organ distribution

Within the body; 5α-R1 are found in sebaceous glands, chest and back skin, liver, adrenal glands and kidney tissues while 5α-R2 are found in the beard, chest skin, liver, seminal vesicles, prostate gland, testis and epididymis, foreskin and scrotum [6, 38]. Within the hair follicle; 5α-R1 is present in the dermal papilla cells, epidermal and follicular keratinocytes while 5α-R2 is present in the inner layer of the outer root sheath, inner root sheath, interfollicular keratinocytes and might be present in the dermal papilla cells [38-39].

2.4.1.3 Biochemical properties

5α-R1 shows a board pH range of 6.5-8 with lower affinity to T (K_m>1μM) while 5α-R2 shows a narrow acidic pH of 5.5 and has a higher affinity for T (K_m<10nM) [19].

2.4.1.4 Differences of 5α-reductase from different sources

Two isoforms of 5α-R have been identified in Homo sapiens and Mus musculus. There general properties are summarized in **Table 1**.

Table 1

General properties of the two isoenzymes from two different sources

Types of 5α-R	Amino acid*	Molecular weight (kDa)**	Isoelectric pH (pI)**
5α-R1-Humans (5α-R1H)	259	29.46	9.19
5α-R1-Mouse (5α-R1M)	255	29.34	8.89
5α-R2-Humans (5α-R2H)	254	28.39	9.47
5α-R2-Mouse (5α-R2M)	254	28.62	9.23

* Based on the information obtained from the NCBI GenBank

**Calculated from ExPASy (http://web.expasy.org/compute_pi/)

Molecular weight and isoelectric pH are properties based on the amino acid composition of the enzymes. The four enzymes or the two isoforms within each species, have different amino acid sequence and their percent homology is summarized in Table 2.

Table 2

Percent amino acid sequence similarities between the two isoenzymes from two different sources

	% Amino acid sequence similarity*			
	5α-R1H	5α-R1M	5α-R2H	5α-R2M
5α-R1H	100	61	47	45
5α-R1M	61	100	45	42
5α-R2H	47	45	100	75
5α-R2M	45	42	75	100

*Calculated using Clone Manager (Scientific & Educational Software USA)

2.4.2 Growth factors

IGF-1, FGF-7, HGF and VEGF are the growth factors produced by the dermal papilla cells and are responsible for maintaining the hair follicle in the anagen phase of the hair cycle. These growth factors are responsible for the proliferation and differentiation of the hair matrix cells into the outer root sheath, inner root sheath and hair shaft. However, the exact pathway of each growth factor related to hair growth has not been studied [20-21].

2.5 Bioassays

2.5.1 5α-reductase based bioassays

2.5.1.1 Cell-free assay system

Cell-free assay have been conducted using 5α-R isolated from rat prostate, rat liver, rat epididymis, human prostate or transfected cell lines. Firstly, tissues or cells are homogenized in a buffer solution composed of tris-hydrochloride, sucrose, dithiothreitol (DTT) and protease inhibitor (PMSF). The homogenate is then subjected to velocity sedimentation in order to obtain the microsomal fraction which is the enzyme solution used for the assay. In general, the reaction is carried out in a test-tube and it consists of buffer (phosphate buffer or trisodium citrate buffer), NADPH, radiolabeled and non-radiolabeled T, test compound and the enzyme solution. The reaction is incubated at 37^{o}C for 10-60 min before it's stopped using either ethyl acetate or dichloromethane. The organic layer is collected, dried, reconstituted and analyzed using TLC or high performance liquid chromatography (HPLC) combined with radioactive detectors [15-16, 18-19, 40-41].

2.5.1.2 Cell-based assay system

Cell-based assay have been conducted on the whole cell expressing the 5α-R such as human prostate cancer cell line or transfected rat cell line. Briefly, the cells are seeded onto 24-well plate at a cell density of 5x10^{4} – 2x10^{5} cells/ml and after 24 hours, they are treated with radiolabeled T in presence or absence of test compound. After incubating it for 2-3 hours, the medium is collected and the steroids are extracted using ethyl acetate. The

organic layer is separated, dried, reconstituted and analyzed using TLC or HPLC combined with radioactive detectors [19, 42-43].

2.5.2 Transcriptional activity assay system

In one of the assay system, the dermal papilla cells are grown to 80% confluency in a 100mm tissue culture dish and were then treated with the potential crude extract for 24 hours. The cells are analyzed for the expression of IGF-1, FGF-7, HGF and VEGF growth factors involved in hair growth using RT-PCR [24, 44]. Another assay system involves in treating the dermal papilla cells with 5α-DHT. In this system, the total RNA is isolated 3-6 hours after the treatment, converted to cDNA and then a microarray study was conducted in order to observe the up-regulation and down-regulation of various growth factors and cytokines presented in the dermal papilla cells [8, 45].

2.6 Bioactive compounds

2.6.1 5α-reductase inhibitory compounds

2.6.1.1 Steroidal inhibitors

The main characteristic of a steroidal compound is the backbone i.e., four-fused carbon ring. The inhibitors are grouped based on the location of the nitrogen (N) atom in the backbone. The steroidal compounds with specific type of inhibition are reviewed in **Table 3**.

Table 3

Steroidal inhibitors

Steroidal compounds	Inhibitors	Type of inhibitors	Reference
4-azasteroid	Finasteride	5α-R2 (IC_{50} = 69 mM)	[6]
	Dutasteride	5α-R1 (IC_{50} = 7 nM), 5α-R2 (IC_{50} = 6 nM)	[6]
	4-MA	5α-R1 (IC_{50} = 1.7 nM), 5α-R2 (IC_{50} = 1.9 nM)	[6]
6- azasteroid	GIIS7669X	5α-R1 and 5α-R2	[6]
10-azasteroid	AS97004	5α-R1	[6]
Androstenecarboxylic acid	Episteride	5α-R2	[6]

2.6.1.2 Non-steroidal inhibitors

Non-steroidal inhibitors can be obtained from either synthetic or natural source. Non-steroidal inhibitors from synthetic source include benzoquinolines, nonsteroidal aryl acids, butanoic acid derivatives, zinc and some other cations [6, 38]. Benzoquinolines, a 5α-R1 inhibitor is derived from the 4-azasteroids [46]. FK143 (4-[3-(3-[bis (4-isobutylphenyl)methylaminobenzoyl)-1H-indol-1-yl]butyric acid), a butanoic acid derivative is a 5α-R1 and 5α-R2 inhibitor [47].

Natural non-steroidal inhibitors are the active compounds isolated from medicinal plant extracts. **Table 4** summarizes the active compounds and/or medicinal plants with 5α-R inhibitory activity.

Table 4

Natural non-steroidal 5α-R inhibitors

No.	Active compounds	Medicinal plants	Type of assay	IC_{50}		Enzyme isolated	Reference
				5α-R1	5α-R2		
1	Oleic, linoleic, palmitic acids	Ethanol extract of *Lygodii Spora*	Cell-free	0.44 ± 0.02mM, 0.37 ± 0.01mM, 1.35 ± 0.03mM		Rat liver	[34]
2	Linoleic, monolinoleic acids	Supercritical Carbondioxide extract of *Brassica rapa* pollen	Cell-free	0.07, 0.18mM		Rat liver	[40]
3	Triolin	Methanol extract of *Torilis japonica*	Cell-free	31.7 ± 4.23 μM		Rat prostate	[41]
4	Epigallocatechin-3-gallate [EGCG]	Camellia sinensis	Cell-free	12 μM	73 μM	Transfected rat cells	[19]
5	Ganoderic acid TR, Ganoderi acid DM, 5α-lanosta-7,9(11),24-triene-15α,26-dihydroxy-3-one	Ethanol extract of *Ganoderma lucidum*	Cell-free	8.6, 10.6, 41.9 μM		Rat liver	[17]
6	Osthenol, Bisabolangenlone	*Angelica Koreana* Max.	Cell-based	0.1, 11.6 μg/ml		Prostate cancer cell line	[42]
7	ND	Methanol extract of *Sophora flavescens*	Cell-free			Rat prostate	[44]
8	Terpenoids, aliphatic alcohols	*Ocimum Basilicum*	Cell-free	ND		Rat prostate	[14]
9	Sterol	Petroleum ether extract of *Cuscuta Reflexa* Roxb.	Cell-free	ND		Human prostate	[15]
10	Soyasaponin 1, Kaikasaponin III	Ethanolic extract of *Pueraria thomsonii*	Cell-free		112, 61 μM	Rat epididymis	[18]

Table 4 (Cont.)

Natural non-steroidal 5α-R inhibitors

No.	Active compounds	Medicinal plants	Type of assay	IC_{50}		Enzyme isolated	Reference
				5α-R1	5α-R2		
11	Oleic acids, Linoleic, lauric acids	Hexane extract of *Serenoa repens*	Cell-free	4 ± 2, 13 ± 3, 17 ± 3 μg/ml	NS, 35 ± 21, 19 ± 9 μg/ml	Transfected insect cells	[16]
12	Myristic acid	Hexane extract of *Serenoa repens*	Cell-free		4 ± 2 μg/ml	Transfected insect cells	[16]
13	ND	Ethanol extract of *Serenoa repens*	Cell-free		2.88 ± 0.45 μg/ml	HEK293 transfected cell line	[48]
14	ND	Ethyl acetate extract of *Thujae occidentalis semen*	Cell-based		2.6 μg/ml	HEK293 transfected cell line	[43]
15	Gossypol, Nordihydroguaiaretic acid, Octyl gallate	NS	Cell-free	7 ,19, 27 μM	21, 50, 58 μM	Transfected rat cells	[19]
			Cell-based	7,19, 7 μM	6,22,18 μM		
16	Genistein, Daidzein	NS	Cell-free		23, 29 μM	Transfected rat cells	[19]
			Cell-based		20, 7μM		

Table 4 (Cont.)

Natural non-steroidal 5α-R inhibitors

No.	Active compounds	Medicinal plants	Type of assay	IC_{50}		Enzyme isolated	Reference
				5α-R1	5α-R2		
17	Bromopyrogallol red	NS	Cell-free	7 μM	84 μM	Transfected rat cells	[19]
18	Caffeic acid phenethyl ester, Biochanin A, Kaempferol	NS	Cell-based	8, 64, 79 μM	7, 5, 20 μM	Transfected rat cells	[19]
19	Anthrarobin	NS	Cell-free Cell-based	4 μM 6 μM		Transfected rat cells	[19]
20	Anthrarobin, Biochanin A, Kaempferol	NS	Cell-free		50, 17,12μM	Transfected rat cells	[19]
21	Alizarin	NS	Cell-free Cell-based	3 μM 6 μM		Transfected rat cells	[19]
22	Myricetin, Quercitin, Baicalein, Fisetin, Pyrogallol red, Caffeic acid phenethyl ester, Purpurogallin, Hydroxydopamine, Dodecyl gallate, Pyrocatechol violet, Pyrogallol and Purpurin	NS	Cell-free	23, 23, 29,57, 15, 25, 30, 42, 43, 48, 70, 2 μM		Transfected rat cells	[19]
23	Emodin, Riboflavin	Ethanol extract of *Polygon i Multiflori Radix*	Cell-free	40, 1.6 μM		Rat prostate	[49]

Table 4 (Cont.)

Natural non-steroidal 5α-R inhibitors

No.	Active compounds	Medicinal plants	Type of assay	IC_{50}		Enzyme isolated	Reference
				5α-R1	5α-R2		
24	NS	Ethanol extract of *Carthamus tinctorius*	Cell-free	NS		Rat liver	[50]
25	(-)-Cubebin,(-)-3,4-dimethoxy,-3,4-desmethylenedioxycubebin, piperine	Ethanol extract of *Piper Nigrum* leaves	Cell-free	0.44, 1.03, 0.48mM		Rat liver	[51]
26	Abietic acid, Pimaric acid and neo abietic acid	Ethanol extract of *Resina Pini*	Cell-free	NS		Rat prostate	[52]
27	Oenothein B	Aqueous extract of *Epilobium parviflorum*	Cell-free	22 μM		Human prostate	[53]
28	Chlorophorin, Artocarpin	Methanol extract of *Artocarpus incisus* heartwood	Cell-free	37, 85 μM		Rat liver	[54]
29	Linoleic, α-linoleic, Palmitic, elaidic, oleic and stearic acid	Acetone extract of *Boehmeria nipononiveo* leaves	NS	NS		NS	[55]
30	1,7-diphenylhept-4-en-3-one, Dihydroyashabushiketol, 5-Hydroxy-7-(4"-hydroxy-3"-methoxyphenyl)-1-phenyl-3-heptanone, 5-Hydroxy-7-(4"-hydroxyphenyl)-1-phenyl-3-heptanone	Acetone extract of *Alpinia officinarum* rhizome	Cell-free	390, 230, 220, 220 μM		Rat prostate	[56]

Table 4 (Cont.)

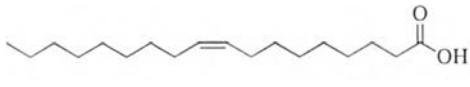
Natural non-steroidal 5α-R inhibitors

No.	Active compounds	Medicinal plants	Type of assay	IC_{50}		Enzyme isolated	Reference
				5α-R1	5α-R2		
31	Impatienol	Ethanol extract of *Impatiens balsamina L.* aerial parts	Cell-free	99.4 μg/ml		Rat prostate	[57]

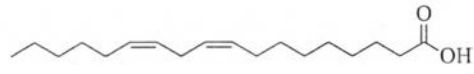
ND- not identified

NS- not specified

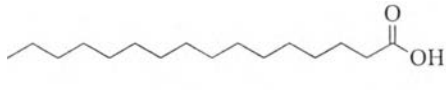
The structures of the compounds with known 5α-R inhibitory activity are shown in **Figure 6**.



Oleic acid



Linoleic acid



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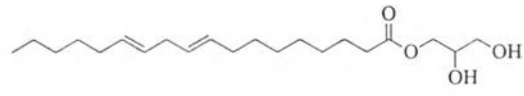

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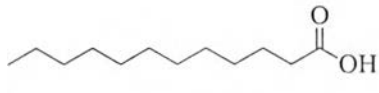
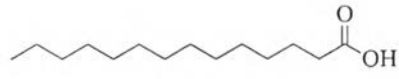
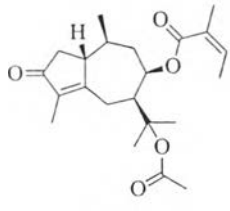


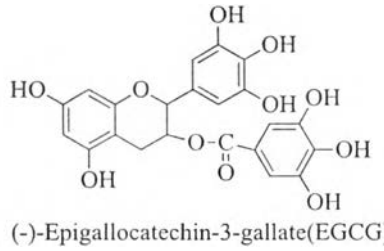
Lauric acid



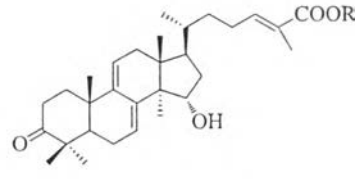
Myristic acid



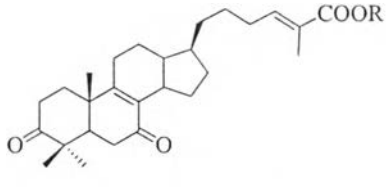
Troilin



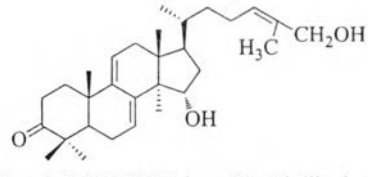
(-)-Epigallocatechin-3-gallate(EGCG)



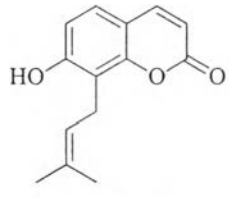
Ganoderic acid TR



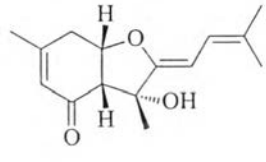
Ganoderic acid DM



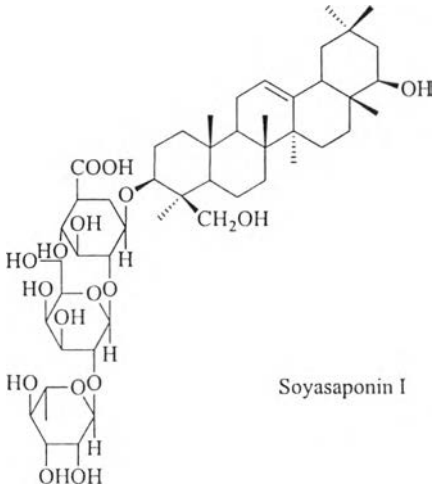
5a-lanosta-7,9(11),24-triene-15a,26-dihydroxy-3-one



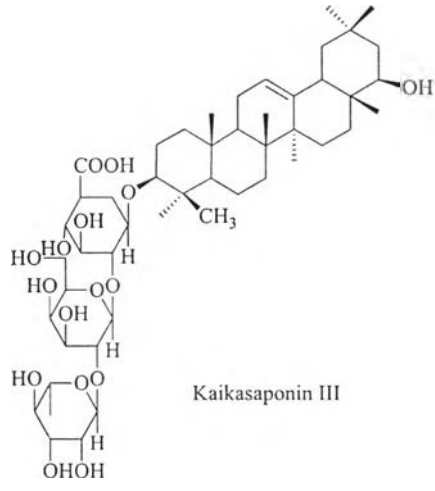
Osthenol



Bisabolangelone



Soyasaponin I



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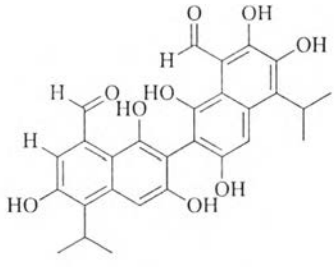
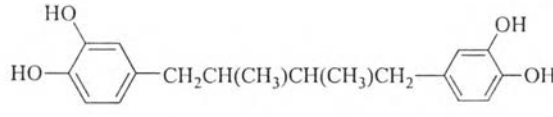
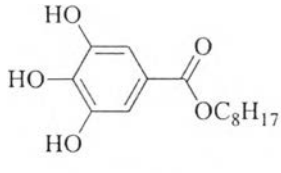


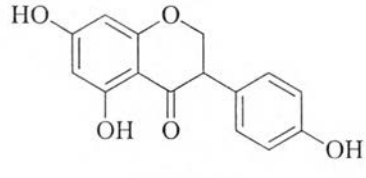
Gossypol



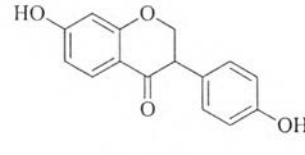
Nordihydroguaiaretic acid



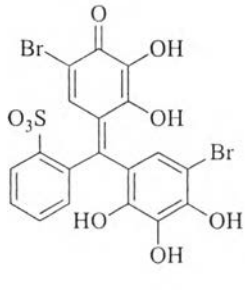
Octyl gallate



Genistein

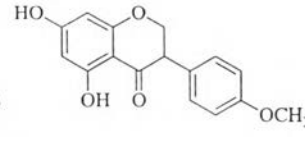


Daidzein

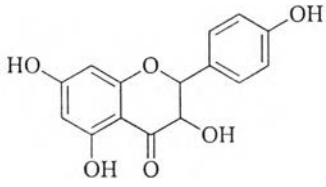


Bromopyrogallol Red

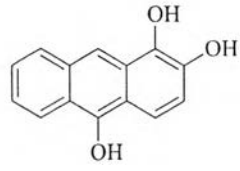
Caffeic acid phenethyl ester



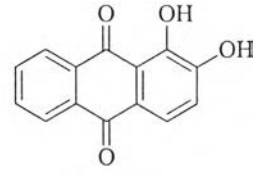
Biochanin A



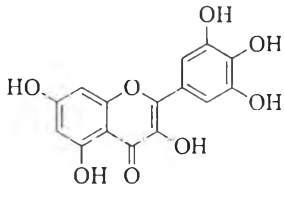
Kaempferol



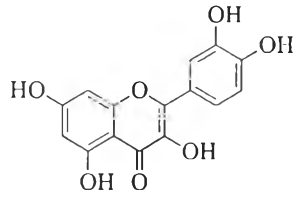
Anthrarobin



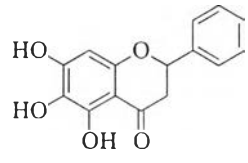
Alizarin



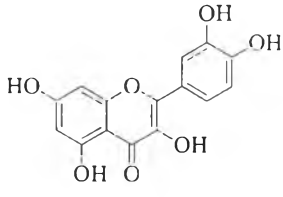
Myricetin



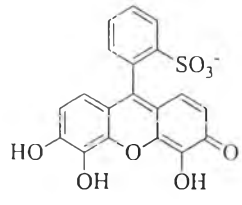
Quercitin



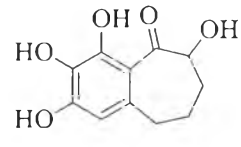
Baicalein



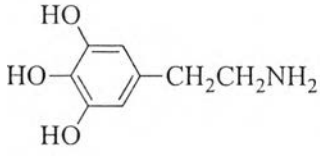
Fisetin



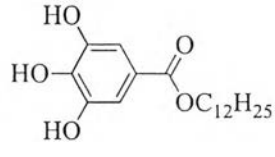
Pyrogallol Red



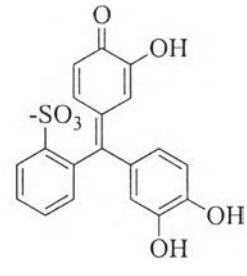
Purpurogallin



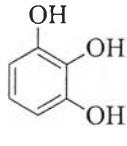
Hydroxydopamine



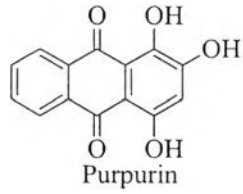
Dodecyl gallate



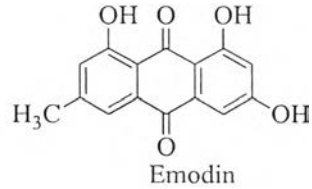
Pyrocatechol violet



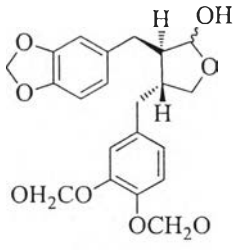
Pyrogallol



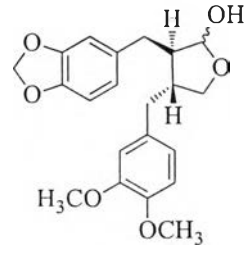
Purpurin



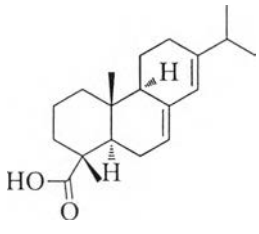
Emodin



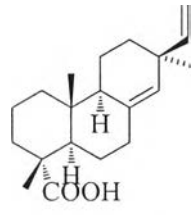
Cubebin



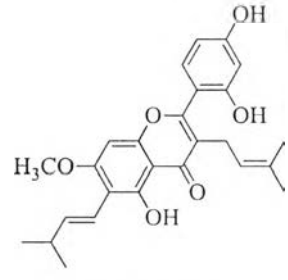
3,4-dimethoxy,-3,4-desmethylenedioxycubebin piperine



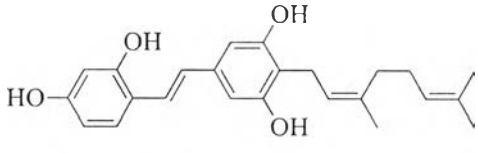
Abietic acid



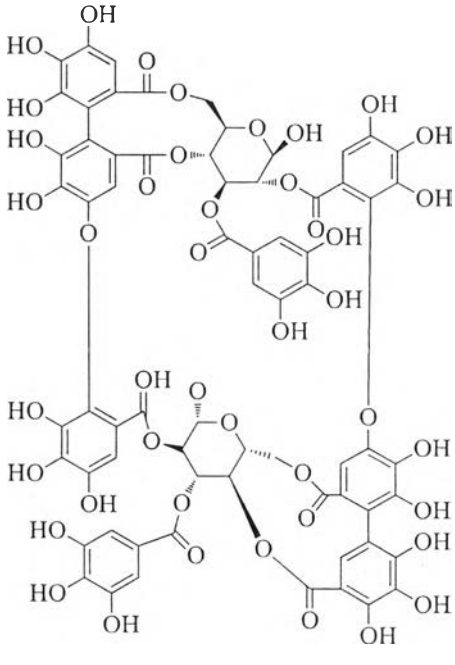
Pimaric acid



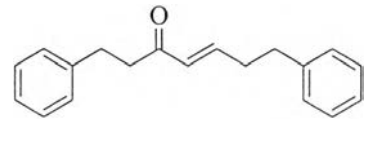
Artocarpin



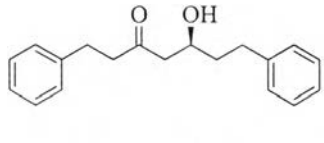
Chlorophorin



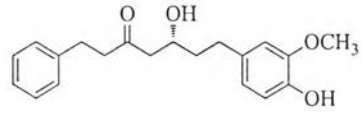
Oenothein B



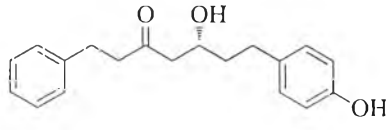
1,7-Diphenylhept-4-en-3-one



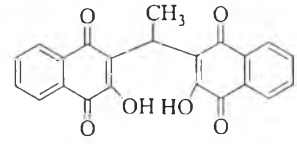
Dihydroyashabushiketol



5-Hydroxy-7-(4"-hydroxy-3"-methoxypheyl)-1-phenyl-3-heptanone



5-Hydroxy-7-(4"-hydroxyphenyl)-1-phenyl-3-heptanone



impatienol

Figure 6. Structures of natural 5α-R inhibitors

Based on the inhibitory activity, structure-activity relationship was conducted for few of the compounds listed in the table above. For example, modification in the structure of troilin showed that complete unsaturation of the guaiane moiety is unfavorable for the inhibition while bulky groups such as acetyl is favorable [41]. Modification of the EGCG structure was conducted through the replacement of the gallate group with aliphatic acid ester, producing a more potent inhibitor. It was concluded that longer fatty acid chain with some degree of unsaturation showed better inhibitory activity than short, saturated fatty aliphatic acids. For flavonoids, it was found that –OH group in the B-ring and the 4-keto group and the 2,3 double bond in the C-ring enhances the inhibitory activity [19].

2.6.2 Growth factors inducing compounds

Ethanolic extract of the root of *Asiasari radix* exhibited an increase in the expression of VEGF growth factor while the methanolic root extract of *Sophora flavescens* increased the expressions of IGF-1 and FGF-7 within the dermal papilla cells in absence of androgens [24, 44]. 5α-DHT treated dermal papilla cells showed an increase in the expression of Dickkopf (DKK-1), causing the keratinocytes to undergo apoptosis [8].

2.6.3 Compounds with dual activities

Till date only the ethanolic root extract of *Asiasari radix* and methanolic root extract of *Sophora flavescens* possess both 5α-R inhibitory activity and growth factor inducing capability in absence of androgens [24, 44].