



**Can plant-derived phytochemicals provide symptom relief
for hair loss?
A critical review**

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Abstract

It is known that hair growth disorders and hair loss can cause personal distress and affect well-being. Whilst clinical conditions remain a target for medical research, current research on hair follicle biology and hair growth control mechanisms also provides opportunities for a range of non-medical and cosmetic interventions that have a modulating effect on the scalp and follicle function. Furthermore, an improvement of the hair fibre characteristics (cuticle structure, cortex size and integrity) could add to the overall positive visual effect of the hair array. Since phytochemicals are a popular choice due to their traditional appeal, this review provides a critical evaluation of the available evidence of their activity for hair benefit, excluding data obtained from animal tests, and offers recommendations on improving study validity and the robustness of data collection in pre-clinical and clinical studies.

Introduction

This consideration of phytochemicals for hair benefit requires an appreciation of hair follicle structural organisation and the context of the stages of the hair growth cycle. This can be related to the most common pathologies of hair growth, as well as non-pathological hair loss conditions that may still raise the perceived need for temporary non-pharmacological treatments. Therefore, we begin with hair follicle and growth cycles and the most common hair loss conditions. We also include an overview of the commonly used pre- and clinical test methods and their contribution to the overall body of evidence regarding treatments for hair loss and we critically review both topical and oral applications in the context of the robustness and relevance of the reported test methods, data produced and interpretation of results. The review concludes with an assessment of the current scientific approaches to phytochemical product evaluation with recommendations for future developments.

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4 **Key words:** phytochemicals, hair growth, hair treatment, hair follicle, alopecia
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7 **Methods**
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10 Various search terms - hair loss, hair growth, human follicle, phytochemicals, plant
11 actives - were used to search the following databases: PubMed, Science Direct, Scopus
12 and Google Scholar. Further manual searches were conducted to access relevant
13 reference sources in the identified articles. Original articles, review articles and opinion
14 pieces published from 1990 to the current date were included.
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21 The following final article selection criteria were applied:
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25 1. Original research including human cell cultures, hair follicles and human volunteer
26 testing was reviewed. Whilst the research value of data, past and current, based on
27 murine cell, follicle and in vivo models is undisputed, differences between human
28 and animal models, as well as the drive to reduce animal testing, have raised the
29 demand for robust human-based data;
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34 2. Phytochemicals including individual or optimised chemical mixtures, as well as plant-
35 derived extracts such as oils and extract mixtures were searched. This criterion
36 reflects the non-medicinal approaches to symptom relief of a range of dermatological
37 and hair related conditions, whereby complementary rather than purely
38 pharmacological activity is sought;
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44 3. Preparations applied topically or for oral use were included, but not those that were
45 applied in combination with medicinal or light therapies. This criterion maintains the
46 focus on complimentary treatments;
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51 4. Review articles and opinion pieces, which were focused on test methods or on
52 phytochemicals/naturally derived mixtures, were included.
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The hair follicle

The hair follicle (HF) is a mini organ of the skin, extending from the epidermis into the lower dermis, tubular in shape and widening into a bulb at its lower end. Its anatomy, structure physiological role and regenerative nature is well described [1-3], although many aspects of its biology remain unclear [4]. Enclosed by the follicle bulb sits the dermal papilla (DP), consisting of spindle-shaped fibroblast cells referred to dermal papilla cells (DPC); connective tissue components and a capillary network. The lower DP is surrounded by the hair bulb containing hair matrix keratinocytes (HMK). During the active hair growth periods, HMK proliferate and cells are 'pushed' upwards, whilst undergoing lineage specific differentiation and keratinisation. This results in the formation of the three structural compartments of the hair fibre, medulla, cortex and cuticle and the follicle inner root sheath (IRS) that plays a role in the shaping of the hair fibre [5], and the companion layer [6]. The follicle is encased by the outer root sheath (ORS), which contains stem cells that play an active role in the initiation of the hair growth cycles [7, 8]. Melanocytes are located in the bulb matrix overlying the DP, where they are actively producing melanin in hair growth, as well as being present in the ORS where they are inactive but contribute to the re-pigmentation of the follicle during a new hair growth cycle. During the active hair growth periods, the melanocytes produce melanosomes which are distributed within the proteins of the cortical cells to afford the variety of hair colour [8]. A healthy human scalp holds between 100 000 - 120 000 HFs.

At any given time, between 80 and 90% of the HFs on a healthy scalp are in the stage of active hair fibre growth. This makes a full head of hair, which is known to present an important factor in individual's well-being. Reduced quality of life and other measures of well-being have been reported in patients with hair loss conditions [9-11].

Hence, in the last two decades, the endogenous and exogenous factors regulating the HF activity, as well as the pathological and age-related changes to hair follicle, have gained further attention from both medical and non-medical researchers.

Hair growth cycle

It is widely accepted that the HF undergoes four phases, resulting in the production of a hair fibre (anagen), regression (catagen), resting (telogen) and fibre shedding (exogen).

The *anagen* or the fibre growth stage is characterised by intense metabolic activity within the DP and the rapid proliferation (mitosis) of HMKs within the bulb. The mitotic rate of HMKs is amongst the highest in the human body [1]. Simultaneously, the bulb melanocytes produce melanin, which is transferred to the cortical cells via melanocyte dendrites. Follicles producing terminal scalp fibres will remain in anagen for 2-6 years, with hair growth maintained at around 1cm per month. The commencement of the regression stage, *catagen*, is marked by the repression of proliferation and an apoptosis-driven HF organ involution, which commences with a switching off of melanogenesis [8, 12]. The remnant HMKs differentiate to form an unpigmented 'club' at the proximal end of the fibre, and the club hair is shed during the telogen or early subsequent anagen. The termination of melanogenesis during the transient anagen-to-catagen phase is accredited to the reduced activity of hair bulb melanocytes and a decrease in the expression and activity of the enzyme tyrosinase (TYR) [8]. At the same time, the DP is protected from apoptosis by the upregulation of immune privilege (IP) [13, 14].

During the *telogen* or resting phase, the HF remains in a quiescent stage for approximately 8-12 weeks, until the commencement of the subsequent anagen, sometimes referred to as *neogen* stage [15]. During early anagen, progenitor cells located within the 'bulge' are activated, and the hair bulb is regenerated. The bulge is an anatomical label mostly used in murine hair growth. In human hair follicles the epithelium

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4 around and immediately below the telogen club hair defines one region of stem cells with
5 the ORS containing further populations [16-18]. The DP is critical in HF regeneration with
6 DP cells having hair regeneration properties [19, 20]. It is also believed that the shape
7 and size of the DP defines the size of the hair fibre [21-24]. The anagen fibre is extruded
8 through the follicle ostium and the remnant club hair from the previous cycle is shed in a
9 process known as *exogen* at an indeterminate stage of the hair cycle [25].

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18 More recently, a suggested adaptation to this model has been published, proposing that
19 the HF organ exhibits a bi-stable equilibrium [15]. According to this theory, the follicles
20 shift between steady states of growth (*anagen*) and rest (*telogen*), with an intermediate
21 transitory phase of either degradation (*catagen*) or neo-morphogenesis (*neogen*). The
22 process is stochastic but autonomous for each follicle, with the roles of different HF
23 compartments and signalling being still insufficiently understood. This type of critical
24 thinking helps to determine whether the strategy for treatment is one of influencing hair
25 growth at the transition points or maintaining the steady state between cyclic transitions.

26 27 28 29 30 31 32 33 34 35 **Hair loss conditions**

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38 It is proposed that 'hair loss' or 'thinning' is a multi-factorial perception of hair density,
39 fibre diameter, hair colour and degree of curl, which together contribute to the overall
40 perception of scalp coverage [26]. Hair loss includes androgen mediated alopecia (AGA)
41 as well as the diffuse hair thinning on the crown more typical of female pattern hair loss.
42 AGA is the most commonly investigated hair loss condition and also presents the
43 broadest range of interventions due to the variations of its manifestations. AGA is
44 mediated by androgen action in susceptible hair follicles, like those in the temple and
45 vertex in male scalp and those of the temple in older females [27-29]. The conversion of
46 testosterone to the more active metabolite dihydrotestosterone (DHT) is catalysed by
47 cytoplasmic 5 α -reductase enzymes within the DP cells [30]. Testosterone and DHT form
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receptor-ligand complexes with the androgen-receptor (AR), but DHT exhibits a greater degree of affinity with the AR than testosterone. It is therefore understood that inhibition of 5 α -reductase will significantly ameliorate AGA and finasteride and dutasteride have emerged as hair growth drugs which inhibit 5 alpha reductase [31] [32, 33].

Hair loss that is not immediately attributable to action of hormones includes auto-immune disorders such as Alopecia Areata (AA) and inflammatory scalp disorders such as Cicatricial Alopecia (CA) which results in scarring and permanent hair loss. Periods of excess hair shedding, which typically presents as a diffuse and transient disturbance of the hair cycle, are referred to as Telogen Effluvium (TE). Hair thinning associated with ageing (Senescent Alopecia) is of concern to older people and hair loss due to excessive mechanical stress on the hair follicle (Traction Alopecia) is common in people with very curly hair who adopt styles involving tight braiding. Poor scalp health such as in dandruff and seborrheic dermatitis can also be linked with temporary shedding. All forms of hair loss are targets for complimentary treatments, as discussed below. A summary of these conditions and their common treatments is presented in Table 1.

Table I: A summary of the different types of alopecia, their aetiology and manifestations as well as the typical proscribed treatment.

<u>Alopecia</u> <u>progenies</u>	<u>Aetiology</u>	<u>Histopathology</u>	<u>Treatment</u>
Androgenic Alopecia (AGA)	Androgen dependant (testosterone/DHT) follicle miniaturisation	Female – diffuse hair loss (Ludwig), often related to hyperandrogenism Male – often manifests as a receding hair-line	Topical Minoxidil (2%) Topical Minoxidil (5%) Oral Finasteride/ Dutasteride

		and hair loss in the vertex region (Hamilton-Norwood)	Hair transplantation
Alopecia Areata (AA)	T-cell dependant auto-immune disorder	Regions of 'patchy' hair-loss, which predominantly manifest in the scalp.	Topical Minoxidil (5%) Topical or oral corticosteroids
Alopecia Totalis (AT)		Complete absence of terminal scalp fibres	Wig/ Hair transplantation
Alopecia Universalis (AU)		Complete absence of scalp and bodily fibres	Wig
Cicatricial Alopecia (CA)	Inflammation of the upper part of the HF leading to irreversible damage of the hair bulge and the loss of stem cells	Permanent loss of hair in the affected patches, visible scarring may occur	Topical or oral antibiotics, corticosteroids; Scalp reduction surgery Hair transplantation
Senescent Alopecia (SA)	Stochastic 'ageing' of the follicle caused by UV, reactive oxygen species and other endogenous and exogenous stressors	Gradual decrease in fibre-diameter and the number of anagen-follicles, resulting in a diffuse hair-loss across the entirety of the scalp (non-androgenic)	Topical Minoxidil Cosmetic intervention Topical antioxidants

Traction Alopecia (TA)	Persistent or significant strain to the follicle, caused by particular grooming practices (Inc. braiding, tight pony-tails)	Scarring of follicles and hair-loss at the frontotemporal margin of the scalp	Acute: anti-inflammatory agents Chronic: Hair transplantation
Telogen Effluvium (TE)	Numerous 'triggers' including physiological and metabolic stress	Non-scarring diffuse hair-loss, characterised by the simultaneous shedding of telogen fibres	Cosmetic interventions: topical antioxidants and 5- α reductase inhibitors

Measurement approaches used to determine treatment benefits for hair loss

The study of the potential benefits of botanicals for common and disease-related hair loss employs many human pre-clinical models and clinical evaluation methods. Understanding the benefits and limitations of such assays is important in the interpretation of the data on treatments.

Hair loss involves alteration to the hair cycle or change to the size of the follicle that affects visible hair, so Phototrichogram (PTG) methods are one of the most useful methods for clinical evaluation. They give an accurate measure of the unit area hair density and the terminal- to-vellus hair ratio using a hair diameter cut off of 40 μ m. PTGs can give an insight to the underlying hair follicle biology, but can underestimate follicle density by not including telogen follicles that have shed the club hair, a sub-phase termed Kenogen, [34]. PTGs are usually used to follow a scalp site at intervals of one month,

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4 however, they are rarely used to follow individual follicle activity. Therefore, PTGs
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6 measure average changes of hairs growing in the site.
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9 The *ex vivo* human hair follicle (HF) model employs intact truncated anagen hair follicles
10 isolated from scalp skin and then placed in organ culture [35]. The development and use
11 of a fully defined medium for *ex vivo* hair growth is an advantage when testing added
12 hair actives. However, while hair fibre formation is observed over a period of a few days,
13 the model reflects maintenance of anagen status, comparing treatments with control.
14 Although treatments can improve maintenance of HF, many authors refer to this as an
15 increase in 'hair growth'. Importantly, as the HFs are usually cultured for periods of only
16 5-16 days, the range of intrinsic pre-programmed factors i.e. gender, scalp location and
17 general health of the donor might have a stronger effect on the growth rate than any
18 treatment [36, 37].
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31 Dermal papilla cells (DPC) have been used *in vitro* for evaluating hair growth benefits of
32 many phytochemicals, recently comprehensively reviewed by Madaan et al. [38].
33 Commonly, anything that encourages or increases DPC expression of Insulin Growth
34 Factor-1 (IGF-1) and Vascular Endothelial Growth Factor (VEGF) are interpreted as
35 anagen-prolonging, whilst any induction of Transforming Growth Factor β (TGF- β)
36 expression is considered as catagen-inducing. The expression of proteins Bcl-2 and Bax
37 and their ratio are also used as markers for apoptosis in DPCs [38]. However, caution
38 must be used when interpreting data as DPC from the more typically used occipital site
39 in men and parietal sites in women do not have androgen receptors [24]; this restricts
40 their use for studies of effects of androgens. Furthermore, DPC very rapidly lose their
41 hair induction phenotype in *in vitro* culture [19] and it is not clear that proliferation plays
42 a role in DPC in normal hair growth *in vivo*, hence studies that use DPC proliferation to
43 predict hair growth may not translate into the *in vivo* situation. The introduction of three-
44 dimensional sphere-like DPC models with or without added ORS cells, has made a major
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4 advance and studies using these models are likely to be more useful to predict *in vivo*
5 benefits [19, 39, 40]. Finally, for topical products to be effective via action in the DP, the
6 actives need to be able to penetrate into the scalp and to reach the DPC directly or via
7 the local circulation. Few studies explore this in detail; however, well-controlled clinical
8 studies that show vehicle only effects vs measurable clinical efficacy can infer some
9 active ingredient penetration necessary to generate the benefit.
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18 **Phytochemicals for hair loss treatment**

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21 Phytochemicals are often the sought-after choice of hair loss treatment; due to the
22 appeal such actives hold with the consumer/clinical patient and their non-drug status.
23 Furthermore, many phytochemicals are recognised for their role in traditional plant-
24 based treatments of skin and hair conditions, and a body of scientific literature related to
25 their medicinal applications may already exist. Those materials that have HF and or DPC
26 biological activity and/or *in vivo* clinical data are reviewed below. A full list of the reviewed
27 studies, meeting the selection criteria, including summaries of the tests applied, key end
28 points and conclusions on activity against targets, has been presented in Table II.
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38 **Caffeine**

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41 Caffeine is a purine alkaloid, derived from several sources including coffee beans,
42 tealeaves and cocoa and kola nuts. Its most well known mechanism of action is a
43 competitive inhibition of the enzyme phosphodiesterase (PDE), which breaks down
44 cyclic adenosine monophosphate (cAMP). By impairing the degradation of cAMP, thus
45 leading to its intracellular replenishment, caffeine has the effect of activating protein
46 kinase A and inhibiting leukotriene synthesis, which in turn reduces micro-inflammation
47 and stimulates cellular proliferation [41, 42]. These mechanisms are of relevance to hair
48 growth modulation. In addition, caffeine's anti-oxidant activity, via scavenging hydroxyl
49 and alkoxy radicals and its ability to inhibit 5 α -reductase, are all of relevance to
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4 stimulating scalp hair growth [43]. Consequently, a wider range of caffeine studies is
5 available, documenting its HF permeation and follicle activity, compared with any other
6 natural/nature-identical compound.
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11 The dose-dependent effect of caffeine combined with testosterone (5µg/ml) on vertex
12 HFs affected by AGA (male donors) was examined by Fischer [42]. After 120 h, the
13 testosterone metabolised by the HFs inhibited HF elongation in comparison with the
14 control growth media, whilst the addition of caffeine at 0.001% and 0.005% counteracted
15 this effect, strongly suggesting inhibition of 5α-reductase. Immunohistochemical staining
16 of the HF within the same study showed increased HMK and ORS cell proliferation in
17 the presence of caffeine. In a later study by the same authors, it was also demonstrated
18 that HF responses to caffeine treatment are gender dependant and *in vitro* tests on
19 ORSCs, demonstrated caffeine's modulating capacity on the growth factors IGF-1,
20 keratinocyte growth factor (KGF) and TGF-β2 that were comparable with minoxidil [44].
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33 A large number of *in vitro* caffeine permeation studies based on human or porcine tissue
34 have been published and the HF 'shunt route' presents an effective penetration route for
35 caffeine, irrespective of the target [45]. *In vivo* investigations involving the HF 'shunt'
36 route of delivery demonstrated caffeine availability in the blood serum and within the HF
37 following a single topical application for 24-48 hours [45-48]. A multi-centre *in vivo* study
38 (n=210) [49] compared the efficacies of a leave-on 0.2% caffeine solution and a 5%
39 minoxidil treatment on the scalp of male AGA patients (Hamilton-Norwood scale: III
40 Vertex to V). A designated dose of treatment product was applied twice daily for 6 months
41 and the primary end-point was the anagen/telogen ratio measured via PTG. The two
42 groups presented similar (not statistically significant) improvements of 11.68% and
43 10.59% in the ratio (baseline vs. 6 months). The authors concluded that a 0.2% caffeine
44 solution was not inferior to 5% minoxidil. However, the study did not include a placebo
45 group, thus it is difficult to ascertain to what degree the interventions might have been
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4 affected by seasonal and other exogenous factors. While these studies are informative,
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6 each potential product type (solution/tonic/shampoo) requires specific investigation,
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8 reflecting more precisely the context of the application technique and caffeine
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10 concentration levels present.
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13 Recently, the caffeine's potential to modulate the HF responses to UV radiation was
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15 demonstrated in a study applying topically 0.1% caffeine to skin specimens including
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17 terminal HF. The authors demonstrated that a combination of high dose UV (A+B)
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19 radiation induced HMK apoptosis, as well as the down-regulation of IGF-1 and the up-
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21 regulation of TGF- β 2 in the proximal (nearest to the scalp) end of the HF, whilst the
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23 application of caffeine for 3 days before and 3 days after radiation alleviated these effects
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25 [50]. The authors suggested that the caffeine's anti-oxidant and DPE-inhibition activities
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27 confer UV-protection properties.
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31 Caffeine is also presented in various cosmetic hair care products, such as shampoos
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33 and conditioners. While not intended for hair loss treatment, product benefits relevant to
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35 consumers concerned with hair thinning have been evidenced [51]. As a small and
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37 hydrophilic molecule, caffeine could contribute to water binding in the hair fibre, thus
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39 improving its plasticity.
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43 In conclusion, caffeine is the most studied phytochemical for hair loss and the various
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45 caffeine studies reported offer reasonable evidence that, when dosed adequately,
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47 caffeine will become present in the HF at levels that support its biological activities and
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49 over a sufficient period of time, could result in ameliorated hair loss in some patients.
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51 Furthermore, caffeine-containing products offer potential for multi-benefit solutions for
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53 consumers suffering hair loss. However, for cosmetic applications, each new formulation
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55 will require clinical and/or consumer testing with appropriate end-points to avoid
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57 generalising about the benefits of caffeine for the hair loss patient or consumer.
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Polyphenols

Polyphenols are a group of naturally occurring bioactive molecules used in a wide range of therapeutic products due to their health promoting effects. Polyphenols protect plants from different biotic and abiotic stresses (UV radiation and ROS-induced lipid peroxidation) due to their ability to scavenge free radicals. They present an interesting opportunity within the hair-growth sector, as they are potent anti-oxidants and anti-inflammatory agents [52, 53] [54]. Epigallocatechin-3-gallate (EGCG) has been identified as an integral polyphenolic catechin (monomeric flavan-3-ol) of green tea (*Camellia sinensis* L.) with well-documented anti-oxidant properties.

As oxidative stress and micro-inflammation have been demonstrated to contribute to the ageing of HFs and the diminishing melanocyte stem cell production [55-59], therefore EGCG is a suitable phytochemical treatment for some hair loss conditions.

An extensive exploratory study of the effect of EGCG on hair growth modulation as conducted *ex vivo* and *in vitro* using HFs in active anagen [60]. EGCG increased HF elongation by up to 123% in comparison with the control growth media. DPCs from HFs from patients treated with EGCG also presented a dose-dependent response of increased expression of survival mediating proteins P-Ekt and Bcl-2, and the apoptosis suppression protein Bax, thus corroborating the *ex vivo* data [60].

In another more recent study, EGCG elicited a dose-dependent improvement in the viability of DHT-treated DPCs [61]. Reduction in cell-senescence due to DHT-mediated elevation of ROS was detected and using a microarray analysis, the investigators identified a range of target genes that were up- or down-regulated in the DHT-treated DPCs in the presence of EGCG. These were associated with the cellular pathways of anti-oxidation, apoptosis, proliferation and cell cycle regulation, which are likely to be important in addressing hair loss.

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4 Recently, the potential benefits of EGCG for treatment of the autoimmune hair loss
5 condition, AA were reported, where the EGCG appeared to preserve immune privilege
6 in an *ex vivo* assay, by inhibiting gamma interferon (IFN- γ) pathways, [62]. Blood
7 samples from female patients with active hair loss and diagnosed AA were compared
8 with age-matched healthy controls. Inflammatory T cells and intracellular cytokines
9 (CD4, IFN- γ , IL-17) in AA patients' samples were increased in comparison to controls,
10 thus confirming their role in the pathogenesis. Both patient PBLs and HKC (HaCat) were
11 incubated with IFN- γ , and treated with 40 μ M EGCG, which specifically inhibited JAK2
12 expression, thus blocking the IFN- γ pathway known to reduce the immune privilege of
13 the HF [62].
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26 It has been demonstrated that EGCG could induce proliferation of aged human
27 epidermal keratinocytes (*in vivo*) in comparison with vehicle (ethanol/propylene glycol)
28 and that EGCG has an anti-apoptotic effect on human epidermal keratinocytes subjected
29 to UV-induced oxidative damage, thus suggesting potential scalp care efficacy [63]. Such
30 results on the epidermis is complementary to the HF-focused studies and would reflect
31 the conditions of clinical studies that include formulations designed for improved product
32 aesthetics, scalp health and user compliance. However, despite all this promising pre-
33 clinical data, actual clinical data on the effects of EGCG on AGA and other non-AGA hair
34 loss conditions is still lacking.
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46 Another polyphenolic phytochemical, procyanidin B-2 isolated from apple juice (*Malus*
47 *pumila*), was reported to ameliorate hair loss in Japanese men, based on its anti-
48 oxidative and anti-inflammatory properties [64, 65]. The effect of 1% active vs placebo
49 was assessed *in vivo* in a 6 month study using PTG and two primary end-points were
50 reported: increases in the total hair density and the A/T ratio (hair diameter > 40 μ m).
51 These studies were conducted only on Japanese males. It is not clear whether 1%
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4 procyanidin products will be effective in FPHL, which highlights the need to adapt the
5 study design to the different presentations of hair loss in different races and genders.
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9 In studies examining the potential effects of polyphenols from other plant sources, Shin
10 et al tested purified polyphenols from *Ecklonia cava* (Ec), sourced from marine brown
11 alga, with enriched eckol content and showed they elicited improved DPC proliferation
12 and increased expression of growth factors (IGF-1 and VEGF) whilst reducing the
13 oxidative stress induced by H₂O₂ *in vitro* [66]. It was concluded that DPC proliferation
14 was dose-dependent and the compound nature of the purified polyphenols + eckol was
15 beneficial in comparison with that of the individual anti-oxidant components. Enhanced
16 *Trifolium Pratense* extract also elicited reduced inflammatory response in DPCs and
17 improved A/T ratio in a pilot clinical study. In another study, a proprietary herbal blend
18 (including camomile and nettle) reduced apoptosis and increased cell viability of DPC
19 from healthy humans [67].
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33 Other actives of interest with clinical data are red clover extract [68]; raspberry ketone
34 [69]; capsaicin from red chilli [70]; rosemary oil [71]; onion juice [72]; saw palmetto [73];
35 nettle/camomile and an undisclosed proprietary herbal blend [74]. Korean red ginseng
36 extract (RGE) contains a range of bioactive constituents. A group of saponins, also
37 referred to as ginsenosides, have been tested for their potential to promote hair growth
38 and overall capacity to prevent oxidative damage in cells; recently reviewed by Choi
39 2018 [75].
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49 In *in vitro* studies, RGE and isolated ginsenoside-Rb1 (G-Rb1) and ginsenoside-Rg3 (G-
50 Rg3) were tested on HMKs and DPCs and isolated HFs. The extract and ginsenoside-
51 Rb1 induced proliferation of HMKs but not of DPCs. In another test, DPCs were pre-
52 treated with the actives, then incubated in DHT for 12 h. Predictably, DHT was found to
53 upregulate the androgen receptor (in comparison with control), whereby the active pre-
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4 treatments mitigated this effect [76]. Panax ginseng ginsenosides were also able to
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6 antagonise the androgen driven anagen-to-catagen transition, suggesting it may prolong
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8 anagen [77]. In a further, study ginsenoside F1 was found to reduce apoptosis in UV-B
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10 radiated human keratinocytes [78]. A range of *in vitro* tests elicited a dose dependent
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12 increase in cell viability, reduced cell DNA fragmentation and counteraction of the UV-B
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14 induced down regulation of Bcl-2. The anti-apoptotic activity of this compound is akin to
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16 that of polyphenols and offers opportunities for scalp care, which could complement the
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18 HF-directed intervention.
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20 21 22 *Oral phytotreatments*

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24 The demand for non-medicinal treatments of mild and age-related conditions of hair loss
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26 lends itself to more holistic approaches, including ingestible treatments. A small number
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28 of *in vivo* oral supplement-based treatments of hair loss have been reported in the
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30 literature. However, in addition to the small test groups, lifestyle and other confounding
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32 variables are typically not fully reported, hence the results are to be treated with caution.
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36 Oral capsaicin and isoflavone improved total hair count in a placebo, double blind study
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38 including AA, AT and AGA patients. The active group also showed increased IGF-1
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40 levels in their blood serum in comparison to baseline values [79]. Saw palmetto was
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42 tested vs. finasteride oral treatment of AGA patients, with hair growth in the vertex area
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44 being comparable to the drug [80]. In another study, saw palmetto and β -sitosterol
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46 improved hair density, based on blind expert score [81]. Pumpkin seed oil, containing
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48 phytosterols [82] was tested in a double-blind, placebo-controlled study of male AGA
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50 patients. The active group demonstrated statistically significant increase in hair count
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52 (density) in comparison with placebo.
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56 More complex blends, such as marine proteins and plant-derived actives, elicited higher
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58 total hair count and density in males with AGA (Norwood scale II and III) [83], as well as
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4 total hair count and the hair diameter of fine hair in healthy females with self-perceived
5 thinning hair [84-87]. These studies lasted 6 months and included placebo groups. The
6 authors concluded that food supplements could provide alternative treatments to hair
7 loss in males with less severe AGA, as well as females who perceive hair loss akin to
8 TE due to life factors.
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11 A common challenge with the interpretation of all *in vivo* data in the context of hair growth
12 cycles is the lack of detail on the timing of tests, as seasonality in hair loss is well
13 documented [88] and could interfere with the results, despite the use of placebo.
14 Furthermore, the grading reported in some of the studies is reliant on one clinical
15 assessor and is not based on any validated scales.
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18 **Discussion**

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20 In summary, whilst the range of phytochemicals proposed for treating hair loss is
21 promising and growing, the number of clinical studies published so far is low and a
22 common set of criteria for evaluating effective interventions with such preparations is
23 generally lacking.
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27 It is notable, however, that all investigated phytochemicals have well-documented
28 pharmacological activities and pathways, which hair researchers have also identified as
29 potential regulators of hair growth using cell and organotypic assays. Several of the
30 above studies have explored the mechanisms and biomarkers in hair follicles or derived
31 cells, based on gene expression and inter-cellular signalling, with the results
32 corroborating the broader data, mostly acquired via animal testing. Comprehensive
33 reviews of *in vitro* HF keratinocyte or DP cell data (human and murine) [38, 89], converge
34 on the following key mechanisms: cellular viability and proliferation in the DP, apoptosis
35 modulation, 5 α -reductase inhibition, inflammation reduction, related to the most
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4 commonly identified outcomes. Caffeine, phenolic compounds and phytosterols are
5 therefore the preferred choices for androgenic hair loss, as evidenced by the available
6 literature. Furthermore, studies using cultured HF demonstrate dose-responses to the
7 above treatments. To improve the relevance of *in vitro* models, it is suggested that DP
8 3D spheroids are adopted, due to their hair inductive ability [19, 90, 91].
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17 Another challenge to current *in vitro* and *ex vivo* data is its focus on the catagen-inducing
18 signalling in anagen HFs. However, according to Bernard [4], *in vivo* data suggests that
19 the responding follicles are not all in anagen, but some are in the resting stage, hence
20 further understanding of the HF responses to these phytochemicals is necessary,
21 specifically on the modulation of telogen to anagen transition.
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30 Another feature of the published *ex vivo* tests is their application of a single
31 phytochemical or simple mixtures. However, the complexity of the HF organ and its self-
32 regulatory processes, including the importance of blood supply, neuroendocrine
33 environment and the stages of the hair cycle, all suggest that a mixture of compounds,
34 targeting more than one process, is likely to be more effective [89]. Further evidence of
35 the positive effects of some actives on HKC proliferation and/or antioxidant efficacy,
36 which expands the range of strategies to conferring optimal conditions for healthy hair
37 growth.
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48 Some phytochemicals (capsaicin, raspberry ketone, onion juice) were identified as
49 stimulators of the perifollicular nerves, inducing immunological reaction and antigenic
50 competition, thus offering potential alternative AA treatments. However, these
51 approaches require more robust clinical evidence of their efficacy.
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4 Finally, when critically reviewing the reports of clinical studies with phytochemicals
5 (topically and orally applied), we conclude that there is an over-reliance on clinician's
6 scales and self-reported patient assessment. No histological data is presented, but the
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8 investigators infer biological activity based on known properties of the plant extract being
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10 tested.
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17 Despite the sparse published peer reviewed data, in a patent review in 2015 of hair and
18 scalp cosmetic treatments, Pawar [92] revealed that natural extracts were included in
19 61% of patents' treatments aimed at the single benefit of hair/loss prevention or alopecia
20 treatment. Some of the patented actives were: (I) extracts: Moringa oleifera, Bamboo
21 sap, Torreya nucifer seed, Mori radix, Scutelleriae radix, (II) phytochemicals such as
22 apeginin and glabranin, with commonly listed activities such as up-regulation of DPC
23 proliferation, inhibition of 5 α -reductase, scalp blood vessel dilation, oxidative stress
24 reduction. This data suggests that phytochemicals will remain an important source of
25 therapeutic and alternative treatments for hair loss. Hence, this critical review concludes
26 with a set of recommendations for improved study validity, which could be of help to
27 researchers.
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42 In summary, a combination of *in vitro* and/or *ex vivo* data with a well-designed clinical
43 trial is recommended. Based on *in vitro* studies to date, it is also desirable that gender
44 specific studies are conducted when androgen-related hair loss is targeted.
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51 *Ex vivo* tests combined with clinical trials would be more helpful in ascertaining the
52 mechanisms of hair cycle modulation by phytochemicals and the magnitude of effects
53 recorded. For *in vivo* clinical studies, placebo control and recognition of the
54 season/month of starting and concluding the study are recommended due to the
55 interference with seasonal moulting [88]. Confounding factors such as nutrition when
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4 topical products are tested, and the use of specific hair care products when nutritional
5 supplements are tested, should be better accounted for. For these studies, PTG offers
6 a better range of end points than clinician's scales, provided the trial is sufficiently long
7 (at least 4-6 months). Conditions such as AA & CA are not candidates for such
8 treatments as they require systematic monitoring of patients undergoing drug therapy,
9 however, phytochemicals as adjuvant to drug therapy is likely to be of benefit to these
10 patients. Finally, whilst HF studies shed light on the follicle's responses to actives, it is
11 uncommon to study scalp epidermis and dermis of sufferers of hair loss. The scalp skin
12 is exposed to a range of exogenous sources of oxidative stress, sensitizers and irritants
13 - solar radiation, pollution, water and grooming products – thus loss of structural integrity
14 of the scalp skin barrier could be contributing to the regulation of the hair growth cycle,
15 with short periods of excess shedding observed with dandruff [93]. Non-invasive studies
16 such as trans epidermal water loss (TEWL) for scalp skin barrier integrity, redness and
17 skin lipid peroxidation, as a proxy for levels of inflammation, as well as biopsy data of
18 general scalp skin/hair morphology would reveal useful complementary information to
19 the PTG-generated data.
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For Peer Review

Table II. A list of all reviewed phytochemical studies, including summaries of tests and assays, endpoint and results.

Phytochemical	Type of tests/substrate	Endpoints & Result	Mechanism of action	Reference
Topical treatments				
Caffeine <i>In vitro/ex vivo</i> <i>/in vivo</i> studies	<i>Ex vivo</i> HF organs (males with AGA Norwood-Hamilton stage III vertex and IV) + testosterone + caffeine in different concentrations (120h) Ki-67 immunostaining	Hair shaft elongation after incubation in testosterone and caffeine: <u>recovery in combined media equal to normal growth media</u> HF growth rate between 24 to 192h treatment with caffeine = <u>dose dependent</u> Ki-67 HMK cells and ORS cells = <u>increase</u>	Stimulation of HFK proliferation and 5 α -reductase inhibition Increased cell proliferation	[1]
	<i>Ex vivo</i> HF organ serum-free culture (healthy females facelift) + testosterone (120h) or + testosterone + caffeine 0.0005% (120h) vs. normal growth media (120h) Hematoxylin-eosin staining Ki67 immunostaining and TUNEL immunostaining	Hair shaft elongation: <u>increase</u> HF cycle score: <u>increase</u> Ki67 HMK cells: <u>increase</u>	5 α -reductase inhibition Increased cell proliferation and reduced apoptosis	[2]

	<p><i>Ex vivo HF</i> organ serum-free culture (males with AGA Norwood-Hamilton stage III vertex and IV) + testosterone (120h) or + testosterone + caffeine 0.0005% or 0.001% (120h) vs normal growth media (120h)</p> <p>Ki67 immunostaining and TUNEL immunostaining</p>	<p>Hair shaft elongation 0.001% caffeine +/- testosterone after 120h = <u>increase</u></p> <p>HF cycle score = <u>increase</u></p> <p>Ki67 positivity (HMK) = <u>increase</u></p>	<p>5α-reductase inhibition and reduced oxidative stress</p> <p>Increased cell proliferation and reduced apoptosis</p>	
	<p><i>In vitro</i> ORS cells (healthy males, eyebrow hair)</p> <p>Treated with caffeine at variable concentrations, minoxidil and tretinoin</p> <p>Ki67 immunostaining</p> <p>TSA immunofluorescence staining</p>	<p>ORS cell proliferation = <u>increase</u></p> <p>Modulating TGF-β2 (catagen inducer) gene expression and protein secretion = <u>decrease</u></p> <p>Modulating IGF-1 and KGF (anagen prolonging) gene expression and protein secretion = <u>increase</u></p>	<p>Stimulation of ORS cell proliferation</p>	
	<p><i>In vitro</i> ORS cells (healthy males, eyebrow hair)</p> <p>Treated with TGF-β2 or anandamide + <u>caffeine 0.001%</u> or IGF-1 or KGF,</p> <p>CyQUANT</p> <p>ELISA and Q-PCR analysis</p>	<p>ORS proliferation = <u>increase</u></p> <p>IGF-1 gene expression and protein secretion = <u>increase</u></p> <p>TGF-β2 gene expression and protein secretion = <u>decrease</u></p> <p>Apoptosis and necrosis after 48hours = <u>decrease</u></p>	<p>Inhibition of apoptosis and necrosis</p>	

	MMP assay & Sytox Green assay			
	<p><i>Ex vivo</i> HF containing skin specimens (2 male donors, occipital scalp)</p> <p>Treated with <u>0.1% caffeine in PEG6000</u> solution vehicle for 6 days (cons) and subjected to combined UVB and UVA radiation on day 3.</p> <p>Hematoxylin staining</p> <p>Melanin staining (Fontana-Masson)</p> <p>Ki-67 immunostaining</p> <p>TUNEL immunostaining</p>	<p>Percentage-fold change in sunburn cells (epidermal) caffeine treated vs. vehicle treated = <u>reduced</u></p> <p>Melanin clumping in the HF in presence of caffeine in comparison with control vehicle = <u>reduced</u></p> <p>ORS cells % change in distal (d), central (c) and proximal (p) position within the HF = differentiated effect of caffeine & UV at different locations</p> <p>IFG-1 and TGF-β2 = increased for HF ORS (p)</p> <p>Percentage of Ki-67 cells and TUNEL HMK cells = <u>reduced</u> apoptosis</p>	<p>UV-induced damage reduction of the HF surrounding epidermis</p> <p>UV protection in the HF epithelium</p> <p>UV mediated damage i.e. apoptosis effect is more prominent in the distal HF, but is elevated by caffeine</p>	[3]
	<i>In vivo</i> , healthy volunteers, n=10. Dosed application of 1% caffeine shampoo (2mins contact time)	Laser scanning microscopy of water-soluble fluorescent dye as a proxy for caffeine after 2min, 24h and 48h = <u>caffeine detected up to 48hours</u>	Water soluble actives can reach depth of 200 μ m, suggested access to the blood capillaries	[4]
	<i>In vivo</i> , healthy female volunteers, n=10. Dosed shampoo application on defined area of the scalp (2mins contact time)	Laser scanning microscopy of water-soluble fluorescent dye as a proxy for caffeine up to 24h after application = <u>caffeine detected</u>	Average penetration depth = 185 μ m, access the blood capillaries	Lademann et al <i>Laser Physics</i> 2010, Vol.

				20, No. 2, pp. 551–556
	<i>In vivo</i> , healthy male volunteers, n=6. 2.5% caffeine solution applied to a defined area of the chest, HFs blocked with waxes vs open HF	Blood serum analysis for caffeine up to 72 hours after application = <u>caffeine detected</u>	Follicular penetration route for caffeine	[5]
	<i>In vivo</i> - open label (males with AGA, Hamilton-Norwood stages III-V) n=210 Leave-on solution application twice a day; 0.2% caffeine vs 5% minoxidil	Percentage change in anagen hairs measured using frontal and occipital trichograms = <u>increase</u>	Stimulation of cell proliferation (HFK) and 5 α -reductase inhibition	[6]
EGCG (<i>Camellia sinensis</i>) <i>In vitro/ex vivo</i> studies	<i>Ex vivo</i> HF, organ culture from the occipital scalp of healthy males, n=3	Hair shaft elongation in 1 μ M EGCG = <u>increased</u>	Prolongation of anagen phase	[7]
	<i>In vitro</i> , DPCs from occipital scalp follicles of healthy males, n=3 MTT assay Western blot analysis	DPC proliferation = <u>increased</u> Erk and Akt = <u>increased</u> Bcl-2 = <u>increased</u> , Bax = <u>decreased</u>	Inhibition of apoptosis	
	<i>In vitro</i> , DHT-treated DPCs from a healthy male after 24h WST-1 Cell cycle analysis	Cell viability, after exposure to EGCG+DHT= <u>increased</u> Cell cycle = <u>extended</u> Cell senescence = <u>reduced</u>	Arresting the DHT Reduction of oxidative cell damage and	[8]

	<p>Cell senescence detection</p> <p>QR-PCR</p> <p>Fluorescence-based oxidation-sensitive probe</p>	<p>Altered miRNA profiles: predicting up and down regulation of IGF-1 and VEGF</p> <p>Intra cellular ROS = <u>reduced</u></p>	<p>related senescence</p> <p>Gene expression</p>	
	<p><i>In vitro and ex vivo</i> using cell culture donors: AA patients n=30 vs healthy control n=30</p> <p>HaCat cells and Jurkat cells (in vitro)</p> <p>PBMC followed by FACS analysis All cells were incubated in IFN-γ and treated with EGCG for variable time and concentrations Western blot analysis <i>in vitro</i> and <i>ex vivo</i> cells</p> <p>ELISA assay and Q-PCR</p>	<p>pSTAT1 = <u>reduced</u>, IRF-1= <u>reduced</u></p> <p>Pro inflammatory T cells and gene expression = <u>reduced</u></p>	<p>IFN-γ signalling inhibition</p> <p>Immunological action</p>	[9]
<p>EGCG</p> <p>Camellia sinensis</p> <p><i>In vivo</i> application/<i>in vitro</i> study</p>	<p><i>In vivo</i>, aged skin n=5, 10% EGCG applied three times a week for 6 weeks</p> <p><i>In vitro</i>, normal human epidermal keratinocytes, different doses applied 2-5 days</p> <p>10%, 1h post treatment</p> <p>Ki-67 immunostaining</p>	<p>Epidermal thickness = <u>increase</u></p> <p>HMK cell proliferation = <u>increase</u></p> <p>Cell proliferation/viability (MTT) = <u>dose dependent increase</u></p> <p>Erk and Akt = <u>increased</u> Bcl-2 increase, Bax = <u>decreased</u></p>	<p>Induced cell proliferation and survival</p>	[10]

	MTT assay			
	ELISA assay			
Procyanadin B-2 <i>Malus pumila</i> In vitro and ex vivo studies	<i>In vivo</i> , male with pattern balding according to Ogata scale, n=29, double blind, placebo controlled, 1% tonic application for 6 months	Phototrichograms used to measure: Total hair count = <u>increased</u> Ratio of terminal/all hair based on diameter measurements of clipped hair = <u>increased</u>	Suppression of inflammation	[11]
Polyphenols (phlorofurofucoecol A, eckol, dieckol) Ecklonia cava In vitro and ex vivo studies	<i>In vitro and ex vivo</i> , 12 healthy male donors for HFs and DPCs (occipital area) MTT assay, minoxidil positive control Q-PCR analysis, polyphenols vs dimethyl sulfoxide DPC and H ₂ O ₂ treatment for ROS (oxidation sensitive and fluorescence-based measurements) HF organ culture in various concentrations of active component vs dimethyl sulfoxide	Cell proliferation - <u>increased</u> mRNA expression of IGF-1 and VEGF = <u>increased</u> Oxidative stress = <u>reduced</u> <u>Increased</u> hair shaft production over 9 days	Growth promotion and ROS scavenging	[12]

<p>Proprietary herbal blend: Chamaemelum nobile, Althaea officinalis, Persea Americana, Rosmarinus Aloe officinalis vera, Urtica dioica, Thymus vulgaris</p> <p>In vivo study</p>	<p><i>In vitro</i>, DPCs obtained from HF organ culture from the occipital scalp of healthy males n=5</p> <p>qRT-PCR assay of 5α-R1 and 5α-R2 expression after treatment with different concentrations of herbal extract</p> <p>MTT and B assays DPC treated with different concentrations of the herbal extract in the presence and absence of Akt1/2 for 48h</p> <p>Cell proliferation (BrdU)</p> <p>DPC treated with different concentrations of the herbal extract</p>	<p>Expression of 5α R2 in DPC = <u>decrease</u></p> <p>DPC viability = <u>increase</u></p> <p>G1 phase progression proteins = <u>increase</u></p> <p>GAPDH, p-ERK and p-Akt = <u>increase</u> Bcl-2 = <u>increase</u> Bax = <u>decrease</u></p>	<p>5α-reductase 2 inhibition</p> <p>Inhibition of apoptosis</p> <p>Cell cycle regulation</p>	<p>[13]</p>
<p>Trifolium pratense flower extract (including isoflavone + biochanin; biometric peptide added in some tests)</p> <p>In vitro and in vivo studies</p>	<p><i>In vitro</i>: normal human dermal fibroblasts stimulated with IL-1α in presence of active variables vs positive and negative control</p> <p><i>In vivo</i>, healthy volunteers with mild to moderate hair loss undefined rating, n=30, placebo controlled 5% active solution, daily application over 4 months</p>	<p>IL-8 secretion = <u>reduced</u></p> <p>Trichoscan recording anagen/telogen ratio = <u>increased</u></p>	<p>Reduced micro-inflammation</p> <p>Combined effects of 5 α-reductase inhibition, reduced microinflammation and stimulation of extracellular matrix</p>	<p>[14]</p>

Rosebery ketone <i>Rubus idaeus</i> In vivo study	<i>In vivo</i> , male and female patients (mixed group AA and AGA), daily application of 0.01% solution vs. vehicle for 5 months Clinician's grading baseline vs. 5 months	Hair growth rates in 50% of active treated group = <u>increased</u>	Stimulation of IGF-1, thus HKC proliferation Increased dermal blood flow Down regulation of androgen receptors in the DPC	[15]
Capsaicin <i>Capsicum annum L</i> In vivo study	<i>In vivo</i> , AA patchy patients (<30% scalp involvement) n=50 capsaicin vs clobestol (positive control), application for 4 weeks, Clinician's assessment after 12 weeks (grading scale)	Hair grading in both groups = <u>statistically not different</u>	Stimulates the perifollicular nerves and the HF immune system	[16]
Rosemary oil <i>Rosmarinus officinalis L</i> In vivo study	<i>In vivo</i> AGA patients (n=50), topical application, positive control = 2% minoxidil Clinician's assessment after 6 months (grading scale) Self-assessment	Hair grading in both groups = <u>statistically not different</u> Rosemary group = <u>self-reported reduced scalp itches and irritation</u>		[17]
Crude onion juice <i>Allium cepa L</i> In vivo study	<i>In vivo</i> , AA patients, n=46 crude juice vs placebo (water) applied to affected area 2 times per day Clinician's assessment in intervals up to 2 months (grading scale)	Hair growth of terminal hair = declared after 2 weeks in active treatment group and increased as a percentage of observations within the group, vs statistically lower % of observations in the placebo group	Induction of immunological reaction and antigenic competition	[18]

<p>Saw palmetto (<i>Serenoa repens</i>)</p> <p><i>In vivo study</i></p>	<p>AGA male patients (Norwood-Hamilton types III, IV, V, VI), n=50, within subject comparison of active lotion after 12 weeks</p> <p>Phototrichogram Clinician assessment Self-assessment (validated scales)</p>	<p>Terminal hair count = <u>increased</u> Vellus hair count = <u>decrease</u> Results 12-24 weeks = <u>inconsistent</u></p>	<p>5 α-reductase inhibition by the fatty acid present in the SP</p>	[19]
<p>Proprietary herbal blend <i>Urtica uren,</i> <i>Urtica dioica,</i> <i>Matricaria chamomilla,</i> <i>Achillea millefolium,</i> <i>Ceratonia siliqua,</i> <i>Equisetum arvense</i></p> <p><i>In vivo study</i></p>	<p>Males AGA grade II-III Hamilton Norwood, and n-66 female with Telogen Effluvium, n=54 assigned to four treatment groups,</p> <p>active shampoo, active leave on solution, a combination of the above, placebo,</p> <p>Blind clinician's assessment – pull test, phototrichogram up to 6 months Self-assessment</p>	<p>Total hair count, Telogen/Anagen ratio after 2,4- and 6-months treatment: <u>increase for each time point in comparison to T0.</u></p> <p>Self-evaluation (questionnaire) scores – higher score for all active treatments vs. placebo</p>	<p>Synergistic combined effect on HF</p>	[20]
<p>Red ginseng extract <i>Panax ginseng</i></p>	<p>DPC cells obtained from non-balding scalp (hair transplantation surgery)</p> <p>DPCs + various concentration of G-Rb1 incubated (2h) with CCK-8</p>	<p>Cell proliferation: <u>increase in a dose-dependent manner</u></p> <p>p-Erk and pAkt expression: <u>increase</u></p>	<p>Increased cell proliferation</p> <p>Reduce DHT</p>	[21]

<p>(RGE)+ ginsenoside Rb1 and ginsenoside Rg3</p> <p><i>In vitro</i> study</p>	<p>ELISA of various concentration of RGE and G Rb-1 incubated with primary antibodies</p> <p>KI-67 assay: DHT pre-treated DPC, incubated with RGE, G-Rb1 and G-Rg3 at different concentration</p> <p>RT-PCR of the androgen receptor expression</p>	<p>Ki-67cell positivity = increase in presence of actives</p> <p>Actives reduced expression</p>	<p>Induced up-regulation of androgen receptor</p>	
<p>Ginsenoside F1 (mixed sources)</p> <p><i>Panax ginseng</i></p>	<p><i>In vitro</i>, UVB-radiated normal human epidermal keratinocytes, different active doses applied for 24h</p> <p>MTT assay TUNEL assay ELISA</p>	<p>Cell proliferation/viability = <u>dose dependent increase</u> for up to 90mJ/cm</p> <p>DNA fragmentation = <u>reduced</u></p> <p>Bcl-2 <u>increased</u>, Bax = <u>decreased</u></p>	<p>Protection against UV-B induced apoptosis</p>	<p>[22]</p>
Oral treatments				
<p>Capsaicin and isoflavone</p> <p>(origin not disclosed)</p> <p><i>In vivo</i> study</p>	<p><i>In vivo</i> patients AGA, AA, AT (n=47), placebo controlled, double blind trial for 5 months</p> <p>Blind clinician's assessment</p> <p>IGF-1 serum levels</p>	<p>IFG-1 level in serum</p> <p>Hair growth assessment based in photographs for patients 5 months vs. base line in active treatment = <u>increased</u></p> <p>IGF-1 serum levels for patients after 5 months vs base line in active treatment = <u>increased</u></p>	<p>Increase in IGF-1 suppresses TGF-β-induced apoptosis</p>	<p>[23]</p>

<p>Saw palmetto + β-sitosterol <i>Serenoa repens</i></p> <p>In vivo study</p>	<p><i>In vivo</i> AGA patients (Norwood - Hamilton types II-VI vertex), n=49 daily supplements for 21 weeks, double blind, placebo controlled</p> <p>Blind clinician's assessment</p> <p>Self-assessment</p>	<p>Improvement ratings = <u>increased</u></p> <p>Self-reported improvement of bold patches=<u>in active group only</u></p>	<p>5 α-reductase inhibition</p>	<p>[24]</p>
<p>Saw palmetto <i>Serenoa repens</i></p> <p>In vivo study</p>	<p><i>In vivo</i>, male AGA patients (Hamilton classification I to IV) n=100,</p> <p>24 monthly daily oral treatment active vs. finasteride (open trial)</p> <p>Global photos, used for baldness score (Hamilton) start and end of treatment</p>	<p>Hair growth in the vertex induced by active finasteride (vertex and occipital area)</p>	<p>5 α-reductase inhibition by the fatty acid present in the SP</p>	<p>[25]</p>
<p>Pumpkin seed oil <i>Cucurbita pepo</i></p> <p>In vivo study</p>	<p><i>In vivo</i>, AGA male patients (Norwood-Hamilton types II, III, III Vertex, IV and V), n=76</p> <p>Placebo controlled, blind trial, 24 weeks</p> <p>Phototrichograms Clinician's grading Self-assessment</p>	<p>Hair count=<u>increase</u>; hair thickness=<u>increase</u> Mean score of clinical grades = <u>increase</u></p>	<p>5 α-reductase inhibition</p>	<p>[26]</p>
<p>Proprietary blend</p> <p><i>Vitamin C</i> <i>(acerola powder and ascorbic</i></p>	<p>AGA males with pattern hair (Norwood-Hamilton scale II,II, III), n=60</p> <p>Placebo controlled for 180 days</p>	<p>Total hair count, total hair density, terminal hair density = <u>increased</u></p> <p>Hair removal = <u>reduced</u></p> <p><u>Improved wellbeing scores</u></p>	<p>Systemic effect</p>	<p>[27]</p>

<p><i>acid), zinc (from zinc oxide), shark powder and mollusk powder, horsetail (stem) extract and flax seed extract</i></p>	<p>Phototrichogram</p> <p>Hair pull test</p> <p>Quality of life questionnaire</p>			
<p>Proprietary blend</p> <p>Including; horsetail derived silica, Vit C from acerola chilly and procyanidin B-2marine complex</p>	<p>Healthy females (n=40), placebo control taking the oral supplement for 180 days</p> <p>Phototrichogram</p> <p>Self-assessment</p>	<p>Terminal hair density = <u>increased</u></p> <p>Hair diameter: <u>increased</u></p> <p>Increased hair diameter</p> <p>Self-reported hair volume, texture, as well as nails and skin related parameters</p> <p>Self-reported hair volume, texture, as well as nails and skin related parameters</p>	<p>Systemic effect</p>	<p>[28]</p>
<p>Proprietary blend including horsetail derived silica, vit C from acerola chilli and procyanidin B-2 marine complex</p>	<p>Healthy females (n=72), placebo controlled, taking the oral supplement for 180 days</p> <p>Phototrichogram</p> <p>Self-assessment</p>	<p>Hair shedding = <u>reduced</u></p> <p>Vellus-like hair diameter = <u>increased</u></p> <p>Increased hair diameter</p> <p>Self-reported hair volume, texture, as well as nails and skin related parameters</p>	<p>Systemic effect</p>	<p>[29]</p>

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13 Table III. Legend of abbreviations and terms used in Table II.

14 AGA	Androgenic alopecia
15 AA	Alopecia areata
16 Akt and pAkt	Protein kinase/phosphorylated Akt, a protein promoting cell survival
17 Bcl-2/Bax	B-cell lymphoma 2, protein regulator inhibiting apoptosis Bcl-2 like protein 4, protein regulating inducing apoptosis
18 CyQUANT	Fluorescence-based assay measuring cell proliferation, based on cellular DNA content
19 DPC	Dermal papilla cells
20 DHT	Dehydrotestosterone, an androgen responsible for follicle miniaturisation
21 EGCG	Epigallocatechin 3-gallate, the most abundant catechin in tea
22 ELISA	Enzyme-linked immunological assay used to detect and quantify the presence of proteins, enzymes and antibodies
23 Erk	Extracellular signal-regulated kinases promoting cell survival
24 FACS	Fluorescence-activated cell sorting of biological cell mixtures
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GAPDH	Glyceraldehyde 3-phosphate dehydrogenase, an enzyme catalysing a step in the glucose break down
HFK	Hair follicle keratinocytes
IFG-1	Insulin-like growth factor-1, a growth mediator
IL-8	Interleukin 8, a proinflammatory cytokine
KGF	Keratinocyte growth factor involved in skin healing
Ki-67	Immunostaining assay (colorimetric) detecting Ki-67 protein expression, associated with cell proliferation, thus providing a marker for the proportion of proliferating cells
MMP	Mitochondrial Membrane Potential Assay measuring the onset of apoptosis
MTT	A colorimetric assay used to measure cell viability/proliferation
ORS	Outer Root Sheath
PBMC	Peripheral blood mononuclear cell used to predict immunosuppressive effects
STAT1/pSTAT1	Signal transducer and activator of transcription (phosphorylated) plays a major role in immune responses
Sytox	Cell viability assay based on fluorescence, also used for cell apoptosis
TUNEL	Terminal Deoxynucleotidyl transferase dUTP nick end labelling, immunostaining assay detecting DNA fragmentation in cells, providing a marker for apoptotic cells
TGF- β 2	Transforming growth factor β 2, a protein suppressing cell proliferation and associated with the hair follicle's entry in catagen

Q-PCR or qRT-PCR	Quantitative (Q) or reverse transcription polymerase chain reaction, measuring the DNA or mitochondrial RNA, used as a marker for expressing growth factors and cycle transition
Western blot analysis	Gel electrophoresis, used to separate a mixture of proteins, concentrations measured via spectrophotometer
WST-1	Colorimetric assay quantifying viable cells/cell proliferation

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