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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

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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.

Key words: phytochemicals, hair growth, hair treatment, hair follicle, alopecia

Methods

Various search terms - hair loss, hair growth, human follicle, phytochemicals, plant actives - were used to search the following databases: PubMed, Science Direct, Scopus and Google Scholar. Further manual searches were conducted to access relevant reference sources in the identified articles. Original articles, review articles and opinion pieces published from 1990 to the current date were included.

The following final article selection criteria were applied:

1. Original research including human cell cultures, hair follicles and human volunteer testing was reviewed. Whilst the research value of data, past and current, based on murine cell, follicle and in vivo models is undisputed, differences between human and animal models, as well as the drive to reduce animal testing, have raised the demand for robust human-based data;
2. Phytochemicals including individual or optimised chemical mixtures, as well as plant-derived extracts such as oils and extract mixtures were searched. This criterion reflects the non-medicinal approaches to symptom relief of a range of dermatological and hair related conditions, whereby complementary rather than purely pharmacological activity is sought;
3. Preparations applied topically or for oral use were included, but not those that were applied in combination with medicinal or light therapies. This criterion maintains the focus on complimentary treatments;
4. Review articles and opinion pieces, which were focused on test methods or on phytochemicals/naturally derived mixtures, were included.

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, apoptosis of some subpopulations, hence the unpigmented club, and a decrease in the expression and activity of the enzyme tyrosinase (TYR) [8, 13]. At the same time, the DP is protected from apoptosis by the upregulation of immune privilege (IP) [14, 15].

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 [16]. 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 around and immediately below the telogen club hair defines one region of stem cells with the ORS containing further populations [17-19]. The DP is critical in HF regeneration with DP cells having hair regeneration properties [20, 21]. It is also believed that the shape and size of the DP defines the size of the hair fibre [22-25]. The anagen fibre is extruded through the follicle ostium and the remnant club hair from the previous cycle is shed in a process known as *exogen* at an indeterminate stage of the hair cycle [26].

More recently, a suggested adaptation to this model has been published, proposing that the HF organ exhibits a bi-stable equilibrium [16]. According to this theory, the follicles shift between steady states of growth (*anagen*) and rest (*telogen*), with an intermediate transitory phase of either degradation (*catagen)* or neo-morphogenesis (*neogen*). The process is stochastic but autonomous for each follicle, with the roles of different HF compartments and signalling being still insufficiently understood. This type of critical thinking helps to determine whether the strategy for treatment is one of influencing hair growth at the transition points or maintaining the steady state between cyclic transitions.

Hair loss conditions

It is proposed that 'hair loss' or 'thinning' is a multi-factorial perception of hair density, fibre diameter, hair colour and degree of curl, which together contribute to the overall perception of scalp coverage [27]. Hair loss includes androgen mediated alopecia (AGA) as well as the diffuse hair thinning on the crown more typical of female pattern hair loss. AGA is the most commonly investigated hair loss condition and also presents the broadest range of interventions due to the variations of its manifestations. AGA is

mediated by androgen action in susceptible hair follicles, like those in the temple and vertex in male scalp and those of the temple in older females [28-30]. The conversion of testosterone to the more active metabolite dihydrotestosterone (DHT) is catalysed by cytoplasmic 5α-reductase enzymes within the DP cells [31]. Testosterone and DHT form 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 5α-reductase will significantly ameliorate AGA and finasteride and dutasteride have emerged as hair growth drugs which inhibit 5 alpha reductase [32] [33, 34].

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), the latter also resulting in 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.

Alopecia progenies	**Aetiology**	**Histopathology**	**Treatment**
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 and hair loss in the vertex region (Hamilton-Norwood)	Topical Minoxidil (2%) Topical Minoxidil (5%) Oral Finasteride/ Dutasteride 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	Permanent loss of hair in the affected patches, visible scarring may occur	Topical or oral antibiotics, corticosteroids; Scalp reduction surgery Hair transplantation

	cells		
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. PTGs are usually used to follow a scalp site at intervals of one month, but they are rarely used to follow individual follicle activity. Therefore, PTGs measure average changes of hairs growing in the site via the accurate measurements of the unit area hair density and the terminal- to-vellus hair ratio using a hair diameter cut off of 30μm [35] or 40μm (Table II). Thus, PTGs are used to infer some modulation to the HF functioning in response to treatments (topical and oral). However, they can underestimate follicle density by not including telogen follicles that have shed the club hair, a sub-phase termed Kenogen, [36].

The *ex vivo* human hair follicle (HF) model employs intact truncated anagen hair follicles isolated from scalp skin and then placed in organ culture [37]. The development and use of a fully defined medium for *ex vivo* hair growth is an advantage when testing added hair actives, but the method has its limitations. Firstly, as hair fibre formation is observed over a period of a few days, the model reflects maintenance of anagen status, not a stimulation of "hair growth". The choices of the HF's sources are critical if androgen-driven hair loss is targeted, including the donor's gender and scalp location [38]. Importantly, as the HFs are usually cultured for periods of only 5-8 days (although longer periods are possible), a range of intrinsic pre-programmed factors such as how long the follicle has been in anagen, may influence HF activity *ex vivo* [39, 40].

Dermal papilla cells (DPC) have been used *in vitro* for evaluating hair growth benefits of many phytochemicals, recently comprehensively reviewed by Madaan *et al.* [41]. Commonly, anything that encourages or increases DPC expression of Insulin Growth Factor-1 (IGF-1) and Vascular Endothelial Growth Factor (VEGF) are interpreted as anagen-prolonging, whilst any induction of Transforming Growth Factor β (TGF-β)

expression is considered as catagen-inducing. The expression of proteins Bcl-2 and Bax and their ratio are also used as markers for apoptosis in DPCs [41]. However, caution must be used when interpreting data as DPC from the more typically used occipital site in men and parietal sites in women do not have androgen receptors [25, 38, 42]; this restricts their use for studies of effects of androgens. Furthermore, DPCs very rapidly lose their hair induction phenotype in *in vitro* culture [20] and it is not clear that proliferation plays a role in DPC in normal hair growth *in vivo*, hence studies that use DPC proliferation to predict hair growth may not translate into the *in vivo* situation. The introduction of three-dimensional sphere-like DPC models with or without added ORS cells, has made a major advance and studies using these models are likely to be more useful to predict *in vivo* benefits [20, 43, 44].

Finally, for topical products to be effective, the actives need to be able to penetrate into the scalp and the HF directly or via the local circulation. Few studies explore this in detail; however, well-controlled clinical studies that show vehicle only effects vs measurable clinical efficacy can infer some active ingredient penetration necessary to generate the benefit.

Phytochemicals for hair loss treatment

Phytochemicals are often the sought-after choice of hair loss treatment; due to the appeal such actives hold with the consumer/clinical patient and their non-drug status. Furthermore, many phytochemicals are recognised for their role in traditional plant-based treatments of skin and hair conditions, and a body of scientific literature related to their medicinal applications may already exist. Those materials that have HF and or DPC biological activity and/or *in vivo* clinical data are reviewed below. A full list of the reviewed studies, meeting the selection criteria, including summaries of the tests

applied, key end points and conclusions on activity against targets, has been presented in Table II.

Caffeine

Caffeine is a purine alkaloid, derived from several sources including coffee beans, tea leaves and cocoa and kola nuts. Its most well known mechanism of action is a competitive inhibition of the enzyme phosphodiesterase (PDE), which breaks down cyclic adenosine monophosphate (cAMP). By impairing the degradation of cAMP, thus leading to its intracellular replenishment, caffeine has the effect of activating protein kinase A and inhibiting leukotriene synthesis, which in turn reduces micro-inflammation and stimulates cellular proliferation [45, 46]. These mechanisms are of relevance to hair growth modulation. In addition, caffeine's anti-oxidant activity, via scavenging hydroxyl and alkoxyl radicals is of relevance to maintaining healthy scalp and stimulating scalp hair growth [47]. However, caffeine's potential for mitigating androgen-induced hair loss has been most widely studied to date, with authors inferring that caffeine can inhibit 5α-reductase, thus reducing the DHT in the HF. The most data-rich studies have been reviewed below.

The dose-dependent effect of caffeine combined with testosterone (5μg/ml) on vertex HFs affected by AGA (male donors) was examined by Fischer [46]. After 120 h, the testosterone metabolised by the HFs inhibited HF elongation in comparison with the control growth media, whilst the addition of caffeine at 0.001% and 0.005% counteracted this effect, thus inferring inhibition of 5α-reductase. Immunohistochemical staining of the HF within the same study showed increased HMK and ORS cell proliferation in the presence of caffeine. In a later study by the same authors, it was also demonstrated that HF responses to caffeine treatment are gender dependant with HF from healthy females responding to testosterone and caffeine at lower

concentration (0.0005%) than the HFs from the AGA males. Finally, *in vitro* tests on ORSCs demonstrated caffeine's modulating capacity on the growth factors IGF-1, keratinocyte growth factor (KGF) and TGF-β2 that were comparable with minoxidil [48].

A large number of *in vitro* caffeine permeation studies based on human or porcine tissue have been published and the HF 'shunt route' presents an effective penetration route for caffeine, irrespective of the target [49]. *In vivo* investigations involving the HF 'shunt' route of delivery demonstrated caffeine availability in the blood serum and within the HF following a single topical application for 24-48 hours [49-52]. A multi-centre *in vivo* study (n=210) [53] compared the efficacies of a leave-on 0.2% caffeine solution and a 5% minoxidil treatment on the scalp of male AGA patients (Hamilton-Norwood scale: III Vertex to V). A designated dose of treatment product was applied twice daily for 6 months and the primary end-point was the anagen/telogen ratio measured via PTG. The two groups presented similar (not statistically significant) improvements of 11.68% and 10.59% in the ratio (baseline vs. 6 months). The authors concluded that a 0.2% caffeine solution was not inferior to 5% minoxidil. However, the study did not include a placebo group, thus it is difficult to ascertain to what degree the interventions might have been affected by seasonal and other exogenous factors. While the above studies are informative and the preclinical data provides insights into the biological activity of caffeine, each potential product type (solution/tonic/shampoo) requires a placebo-controlled clinical trial reflecting more precisely the context of the application technique and caffeine concentration levels present.

Recently, the caffeine's potential to modulate the HF responses to UV radiation was demonstrated in an *ex vivo* study applying topically 0.1% caffeine to full thickness skin specimens including terminal HF. Firstly, the authors demonstrated that a combination of high dose UV (A+B) radiation induced HMK apoptosis, as well as the down-regulation of IGF-1 and the up-regulation of TGF-β2 in the proximal (nearest to the

scalp) end of the HFs, hence full thickness skin organ culture offers an alternative test model. In follow up tests, the application of caffeine to the skin samples for 3 days before and 3 days after irradiation alleviated the deleterious effects and the authors suggested that the caffeine's anti-oxidant and DPE-inhibition activities confer some UV-protection properties. However, in line with previous studies, the authors stressed the dose-dependent effect, as caffeine-induced apoptosis of HMCs occurred at certain doses. This confirms the need for vehicle formulations and active concentrations testing as stated already [54].

Caffeine is also presented in various cosmetic hair care products, such as shampoos and conditioners. There is no published data on caffeine's specific impact on the hair shaft's properties, although as a small and hydrophilic molecule, caffeine could contribute to water binding in the hair fibre, thus improving its plasticity. A leave-on product, containing caffeine, niacinamide and a specific polymer, and benefiting consumers concerned with hair thinning, was found to successfully deposit radio labelled caffeine in the hair fibres. Improved mechanical properties such as break stress and shear modulus of the fibres were reported, thus inferring the caffeine's (and other actives') effect on structure. The authors concluded that fibre condition improvements via water-binding molecules (and the selected polymer) could be beneficial to thinning hair [55].

In conclusion, caffeine is the most studied phytochemical for hair loss and the various caffeine studies reported offer reasonable evidence that, when dosed adequately, caffeine will become present in the HF at levels that support its biological activities and over a sufficient period of time, could result in ameliorated hair loss in some patients. Furthermore, caffeine-containing products offer potential for multi-benefit solutions for consumers suffering hair loss. However, for cosmetic applications, each new

formulation will require clinical and/or consumer testing with appropriate end-points to avoid generalising about the benefits of caffeine for the hair loss patient or consumer.

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 [56, 57] [58]. 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 [59-63], 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 [64]. 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 [64].

In another more recent study, EGCG elicited a dose-dependent improvement in the viability of DHT-treated DPCs [65]. 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.

Recently, the potential benefits of EGCG for treatment of the autoimmune hair loss condition, AA were reported, where the EGCG appeared to preserve immune privilege in an *ex vivo* assay, by inhibiting gamma interferon (IFN-γ) pathways, [66]. Blood samples from female patients with active hair loss and diagnosed AA were compared with age-matched healthy controls. Inflammatory T cells and intracellular cytokines (CD4, IFN-γ, IL-17) in AA patients' samples were increased in comparison to controls, thus confirming their role in the pathogenesis. Both patient PBLs and HKC (HaCat) were incubated with IFN-γ, and treated with 40μM EGCG, which specifically inhibited JAK2 expression, thus blocking the IFN-γ pathway known to reduce the immune privilege of the HF [66].

It has been demonstrated that EGCG could induce proliferation of aged human epidermal keratinocytes (*in vivo*) in comparison with vehicle (ethanol/propylene glycol) and that ECGC has an anti-apoptotic effect on human epidermal keratinocytes subjected to UV-induced oxidative damage, thus suggesting potential scalp care efficacy [67]. Such results on the epidermis is complementary to the HF-focused studies and would reflect the conditions of clinical studies that include formulations designed for improved product aesthetics, scalp health and user compliance. However, despite all this promising pre-clinical data, actual clinical data on the effects of EGCG on AGA and other non-AGA hair loss conditions is still lacking.

Another polyphenolic phytochemical, procyanidin B-2 isolated from apple juice (*Malus pumila),* was reported to ameliorate hair loss in Japanese men, based on its anti-oxidative and anti-inflammatory properties [68, 69]. The effect of 1% active vs placebo was assessed *in vivo* in a 6 month study using PTG and two primary end-points were

reported: increases in the total hair density and the A/T ratio (hair diameter > 40μm). These studies were conducted only on Japanese males. It is not clear whether 1% procyanidin products will be effective in FPHL, which highlights the need to adapt the study design to the different presentations of hair loss in different races and genders.

In studies examining the potential effects of polyphenols from other plant sources, Shin et al tested purified polyphenols from *Ecklonia cava* (Ec), sourced from marine brown alga, with enriched eckol content and showed they elicited improved DPC proliferation and increased expression of growth factors (IGF-1 and VEGF) whilst reducing the oxidative stress induced by H_2O_2 *in vitro* [70]. It was concluded that DPC proliferation was dose-dependent and the compound nature of the purified polyphenols + eckol was beneficial in comparison with that of the individual anti-oxidant components. Enhanced *Trifollium Pratense* extract also elicited reduced inflammatory response in DPCs and improved A/T ratio in a pilot clinical study. In another study, a proprietary herbal blend (including camomile and nettle) reduced apoptosis and increased cell viability of DPC from healthy humans [71].

Other actives of interest with clinical data are red clover extract [72]; raspberry ketone [73]; capsaicin from red chilli [74]; rosemary oil [75]; onion juice [76]; saw palmetto [77]; nettle/camomile and an undisclosed proprietary herbal blend [78]. Details of the test methods and end-points are listed in Table II. However, small cohorts and variable approaches to clinical study design make the data difficult to interpret even when techniques such as PTG are used. Further critique of these studies is provided in the discussion section of this review.

Korean red ginseng extract (RGE) contains a range of bioactive constituents. A group of saponins, also referred to as gingsenosides, have been tested for their potential to

promote hair growth and overall capacity to prevent oxidative damage in cells; recently reviewed by Choi 2018 [79].

In *in vitro* studies, RGE and isolated ginsenoside-Rb1 (G-Rb1) and ginsenoside-Rg3 (G-Rg3) were tested on HMKs and DPCs and isolated HFs. The extract and ginsenoside-Rb1 induced proliferation of HMKs but not of DPCs. In another test, DPCs were pre-treated with the actives, then incubated in DHT for 12 h. Predictably, DHT was found to upregulate the androgen receptor (in comparison with control), whereby the active pre-treatments mitigated this effect [80]. Panex ginseng ginsenosides were also able to antagonise the androgen driven anagen-to-catagen transition, suggesting it may prolong anagen [81]. In a further, study ginsenoside F1 was found to reduce apoptosis in UV-B radiated human keratinocytes [82]. A range of *in vitro* tests elicited a dose dependent increase in cell viability, reduced cell DNA fragmentation and counteraction of the UV-B induced down regulation of Bcl-2. The anti-apoptotic activity of this compound is akin to that of polyphenols and offers opportunities for scalp care, which could complement the HF-directed intervention. However, clinical data for RGE is lacking.

Oral phytotreatments

The demand for non-medicinal treatments of mild and age-related conditions of hair loss lends itself to more holistic approaches, including ingestible treatments. A small number of *in vivo* oral supplement-based treatments of hair loss have been reported so far in the literature and, similarly to the clinical studies of topical treatments, the robustness of the methods and data is variable. In this review, only studies including placebo or drug-based control have been reviewed

Oral capsaicin and isoflavone improved total hair count in a placebo, double blind study including AA, AT and AGA patients. The active group also showed increased IGF-1

levels in their blood serum in comparison to baseline values [83]. Saw palmetto was tested vs. finasteride oral treatment of AGA patients, with hair growth in the vertex area being comparable to the drug [84]. In another study, saw palmetto and *β*-sitosterol improved hair density, based on blind expert score [85]. Pumpkin seed oil, containing phytosterols [86] was tested in a double-blind, placebo-controlled study of male AGA patients. The active group demonstrated statistically significant increase in hair count (density) in comparison with placebo. The details of these studies are listed in Table II.

More complex blends, such as marine proteins and plant-derived actives, elicited higher total hair count and density in males with AGA (Norwood scale II and III) [87], as well as total hair count and the hair diameter of fine hair in healthy females with self-perceived thinning hair [88-91]. These studies lasted 6 months, were based on 40-72 participants and included placebo groups. The authors concluded that food supplements could provide alternative treatments to hair loss in males with less severe AGA, as well as females who perceive hair loss akin to TE due to life factors. However, not all studies report the criterion for determination of terminal hair. A common challenge with the interpretation of all reviewed *in vivo* data in the context of hair growth cycles is the lack of detail on the timing of tests, as seasonality in hair loss is well documented [92] and could interfere with the results, despite the use of placebo.

Discussion

In summary, whilst the range of phytochemicals proposed for treating hair loss is promising and growing, the number of clinical studies published so far is low and a common set of criteria for evaluating effective interventions with such preparations is generally lacking.

It is notable, however, that all investigated phytochemicals have well-documented pharmacological activities and pathways, which hair researchers have also identified as potential regulators of hair growth using cell and organotypic assays. Several of the above studies have explored the mechanisms and biomarkers in hair follicles or derived cells, based on gene expression and inter-cellular signalling, with the results corroborating the broader data, mostly acquired via animal testing. Comprehensive reviews of *in vitro* HF keratinocyte or DP cell data (human and murine) [41, 93], converge on the following key mechanisms: cellular viability and proliferation in the DP, apoptosis modulation, 5α-reductase inhibition, inflammation reduction, related to the most commonly identified outcomes. Caffeine, phenolic compounds and phytosterols are therefore the preferred choices for androgenic hair loss, as evidenced by the available literature. Furthermore, studies using cultured HF demonstrate dose-responses to the above treatments. To improve the relevance of *in vitro* models, it is suggested that DP 3D spheroids are adopted, due to their hair inductive ability [20, 94, 95].

Another challenge to current *in vitro* and *ex vivo* data is its focus on the catagen-inducing signalling in anagen HFs. However, according to Bernard [4], *in vivo* data suggests that the responding follicles are not all in anagen, but some are in the resting stage, hence further understanding of the HF responses to these phytochemicals is necessary, specifically on the modulation of telogen to anagen transition.

Another feature of the published *ex vivo* tests is their application of a single phytochemical or simple mixtures. However, the complexity of the HF organ and its self-regulatory processes, including the importance of blood supply, neuroendocrine environment and the stages of the hair cycle, all suggest that a mixture of compounds, targeting more than one process, is likely to be more effective [93]. Further evidence

of the positive effects of some actives on HKC proliferation and/or antioxidant efficacy, which expands the range of strategies to conferring optimal conditions for healthy hair growth.

Some phytochemicals (capsaicin, raspberry ketone, onion juice) were identified as stimulators of the perifollicular nerves, inducing immunological reaction and antigenic competition, thus offering potential alternative AA treatments. However, these approaches require more robust clinical evidence of their efficacy, not found in the reviewed papers.

Finally, when critically reviewing the reports of clinical studies with phytochemicals (topically and orally applied), we conclude that there is an over-reliance on clinician's scales and self-reported patient assessment. No histological data is presented although, some photographic evidence is published, and hence the investigators infer biological activity based on known properties of the plant extract being tested and/or *in vitro* data.

Despite the sparse published peer reviewed data, in a patent review in 2015 of hair and scalp cosmetic treatments, Pawar [96] revealed that natural extracts were included in 61% of patents' treatments aimed at the single benefit of hair/loss prevention or alopecia treatment. Some of the patented actives were: (I) extracts: Moringa oleifera, Bamboo sap, Torreya nucifer seed, Mori radix, Scutelleriae radix, (II) phytochemicals such as apeginin and glabranin, with commonly listed activities such as up-regulation of DPC proliferation, inhibition of 5α-reductase, scalp blood vessel dilation, oxidative stress reduction. This data suggests that phytochemicals will remain an important source of therapeutic and alternative treatments for hair loss. Hence, this critical

review concludes with a set of recommendations for improved study validity, which could be of help to researchers.

In summary, a combination of *in vitro* and/or *ex vivo* data with a well-designed clinical trial is recommended. Based on *in vitro* studies to date, it is also desirable that gender specific studies are conducted when androgen-related hair loss is targeted.

Specifically, *ex vivo* tests combined with clinical trials would be more helpful in ascertaining the mechanisms of hair cycle modulation by phytochemicals and the magnitude of effects recorded. For *in vivo* clinical studies, placebo control and recognition of the season/month of starting and concluding the study are recommended due to the interference with seasonal moulting [92]. Confounding factors such as nutrition when topical products are tested, and the use of specific hair care products when nutritional supplements are tested, should be better accounted for. For these studies, PTG offers a better range of end points than clinician's scales or self-assessment, provided the trial is sufficiently long (at least 4-6 months). Furthermore, global hair growth photographic evidence should be provided thus allowing for the assessment of the relative magnitude of the improvements. Conditions such as AA & CA are not candidates for such treatments as they require systematic monitoring of patients undergoing drug therapy, however, phytochemicals as adjuvant to drug therapy is likely to be of benefit to these patients. Finally, whilst HF studies shed light on the follicle's responses to actives, it is uncommon to study scalp epidermis and dermis of sufferers of hair loss. The scalp skin is exposed to a range of exogenous sources of oxidative stress, sensitizers and irritants - solar radiation, pollution, water and grooming products – thus loss of structural integrity of the scalp skin barrier could be contributing to the regulation of the hair growth cycle, with short periods of excess shedding observed with dandruff [97]. Non-invasive studies such as trans epidermal

water loss (TEWL) for scalp skin barrier integrity, redness and skin lipid peroxidation, as a proxy for levels of inflammation, as well as biopsy data of general scalp skin/hair morphology would reveal useful complementary information to the PTG-generated data.

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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 ***In vitro/ex vivo /in vivo* studies**	*Ex vivo* 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: recovery in combined media equal to normal growth media HF growth rate between 24 to 192h treatment with caffeine = dose dependent Ki-67 HMK cells and ORS cells = increase	Stimulation of HFK proliferation and 5α-reductase inhibition Increased cell proliferation	[1]

	Ex vivo 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>	5α-reductase inhibition Increased cell proliferation and reduced apoptosis	[2]
	Ex vivo HF 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) Ki67 immunostaining and TUNEL immunostaining	Hair shaft elongation 0.001% caffeine +/- testosterone after 120h = <u>increase</u> HF cycle score = <u>increase</u> Ki67 positivity (HMK) = <u>increase</u>	5α-reductase inhibition and reduced oxidative stress Increased cell proliferation and reduced apoptosis	

	In vitro ORS cells (healthy males, eyebrow hair) Treated with caffeine at variable concentrations, minoxidil and tretinoin Ki67 immunostaining TSA immunofluorescence staining	ORS cell proliferation = <u>increase</u> Modulating TGF-β2 (catagen inducer) gene expression and protein secretion = <u>decrease</u> Modulating IGF-1 and KGF (anagen prolonging) gene expression and protein secretion = <u>increase</u>	Stimulation of ORS cell proliferation	
	In vitro ORS cells (healthy males, eyebrow hair) Treated with TGF-β2 or anandamide + <u>caffeine 0.001%</u> or IGF-1 or KGF, CyQUANT ELISA and Q-PCR analysis	ORS proliferation = <u>increase</u> IGF-1 gene expression and protein secretion = <u>increase</u> TGF-β2 gene expression and protein secretion = <u>decrease</u> Apoptosis and necrosis after 48hours = <u>decrease</u>	Inhibition of apoptosis and necrosis	

	MMP assay & Sytox Green assay			
	Ex vivo HF containing skin specimens (2 male donors, occipital scalp) Treated with 0.1% caffeine in PEG6000 solution vehicle for 6 days (cons) and subjected to combined UVB and UVA radiation on day 3. Hematoxylin staining Melanin staining (Fontana-Masson) Ki-67 immunostaining TUNEL immunostaining	Percentage-fold change in sunburn cells (epidermal) caffeine treated vs. vehicle treated = reduced Melanin clumping in the HF in presence of caffeine in comparison with control vehicle = reduced ORS cells % change in distal (d), central (c) and proximal (p) position within the HF = differentiated effect of caffeine &UV at different locations IFG-1 and TGF-β2 = increased for HF ORS (p) Percentage of Ki-67 cells and TUNEL HMK cells = reduced apoptosis	UV-induced damage reduction of the HF surrounding epidermis UV protection in the HF epithelium UV mediated damage i.e. apoptosis effect is more prominent in the distal HF, but is elevated by caffeine	[3]

	In vivo, 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 = caffeine detected up to 48hours	Water soluble actives can reach depth of 200 μm, suggested access to the blood capillaries	[4]
	In vivo, 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= caffeine detected	Average penetration depth = 185 μm, access the blood capillaries	Lademann et al *Laser Physics 2010, Vol. 20, No. 2, pp. 551–556*
	In vivo, 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 = caffeine detected	Follicular penetration route for caffeine	[5]
	In vivo - 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 Frontal and occipital PTG (anagen hair diameter > 40μm)	Percentage change in anagen hairs measured using frontal and occipital trichograms = increase	Stimulation of cell proliferation (HFK) and 5 α-reductase inhibition	[6]

EGCG **(**Camellia sinensis) ***In vitro/ex vivo* studies**	*Ex vivo* HF, organ culture from the occipital scalp of healthy males, n=3	Hair shaft elongation in 1µM ECGC = increased	Prolongation of analgen phase	[7]
	In vitro, DPCs from occipital scalp follicles of healthy males, n=3 MTT assay Western blot analysis	DPC proliferation = increased Erk and Akt = increased Bcl-2 = increased, Bax = decreased	Inhibition of apoptosis	
	In vitro, DHT-treated DPCs from a healthy male after 24h WST-1 Cell cycle analysis Cell senescence detection	Cell viability, after exposure to EGCG+DHT= increased Cell cycle = extended Cell senescence = reduced Altered miRNA profiles: predicting up and down regulation of IGF-1 and VEGF	Arresting the DHT Reduction of oxidative cell damage and related senescence Gene expression	[8]

	QR-PCR Fluorescence-based oxidation-sensitive probe	Intra cellular ROS = reduced		
	In vitro and ex vivo using cell culture donors: AA patients n=30 vs healthy control n=30 HaCat cells and Jurkat cells (in vitro) PBMC followed by FACS analysis All cells were incubated in IFN-γ and treated with EGCG for variable time and concentrations Western blot analysis *in vitro* and *ex vivo* cells ELISA assay and Q-PCR	pSTAT1 = reduced, IRF-1= reduced Pro inflammatory T cells and gene expression = reduced	IFN-γ signalling inhibition Immunological action	[9]
EGCG **Camellia sinensis**	*In vivo*, aged skin n=5, 10% EGCG applied three times a week for 6 weeks	Epidermal thickness =increase	Induced cell proliferation and survival	[10]

***In vivo* application/in vitro study**	*In vitro*, normal human epidermal keratinocytes, different doses applied 2-5 days 10%, 1h post treatment Ki-67 immunostaining MTT assay ELISA assay	HMK cell proliferation = increase Cell proliferation/viability (MTT) = dose dependent increase Erk and Akt = increased Bcl-2 increase, Bax = decreased		
Procyanadin B-2 *Malus pumila* ***In vitro* and *ex vivo* studies**	*In vivo*, male with pattern balding according to Ogata scale, n=29, double blind, placebo controlled, 1% tonic application for 6 months PTG (terminal hair diameter > 60μm)	Phototrichograms used to measure: Total hair count density = increased Ratio of A/TTerminal hair hair count based on diameter measurements of clipped hair = increased	Suppression of inflammation	[11]

Polyphenols (phlorofurofucoeckol A, eckol, dieckol) ***Ecklonia cava*** ***In vitro* and *ex vivo* studies**	*In vitro and ex vivo,* 12 healthy male donors for HFs and DPCs (occipital area) MTT assay, minoxidil positive control Q-PCR analysis, polyphenols vs dimethyl sulfoxide DPC and H2O2 treatment for ROS (oxidation sensitive and fluorescence-based measurements) HF organ culture in various concentrations of active component vs dimethyl sulfoxide	Cell proliferation - increased mRNA expression of IGF-1 and VEGF = increased Oxidative stress = reduced Increased hair shaft production over 9 days	Growth promotion and ROS scavenging	[12]
Proprietary herbal blend: Chamaemelum nobile, Althaea officinalis, Persea Americana, Rosmarinus	*In vitro,* DPCs obtained from HF organ culture from the occipital scalp of healthy males n=5 qRT-PCR assay of 5α-R1 and 5α-R2 expression after treatment with different concentrations of herbal	Expression of 5α R2 in DPC = decrease DPC viability = increase	5α-reductase 2 inhibition Inhibition of apoptosis	[13]

Aloe officinalis vera, Urtica dioica , Thymus vulgaris ***In vivo* study**	extract MTT and B assays DPC treated with different concentrations of the herbal extract in the presence and absence of Akt1/2 for 48h Cell proliferation (BrdU) DPC treated with different concentrations of the herbal extract	G1 phase progression proteins = increase GAPDH, p-ERK and p-Akt = increase Bcl-2 =increase Bax = decrease	Cell cycle regulation	
Trifolium pratense flower extract (including isoflavone + biochanin; biometric peptide added in some tests)	*In vitro*: normal human dermal fibroblasts stimulated with IL-1α in presence of active variables vs positive and negative control	IL-8 secretion = reduced	Reduced micro-inflammation	[14]
	In vivo, healthy volunteers with mild to moderate hair loss undefined rating, n=30, placebo controlled 5% active solution, daily application over	Trichoscan recording anagen/telogen ratio = increased	Combined effects of 5 α-reductase inhibition, reduced	

***In vitro* and *in vivo* studies**	4 months PTG (anagen hair identified via growth rate >0.3mm/day)		microinflammation and stimulation of extracellular matrix	
Rosebery ketone *Rubus idaeus* ***In vivo* study**	*In vivo,* 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 = increased	Stimulation of IGF-1, thus HKC proliferation Increased dermal blood flow Down regulation of androgen receptors in the DPC	[15]
Capsaicin *Capsicum annuum L* ***In vivo* study**	*In vivo,* 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 = statistically not different	Stimulates the perifollicular nerves and the HF immune system	[16]
Rosemary oil Rosmarinus	*In vivo* AGA patients (n=50), topical application, positive control = 2% minoxidil	Hair grading in both groups = statistically not different		[17]

officinalis L ***In vivo* study**	Clinician's assessment after 6 months (grading scale) Self-assessment	Rosemary group = <u>self-reported reduced scalp itches and irritation</u>		
Crude onion juice Allium cepa L ***In vivo* study**	*In vivo,* 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]
Saw palmetto *(Serenoa repens)* ***In vivo* study**	AGA male patients (Norwood-Hamilton types III, IV, V, VI), n=50, within subject comparison of active lotion after 12 weeks Phototrichogram Clinician assessment Self-assessment (validated scales)	Terminal hair count = <u>increased</u> Vellus hair count = <u>decrease</u> Results 12-24 weeks = <u>inconsistent</u>	5 α-reductase inhibition by the fatty acid present in the SP	[19]

Proprietary herbal blend *Urtica uren, Urtica dioica, Matricaria chamomilla, Achillea millefolium, Ceratonia sili-qua, Equisetum arvense* ***In vivo* study**	Males AGA grade II-III Hamilton Norwood, and n-66 female with Telogen Effluvium, n=54 assigned to four treatment groups, active shampoo, active leave on solution, a combination of the above, placebo, Blind clinician's assessment – pull test, phototrichogram up to 6 months Self-assessment PTG up to 6 months (anagen hair identification criterion not reported)	Total hair countdesnity, Telogen/ Anagen/Telogen ratio after 2,4- and 6-months treatment: <u>increase for each time point in comparison to T0.</u> Self-evaluation (questionnaire) scores – higher score for all active treatments vs. placebo	Synergistic combined effect on HF	[20]
Red ginseng extract *Panax ginseng* (RGE)+ ginsenoside Rb1 and ginsenoside Rg3 ***In vitro* study**	DPC cells obtained from non-balding scalp (hair transplantation surgery) DPCs + various concentration of G-Rb1 incubated (2h) with CCK-8 ELISA of various concentration of RGE and G Rb-1 incubated with	Cell proliferation: <u>increase in a dose -dependent manner</u> p-Erk and pAkt expression: <u>increase</u> Ki-67cell positivity = increase in presence of actives	Increased cell proliferation Reduce DHT Induced up-regulation of androgen	[21]

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

***In vivo* study**	Blind clinician's assessment IGF-1 serum levels	vs. base line in active treatment = increased IGF-1 serum levels for patients after 5 months vs base line in active treatment = increased		
Saw palmetto + β-sitosterol *Serenoa repens* ***In vivo* study**	*In vivo* AGA patients (Norwood - Hamilton types II-VI vertex), n=49 daily supplements for 21 weeks, double blind, placebo controlled Blind clinician's assessment Self-assessment	Improvement ratings = increased Self-reported improvement of bold patches=in active group only	5 α-reductase inhibition	[24]
Saw palmetto *Serenoa repens* ***In vivo* study**	*In vivo,* male AGA patients (Hamilton classification I to IV) n=100, 24 monthly daily oral treatment active vs. finasteride (open trial) Global photos, used for baldness score (Hamilton) start and end of treatment	Hair growth in the vertex induced by active finasteride (vertex and occipital area)	5 α-reductase inhibition by the fatty acid present in the SP	[25]

Pumpkin seed oil *Cucurbita pepo* ***In vivo* study**	*In vivo,* AGA male patients (Norwood-Hamilton types II, III, III Vertex, IV and V), n=76 Placebo controlled, blind trial, 24 weeks Phototrichograms PTG Clinician's grading Self-assessment	Hair count (density)=increase; hair thickness=increase Mean score of clinical grades = increase	5 α-reductase inhibition	[26]
Proprietary blend *Vitamin C (acerola powder and ascorbic acid), zinc (from zinc oxide), shark powder and mollusk powder, horsetail (stem) extract and flax seed extract*	AGA males with pattern hair (Norwood-Hamilton scale II,II, III), n=60 Placebo controlled for 180 days Phototrichogram PTG Hair pull test	Total hair count, total hair density, terminal hair density = increased Hair removal = reduced	Systemic effect	[27]

	Quality of life questionnaire	Improved wellbeing scores		
Proprietary blend Including; horsetail derived silica, Vit C from acerola chilly and procyanidin B-2marine complex	Healthy females (n=40), placebo control taking the oral supplement for 180 days Phototrichogram PTG Self-assessment	Terminal hair density = increased Hair diameter: increased Increased hair diameter Self-reported hair volume, texture, as well as nails and skin related parameters Self-reported hair volume, texture, as well as nails and skin related parameters	Systemic effect	[28]
Proprietary blend including horsetail derived silica, vit C from acerola chilli and	Healthy females (n=72), placebo controlled, taking the oral supplement for 180 days Phototrichogram PTG	Hair shedding = reduced Vellus-like hair diameter = increased Increased hair diameter	Systemic effect	[29]

procyanidin B-2 marine complex	Self-assessment	Self-reported hair volume, texture, as well as nails and skin related parameters		

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

AGA	Androgenic alopecia
AA	Alopecia areata
Akt and pAkt	Protein kinase/phosphorylated Akt, a protein promoting cell survival
Bcl-2/Bax	B-cell lymphoma 2, protein regulator inhibiting apoptosis Bcl-2 like protein 4, protein regulating inducing apoptosis
CyQUANT	Fluorescence-based assay measuring cell proliferation, based on cellular DNA content
DPC	Dermal papilla cells
DHT	Dehydrotestosterone, an androgen responsible for follicle miniaturisation
EGCG	Epigallocatechin 3-gallate, the most abundant catechin in tea
ELISA	Enzyme-linked immunological assay used to detect and quantify the presence of proteins, enzymes and antibodies
Erk	Extracellular signal-regulated kinases promoting cell survival
FACS	Fluorescence-activated cell sorting of biological cell mixtures
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-β2	Transforming growth factor β2, 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