FROM FOLLICLE TO FORMULATION: EXPLORING SAW PALMETTO AND CAFFEINE SHAMPOOS FOR ALOPECIA MANAGEMENT

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Abstract

Alopecia is a common hair disorder characterized by excessive hair loss and reduced hair density, with androgenetic alopecia (AGA) being strongly associated with genetic factors and dihydrotestosterone (DHT). Limitations of conventional treatments have encouraged the exploration of alternative topical formulations. Saw palmetto (Serenoa repens), rich in fatty acids and phytosterols, may influence 5α-reductase activity, while caffeine may promote hair follicle activity and regulate hair growth. This review explores the potential of combining saw palmetto and caffeine in shampoo formulations for alopecia management, focusing on their mechanisms of action, formulation development, physicochemical characterization, stability, safety, and quality control. Although both ingredients demonstrate promising biological properties, their combined clinical efficacy and potential synergistic effects remain insufficiently established. Further standardized formulations and controlled clinical studies are needed to validate their effectiveness as an adjunctive approach to AGA management.

Keywords: *Alopecia; Androgenetic alopecia; Serenoa repens; Saw palmetto; Caffeine; Hair growth; Hair follicle; 5α-reductase; Dihydrotestosterone; Herbal shampoo.*

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1. Introduction

1.1 Background of Alopecia

Alopecia is a heterogeneous group of disorders that can manifest as abnormal hair shedding, reduced hair density, impaired follicular growth, or permanent destruction of hair follicles. Hair follicles are highly specialized mini-organs that undergo continuous cycles of growth, regression, resting and shedding during the life. A normal hair density is maintained by a coordinated interaction between epithelial cells, dermal papilla cells, follicular stem cells, immune mediators, growth factors, hormones, and the extracellular matrix. Whiting (1993) Alteration of these interactions may change follicular cycling and produce clinically different patterns of hair loss. Causes of hair loss may be transient or progressive and may be genetic, hormonal, inflammatory, autoimmune, nutritional, metabolic, pharmacologic, or environmental in origin. Sinclair (1998) The clinical appearance is dependent on the underlying mechanism and may be diffuse shedding, thinning in a specific pattern, discrete patches, or scarring with permanent destruction of the hair follicle. Hence, the biological basis of the specific type of alopecia needs to be understood for the selection of an appropriate therapeutic strategy. Trueb (2002)

1.2 Androgenetic Alopecia

Androgenetic alopecia (AGA), or male/female pattern hair loss, is the most common type of progressive hair loss. Randall (2008) It occurs in both sexes, but the pattern and distribution are different. In men, hair loss starts in the frontal and temporal regions and may spread to the vertex, whereas women usually have diffuse thinning over the central scalp with preservation of the frontal hairline. Inui and Itami (2013) The characteristic pathologic feature of AGA is progressive miniaturization of susceptible terminal hair follicles. Ellis et al. (2002) In this process, terminal hairs become progressively thinner, shorter and less pigmented, and the duration of the anagen phase shortens. Repeated cycling of the follicles results in a higher proportion of small vellus-like hairs and a clinically apparent reduction in hair density. Rathnayake and Sinclair (2010)

1.3 Function of Androgens and Dihydrotestosterone

Androgen signaling is an integral part of the pathophysiology of AGA. Testosterone is converted to the more potent androgen dihydrotestosterone (DHT) by the enzyme 5α-reductase. Adil and Godwin (2017) DHT binds to androgen receptors in susceptible follicular cells and alters the expression of genes involved in follicular growth and differentiation. Kanti et al. (2018) DHT has a strong dependence on follicular susceptibility. Thus, AGA is not only due to increased circulating androgen levels but also involves local androgen

metabolism and genetically mediated follicular sensitivity. Manabe et al. (2018) Elevated androgen-mediated signaling can affect dermal papilla-cell function and the production of paracrine factors that regulate nearby epithelial cells, leading to progressive miniaturization. Meidan and Touitou (2001)

1.4 Limitations of Present Therapies

There are a number of pharmacological treatment options for AGA including topical minoxidil and 5α-reductase inhibitors such as finasteride and dutasteride. Suchonwanit et al. (2019) These treatments can provide clinically significant benefits, but treatment generally needs to be continued if one wants to maintain the response. Treatment adherence may therefore be an important determinant of long-term efficacy. Messenger and Rundegren (2004), Rossi et al. (2012) This has generated considerable interest in plant-derived ingredients that may influence androgen metabolism, inflammation, oxidative stress, or follicular growth. Moreover, concerns about adverse effects, contraindications, individual variability in treatment response, and patient preference for cosmetic or botanical products have prompted the exploration of alternative topical approaches.

1.5 Why Caffeine and Saw Palmetto?

Saw palmetto (Serenoa repens) is a lipid-rich herbal extract containing fatty acids and phytosterols. Experimental evidence indicates that saw palmetto components can inhibit 5α-reductase, and may thus have a role in androgen-dependent pathways involved in AGA. Mysore (2012) The other anti-inflammatory and antioxidant properties may have complementary effects in the follicular microenvironment. Mysore and Shashikumar (2016) Caffeine is also being touted as a topical hair growth ingredient. Experimental studies on human hair follicles have demonstrated stimulatory effects on follicular growth, and suggested that caffeine may antagonize testosterone-associated suppression of follicular activity. Kaufman et al. (1998) Clinical investigations have subsequently been conducted on caffeine-containing topical formulations, including shampoos. Price et al. (2000) The combination of saw palmetto and caffeine is particularly interesting because the two ingredients may affect different components of follicular biology. Saw palmetto may act primarily on androgen-related mechanisms, while caffeine may impact follicular cell activity and hair-cycle regulation. However, direct evidence showing clinical superiority or true synergy between the two ingredients is limited and requires systematic investigation.

1.6 Objectives and Scope of the Review

This review discusses the scientific rationale for the development of a saw-palmetto caffeine shampoo for the management of alopecia. The discussion moves from the biology of the hair

follicle and the pathophysiology of alopecia to the botanical and pharmacologic properties of saw palmetto and caffeine. Particular attention is paid to their mechanism of action, synergistic biological effects, topical delivery via shampoo, formulation development, quality evaluation, analytical characterization, preclinical and clinical evaluation, safety, regulatory aspects and future research avenues.

2. Biology of Hair Follicles and Mechanisms Related to Alopecia

2.1 Morphology and Physiology of the Hair Follicle

The hair follicle is a complex epithelial-mesenchymal structure, located in the skin, that produces the hair shaft. Its main parts are the hair bulb, matrix, dermal papilla, dermal sheath, inner root sheath, outer root sheath and bulge region. Shapiro and Kaufman (2003) Each part has a specialized function, but continuous communication between the parts controls follicular growth and cycling. The hair matrix is formed by rapidly dividing keratinocytes which differentiate to form the hair shaft and inner root sheath. Gupta and Charrette (2014) Melanocytes within the hair bulb synthesize melanin and transfer pigment to developing hair-fiber keratinocytes. The size of the matrix and the activity is closely related to the diameter and length of the resulting hair shaft. Roehrborn et al. (2002)

Table 1. Hair-follicle structures relevant to hair growth and alopecia

Structure	Major function	Relevance
Bulge region	Stem-cell niche	Follicular regeneration
Dermal papilla	Mesenchymal signaling center	Controls matrix proliferation and hair growth
Hair matrix	Rapidly proliferating epithelial cells	Produces hair shaft
Outer root sheath	Epithelial support and regeneration	Maintains follicular architecture
Inner root sheath	Guides developing shaft	Determines fiber shape
Hair shaft	Keratinized fiber	Clinical measure of density and diameter
Sebaceous gland	Produces sebum	Influences follicular/scalp environment
Arrector pili	Smooth muscle attachment	Anatomical landmark of follicular unit

2.2 Dermal Papilla and Follicle Signaling

At the base of the follicle is a specialized population of mesenchymal cells called the dermal papilla. It plays an important role in controlling hair-follicle growth by communicating with

epithelial cells. Oliveira-Soares et al. (2013) Dermal papilla cells secrete a number of growth factors and signaling molecules that control the proliferation, differentiation, and maintenance of matrix cells during the anagen phase.Gupta et al. (2018) Hair production occurs normally only when dermal papilla cells interact with epithelial follicular cells. Changes in dermal papilla-cell behavior may be involved in follicular miniaturization in AGA and are an important target for experimental hair-growth therapies. Suchonwanit et al. (2014)

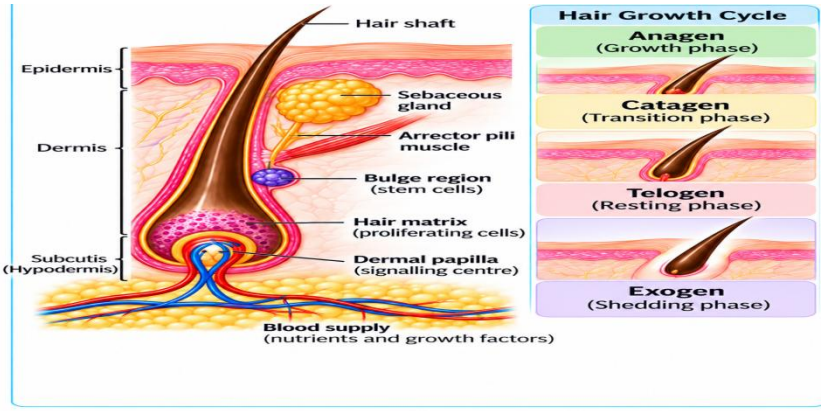


Figure 1: Structure of the Hair Follicle and Hair-Growth Cycle

2.3 Stem cells of the hair follicle.

Hair-follicle stem cells are mainly localized in the bulge region and are a cellular reservoir for regeneration of the follicular epithelium. Piraccini et al. (2022) Their activation in the transition from telogen to anagen plays a role in regeneration of the lower follicular structures. Suchonwanit et al. (2018) Wnt/β-catenin signaling is one of the main pathways involved in stem-cell activation and follicular regeneration. Lee et al. (2013) Abnormal regulation of this pathway may interfere with follicular cycling and contribute to impaired hair growth.

Table 2. Hair-cycle phases and their relevance to alopecia

Phase	**Principal event**	**Clinical relevance**
Anagen	Active hair production	Shortening contributes to miniaturization
Catagen	Follicular regression	Premature entry reduces productive growth
Telogen	Resting phase	Increased proportion occurs in telogen effluvium
Exogen	Hair shedding	Excessive shedding is perceived as hair fall
Kenogen	Follicle temporarily without visible fiber	May contribute to reduced visible density
Botanical approaches	Multiple proposed pathways	Variable evidence and standardization

2.4 Hair Growth Cycle

The hair follicle goes through anagen, catagen and telogen with subsequent shedding during exogen. Sinclair (2009) Anagen is the active growth phase and can last years for the scalp follicles. During this phase, matrix-cell proliferation and differentiation are very active. Rebora (2016) Catagen is a relatively short phase of involution associated with apoptosis-related regression of the lower follicle. Malkud (2015) Telogen is the resting phase, in which the follicle is relatively inactive until reentering anagen. Suchonwanit et al. (2024) Alterations in the length of these phases or transition between these phases can result in abnormal hair shedding or decreased hair density.

2.5 Regulation of Hair-Follicle Development

The cycling of hair follicles is regulated by a complex network of signaling pathways. Wnt/β-catenin signaling promotes anagen initiation and follicular regeneration, while bone morphogenetic proteins and transforming growth factor-β regulate follicular differentiation and regression. Grover and Khurana (2010)

Other important mediators are fibroblast growth factors, insulin-like growth factor-1, vascular endothelial growth factor, Sonic hedgehog, epidermal growth factor and various cytokines. Headington (1993) The balance between these signals determines if the follicle remains in an active growth phase or progresses to regression.

2.6 5α-Reductase and the Effect of DHT

5α-Reductase catalyzes the conversion of testosterone to dihydrotestosterone (DHT), a potent androgen involved in the development and progression of AGA. Cotsarelis and Millar (2001) The enzyme has two major isoforms, type I and type II 5α-reductase, which are expressed differentially in various tissues of the body and in different follicular compartments. Stenn and Paus (2001) DHT-mediated activation of the androgen receptor may alter dermal papilla-cell signaling and influence the synthesis of growth regulatory factors. Paus and Cotsarelis (1999) Increased local androgen activity is thus considered as one of the main biological mechanisms involved in progressive follicular miniaturization.

2.7 Hair Growth's Molecular Pathways

Molecular pathways involved in hair-follicle cycling and androgen signaling. Wnt/β-catenin signaling induces follicular stem-cell activation and anagen development, whereas DKK-1 has been implicated in suppression of Wnt-associated activity in androgen-sensitive follicles. Alonso and Fuchs (2006) Additional pathways involving transforming growth factor-β, fibroblast growth factors, vascular endothelial growth factor, and inflammatory mediators may also contribute to follicular growth and regression. Schneider et al. (2009) The

multifactorial nature of these pathways provides a rationale for investigating multi-target topical formulations rather than relying on a single molecular target alone.

3. Alopecia: Classification, Etiology and Current Therapeutic Options

3.1 Types of hair loss

Alopecia is typically classified into non-scarring (nonscarring) and scarring (cicatricial) alopecia, based on the maintenance of the hair follicle architecture. Stenn et al. (1996) In nonscarring alopecia, the follicular architecture is generally maintained and hair growth may be restored when the underlying cause is addressed. Scarring Alopecia is described as the irreversible destruction of the follicular unit with the replacement of the follicular structures by fibrotic tissue. Millar (2002) The nonscarring alopecias include androgenetic alopecia, alopecia areata, telogen effluvium, anagen effluvium, and various types of drug- or disease-associated hair loss. Fuchs (2007) These conditions vary widely in etiology, clinical presentation, follicular biology, and therapeutic requirements. Therefore, accurate classification prior to treatment initiation is important.

Table 3. Classification of major alopecia

Type	Typical presentation	Major associations
Androgenetic alopecia	Patterned progressive thinning	Androgen sensitivity, genetics, aging
Alopecia areata	Patchy or diffuse nonscarring loss	Autoimmune mechanisms
Telogen effluvium	Diffuse shedding	Stress, illness, nutritional/hormonal triggers
Anagen effluvium	Rapid active-phase loss	Chemotherapy/toxic exposure
Scarring alopecia	Permanent loss with fibrosis	Inflammatory, autoimmune or infectious disease
Traction alopecia	Localized loss in traction areas	Tight hairstyles/chronic tension

3.2. Androgenetic Hair Loss

Androgenetic alopecia is the most frequent progressive hair loss disorder in males and females. Blanpain and Fuchs (2009) The condition develops in genetically susceptible follicles that are exposed to androgen-mediated signaling. Progressive miniaturization leads to thinning and shortening of terminal hairs with a progressive decrease in visible hair density. Nowak et al. (2008) In men, the condition usually starts with recession of the frontal

and temporal hairlines, then progresses to thinning over the vertex. Rompolas et al. (2013) In women, the typical pattern is diffuse thinning over the central scalp, with relative preservation of the frontal hairline.Blanpain et al. (2004) Severity and progression are determined by genetic factors, age, hormonal environment, and individual follicular sensitivity to androgens.

3.3 Alopecia Areata

Alopecia areata is an autoimmune disease that causes nonscarring hair loss and classically presents as well-demarcated patches of hair loss. Greco et al. (2009) The disease is associated with immune-mediated disruption of the normal immune privilege of the hair follicle, especially during the anagen phase. Jahoda (2003) CD8+ T lymphocytes and associated inflammatory mediators have been implicated in follicular attack. Rendl et al. (2005) Spontaneous regrowth may occur but recurrent or extensive disease may require systemic or targeted immunomodulatory treatment. The pathophysiology of alopecia areata is quite different from that of AGA, thus treatments that target androgen metabolism should not be generalized to autoimmune alopecia.

3.4 Telogen Effluvium

Telogen effluvium is an excessive loss of telogen hairs after disruption of the normal hair growth cycle. Driskell et al. (2011) The most frequent etiologies include acute illness, psychological or physiological stress, nutritional deficiencies, major surgery, hormonal changes, and certain drugs. Jahoda and Reynolds (1996) As opposed to AGA, telogen effluvium usually presents with diffuse shedding, rather than a characteristic patterned distribution. Hardy (1992) The condition can be acute or chronic, depending on how long the inciting factor persists. Management continues to focus on identifying and correcting the root cause.

3.5 Anagen Effluvium

Anagen effluvium results from an abrupt damage of the actively growing anagen follicles. Botchkarev and Kishimoto (2003) It can be precipitated by cytotoxic chemotherapy, radiation, or other toxic agents that rapidly affect the proliferating matrix cells. Botchkarev and Fessing (2005) The hair follicles affected are in the active growing phase and hair loss can occur relatively quickly. Often the follicular structure is preserved and regrowth can occur after removal of the causative factor. Millar (2002)

3.6 Scarring Hair Loss

Scarring alopecias are a set of inflammatory conditions characterized by the irreversible destruction of hair follicles. Lim and Nusse (2013) The follicular epithelium is gradually replaced by fibrosis, causing permanent loss of hair in the affected areas. Ouji et al. (2006) Examples include lichen planopilaris, frontal fibrosing alopecia, central centrifugal cicatricial alopecia, and discoid lupus erythematosus. Enshell-Seijffers et al. (2010a) Early diagnosis is important because treatment is aimed at suppressing inflammation and preventing further follicular destruction, not simply stimulating hair growth.

3.7 Causes

Alopecia can be caused by multiple factors such as genetic, endocrine, autoimmune, nutritional, inflammatory, infectious, pharmacological and environmental. Enshell-Seijffers et al. (2010b) In AGA, genetic predisposition plays an important role whereas autoimmune mechanisms are the main pathophysiology of alopecia areata. Reddy et al. (2001) Nutritional deficiencies such as iron, zinc, vitamin D and other nutrients have been assessed in relation to diffuse hair loss, though the clinical significance is dependent on the individual and the presence of a true deficiency. Ouji et al. (2006) Endocrine disorders such as thyroid dysfunction and hormonal changes can also affect the hair-growth cycle. Kwack et al. (2012) Moreover, oxidative stress and inflammation have been proposed as contributors to follicular dysfunction. Kwack et al. (2008) These mechanisms are of particular relevance when considering multifunctional topical formulations containing botanical ingredients with antioxidant or anti-inflammatory properties.

3.8 Existing Pharmacological Treatments

Topical minoxidil remains one of the main pharmacologic treatments for AGA. Lee et al. (2011) It can extend or maintain the anagen phase and increase the percentage of actively growing follicles. Inui et al. (2002) The exact molecular mechanism is complex and involves effects on potassium channels, vascular signaling, and follicular cellular activity. Finasteride is a type II 5-alpha reductase inhibitor that selectively inhibits the enzyme and reduces the conversion of testosterone to DHT. Rogers and Avram (2008) Finasteride can reduce follicular miniaturization and help to preserve terminal hair by reducing androgenic stimulation of susceptible follicles. Blume-Peytavi and Vogt (2010) Dutasteride inhibits both type I and type II 5α-reductase and therefore has more extensive suppression of DHT synthesis. Pratt et al. (2017) Although it has shown efficacy in AGA, its use and regulatory status are different in countries and clinical settings. Islam et al. (2015) Other strategies include ketoconazole formulations, low level light therapy, platelet rich plasma,

microneedling, hair transplantation and a variety of nutraceutical or botanical treatments. Messenger et al. (2012)

3.9 Shortcomings of Current therapies

However, the management of AGA remains challenging despite the availability of established therapies, as treatment often requires prolonged use and responses vary considerably among individuals. Strazzulla et al. (2018) If effective therapy is discontinued, there may be a gradual loss of the treatment-associated benefit, as the underlying biological susceptibility remains present. Vano-Galvan et al. (2020)

Topical treatments may also have local irritation or cosmetic acceptability issues that may adversely affect adherence. Requena and Sangueza (2015a) Other contraindications or considerations for adverse effects may be present with systemic therapies and treatment choice may depend on patient characteristics and clinical circumstances. Requena and Sangueza (2015b) Botanical products, however, should not be assumed to be automatically equivalent to established pharmacological treatments and their efficacy must be demonstrated using appropriately controlled studies. Bolduc et al. (2016) Therefore standardized saw palmetto and caffeine delivered by a carefully optimized shampoo formulation are an area worthy of systematic investigation especially for supportive management of androgen related hair loss.

Table 4. Conventional and emerging approaches for androgenetic alopecia

Therapy	Main rationale	Important limitation
Minoxidil	Supports follicular growth/anagen	Irritation, hypertrichosis, adherence
Finasteride	Type-2 5α-reductase inhibition	Sex-specific/reproductive safety considerations
Dutasteride	Type-1 and type-2 5α-reductase inhibition	Regulatory and systemic exposure considerations
Ketoconazole shampoo	Antifungal/anti-inflammatory adjunct	Not primary AGA therapy
Low-level laser therapy	Photobiomodulation	Repeated treatment required
Platelet-rich plasma	Growth-factor signaling	Protocol heterogeneity
Hair transplantation	Redistribution of donor follicles	Invasive; donor limitations

4. Saw Palmetto (Serenoa repens): Botanical Description and Phytochemicals

4.1 Botanical Description

Saw palmetto (Serenoa repens (W. Bartram) Small) is a small, slow-growing palm in the family Arecaceae. It has fan-shaped leaves and branched stems and produces dark purple to black fruits when mature. Rogers (2012) Ripe fruits are the main plant material used for preparation of medicinal and phytopharmaceutical extracts. The fruits contain a lipid-rich fraction which has received considerable pharmacological attention. Unlike many conventional aqueous botanical extracts, saw-palmetto preparations used in pharmacological studies are typically prepared using lipid-extraction methods that concentrate fatty acids, sterols, and other lipophilic constituents. Trueb (2002)

4.2 Distribution and Classification Serenoa repens is indigenous mainly to the southeastern

United States and is particularly associated with the areas of Florida and neighboring southeastern states. Trueb (2013) It is a plant of warm climates and sandy soils and is common in coastal and sub-tropical environments. When producing standardized extracts, the botanical identity and the geographical origin of the raw material are important considerations, as environmental conditions, stage of harvesting, processing and storage can influence phytochemical composition. Almohanna et al. (2019) Hence, a correct botanical identification is an essential preliminary step in quality control.

4.3 Uses Traditional and medicinal uses

The fruit of saw-palmetto has a long tradition of use by native people and has been subsequently studied for a variety of uses. Guo and Katta (2017) Its modern pharmacological research has focused in particular on disorders associated with androgen metabolism, for example benign prostatic hyperplasia and androgen-related conditions. The proposed antiandrogenic activity of the lipid extract raised interest in hair-loss management. Rushton (2002) The ability of saw-palmetto ingredients to alter the activity of 5α-reductase has offered a mechanistic basis for the investigation of its use in hair-care products, as androgen signaling and the formation of DHT are important elements of AGA.

4.4 Phytochemical Composition

The pharmacologically active fraction of saw palmetto consists mainly of lipophilic constituents. Celleno et al. (2025) The chemical profile comprises fatty acids, phytosterols and other unsaponifiable compounds in lower amounts. The biological activity of the extract is probably due to the synergistic contribution of several constituents rather than one isolated molecule. Randall (2008) Thus, the characterization of the full extract has a big impact on the quality and reproducibility of the final formulation. Imperato-McGinley et al. (1974)

4.5 Fatty acid

Fatty acids are an important component of lipid fraction of S. repens fruit. Fatty acids most commonly reported are lauric acid, oleic acid, myristic acid, palmitic acid and linoleic acid. Andersson et al. (1991)

The relative concentration of these fatty acids can vary considerably between extracts depending on the conditions of extraction and the characteristics of the raw material. Russell and Wilson (1994) Such variation may account for the differences in biological activity observed among commercially available preparations. Experimental studies have shown that some of these fatty acids are biologically active in relation to androgen metabolism. Jenkins et al. (1991) The activity of a single fatty acid should not be automatically assumed to be representative of the activity of the complete standardized extract.

4.6. Phytosterols

Phytosterols are another important ingredient of saw-palmetto extracts. Thigpen et al. (1993) Sterol constituents of S. repens were identified as β-sitosterol, campesterol and stigmasterol. Bruchovsky and Wilson (1968) The pharmacological profile of the extract may be due to the presence of phytosterols that could act on lipid-associated cellular processes and possibly on androgen-related pathways. Wilson (1991) β-Sitosterol has been of particular interest in studies on the biological effects of saw palmetto. Therefore, the presence and the concentration of phytosterols can be included in the chemical characterization and standardization strategy of saw-palmetto preparations. Ueki et al. (1997)

4.7 Standardization of Saw-Palmetto Extracts

Standardization is especially critical if saw palmetto is to be used in a pharmaceutical or cosmetic formulation. Randall et al. (2001) Two products labeled to contain Serenoa repens could have significantly different chemical compositions depending on the extraction solvents, plant materials, or processing procedures used in their preparation. A standardized extract should therefore have specified values for its major fatty acids and phytosterol content where appropriate. Hibberts et al. (1998) Chromatographic fingerprinting may be an additional means of confirming identity and batch to batch consistency.

Standardization of a saw-palmetto-caffeine shampoo is important for reproducibility and for interpretation of efficacy data. Without proper chemical characterization, differences in biological activity between batches of a product could be erroneously attributed to formulation performance rather than variation in the botanical ingredient. Choudhry et al. (1992)

4.8 Extraction of Quality Factors

Several factors including the plant variety, geographical origin, fruit maturity, harvesting conditions, extraction solvent, extraction temperature, processing conditions and storage may influence the phytochemical quality of S. repens extracts. Shima et al. (1996) Lipid oxidation is another important consideration as unsaturated fatty acids can be oxidatively degraded during prolonged exposure to heat, oxygen and light. Inui and Itami (2011) Oxidation may change the chemical composition and can potentially influence the stability, smell, appearance and biological activity of the extract. Thus, saw-palmetto extract should be stored under controlled conditions for formulation development and incorporated in a shampoo system that minimizes degradation. The quality of the botanical fraction can be preserved through the use of suitable antioxidants, packaging materials and stability testing protocols. Garza et al. (2011)

4.9 Phytochemistry in the Management of Alopecia

The phytochemical profile of saw palmetto is directly relevant to its proposed use in AGA. Its fatty acids and phytosterols constitute a chemically heterogenous mixture and can interact with biological targets involved in androgen metabolism. Qi et al. (2020) Experimental data suggest that lipid-rich S. repens extracts can modify the 5α-reductase activity, thus offering a possible connection between its phytochemical constituents and androgen-dependent hair follicle biology. Purba et al. (2017)

Table 5. Botanical and phytochemical profile of Serenoa repens

Feature	Description	Formulation significance
Botanical name	Serenoa repens	Authentication required
Family	Arecaceae	Botanical identity control
Plant material	Ripe fruits/berries	Source affects composition
Extract	Lipid-rich/liposterolic preparations	Solubility and stability must be addressed
Major fatty acids	Lauric, oleic, myristic, palmitic, linoleic acids	Useful for standardization
Phytosterols	β-Sitosterol, stigmasterol and related sterols	Useful chemical markers
Quality control	Fatty-acid/phytosterol profile and fingerprint	Reduces batch variability

However, the exact contribution of individual constituents is ill-defined. Future formulation studies should therefore not limit themselves to reporting percentage of saw-palmetto extract but should include data on chemical standardization, quantification of marker compounds,

chromatographic fingerprinting, and batch-to-batch consistency. Such characterization will be critical in establishing a reproducible relationship between formulation composition, biological activity and clinical efficacy.

Table 6. Major Phytochemical Constituents of Saw Palmetto Relevant to Formulation Development

Phytochemical group	Representative constituents	Potential relevance
Fatty acids	Lauric acid, oleic acid, myristic acid, palmitic acid, linoleic acid	Potential contribution to 5α-reductase modulation and biological activity
Phytosterols	β-Sitosterol, campesterol, stigmasterol	Potential contribution to androgen-related and cellular effects
Unsaponifiable fraction	Sterols and other lipophilic constituents	May contribute to overall biological activity
Lipid fraction	Complex mixture of fatty acids and sterols	Principal pharmacologically investigated fraction

5. Mechanisms of Saw Palmetto in the Treatment of Alopecia

5.1 5α-reductase inhibition

The lipid rich extract of saw palmetto (Serenoa repens) contains fatty acids and phytosterols, which can interfere with androgen metabolism, and is being investigated mainly in alopecia. In vitro studies have shown that extracts from saw-palmetto may inhibit 5α-reductase activity, the enzyme that converts testosterone to dihydrotestosterone (DHT). This mechanism is especially relevant to androgenetic alopecia (AGA) where androgen-sensitive follicles undergo progressive miniaturization. The extent of inhibition depends on the composition of the extract, the extraction procedure, concentration and standardization; thus, the activity of one saw-palmetto preparation should not be automatically attributed to all commercial extracts. Schneider (2019)

5.2 Androgen signaling modulation

In addition to its direct effects on 5α-reductase, saw palmetto may also act on androgen-dependent signaling in the hair follicle. DHT exerts its effects on susceptible follicular cells through the androgen receptor, leading to downstream changes characterized by a reduced anagen phase and progressive follicular miniaturization. The constituents of saw-palmetto may help to attenuate these processes by decreasing androgenic stimulation or by altering the

local availability of androgens. However, evidence for direct androgen receptor modulation is less well established than the proposed 5α-reductase pathway, and further mechanistic studies are required to determine whether observed effects are primary or secondary to reduced DHT formation. Lim et al. (2018)

5.3 Effect on the formation of DHT

Inhibition of local DHT formation is a potential important mechanism of action of saw palmetto in hair growth management. Testosterone is converted to DHT by type I and type II 5α-reductase enzymes and their relative contribution varies between tissues. The major role of excess androgen signaling in androgen-sensitive scalp follicles is the development of follicular miniaturization and changes in the hair growth cycle. Lipid-soluble fractions of saw-palmetto have shown inhibitory activity against 5α-reductase in experimental systems, and it is suggested that topical delivery may alter the follicular microenvironment. However, there is limited clinical evidence that topical saw-palmetto application consistently decreases scalp DHT. Silva-Vargas et al. (2005)

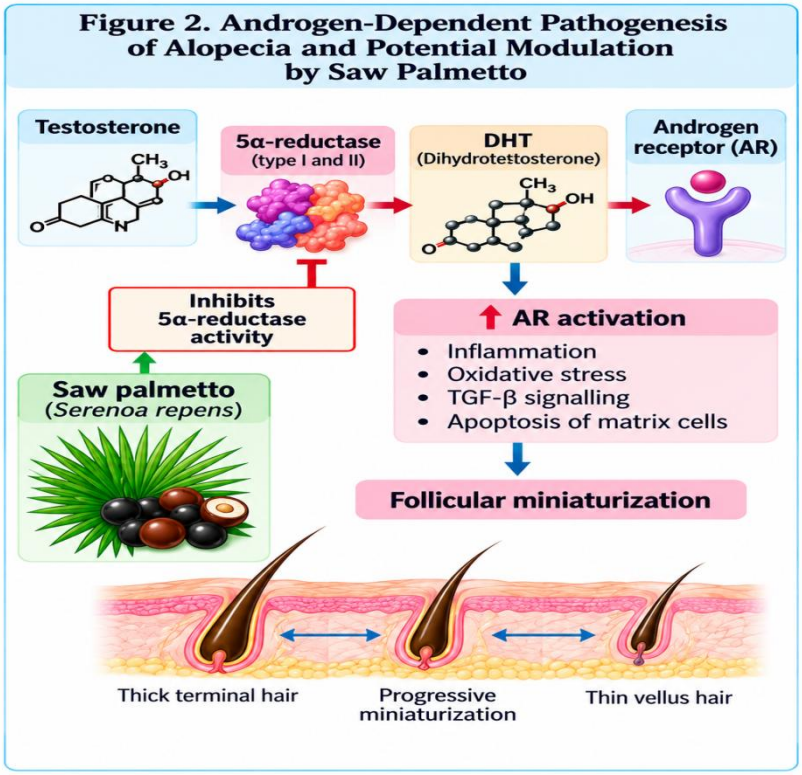


Figure 2: Androgen-Dependent Pathogenesis of Alopecia and Potential Modulation by Saw Palmetto

5.4 Anti-inflammatory activity

Chronic low-grade perifollicular inflammation has been suggested as one factor that might be associated with follicular miniaturization in AGA. Saw-palmetto constituents have exhibited

anti-inflammatory effects in experimental models and can modulate inflammatory mediators important to tissue responses. This activity could complement the androgen-related mechanism by decreasing an inflammatory environment that might negatively affect follicular function. However, how these effects relate to clinical hair regrowth is unclear, as anti-inflammatory activity in cellular or biochemical models does not necessarily translate into detectable increases in scalp hair density. Enshell-Seijffers et al. (2007)

5.5 Activity of antioxidant

Follicular cells can be affected by oxidative stress via increased reactive oxygen species and changes in cellular signaling, membrane integrity and inflammatory responses . Saw palmetto contains bioactive lipid and phytosterol fractions that have been studied for antioxidant and protective effects. Such activity may be involved in maintaining a favorable follicular microenvironment and protecting cells from oxidative damage. Thus, antioxidant action should be considered as a secondary mechanism of action and not as a proven primary pathway for hair regrowth, particularly because of the need for further standardization of the relationship between oxidative biomarkers and clinical AGA outcomes. Botchkarev (2003)

5.6 Influence on follicular miniaturization

Follicular miniaturization is a hallmark of AGA and is associated with progressively thinner, shorter and less pigmented terminal hairs. The proposed actions of saw palmetto such as decreased 5α-reductase activity, potential modulation of androgen signaling and anti-inflammatory effects may cumulatively reduce factors related to miniaturization. Improvements in hair counts or reductions in hair loss have been reported in some populations in clinical studies of topical or oral saw-palmetto preparations, but study designs, extract concentrations, treatment durations, and outcome measures vary considerably. Therefore, saw palmetto should be considered a promising adjunctive botanical intervention, rather than a proven alternative to evidence-based pharmacological therapies. Paus et al. (1999)

5.7 Other possible mechanisms

Mechanisms other than the above have been proposed for the activity of saw palmetto in hair disorders, such as effects on cellular proliferation, sebaceous activity, inflammatory signaling, and the follicular microenvironment. The composition of the fatty acid-rich extract may also affect signaling associated with membranes and local lipid metabolism. These mechanisms are still on the exploratory phase and should be interpreted according to the chemical complexity of saw-palmetto preparations. Importantly, standardization of total fatty acids, phytosterols, extraction method and chemical fingerprint is essential when comparing

mechanistic or clinical findings across studies. Al-Nuaimi et al. (2010) To summarize, the available evidence supports a biologically plausible multi-target role for saw palmetto. However, the clinical relevance of individual mechanisms and their contribution to sustained hair growth deserve better-controlled studies using chemically characterized preparations.

Table 7. Proposed mechanisms of saw palmetto in alopecia

Mechanism	Potential effect	Evidence interpretation
5α-Reductase inhibition	May reduce DHT formation	Experimental/limited clinical support
Androgen-pathway modulation	May reduce androgen-dependent effects	Mechanistically plausible
Anti-inflammatory action	May reduce inflammatory signaling	Mainly experimental support
Antioxidant action	May reduce oxidative stress	Supportive preclinical evidence
Miniaturization modulation	Potential preservation of follicular growth	Clinical evidence remains limited
Lipid-mediated activity	Fatty acids/sterols may contribute	Constituent-dependent

6. Caffeine: Pharmacological and Cosmetic Aspects

6.1 Profile of chemical and pharmacological

Caffeine is a naturally occurring methylxanthine alkaloid found in coffee, tea, cocoa and a number of plants. It is chemically 1,3,7-trimethylxanthine and is known for its relatively high water solubility compared to many lipophilic botanical constituents. Its known pharmacological actions include antagonism of adenosine receptors (especially A1 and A2A) and effects on intracellular signaling and cellular metabolism. These properties have generated interest in caffeine as a topical active for scalp and hair-care applications in cosmetic formulations. The relatively small molecular size and well-known cosmetic application of caffeine make it particularly suitable for incorporation into aqueous or hydroalcoholic topical systems. Schneider and Paus (2014)

6.2 Mechanism of action.

It is thought that the biological effects of caffeine relevant to hair growth are mediated through several complementary pathways. At the cellular level , caffeine can antagonize adenosine receptors and affect intracellular cyclic AMP signaling . Experimental studies have also suggested effects on phosphodiesterase activity, cellular metabolism and responses to hormonal or stress related signals. These mechanisms might be involved in the maintenance

of follicular activity and in the lengthening of the growth phase in hair follicles. However, the exact contribution of each pathway in human scalp follicles remains incompletely defined and experimental concentrations should not be directly compared with concentrations achieved clinically after cosmetic application. Blume-Peytavi et al. (2008)

6.3 Impact on hair follicles

Investigations of isolated human hair follicles and follicular cells have provided evidence that caffeine can act directly on follicle growth. 34,35 The experimental exposure was associated with stimulation of hair-shaft elongation and cellular activity in the follicular unit. The dermal papilla and matrix areas are particularly important as they are responsible for the proliferative and signaling functions leading to anagen hair production. These findings biologically support topical caffeine use, but do not by themselves prove clinical efficacy as the conditions of follicular organ culture are very different from the environment of the scalp and from the use of rinse-off cosmetics. Tosti et al. (2009)

6.4 Effects on anagen phase

Anagen is the active phase of the hair cycle and defines the length of time matrix keratinocyte production of the hair shaft occurs. Caffeine has been studied for its ability to promote anagen-associated activity in follicles and to oppose processes that lead to premature transition to catagen. Experimental data have shown that caffeine can promote hair follicle growth and cell viability under some circumstances. This potential effect is important in AGA, where progressive shortening of anagen contributes to the production of increasingly miniaturized hairs. However, clinical interpretation requires standardized measurement of anagen-to-telogen ratio, hair density and shaft diameter instead of relying on laboratory endpoints alone. Tosti and Piraccini (2007)

6.5 Cellular and molecular mechanisms

Several cellular mechanisms have been proposed to explain the follicular actions of caffeine. These include modulation of adenosine signaling, intracellular cyclic AMP, phosphodiesterase-related signaling and pathways involved in follicular cell proliferation and survival . Caffeine has also been examined in relation to insulin-like growth factor-1 and other growth-associated mediators in the follicular microenvironment . Overall, the evidence suggests that caffeine may exert its effects through multiple interacting pathways rather than through a single molecular target. However, their relative importance in vivo is unknown,

highlighting the need for studies combining molecular biomarkers with clinically meaningful hair growth endpoints. Rossi et al. (2016)

6.6 Topical caffeine in hair care products

Topical caffeine has been added to shampoos, lotions, tonics, foams and leave-on scalp preparations. Shampoo is an attractive vehicle because it can be incorporated into a familiar hair care routine and can deliver the active directly to the scalp during washing. However, the rinse-off nature of the product limits contact time and may reduce the fraction of caffeine available for follicular interaction . Therefore, formulation variables such as concentration, pH, surfactant system, viscosity, deposition characteristics, washing frequency and scalp contact time may influence performance. A recent systematic review found clinical evidence for the use of topical caffeine in hair-loss preparations, but noted a large heterogeneity in study designs, caffeine concentrations, comparators, and methodological quality. Piraccini and Tosti (2018)

6.7 Clinical evidence: caffeine

The clinical evidence for topical caffeine is heterogeneous but promising. Randomized and controlled studies have evaluated caffeine-containing liquids, shampoos, and combined formulations in subjects with androgenetic or pattern hair loss. One randomized multicenter study compared a topical liquid containing 0.2% caffeine with 5% minoxidil and found comparable improvement of selected hair-cycle outcomes over 6 months; however, the formulation was a leave-on liquid and not a rinse-off shampoo, and the open-label design limits direct interpretation as evidence of equivalence. Rakowska et al. (2008) A double-blind study of a phyto-caffeine shampoo in women with androgenetic alopecia demonstrated a significant improvement in hair-pull test results and favorable secondary outcomes after 6 months with good tolerability. Inui (2011) More recent controlled studies of shampoos containing caffeine have also demonstrated improvements in hair loss-related outcomes, but differences in formulation and study design preclude product-to-product comparisons. Rudnicka et al. (2013) A 2025 systematic review of topical caffeine found nine clinical studies and concluded that the overall findings were positive but with variable quality of evidence and a need for larger, standardized randomized trials. Rudnicka et al. (2008a) Caffeine can therefore be considered a scientifically plausible and clinically promising cosmetic active, but its efficacy is strongly dependent upon formulation, exposure conditions and quality of supporting clinical evidence.

Table 8. Pharmacological and follicular effects of caffeine

Effect	Proposed mechanism	Potential implication
PDE inhibition	Increased intracellular cAMP	Supports cellular signaling
Follicular cell activity	May promote proliferative/metabolic activity	Potential hair-growth support
Anagen support	May counter premature regression	Potential prolongation of active growth
Follicular penetration	Can reach follicular structures after topical use	Supports topical delivery
Androgen-related effects	May counter some testosterone-associated inhibition in experimental models	Potential adjunctive action

7. Complementary Mechanisms of Caffeine and Saw Palmetto

7.1 Scientific rationale for the combination

The rationale behind combining saw palmetto and caffeine is that the two actives may affect different biological processes involved in hair loss. Saw palmetto is mostly studied for effects on androgen metabolism, especially pathways involving 5α-reductase and dihydrotestosterone (DHT), while caffeine has been studied for direct effects on follicular activity and maintenance of anagen-associated growth. Therefore, combining these activities may offer wider biological coverage than depending on one mechanism. This rationale is scientifically plausible but mechanistic complementarity should not be interpreted as evidence of clinical superiority or established pharmacological synergy. Rudnicka et al. (2008b)

7.2 Mechanisms that may complement each other

Saw palmetto and caffeine may act at different levels of the hair follicle environment. The saw palmetto may reduce the androgenic signaling by preventing local conversion of testosterone to DHT. The caffeine may increase the follicular cell activity through adenosine related and intracellular signaling. These pathways may theoretically converge on preservation of follicular function, maintenance of anagen activity and reduction of progressive miniaturization. The potential advantage of combining agents with different mechanisms is relevant for multifactorial disorders such as androgenetic alopecia, but their interaction has not been properly characterized in controlled human studies. Rossi et al. (2020)

7.3 Saw Palmetto-DHT Pathway

The main rationale for inclusion of saw palmetto is the androgenic component of androgenetic alopecia. 5α-reductase converts testosterone to DHT, which can activate androgen receptors in genetically susceptible scalp follicles. The progressive shortening of anagen and the transformation of terminal hairs into finer miniaturized hairs are associated with a persistent androgen signaling. Lipid-rich preparations of saw palmetto have been shown to have 5α-reductase-inhibiting activity in experimental systems, supporting the investigation of these preparations as botanical antiandrogenic agents. The extent of change in follicular DHT concentrations by topical saw palmetto in humans is however uncertain and depends on the composition of the extract, the dose, the delivery system, and follicular exposure. Bussoletti et al. (2020)

7.4 Caffeine folliculogenesis pathway

Caffeine provides a complementary growth mechanism. Experimental studies have documented effects of caffeine on isolated human hair follicles, such as stimulation of hair-shaft elongation and support of follicular activity under certain culture conditions. Proposed mechanisms include antagonism of adenosine receptors, modification of cyclic AMP signaling and effects on cellular proliferation and survival. Clinical studies of topical caffeine preparations have also reported favorable changes in selected hair-growth outcomes, although the quality of evidence varies and formulations differ considerably. These results do suggest a role for caffeine as a follicular supportive agent and not as a replacement for established antiandrogenic therapy. Dhurat et al. (2017)

7.5 Potential additive/synergistic effects

From a formulation-development perspective, the two actives could theoretically provide additive effects, as they target partially distinct biological processes: saw palmetto might address androgen-mediated follicular miniaturization, while caffeine could support follicular growth activity. An additive effect means the combined formulation provides the expected contribution of both actives, whereas true synergy means there is an effect greater than the sum of their individual effects. To demonstrate synergy, one must perform experiments with proper design, defined concentrations, validated biological endpoints and comparison with each active alone. Currently, there is not enough direct evidence to demonstrate true saw-palmetto-caffeine synergy in human scalp follicles or in clinical alopecia populations. Dressler et al. (2017)

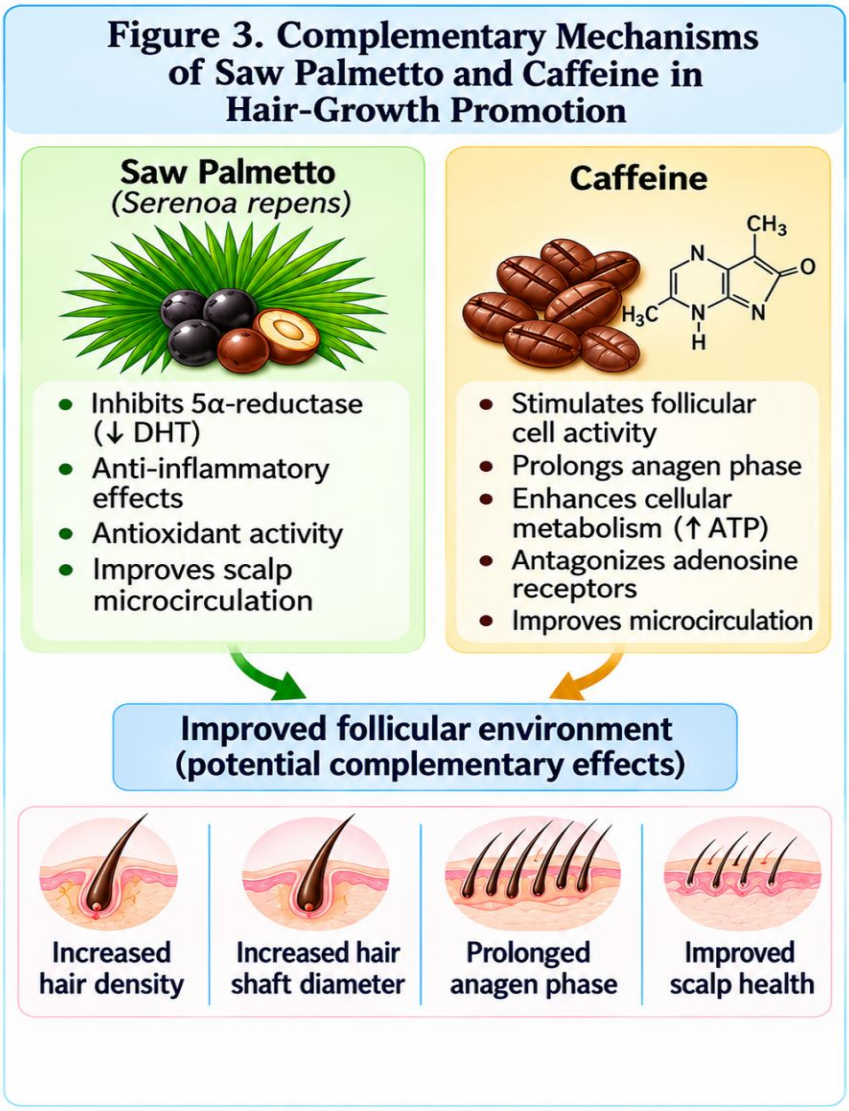


Figure 3: Complementary Mechanisms of Saw Palmetto and Caffeine in Hair-Growth Promotion

7.6 Existing evidence and limitations

There is more evidence for the individual ingredients than there is for the combination. Topical saw palmetto has been shown to decrease hair loss or increase hair measurements in selected populations and controlled studies of topical caffeine-containing products have shown improvements in hair-pull, hair-cycle or phototrichographic outcomes. Differences in extract standardization, caffeine concentration, formulation type, treatment duration, participant characteristics and outcome measures make comparisons between studies difficult Chen et al. (2024). Evidence from caffeine leave-on liquids can't be directly applied to rinse-off shampoos, and findings from one standardized saw-palmetto preparation can't necessarily be generalized to all commercial extracts. Future studies should compare saw palmetto alone, caffeine alone, the combination, and an appropriate vehicle control, using standardized

preparations and objective hair-growth endpoints. In the absence of such studies, the combination may be considered mechanistically promising but not clinically proven to be synergistic. Welzel et al. (2022)

Table 9. Representative clinical evidence for caffeine and saw palmetto

Intervention	Study design / population	Main finding	Key limitation
Phyto-caffeine shampoo	Randomized double-blind study in women with AGA	Improved hair-pull and secondary outcomes	Product-specific evidence
0.2% caffeine topical liquid	Randomized multicenter study in men	Anagen ratio improved; comparator was minoxidil	Liquid, not shampoo; open-label
Caffeine + adenosine shampoo	Randomized controlled study	Improved hair-loss-related outcomes	Combination-specific formulation
Topical Serenoa repens	Prospective pilot in men	Hair counts increased during follow-up	No placebo control
Standardized saw-palmetto oil	Randomized placebo-controlled 16-week study	Reduced hair fall and increased density	Limited duration; extract-specific
Caffeine systematic review	Multiple clinical studies	Overall favorable direction with variable evidence quality	Need larger standardized trials

8. Shampoo as a Local Delivery Tool in the Treatment of Baldness

8.1 Benefits of shampoo delivery

Shampoo is a convenient topical vehicle for interventions in scalp-directed hair care, as it combines treatment with routine cleansing and allows direct application over the scalp. A formulation of saw palmetto and caffeine is able to deliver the active ingredients over a relatively large area of scalp and at the same time remove sebum, particulate matter and cosmetic residues. This approach may improve user acceptability and adherence as it does not necessarily require an additional leave-on treatment step. Shampoo systems may also contain a variety of functional ingredients, including surfactants, conditioning agents, humectants, preservatives, and botanical actives, to allow development of a cosmetically acceptable product. Ly et al. (2025)

8.2 Limitations of rinse-off products

The main drawback of shampoo as a therapeutic delivery system is its short residence time. Once applied, this formulation is massaged into the scalp and washed with water, limiting the time available for active ingredients to partition into the stratum corneum or reach follicular structures. Surfactants may also affect the solubility, partitioning and deposition of active compounds. Therefore a shampoo containing an active ingredient does not necessarily mean that the follicle has been adequately exposed to that ingredient. Therefore, in the formulation design of a rinse-off formulation intended to support hair-growth related activity, optimization of concentration, vehicle composition, viscosity, application procedure and contact time is important. Szendzielorz and Spiewak (2025)

8.3 Penetration through skin

The delivery of a topical to the scalp requires the active ingredient to cross or circumvent the stratum corneum and where appropriate to enter the follicular openings. Hair follicles are specialized appendageal structures that can act as reservoirs for topically applied compounds. The penetration is dependent on molecular size, lipophilicity, aqueous solubility, formulation composition, skin condition, sebum content and duration of contact. Caffeine is comparatively soluble in water. The lipid rich components of saw palmetto have different physicochemical characteristics and may require appropriate solubilization or dispersion techniques. Hence a combined formulation needs to take into account the different physicochemical requirements of both actives. Herman and Herman (2016)

8.4 Targeting the hair follicle

Follicular targeting is particularly important in alopecia as the hair follicle is the primary biological structure responsible for hair production. Particulate systems, including lipid-based carriers and other advanced delivery approaches have been explored to enhance deposition of topical actives around follicular openings. However, one should not assume that a conventional shampoo is a follicle-targeted delivery system. Its performance is dependent on the formulation being able to deposit and retain actives during the short wash period. Follicular deposition, rather than total scalp deposition, may thus provide more meaningful information in formulation development. Prager et al. (2002)

8.5 Contact time and active retention

Contact time is a key determinant of performance for rinse off formulations. A longer contact time of a shampoo with the scalp may increase the opportunity for active deposition. However, the increased exposure time to surfactants may also increase the potential for dryness or irritation in susceptible individuals. The active retention can be affected by

surfactant concentration, polymeric thickeners, conditioning agents, solubilizers, and interaction of ingredients with hair and scalp surfaces. Therefore a practical formulation should provide adequate active exposure, acceptable cleansing properties and user comfort. Standardized instructions for application and rinsing are also important in the evaluation of clinical or experimental findings. Fischer et al. (2011)

8.6 Factors influencing topical delivery

The delivery of saw palmetto and caffeine from shampoo can be influenced by a number of formulation and biological factors. These include active concentration, extract standardization, particle size or dispersion state, solvent system, surfactant choice, pH, viscosity, temperature stability, washing frequency, scalp sebum, skin condition, and contact time. The compatibility of ingredients is especially important as the caffeine and complex lipid fraction of saw palmetto possess different physicochemical properties. The formulation should be chemically stable and uniformly distributed throughout its shelf life with minimum precipitation, phase separation, degradation and microbial contamination. Thus, development should include physicochemical characterization in combination with in vitro release, deposition or permeation studies and appropriate stability studies prior to clinical evaluation. Fischer et al. (2007a)

9. Development of Saw Palmetto-Caffeine Shampoo Formulation

9.1 Selection of active substances

The first crucial step in development of a saw-palmetto-caffeine shampoo is selection of the active ingredients. Saw palmetto should ideally be used as a chemically defined extract with a specific profile of fatty acids and phytosterols rather than as an undefined crude botanical material. Caffeine should be chosen in a suitable cosmetic or pharmaceutical grade with documented purity and stability. Setting concentrations should be based on available evidence and noting the limitations of rinse-off delivery. Since direct evidence for the combination is still limited, one should not assume that increasing the concentration of either active will lead to a proportional increase in hair-growth activity. Fischer et al. (2007b)

9.2 Choice of shampoo base

The base of the shampoo must enable efficient cleansing, be compatible with the scalp and provide physical and chemical stability to the actives. Acceptable base should have acceptable appearance, viscosity, spreadability, foaming characteristics , rinsability and microbial stability. The selection should also consider the physicochemical differences between caffeine and the lipid-rich saw-palmetto fraction. Over - cleansing regimes may lead

to increased scalp dryness or irritation and thus reduced consumer acceptance. A mild base, well tolerated is therefore desirable for repeated application on the scalp. Topal et al. (2019)

9.3 Surfactants

Surfactants lower surface tension, improve wetting of the skin, emulsify sebum, and lift particles and oily materials from the scalp and hair. Anionic surfactants allow cleaning and foaming, amphoteric and nonionic surfactants can help to make the product milder and to balance the formula. The surfactant system should be selected for cleansing strength, scalp tolerance, compatibility with botanical ingredients, and effects on active retention. High surfactant activity might enhance the removal of deposited actives and might influence the sensory characteristics negatively. Trueb (2016)

9.4 Co-surfactants and solubilizing agents

Co-surfactants and solubilizers can be used to enhance incorporation and physical stability of ingredients with limited solubility in the aqueous phase. This is especially true for the lipid soluble constituents of saw palmetto which may require strategies of dispersion, emulsification or solubilization. The chosen excipient should not cause undue irritation or destabilize the shampoo. The solubilizer concentration has to be optimized, since excessive amounts can affect viscosity, foaming, cleansing performance or active partitioning. Varothai and Bergfeld (2014)

9.5 Moisturizers

Humectants help to retain moisture in the formulation, and may improve the sensory properties of the shampoo and the condition of the scalp and hair. The commonly used polyols are glycerol, propylene glycol and the like. Their concentration should be based on compatibility, viscosity, preservative performance and active stability. Therefore excessive levels can change rheological behavior and sensory properties and should be optimized. Ramos and Miot (2015)

9.6 Conditioning agents

Conditioning agents reduce friction, improve wet and dry combing and improve the feel of hair. Consideration may be given to cationic polymers, silicones, proteins, and selected lipid-based materials. These should be included with compatibility assessment since conditioning agents can affect the deposition of other ingredients and change viscosity or appearance. Conditioning should be sufficient without excessive build-up that may interfere with scalp cleansing. Vano-Galvan et al. (2017)

9.7 Conservantes

As shampoo is composed of water and organic materials, an effective preservation system is required to control bacterial and fungal contamination during shelf life. The preservative should be effective in the pH range of the formulation and should be compatible with surfactants, botanical constituents, caffeine, polymers and other excipients . The preservative efficacy should be proven by a suitable challenge test supported by routine microbial quality testing. Blume-Peytavi et al. (2011)

9.8 Modifiers of pH

The pH of the shampoo influences the compatibility with the scalp, the behavior of surfactants, the activity of preservatives, the viscosity and stability of ingredients. The use of a mild acidic formulation may be preferred for hair-care products, since it may support acceptable scalp tolerance and hair-surface condition. The proper pH adjustment or buffering should be selected after evaluation of the active stability, preservative performance, viscosity, and sensory characteristics. Fabbrocini et al. (2018)

9.9 Viscosity Enhancers

Viscosity impacts the look of the product, its pourability, its application, how long it stays on the scalp during washing, and how consumers perceive it. Cellulose derivatives, natural gums, acrylic polymers and other rheology modifiers may be considered. Selected polymer should be stable with surfactants, electrolytes, botanical extracts, and the target pH. Optimization should consider ease of dispensing, spreadability and potential for active deposition. Birch et al. (2001)

9.10 Compatibility and stability

Compatibility studies are important because saw palmetto is a chemically complex botanical extract and caffeine has unique physicochemical properties. They can interact with surfactants, preservatives, polymers, fragrances, pH modifiers, or packaging materials. The stability assessment should include monitoring of appearance, color, odor, pH, viscosity, phase separation, microbial quality and active content under appropriate conditions. Visual inspection alone is a less rigorous measure of chemical stability than analytical monitoring of caffeine and representative saw-palmetto markers. Sinclair et al. (2004)

9.11 Formulation optimization

Optimization of formulations should be a systematic approach rather than changing several variables without a defined experimental design. The responses may include active content, physical stability, viscosity, foam properties, cleansing performance, microbial quality and in vitro deposition or release. Design-of-experiments approaches can identify interactions

between formulation variables and minimize unnecessary experimental batches. Sinclair (2005)

9.12 Sophisticated delivery methods

Advanced delivery systems can be attempted when conventional shampoo does not give adequate active retention or follicular exposure. Liposomes, nanoemulsions, lipid-based carriers, polymeric systems and other colloidal approaches could potentially improve solubilization, deposition or controlled release. Greater complexity in formulation also requires additional characterization, manufacturing control, safety evaluation, stability testing and regulatory assessment. Whiting (1996)

Table 10. Proposed composition of saw-palmetto-caffeine shampoo

Component	Role	Key consideration
Standardized saw-palmetto extract	Botanical active	Standardization, oxidation and dispersion
Caffeine	Primary active	Assay, solubility and stability
Primary surfactant	Cleansing	Mildness and compatibility
Secondary surfactant	Foam/mildness	Compatibility
Solubilizer/co-surfactant	Active dispersion	Physical stability
Humectant	Moisture retention	Hair/scalp feel
Conditioning polymer	Manageability	Surfactant compatibility
Preservative	Microbial protection	Challenge testing
Chelator	Controls trace metals	Stability
Antioxidant	Protects oxidation-sensitive lipids	Compatibility
Viscosity modifier	Controls rheology	Storage stability
pH modifier	Final pH adjustment	Scalp compatibility

10. Evaluation of the developed shampoo quality.

10.1 Appearance

The first quality attribute assessed during development of a shampoo formulation is physical appearance. The product must be homogeneous and free from any visible particles, phase separation, sedimentation or other evidence of instability. The evaluation should be for uniformity of the formulation, clarity or opacity as appropriate, consistency and evidence of precipitation of botanical constituents. Any changes in appearance during storage may indicate chemical degradation, incompatibility, microbial growth or physical instability and should therefore be systematically recorded. Malkud (2015)

10.2. Color and Odor

Color and odor should be consistent with the intended product profile and should not change unacceptably during storage. Botanical extracts may be added to impart characteristic color and odor and fragrances may be added to improve consumer acceptability. Significant alterations might be caused by oxidation, degradation of volatile components, interaction between components of the formulation or microbial contamination. Therefore, the organoleptic evaluation should be carried out at the beginning and during the stability studies under standardized observation conditions. Shrivastava (2009)

10.3 pH

Measure the pH of the formulated shampoo using a calibrated digital pH meter at the specified temperature. pH is important because it can affect scalp compatibility, hair surface characteristics, preservative activity, surfactant behavior, viscosity and stability of active ingredients. Measurements should be made on representative samples and with a consistent dilution or direct-measurement procedure. The chosen formulation should maintain the target pH during the intended shelf-life. Vano-Galvan and Camacho-Martinez (2010)

10.4 Viscosity

The viscosity influences the flow characteristics, dispensing properties, spreadability and sensory properties of a shampoo. It is determined using a suitable rotational viscometer under standard conditions of spindle, speed, temperature and sample. The viscosity should be checked during formulation optimization and stability testing since changes in viscosity may indicate polymer degradation, phase separation, electrolyte interactions or other formulation changes. The target range should allow for convenient dispensing while providing adequate residence and spread on the scalp. King et al. (2023)

10.5 Foam formation and foam stability

Shampoo is a consumer product whose important properties are foamability and foam stability . The foam can be assessed by a standard cylinder-shaking or other similar procedure and the initial foam volume and the foam retention over a specified time period are measured. Foam volume isn't a direct measure of cleansing or hair-growth efficacy, but insufficient or excessive foaming can influence consumer acceptance and perception of product performance. Thus the evaluation should be seen in conjunction with scalp tolerability and cleansing efficiency. King et al. (2022a)

10.6 Ability to wet

Wetting ability is the capacity of the formulation to spread rapidly over and wet hair or a standardized test surface. Shorter wetting time usually means that aqueous formulation is in

better contact with the substrate. The property is affected by surfactant composition, surface tension, viscosity and other excipients. Standardized wetting tests can be useful for comparing formulations during optimization and confirming batch-to-batch consistency. King et al. (2022b)

10.7 Test for dirt dispersal

The dirt-dispersion test is a common test to determine whether a shampoo can remove oily or particulate material without undesirable redistribution of dirt in the foam. A fixed amount of test soil can be added to a fixed shampoo solution and evaluated after controlled agitation. Excessive dirt remaining in the foam indicates unfavorable surfactant characteristics or inadequate cleaning behavior. It is primarily a comparative formulation-screening tool and cannot be interpreted as a direct measure of clinical hair-growth activity. King et al. (2023)

10.8 Solids content

Solid content is the amount of non-volatile material left after removing water and other volatile components. It can be determined by accurately weighing a defined quantity of formulation and drying it under controlled conditions until a constant or specified end point is reached. Information on the concentration of surfactants, polymers, conditioning materials, botanical extract components and other non-volatile ingredients. Consistent values are useful to monitor the reproducibility of manufacturing and the stability of formulations. Kennedy Crispin et al. (2016)

10.9. Cleanability

Rinsability refers to how easily shampoo can be rinsed from hair and scalp after application. A good formulation should be effective in cleansing the skin but should not leave too much residue, greasy or heavy after rinsing. Rinsability can be evaluated by means of standardized hair tresses or controlled consumer procedures. Especially if the formulation contains lipid-rich saw-palmetto extract or conditioning agents that can affect residue and post-wash sensory attributes. Craiglow and King (2017)

10.10 Content of active substance

The active ingredients have to be quantified to be able to establish dose uniformity and chemical stability. Caffeine can be measured using a validated chromatographic method such as HPLC with appropriate detection and sample preparation. Saw palmetto is more complex, containing several fatty acids and phytosterols. Representative chemical markers and/or a validated chromatographic fingerprint can be used for standardization. Active-content testing should be carried out during formulation development, batch release and stability studies to confirm that the product remains within predefined specifications. Strazzulla et al. (2018)

10.11 Microbial testing

Microbial quality testing is important for an aqueous cosmetic formulation as contamination can impact the safety and stability of the product. Tests should be suitable for the enumeration of the microbial load and for the detection of specified objectionable microorganisms in accordance with applicable pharmacopeial or cosmetic standards. The efficacy of the preservative should be demonstrated also during formulation development. Microbial testing should be conducted according to validated procedures and interpreted based on the requirements that are relevant to the intended market and product category. Strazzulla et al. (2018)

Table 11. Quality evaluation of the formulated shampoo

Parameter	**Purpose**	**Typical method**
Appearance	Detect separation/instability	Visual inspection
Colour/odour	Batch consistency/degradation	Organoleptic assessment
pH	Compatibility and stability	Calibrated pH meter
Viscosity	Pourability and acceptability	Brookfield/equivalent
Foamability	Consumer performance	Standard foam test
Foam stability	Persistence of foam	Time-based foam measurement
Wetting ability	Spreading/cleansing	Standard wetting test
Dirt dispersion	Redeposition tendency	Standard shampoo test
Solid content	Nonvolatile fraction	Gravimetric method
Rinsability	Ease of removal	Standardized washing
Stability	Shelf-life assessment	Accelerated/long-term study

10.12 Stability investigations

Stability studies are performed to determine if the shampoo maintains its physical, chemical, microbiological and performance properties throughout the expected shelf life. Studies should follow appearance, color, odor, pH, viscosity, foam characteristics, microbial quality, active content and evidence of phase separation or precipitation under appropriate storage conditions. Accelerated and long-term studies may be employed in accordance with applicable stability protocols. For a saw palmetto-caffeine formulation, chemical monitoring is particularly important since the botanical constituents may undergo oxidation or other changes that cannot be detected by visual examination. Craiglow and King (2017)

11. Evaluation of hair growth activity

11.1 Pull Test for Hair

The hair-pull test is a simple clinical procedure to estimate active hair shedding. A bundle of scalp hairs is gently pulled & the number of hairs pulled out is recorded. Repeated measurements may give some indication of changes in shedding during treatment. The test is useful as a supportive endpoint but is operator dependent and can be influenced by frequency of washing and interval between assessments. It should thus be combined with objective measures of density and hair-cycle status. Fischer et al. (2007a)

11.2 Hair count evaluation

Counting hairs provides a direct measure of the number of hairs visible in a defined area of the scalp. Depending on the study design standardized photographs, clipped area counts or trichoscopy systems can be used. Measure on the same area of scalp with the same magnification, lighting, and assessment procedure for repeat measurements. Hair counts are especially useful for detecting treatment-induced changes when baseline and follow-up measurements are taken under controlled conditions. Rossi et al. (2016)

11.3. Hair density measurement

Hair density, the number of hairs per unit of scalp area, is an important efficacy endpoint in alopecia studies. Objective photographic or digital trichoscopy may minimize observer variability. To improve the understanding of the contribution of terminal and miniaturized hairs to clinical appearance, it is preferable to give information about hair diameter and hair type in addition to density. Standardization of the target scalp area is needed for reliable longitudinal assessment. Birch et al. (2001)

11.4 Diameter of hair shaft

The diameter of the hair-shaft informs about follicular miniaturization and the transition between terminal and vellus-like hairs. Androgenetic alopecia is characterized by progressive reduction of the shaft diameter. It can be measured by trichoscopy or microscopic methods. Total hair number changes only modestly , but an increase in mean shaft diameter might be indicative of improved terminal-hair characteristics.Birch et al. (2001)

11.5 Trichoscopy examination

Trichoscopy is a non-invasive method for magnified visualization of scalp and hair structures. It can identify hair diameter variability, follicular openings, yellow dots, perifollicular changes and other features of importance for differential diagnosis and treatment follow-up. In AGA, trichoscopic parameters can assist in documenting

miniaturization and treatment-related changes. Digital image capture also facilitates longitudinal comparison and quantitative analysis. Inui (2011)

11.6 Phototrichogram

Phototrichography employs standardized photography and computer assisted analysis to estimate hair number, density, diameter, growth rate and the proportion of hairs in different phases of the cycle. This is especially useful in controlled clinical studies, where measurements can be repeated at specified time intervals. The technique requires consistent imaging, scalp preparation and analysis algorithms to minimize measurement error. Rossi et al. (2016)

11.7 Anagen to telogen ratio

The ratio of anagen and telogen hairs provides information about the dynamics of the hair cycle. AGA can be associated with progressive shortening of anagen and relative increase in telogen or miniaturized hairs. Thus treatment-associated increases in anagen proportion may be supportive of a biological effect on the hair cycle. This endpoint should be interpreted in conjunction with hair density and diameter, as changes in cycle distribution do not necessarily translate directly into clinically meaningful regrowth. Blume-Peytavi et al. (2011)

11.8 World photo assessment

Global standardized photography is used to document visible changes in hair coverage. Take pictures with controlled light, camera distance, background, hair style, hair length, head position. Independent blind assessment may reduce investigators' bias. Global photography is especially useful as a complementary endpoint, since it captures the overall cosmetic appearance, but is still sensitive to differences in image quality and styling. Rossi et al. (2016)

11.9 Patient reported outcomes

Patient reported outcomes capture perceptions that may not be fully captured by instrumental measurements and include perceived shedding, hair volume, scalp comfort, cosmetic satisfaction and treatment convenience. Where available, validated questionnaires are preferred. Such measures should be used in conjunction with objective endpoints since subjective improvement can be influenced by expectations, hair-care practices and changes in styling. Bussoletti et al. (2020)

12. Preclinical Testing

12.1 Models in vitro

In vitro models can be used to evaluate early the biological activity and safety of saw palmetto and caffeine and their combinations. For example, cell-based assays provide the

opportunity to examine proliferation, viability, inflammatory signaling, oxidative stress, and responses to pathway-specific stimuli prior to more complex models. These systems allow for the controlled performance of concentration-response experiments but are not able to fully recapitulate the multicellular follicular environment of human scalp. Fischer et al. (2007b)

12.2 Models of dermal papilla cells

Dermal papilla cells are a key regulator of hair follicle growth and cycling. Thus, cultured dermal papilla cells can be used to study the responses to caffeine, saw-palmetto extracts or formulated preparations. End points can include cell viability, proliferation, growth factor expression, and pathway associated biomarkers. Passage number, culture conditions and donor variability must be controlled, since these factors can influence phenotype and experimental reproducibility. Schneider (2019)

12.3 Culture of hair-follicles as an organ

Culture of human hair follicle organ offers a more physiologically relevant model than isolated cell systems because the follicular structure and the interactions between multiple cell populations are partially preserved. After exposure to test formulations or actives, hair-shaft elongation, anagen maintenance and morphological changes can be monitored. The model is useful for mechanistic screening, but has limitations for tissue viability, donor variability and the short experimental period. Fischer et al. (2007a)

12.4 Studies on cytotoxicity

It is necessary to assess the cytotoxicity to identify concentrations that do not adversely affect follicular or scalp-related cells. Suitable assays may include metabolic viability assays, membrane integrity assays, or microscopic examination. Testing should be undertaken on the individual actives, the combination and appropriate excipients or finished formulations. Biological activity should be interpreted in combination with relevant therapeutic or cosmetic window and cytotoxicity.

12.5 5*α*-reductase inhibition assay

The antiandrogenic potential of saw-palmetto preparations can be investigated by a 5 alpha-reductase inhibition assay. The assay should employ a defined source of enzyme and a validated substrate and detection methodology. Interpretation requires concentration-response relations and appropriate positive and negative controls. Results may provide support for mechanistic claims, but should not be equated directly with clinical efficacy, as enzyme inhibition in vitro does not establish follicular DHT reduction in humans. Russell and Wilson (1994)

12.6 Ex vivo studies .

Simple cell culture is less representative of human tissue than ex vivo skin or scalp models which can provide data on formulation deposition, penetration, irritation and follicular localization. Such models could be especially useful for comparing conventional shampoo with advanced delivery systems. The need for appropriate controls and validated analytical approaches to distinguish between surface deposition and true follicular penetration has been emphasized. Fischer et al. (2007a)

12.7 Hair growth models in animals

Animal models can be used to study topical tolerance, hair-cycle effects and preliminary efficacy. Common measures assess changes in hair regrowth, follicular morphology and anagen induction. However, differences in follicular biology, architecture of the skin, grooming practices and drug metabolism preclude easy translatability to alopecia in humans. Hence animal studies should complement rather than replace human clinical evaluation. Blume-Peytavi et al. (2011)

12.8 Histopathological assessment

Histopathology can provide structural evidence of changes of the follicle after treatment. Parameters may include follicular density, follicular diameter, anagen to catagen features, epidermal changes, and inflammatory cell infiltration. Appropriate blinding and standardized tissue processing are essential to minimize interpretation bias. Histological findings have the greatest value if correlated with functional hair-growth and molecular endpoints. Whiting (1993)

13. Saw Palmetto and Caffeine Formulations: Clinical Evaluation

13.1 Planning clinical trials

Clinical assessment should be carried out in a well defined population, using a standardized intervention, a suitable control, prespecified endpoints, and an appropriate duration of treatment. Randomized controlled designs are more convincing than uncontrolled studies and blinding may reduce assessment bias. Standardization of treatment instructions, washing frequency, contact time, concomitant hair products, and adherence is needed for a rinse-off saw-palmetto-caffeine shampoo. Kanti et al. (2018)

13.2 Saw-palmetto and clinical trials

Clinical studies of saw-palmetto preparations have found reductions in hair loss and improvements in selected hair-growth measures in people with pattern hair loss. Preparation heterogeneity remains with preparations differing in extraction method, fatty-acid content, concentration, route of administration and duration. Some topical studies have shown

increased hair counts but the lack of placebo controls in several studies limits interpretation. Herman and Herman (2016)

13.3 Clinical studies on caffeine

Overall, clinical trials with topical caffeine have demonstrated predominantly positive changes in hair loss-related outcomes, including hair-pull findings, hair-cycle measures, and photographic or phototrichographic endpoints. Evidence includes shampoos and leave-on products, but these dosage forms are not to be considered interchangeable. A promising overall evidence has been reported in recent systematic reviews, but heterogeneity and the need for larger and better controlled trials have been underlined. Bussoletti et al. (2020)

13.4 Combination studies of saw palmetto and caffeine

There is less direct clinical evidence for a standardized saw-palmetto-caffeine shampoo than for the individual ingredients. Thus, a combination study should be designed to differentiate the contribution of each active by including appropriate comparator groups. Better results with the combination alone would not demonstrate synergy unless the design permits comparison with each of the individual treatments at the same exposure. Bussoletti et al. (2020)

13.5 Endpoints for efficacy

Objective measures such as hair density, terminal-hair count, shaft diameter, phototrichographic parameters, and standardized photography should be used, in the ideal setting of clinical efficacy. Hair-pull testing and patient reported outcomes may be supportive. A combination of complementary endpoints is preferred as no single measure captures the full biological and cosmetic dimensions of alopecia. Rossi et al. (2016)

13.6 Safety end-points

The safety evaluation should include adverse events related to the scalp or hair such as local irritation, erythema, burning, itching, dryness, scaling and sensitization. Treatment-emergent events should be documented by investigators throughout the study and assessed for severity, duration, and relationship to the investigational product. Depending on the risk of the study and regulatory expectations, appropriate dermatological examinations and laboratory tests may be included. Bussoletti et al. (2020)

13.7 Limitations of present clinical evidence

Existing evidence is limited by small sample sizes, heterogeneous formulations, variable active concentrations, inconsistent outcome measures, short follow-up and incomplete reporting of standardization. Many studies focus on individual ingredients rather than the actual combination or shampoo dosage form. These limitations compromise quantitative

comparisons and highlight the necessity of well-powered, randomized, standardized clinical trials. Rossi et al. (2016)

14.1.1 Analysis of Caffeine by HPLC

The chemical stability, aqueous solubility, and well-characterized chromatographic behaviour of caffeine make it suitable for quantitative determination using high-performance liquid chromatography (HPLC). Caffeine has been investigated in relation to hair follicle growth and its potential application in hair-loss formulations (Fischer et al., 2007; Fischer et al., 2011). The analytical method should demonstrate specificity, linearity, accuracy, precision, range, detection capability, and robustness. Sample preparation must consider the presence of surfactants, polymers, botanical extracts, and other formulation components to minimize matrix interference.

14.2 GC-FID/GC-MS Analysis of Saw Palmetto Oil

Gas chromatography coupled with flame-ionization detection (GC-FID) or mass spectrometry (GC-MS) is applicable to the characterization of volatile and derivatized fatty-acid constituents of saw palmetto oil. GC-MS provides structural information about individual constituents, whereas GC-FID is useful for quantitative profiling. Saw palmetto-derived constituents have been investigated for their potential inhibitory effects on 5-alpha-reductase, supporting their relevance in androgenetic alopecia research (Prager et al., 2002; Herman and Herman, 2016). Standardization of sample preparation and derivatization procedures is essential for obtaining reproducible measurements of the lipid fraction.

14.3 Phytosterol Analysis

Phytosterols are relevant chemical markers for the characterization and standardization of botanical extracts, including saw palmetto. Chromatographic analysis of β-sitosterol, campesterol, stigmasterol, and related constituents can provide information regarding extract identity, purity, and batch-to-batch consistency. Botanical constituents and their potential mechanisms of action in hair-loss management have been discussed by Herman and Herman (2016). Since no single constituent necessarily represents the complete biological activity of an extract, phytosterol analysis should be interpreted alongside fatty-acid composition and other chemical characteristics.

14.4 LC-MS Analysis

Liquid chromatography-mass spectrometry (LC-MS) provides detailed chemical profiling of complex botanical extracts and facilitates the identification of minor constituents that may not be adequately characterized by conventional analytical techniques. It can be employed for authentication, comparative chemical profiling, degradation studies, and investigation of

formulation-related changes. The characterization of multiple active constituents is particularly relevant when evaluating botanical ingredients used in hair-loss formulations (Herman and Herman, 2016).

14.5 Chromatographic Fingerprinting

Chromatographic fingerprinting provides a comprehensive representation of the chemical composition of botanical extracts compared with the analysis of individual marker compounds. Comparison of chromatographic fingerprints among different batches helps assess consistency, identify possible adulteration, and monitor chemical changes during storage. For saw palmetto extracts, combining chromatographic fingerprinting with quantitative marker analysis can strengthen quality-control procedures (Prager et al., 2002; Herman and Herman, 2016).

Table 12. Analytical and characterization strategy

Analyte/attribute	Technique	Purpose
Caffeine	HPLC-UV/DAD	Quantitative assay
Fatty acids	GC-FID/GC-MS	Saw-palmetto lipid profile
Phytosterols	GC or HPLC	Marker characterization
Extract fingerprint	HPLC/UPLC or GC	Identity and batch consistency
Unknown constituents	LC-MS/MS	Chemical profiling
Finished-product stability	Validated chromatography	Monitor active retention/degradation

15. Molecular Biomarkers and Mechanism of Action Evaluation

15.1 5α-reductase

The measurement of 5α-reductase expression or activity may help investigate the postulated antiandrogenic mechanism of saw palmetto. Depending on the model, assays may be based on gene expression, protein abundance, enzyme activity or downstream androgen metabolites. Indeed, a reduction in enzyme activity is stronger mechanistic evidence than expression changes alone, since expression and functional activity are not necessarily equivalent. Russell and Wilson (1994)

15.2 Receptor for androgen

The androgen receptor mediates cellular responses to testosterone and dihydrotestosterone. Its expression and downstream signaling can be exploited to determine if a formulation alters androgen responsiveness. Such measurements are particularly relevant in models of

androgen-sensitive follicular cells, but receptor expression changes should be interpreted in conjunction with functional signaling endpoints. Choudhry et al. (1992)

15.3 DHTs

DHT is the major androgen involved in follicular miniaturization in susceptible scalp sites. Measurement of DHT in appropriate experimental systems can be used to determine whether a treatment alters androgen metabolism. Tissue or follicular measurements are more mechanistically informative than non-specific systemic measurements for the assessment of a topical product, although technically robust sampling is needed. Trueb (2002)

15.4 β-catenin signaling pathway

Follicular development and cycling involves Wnt/β-catenin signaling and this pathway has been investigated as a pathway supporting anagen-associated activity. Thus, changes in β-catenin or elements of the associated pathway may provide mechanistic insight into the follicular response to therapy. However, modulation of pathways should be interpreted with caution because Wnt signaling is context dependent and alone does not establish clinical hair regrowth. Millar (2002)

15.5 DKK

Dickkopf-1 (DKK-1) is an inhibitor of Wnt signaling and has been studied in the setting of androgen-mediated miniaturization of follicles. Androgen-sensitive follicular systems in experiments have shown increased expression of DKK-1. The measurement of DKK-1 along with Wnt-associated markers can help define whether a formulation affects pathways related to follicular regression. Kwack et al. (2012)

15.6 VEGF

VEGF stands for vascular endothelial growth factor. Vascular endothelial growth factor (VEGF) is involved in angiogenic processes in the developing hair follicle. Changes of VEGF expression may be evaluated as an exploratory marker of follicular vascular and growth-related response. VEGF plays a role in different physiological processes, so its assessment must be linked with morphological and functional hair growth endpoints. Randall et al. (2001)

15.7 IGF-1 (Insulin-like growth factor-1)

Insulin-like growth factor-1 (IGF-1) plays a role in the regulation of follicular growth, differentiation and survival. Changes in IGF-1 signaling experimentally might therefore offer corroborating evidence for growth-promoting activity. Depending on the experimental model, the assessment may be gene expression, protein levels or cellular responses related to the pathways. Randall et al. (2001)

15.8 FGF-7

Fibroblast growth factor-7 (FGF-7), also known as keratinocyte growth factor, has been implicated in epithelial and follicular growth processes. Alterations in FGF-7 expression may provide an insight into effects on follicular epithelial activity. As with other biomarkers the interpretation should be based on a panel of markers and not a single molecular measurement. Headington (1993)

15.9 Markers of inflammation

The anti-inflammatory component of a formulation can be evaluated by investigating inflammatory mediators such as selected interleukins, tumor necrosis factor related markers and chemokines. Such measurements could be particularly useful in the study of scalp irritation or perifollicular inflammation. Biomarker selection should be hypothesis-driven and supported by the appropriate controls. Inui et al. (2002)

15.10 Markers of Oxidative Stress

Oxidative stress assessment may include measurement of lipid peroxidation products, reactive oxygen species-dependent parameters, glutathione status and antioxidant enzymes. These endpoints may be useful in determining whether treatment affects the oxidative environment surrounding the follicular cells. Since oxidative biomarkers are sensitive to sample handling and tissue conditions, a standardized protocol for collection and processing is essential. Trueb (2002)

16. Safety and regulatory issues

16.1 Skin safety

A topical shampoo for repeated use on the scalp must have an acceptable safety profile under the proposed conditions of exposure. “We should be evaluating the final formulation, not just the safety profiles of the individual ingredients.” Repeated-use studies can provide information on cumulative irritation and changes in scalp condition. Rossi et al. (2012)

16.2 Irritation to skin

The irritation assessment should determine whether the formulation produces erythema, burning, itching, dryness, or other local reactions. Where appropriate, testing should be performed using validated approaches and appropriate ethical and regulatory procedures. Potential contributors to irritation include the choice of surfactant, pH, fragrance, preservatives and botanical ingredients. Rossi et al. (2012)

16.3 Sensibilization

Sensitization is an important consideration in botanical extracts, fragrances, preservatives and other formulation components. A product may be well tolerated in a single exposure but

allergic responses can occur on repeated exposure in sensitized individuals. Thus, ingredient-specific safety information and suitable finished-product evaluation should be incorporated into development. Rossi et al. (2012)

16.4 Long-term safety

The cumulative exposure over the long term, repeated washings, chronic contact with the scalp and possible changes in skin barrier function must be taken into account. The relatively short contact time with shampoo is intended but users may be exposed repeatedly over months or years. Therefore, long-term safety assessment should be representative of the proposed frequency and duration of use . Bussoletti et al. (2020)

16.5 Botanical Extracts Standardization

Standardization of botanicals is necessary for reproducibility and safety as the chemical composition may vary with plant source, harvesting, processing, extraction solvent, storage and adulteration. Saw-palmetto extracts should be characterized by relevant fatty-acid and phytosterol markers and, where appropriate, chromatographic fingerprints. Standardization also improves interpretability of efficacy studies. Herman and Herman (2016)

16.6 Quality Assurance

Quality assurance of shampoo formulations should include the identification and authentication of raw materials, active ingredient content, microbial quality, physicochemical properties, preservative effectiveness, packaging compatibility, and stability of the finished product. Before routine manufacturing, appropriate product specifications should be established and supported by validated analytical procedures. Batch-to-batch consistency is particularly important for botanical formulations because variations in the chemical composition of plant extracts may influence product quality and performance (Herman and Herman, 2016).

16.7 Regulatory Aspects of Cosmetic Products

The regulatory classification of a product depends on its intended use, composition, claims, and applicable jurisdiction. Cosmetics marketed as shampoos must comply with relevant cosmetic safety, labelling, manufacturing, and ingredient requirements. Product claims should be supported by appropriate scientific evidence and must not inadvertently or deliberately classify a cosmetic product as a medicinal treatment, which may require compliance with separate regulatory frameworks.

16.8 Therapeutic and Cosmetic Claims

The distinction between cosmetic and therapeutic claims is particularly important for products intended for alopecia management. Claims related to cleansing, conditioning, scalp

care, and cosmetic improvement may fall within cosmetic positioning, whereas claims involving the diagnosis, treatment, prevention, or cure of diseases may trigger medicinal or drug-related regulatory requirements, depending on the jurisdiction. Therefore, product claims should be scientifically substantiated, appropriately worded, and consistent with the regulatory classification of the formulation.

17. Alopecia: A Comparison of Conventional and Emerging Therapies

17.1 Minoxidil

Minoxidil is an established topical treatment for androgenetic alopecia and has considerably stronger clinical evidence than most botanical cosmetic ingredients. Its proposed mechanisms include potassium channel modulation, vascular effects, and regulation of hair follicle cycling. Therefore, a shampoo containing saw palmetto and caffeine should be positioned as a potential adjunctive cosmetic approach unless comparative clinical evidence demonstrates therapeutic efficacy (Suchonwanit et al., 2019).

17.2 Finasteride

Finasteride is a selective type II 5α-reductase inhibitor that reduces dihydrotestosterone (DHT) formation and subsequently decreases androgen-mediated effects associated with male androgenetic alopecia. Its use involves systemic safety considerations, and it has a more established clinical evidence base than saw palmetto for appropriate therapeutic indications. Finasteride and saw palmetto have conceptually overlapping mechanisms involving androgen metabolism, whereas caffeine acts through different proposed biological pathways (Mysore and Shashikumar, 2016).

17.3 Dutasteride

Dutasteride inhibits both type I and type II 5α-reductase enzymes, resulting in a greater reduction in DHT formation than selective type II inhibition. Its use should be based on appropriate clinical indications and safety considerations. Comparisons between dutasteride and saw palmetto should consider their mechanisms of action, quality of clinical evidence, routes of administration, and safety profiles rather than assuming that botanical 5α-reductase inhibition is equivalent to pharmacological inhibition (Gupta and Charrette, 2014).

17.4 Ketoconazole

Ketoconazole-containing shampoos are primarily used for scalp conditions associated with fungal infections and inflammation. They may be incorporated into hair-loss management in selected clinical situations. Their mechanism of action differs from those of saw palmetto and caffeine. This distinction highlights the importance of selecting formulation ingredients and

defining product claims according to the underlying cause of hair loss (Rogers and Avram, 2008).

Reference note: Rogers and Avram (2008) provide a general review of medical treatments for pattern hair loss; a dedicated reference on ketoconazole shampoo would strengthen this subsection.

17.5 Other Botanical Therapies

Other botanical ingredients investigated for hair-loss management include plant-derived extracts with potential antiandrogenic, anti-inflammatory, antioxidant, and follicular-supportive properties. However, the available evidence varies considerably in terms of scientific quality, extract standardization, and clinical validation. Consequently, comparisons should consider extract characterization, clinical trial design, active constituent exposure, and objective evaluation endpoints rather than botanical origin alone (Herman and Herman, 2016).

17.6 Nutraceutical Approaches

Nutraceutical approaches may help address nutritional deficiencies, oxidative stress, and metabolic factors associated with hair health. Such interventions are particularly relevant when an underlying nutritional or metabolic abnormality is identified. However, nutritional supplementation cannot be considered a universal substitute for appropriate diagnosis and evidence-based treatment of established alopecia (Trueb, 2013).

18. Challenges, Research Gaps and Future Outlook

18.1 Absence of Standardized Saw Palmetto Preparations

One of the major limitations in the existing literature is the variability in saw palmetto preparations. Differences in extraction techniques, fatty acid composition, phytosterol profiles, concentration, and formulation characteristics may result in substantial variations in product quality and biological activity. Future investigations should include detailed chemical characterization and employ standardized botanical preparations to improve reproducibility and ensure consistency across studies (Herman and Herman, 2016).

18.2 Limited Combination Studies

The mechanistic rationale for combining saw palmetto and caffeine is scientifically plausible; however, direct clinical evidence supporting the standardized combination remains limited. Further controlled investigations are required to compare the individual effects of each active ingredient with their combined effects. Such studies would help establish whether the combination produces additive or synergistic benefits in hair-loss management (Bussoletti et al., 2020).

18.3 Delivery Challenges Associated with Rinse-Off Formulations

The relatively short contact time of shampoo on the scalp represents a significant formulation challenge. Rinse-off products require optimized deposition, follicular retention, and active ingredient stability to achieve potentially beneficial effects on hair follicle biology. Future research should quantify actual scalp deposition and follicular exposure rather than relying exclusively on the nominal concentration of active ingredients in the formulation. Standardized analytical methods and appropriate formulation evaluation are essential for establishing reproducible results (Prager et al., 2002; Szendzielorz and Spiewak, 2025).

18.4 Limited Evidence Regarding Long-Term Clinical Use

Most available clinical investigations have relatively short treatment durations, making it difficult to determine whether the observed improvements are maintained following prolonged use. Long-term clinical studies are therefore necessary to evaluate the durability of treatment outcomes, patient adherence, cumulative tolerability, and changes in therapeutic response over time. Extended follow-up would also provide valuable information regarding the long-term suitability of caffeine-based and botanical hair-care preparations (Ly et al., 2025).

18.5 Need for Standardized Clinical Outcomes

Variations in hair-counting techniques, photographic assessment methods, trichoscopic evaluation, hair-pull testing, and patient-reported outcomes make comparisons between clinical studies difficult. Future investigations should employ validated and standardized assessment methods, predefined clinical endpoints, and appropriate statistical analysis plans. Standardization would improve the reliability, comparability, and interpretation of clinical findings related to hair-loss treatments (Rossi et al., 2016).

18.6 Advanced Follicular Delivery Systems

Follicular targeting represents an important opportunity for improving topical drug and active ingredient delivery. Advanced delivery systems may enhance residence time near follicular openings and improve the local exposure of poorly soluble botanical constituents. However, improvements in follicular delivery should be supported by quantitative analytical evidence and correlated with relevant biological and clinical outcomes (Topal et al., 2019).

18.7 Personalized Alopecia Therapy

Alopecia is a heterogeneous condition, and treatment responses may vary according to the underlying diagnosis, androgen sensitivity, age, sex, disease duration, scalp condition, and genetic factors. Future investigations may benefit from stratifying participants according to clinically relevant phenotypes and molecular characteristics rather than grouping all

individuals experiencing hair loss into a single population. Such an approach could contribute to the development of more individualized and effective treatment strategies (Inui and Itami, 2013).

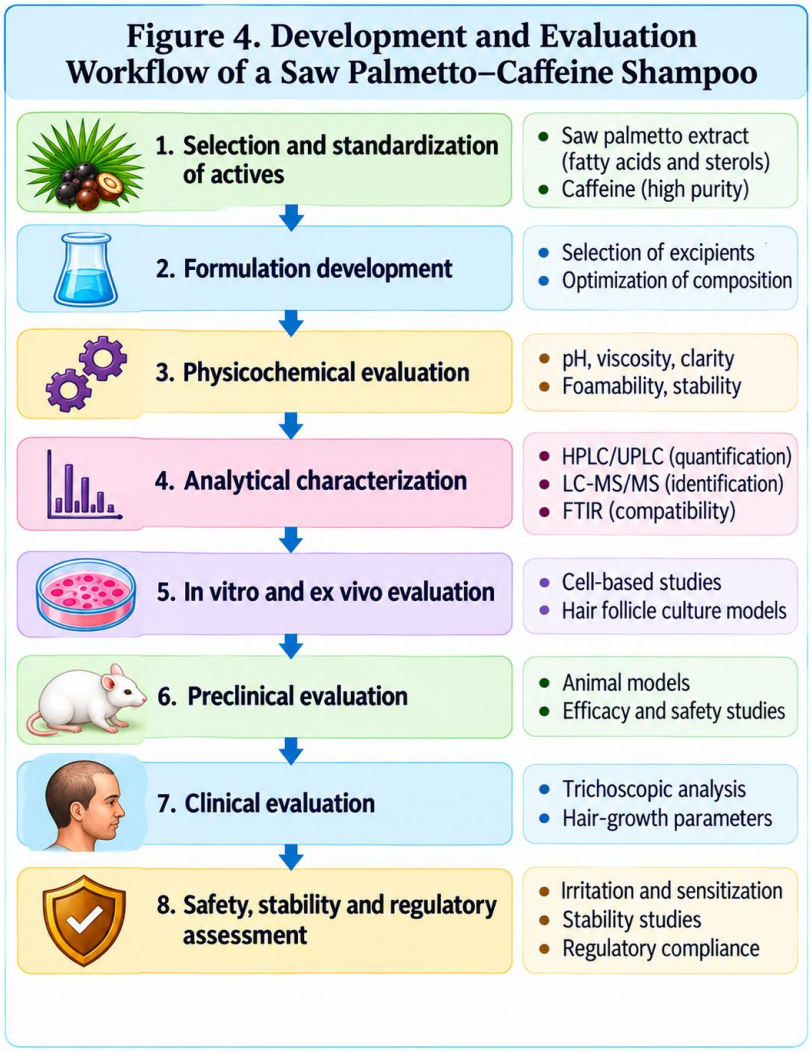


Figure 4: Development and Evaluation Workflow of a Saw Palmetto-Caffeine Shampoo

19. Conclusion

Both saw palmetto and caffeine are mechanistically distinct but potentially complimentary ingredients to be included in topical hair-care formulations. Saw palmetto has been primarily studied for its potential impact on androgen pathways, while caffeine has been studied for its impact on follicular activity and hair-cycle biology. While shampoo offers a convenient and cosmetically acceptable delivery system for agents to the scalp, its rinse-off nature presents significant hurdles concerning active retention and follicular exposure. Thus, successful development calls for careful selection and standardization of the active ingredients, optimization of the shampoo base, analytical characterization, stability evaluation, and appropriate assessment of hair-growth activity. At present, the combination should be

considered a promising formulation concept rather than a clinically established synergistic therapy. Future standardized preclinical and clinical studies will be key to establish its efficacy, safety, optimal composition and translational value in the management of alopecia.

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17. Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this review.

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