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# The Development of Medical Shampoo with a Plant-Based Substance for the Treatment of Seborrheic Dermatitis †

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**Abstract:** Dermatological diseases of the scalp such as seborrheic dermatitis (SD) significantly affect the quality of life of a population. Medical shampoos with synthetic substances have adverse effects that can be alleviated by use of medical shampoos with plant-based substances. Therefore, the aim of this research was to develop a natural medical shampoo with a plant-based substance that is effective for the treatment of SD. The natural-based surfactants helped to achieve proper characteristics and pH-stability. The medical shampoo formulation with the novel substance, made from *Melaleuca alternifolia* leaf oil, 1,8-cineole and (-)-α-bisabolol, at a concentration of 0.75% exhibited high antibacterial activity against *Staphylococcus epidermidis* and *Staphylococcus aureus* with log10CFU reduction >1.0. Interestingly, the antifungal activity against *Candida albicans* (a model host of *Malassezia* species) was comparable to that of ketoconazole, climbazole and piroctone olamine when using the broth microdilution method. The shampoo formulation with a combination of natural-based surfactants possessed the optimal characteristics, such as physical appearance, pH-stability and density foam stability. Therefore, the developed natural-based medical shampoo with a substance of plant origin could be used for the prophylaxis and alleviation of SD.

**Keywords:** seborrheic dermatitis; dandruff; medical shampoo; antimicrobial activity; phytochemicals; surfactants



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## 1. Introduction

Nowadays, people pay more attention to proper cleansing and the choice of natural shampoo for scalp health. It has been established that seborrheic dermatitis (SD) has a high prevalence worldwide and occurs in up to 50% of the adult population [1]. For this reason, medical shampoos for the treatment of SD are becoming popular in order to clean the scalp, remove excessive sebum with fatty acids, exfoliate stratum corneum, relieve itching, alleviate inflammation and regulate scalp microflora involved in the pathogenesis of SD. The detailed pathogenesis of SD is not well understood, but there are various intrinsic and extrinsic factors that worsen the clinical manifestations and increase the severity of SD. Normally, the scalp microflora, such as *Malassezia* species, *Staphylococcus* species and other bacteria, maintains a pH balance, constant renewal of the stratum corneum and moisturizing properties of the scalp. However, excessive sebaceous gland secretion and a related abundance of *Malassezia* sp. causes scalp irritation, itching, dryness and visible flakes [2] that negatively impacts patients' well-being and psychological comfort [3]. It has been established that *Staphylococcus epidermidis* (*S. epidermidis*), *Staphylococcus aureus* (*S. aureus*), *Malassezia furfur* (*M. furfur*) and *Candida albicans* (*C. albicans*) are a part of scalp microflora that exacerbate the pathogenesis of SD [4]. However, current shampoos do not target these scalp microorganisms.

The treatment of SD and dandruff with prolonged cleanliness of the scalp is needed for improvement of clinical manifestations and remission. However, dermatologists and trichologists currently recommend only approved drugs for use against fungal colonization and immunity-mediated inflammation. The common medical shampoos and other products for external use contain topical synthetic agents such as antifungals, antibacterials and anti-inflammatory substances alone or in combination. However, synthetic substances may cause serious hair and scalp problems [5–8]. Antimicrobial resistance becomes one of the serious public health concerns in the treatment of SD due to the increase in azoles-resistant strains of *Malassezia* species [9].

Therefore, natural shampoo with an effective plant-based substance for proper scalp treatment can be beneficial for scalp appearance and health in order to prevent and treat SD. Plant-based substances, such as essential oils or plant extracts, have comprehensive compositions, multiple properties, different activities and low irritancy potential [10]. In an investigation by the authors, the synergetic combination of *Melaleuca alternifolia* (*M. alternifolia*) leaf oil, 1,8-cineole (eucalyptol) and (-)-α-bisabolol in a ratio of 1:1:1 was found to exhibit a documented therapeutic antimicrobial potential against pathogenic microflora related to SD [11].

Most commercial medical shampoos consist of synthetic surfactants and other additives. Nevertheless, patients have recently become more focused on the naturalness of medical shampoo, its clear composition and its development in accordance with dermatologists' recommendations. The aim of this research was to develop a natural medical shampoo with an investigated plant-based substance based on *M. alternifolia* essential oil, 1,8-cineole and (-)-α-bisabolol for the treatment of SD. Additionally, shampoo formulations containing the plant-based substance were characterized.

## 2. Results and Discussion

### *2.1. Development of the Shampoo Formulation*

Four shampoo formulations with the plant-based substance were developed for antimicrobial susceptibility testing and physical characteristics assessment. All formulations were transparent with medium viscosity and homogenous textures. For better shampoo properties, the formulations had an average pH value of 5.50–5.56. Moreover, the mild acidic pH values of medical shampoos could increase hair cleansing, decrease eye irritation and keep the pH balance of the scalp suitable for microflora regulation [12]. The foam characteristics depending on the concentration of lauryl glucoside in the formulations are presented in Table 1. The different concentrations of lauryl glucoside helped to regulate foam characteristics and viscosity. The addition of lauryl glucoside at a concentration of 5.0% produced the desired foam number and foam stability that are crucial properties of medical shampoos. The concentration of lauryl glucoside above 5.0% significantly increased the viscosity in the shampoo formulations and affected the stability over 14 days. Thus, the formulation C had the most optimal characteristics for further research and assessment for its antimicrobial potential.

**Table 1.** Characterization of shampoo formulations A–D.

| Formulation | pH (10% *w*/*w*) [a] | Density, g/mL | Viscosity, mPa·s [a] | Foam Number, mm [a] | Foam Stability, mm [a] |
|---|---|---|---|---|---|
| A | 5.50 ± 0.05 | 1.025 | 1540 ± 13 | 182 ± 2 | 0.86 ± 0.02 |
| B | 5.52 ± 0.05 | 1.020 | 3164 ± 26 | 220 ± 5 | 0.85 ± 0.02 |
| C | 5.56 ± 0.05 | 1.018 | 5764 ± 27 | 222 ± 3 | 0.87 ± 0.02 |
| D | 5.53 ± 0.05 | 1.020 | 8257 ± 35 | 223 ± 6 | 0.86 ± 0.02 |

[a] All measurements were carried out in triplicate.

The formulation C showed the pH stable profile and viscosity parameters over 14 days during the accelerated stability test. The data of this formulation are shown in Table 2. Nevertheless, the viscosity of the sample varied with a slight decrease. Therefore, shampoo formulation C was chosen for incorporation into the developed substance at a total phytochemical's concentration of 0.75% for further antimicrobial research.

**Table 2.** Accelerated stability of shampoo formulation C.

| Characteristic | Initial | 1 Day | 7 Days | 14 Days |
|---|---|---|---|---|
| pH value (10% *w*/*w*) [a] | 5.54 ± 0.05 | 5.55 ± 0.05 | 5.57 ± 0.05 | 5.60 ± 0.05 |
| Viscosity, m·Pas [a] | 5789 ± 30 | 5667 ± 25 | 5600 ± 27 | 5608 ± 30 |

[a] All measurements were carried out in triplicate.

### *2.2. Evaluation of the Antimicrobial Effect for Shampoo with the Plant-Based Substance*

The substance of plant origin enriched with *M. alternifolia* essential oil, 1,8-cineole and (-)-α-bisabolol at a specific chosen ratio of 1:1:1 was previously investigated for the balancing of the skin microflora [11] and incorporated into the shampoo formulation C. The tested shampoo formulation contained *M. alternifolia* essential oil, 1,8-cineole and (-)-α-bisabolol at a concentration of 0.25%, 0.25% and 0.25%, respectively. The results showed that this shampoo formulation inhibited the growth of standard strains of microorganisms compared to the formulations with antifungal substances recommended for the treatment of SD (Table 3). Although the Log10CFU reduction depends on the concentration of the substances and tested strains, the shampoo with the novel substance at a total concentration of 0.75% decreased the Log10CFU value of 2.0 and more.

**Table 3.** Antimicrobial effect against standard strains participating in the pathogenesis of SD.

| No. | Strain [b] | Log10CFU, Mean ± SD [a] | | | | |
|---|---|---|---|---|---|---|
| | | Negative Control | Plant-Based Substance at a Ratio of 1:1:1 | Climbazole | Ketoconazole | Piroctone Olamine |
| 1 | *S. epidermidis* ATCC 14990 | 6.90 | 4.83 | 5.57 | 6.57 | 6.44 |
| 2 | *S. aureus* ATCC 29213 | 6.90 | 4.81 | 6.17 | 4.51 | 5.51 |
| 3 | *C. albicans* ATCC 10231 | 5.20 | 2.86 | 2.82 | 1.79 | 2.86 |

[a] The Log10 CFU value is characterized by a decrease in viable cell count after 24 h in the presence of active ingredients and active controls by means of a broth dilution method. All experiments were carried out in triplicate. [b] Bacteria and fungi strains from the American Type Culture Collection (ATCC) were used for this study.

It was found that the botanical substance based on *M. alternifolia* essential oil, 1,8-cineole and (-)-α-bisabolol at a specific ratio of 1:1:1 showed a comparable antimicrobial effect against *S. epidermidis*, *S. aureus* and *C. albicans* at concentrations lower than climbazole (1.0%), piroctone olamine (1.0%) and ketoconazole (2.0%). Detailed analysis of the antimicrobial activity of the formulation with the plant-based substance showed significant inhibition in *S. epidermidis*, *S. aureus* and *C. albicans* growth with an average efficiency of 99.1%, 99.2% and 99.5%, respectively. Climbazole and piroctone olamine are widely used in antidandruff shampoos but reveal more antifungal activity than antibacterial activity. The effect of climbazole and piroctone olamine against *C. albicans* was comparable to the botanical substance with an average efficiency of 99.6% and 99.5% (equivalent to a 2.38 and 2.34 Log10CFU reduction), respectively. Ketoconazole, a recommended antifungal drug for the first-choice treatment of dandruff and SD, had the highest antifungal effect

and showed a 3.41 Log10CFU reduction (equivalent to a 99.96% effect) but possessed the lowest antimicrobial effect against *S. epidermidis* with a 0.33 Log10CFU reduction.

The plant-based substance is enriched with different phytomolecules, allowing it to have the highest antimicrobial efficiency in a short-term period following application. Recent in vitro research data have shown that formulations with natural ingredients should be effective against both fungi and bacteria for the treatment of dandruff and SD [4]. The combination of *M. alternifolia* essential oil, 1,8-cineole and (-)-α-bisabolol at an equivalent ratio exhibited a synergetic antibacterial and antifungal effect against microorganisms involved in the etiopathogenesis of SD [11]. Based on study data and analysis, the biologically active phytoconstituents can be described as having antimicrobial, anti-inflammatory and anti-irritating properties [11]. This antimicrobial effect is higher than climbazole- and piroctone olamine-mediated fungi reduction and is comparable to ketoconazole efficacy. Antimicrobial resistance of the chosen microorganisms to the phytoconstituents of the plant-based substance—*M. alternifolia* essential oil, 1,8-cineole and (-)-α-bisabolol—is unknown and such information would help in the near future in the development of new formulations for prolonged external use. Additional research should be performed regarding the toxicological properties, skin sensitization and dermatologist-approved clinical tolerance of the developed shampoo formulation with the novel plant-based substance.

## 3. Materials and Methods

### *3.1. Chemicals and Materials*

*Melaleuca alternifolia* leaf essential oil (CAS 68647-73-4), eucalyptol (CAS 470-82-6) and natural (-)-α-bisabolol (CAS 23089-26-1) from ERNESTO VENTÓS S. A. (Barcelona, Spain) with purity of at least 99.0% were purchased. The developed shampoo formulations contained basic ingredients, such as anionic, amphoteric and nonionic surfactants of plant origin. Additionally, humectant, solubilizing agent, conditioning agent, preservatives, chelating agent, antioxidant and pH regulator were used for the final formulations. The ingredients of each shampoo formulation are shown in Table 4.

**Table 4.** Formulations of antidandruff shampoos in this research study.

| No. | Ingredient | Function | Formulation (% *w*/*w*) | | | |
|---|---|---|---|---|---|---|
| | | | A | B | C | D |
| 1 | Sodium coco-sulfate (Sulfopon 1216 G) | Anionic surfactant | 6.0 | 6.0 | 6.0 | 6.0 |
| 2 | Coco glucoside (Plantacare 818 UP) | Nonionic surfactant | 7.5 | 7.5 | 7.5 | 7.5 |
| 3 | Cocamidopropyl betaine (Dehyton K45) | Amphoteric surfactant | 5.0 | 5.0 | 5.0 | 5.0 |
| 4 | Lauryl glucoside (Plantacare 1200 UP) | Nonionic surfactant | - | 3.0 | 5.0 | 7.0 |
| 5 | Polyquaternium-67 | Conditioning agent | 0.3 | 0.3 | 0.3 | 0.3 |
| 6 | Glycerin | Humectant | 3.0 | 3.0 | 3.0 | 3.0 |
| 7 | Tetrasodium glutamate diacetate salt | Chelating agent | 0.5 | 0.5 | 0.5 | 0.5 |
| 8 | *M. alternifolia* essential oil | Active ingredient of the plant-based substance | 0.45 | 0.45 | 0.45 | 0.45 |
| 9 | 1,8-Cineole | Active ingredient of the plant-based substance | 0.15 | 0.15 | 0.15 | 0.15 |
| 10 | (-)-α-Bisabolol | Active ingredient of the plant-based substance | 0.15 | 0.15 | 0.15 | 0.15 |

**Table 4.** *Cont.*

| No. | Ingredient | Function | Formulation (% *w*/*w*) | | | |
|---|---|---|---|---|---|---|
| | | | **A** | **B** | **C** | **D** |
| 11 | Betaine | Active ingredient | 1.0 | 1.0 | 1.0 | 1.0 |
| 12 | Panthenol | Active ingredient | 0.5 | 0.5 | 0.5 | 0.5 |
| 13 | Tocopheryl acetate | Antioxidant | 0.05 | 0.05 | 0.05 | 0.05 |
| 14 | Potassium sorbate, sodium benzoate (Euxyl K712) | Preservative | 0.7 | 0.7 | 0.7 | 0.7 |
| 15 | Citric acid monohydrate | pH regulator | 0.6 | 0.71 | 0.75 | 0.80 |
| 16 | Water q.s. | Diluent | up to 100 | up to 100 | up to 100 | up to 100 |

### *3.2. Characterization of Shampoo Formulations A–D*

The developed shampoos with the plant-based substance were characterized by inspection using several parameters in terms of physical appearance, pH-stability, viscosity and density foam characteristics. Each formulation was assessed for organoleptic characteristics such as color, odor, appearance and homogeneity. Determination of density foam characteristics was carried out using the cylinder shake method [12] in triplicate. The pH stability of the final formulations with concentration of 10% (*w*/*w*) was evaluated by using a pH meter at a temperature of 22–24 °C ($n$ = 3). The Brookfield Rheometer (Model DV1M, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with spindle 4 and 10 rpm at 23 °C was used to measure viscosity in triplicate. The stability test of shampoo formulations was performed in a hot room cycle. The shampoo formulations were kept in a thermal cabinet at 42 °C for 14 days as an approach to evaluating accelerated stability. After that, the parameters described above such as appearance, pH stability, density foam characteristics and viscosity were evaluated in triplicate ($n$ = 3). The data are presented as averages with standard deviations (SD).

### *3.3. Antimicrobial Activity of the Shampoo Formulations by Determining the Log10CFU Reduction*

*S. epidermidis* ATCC 14990, *S. aureus* ATCC 29213 and fungi *C. albicans* ATCC 10231, which participate in the pathogenesis of SD, were selected from the American Type Culture Collection (ATCC) in order to evaluate antimicrobial activity. The standard medium, such as Mueller Hinton broth (MHB) and tryptone soy agar (TSA), were chosen for initial cultivation and growth of the tested bacteria at a temperature of 37 °C for 24 h. The strain *C. albicans* was initially cultivated in a selective culture medium such as Sabouraud dextrose agar (SDA) at a temperature of 37 °C for 24 h and then cultivated in a double-concentrated Sabouraud dextrose broth (SDB) medium suitable for the stable conditions of fungi growth and survival. Antimicrobial susceptibility testing in accordance with EUCAST rules [13] was performed by using the standard broth microdilution method. The Log10CFU reduction in bacteria and fungi was used to determine the antimicrobial effect of the tested shampoo formulation with the substances through quantitative evaluation of viable total colony counts. The active controls for antifungal activity were formulations C with climbazole at a concentration of 1.0%, piroctone olamine at a concentration of 1.0% and ketoconazole at a concentration of 2.0%. Firstly, 9 mL of each shampoo formulation with the novel plant-based substance was mixed with MHB and then added to 1 mL of each microorganism at a $1.0 \times 10^6$ CFU/mL concentration. The incubation of the sample with microorganisms was performed at 37 °C for 24 h. After the incubation period, the viable cells of the chosen standard bacteria and fungi were counted on specific agar plates and CHROMagar Malassezia, respectively. Additionally, the negative control for this study was the suspension of the microorganisms without formulation and any substances. Three biological repetitions were performed for the study. Finally, results were evaluated using the calculation of a decimal logarithm of reduction in colony-forming units per mL

(Log10CFU/mL); 1 and 2 Log10CFU reduction corresponded to an antimicrobial effect of 90.0% and 99.0%, respectively.

## 4. Conclusions

The natural medical shampoo formulations with the investigated substance based on *Melaleuca alternifolia* leaf oil, 1,8-cineole and (-)-α-bisabolol for the prevention and treatment of dandruff and SD were developed. The natural surfactants such as sodium coco-sulfate, cocamidopropyl betaine, lauryl glucoside and coco glucoside were used to achieve a proper physical appearance, pH-stability and foam characteristics. The optimal formulation passed the accelerated stability test and retained their viscosity and pH values over 14 days. Additionally, the developed formulation with the novel botanical substance at a concentration of 0.75% demonstrated a high antimicrobial effect against *S. epidermidis*, *S. aureus* and *C. albicans* that are triggers for the development of SD clinical manifestations. The antimicrobial effect of the tested formulation was comparable to that of piroctone olamine, climbazole and ketoconazole. Thus, the developed shampoo formulation C with this novel plant-based substance may be a promising drug for the prevention and treatment of dandruff and SD after additional research.

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