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

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

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

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1. Introduction

Nowadays, people pay more attention to proper cleansing and choice of natural shampoo for scalp health. It is established that seborrheic dermatitis (SD) have a high prevalence worldwide and occurs in up to 50% of 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 process, and regulate scalp microflora involved in pathogenesis of SD. The detailed pathogenesis of SD isn't well understood, but there are various intrinsic and extrinsic factors that worse the course of the SD and affect the severity of clinical manifestations. Normally, the scalp microflora, such as *Malassezia* species, *Staphylococcus* species and other bacteria, maintains a pH balance, constant renewal of stratum corneum and moisturizing properties of the scalp. However, excessive sebaceous gland secretion and related abundance of *Malassezia* sp. causes the scalp irritation, itching, dryness, visible flakes [2] that negatively impacts patients' well-being and psychological comfort [3]. It was established that *Staphylococcus epidermidis* (*S. epidermidis*), *Staphylococcus aureus* (*S. aureus*), *Candida albicans* (*C. albicans*) are involved in the pathogenesis of SD [4]. However, current shampoos don't focus on these scalp microorganisms.

The treatment of SD and dandruff focuses on alleviating symptoms and maintaining the remission with long-term cleanliness of the scalp, but only targets fungal colonization and immunity-mediated inflammation. The most common medical shampoos contain topical synthetic agents as antifungals and anti-inflammatory substances in accordance with clinical recommendations of different countries. However, these 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 increase of azoles-resistance strains of *Malassezia* species [9].

Therefore, natural shampoo with effective plant-based substance for proper scalp treatment can be helpful for the treatment of SD. Plant-based substances, such as essential oils or plant extracts, have a comprehensive compositions, multiple properties, different activities, and low irritancy potential [10]. As it was discovered by the authors, the most promising substance for the treatment of SD are synergetic combination of *Melaleuca alternifolia* (*M. alternifolia*) leaf oil, 1,8-cineole (eucalyptol) and (-)-α-bisabolol in a ratio of 1:1:1 with documented therapeutic antimicrobial potential [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, clear composition, and development in accordance with dermatologists' recommendations. In this context, the aim of research was to develop the natural medical shampoo with the 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 properties of shampoo, the formulations had an average pH value of 5.50–5.56. Moreover, the mild acidic pH values of medical shampoos could increase the hair cleansing, decrease eye irritation, and keep pH balance of the scalp for microflora regulation [12]. The foam characteristics depended on 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% allowed to achieve the desired foam number and foam stability that are crucial properties of medical shampoos. The concentration of lauryl glucoside above 5.0% significantly increased viscosity of shampoo formulations and affected the stability in 14 days. Thus, the formulation C had the optimal characteristics for further research and assessment of 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 for 14 days during accelerated stability test. The data of this formulations is shown in Table 2. Never-

theless, the viscosity of sample varied with a slight decrease. Therefore, shampoo formulation C was selected for the incorporation of the plant-based 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. Antimicrobial Activity of Shampoo with the Plant-Based Substance

The plant-based substance based on *M. alternifolia* essential oil, 1,8-cineole and (-)-α-bisabolol at a ratio of 1:1:1 was previously investigated for the regulation of the skin microflora [11] and incorporated to 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 plant-based substance at a total concentration of 0.75% decreased the Log10CFU value of 2.0 and more.

Table 3. Antimicrobial activity against strains involved 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 was determined after 24 h of treatment via broth dilution method. All experiments were carried out in triplicate. [b] Bacteria and fungi were purchased from American Type Culture Collection (ATCC).

The plant-based substance based on *M. alternifolia* essential oil, 1,8-cineole and (-)-α-bisabolol at a ratio of 1:1:1 showed comparable antimicrobial activity against *S. epidermidis*, *S. aureus*, *C. albicans* at concentrations lower than climbazole (1.0%), piroctone olamine (1.0%) and ketokonazole (2.0%). Detailed analysis of the antimicrobial activity of the formulation with the plant-based substance showed the significant ihibition of *S. epidermidis*, *S. aureus*, *C. albicans* growth with an average eficiency of 99.1%, 99.2% and 99.5% resceptively. Climbazole and piroctone olamine are widely used in antidandruff shampoos, but reveal more antifungal activity than atibacterial activity. The effect of climbazole and piroctone olamine against *C. albicans* was comparable to the plant-based substance with an average efficiecy of 99.6% and 99.5% (equivalent to a 2.38 and 2.34 Log10CFU reduction) respectively. Ketoconazole, recommended antifungal drug for the treatment of SD, had a highest antifugal effect with an average efficiecy of 99.96% (equivalent to a 3.41 Log10CFU reduction), but possessed a lowest antimicrobial effect against *S. epidermidis* with 0.33 Log10CFU reduction.

The comprehensive phytochemical composition of the plant-based substance allowed to have highest antimicrobial efficiency. New data show that new formulations for

the treatment of SD should be effective against both fungi and bacteria [4]. The complex of *M. alternifolia* essential oil, 1,8-cineole and (-)-α-bisabolol at a ratio of 1:1:1 showed the synergetic antimicrobial effect against microorgaisms [11] involved in the ethiopathogenesis of SD. Based on literaly data, the biologically active phytoconstituents have an antimicrobial, antiinflammatory, antiirritating properties [11]. This antimicrobial effect is a higher than climbazole- and piroctone olamine-mediated Log10CFU reduction and comparable to ketoconazole efficiency. There are no recent data about antimicrobial resistance of chosen microorganisms to the main phytoconstituets of the plant-based substance—*M. alternifolia* essential oil, 1,8-cineole and (-)-α-bisabolol—that could be useful for the treatment of SD in the nearest future. There is needed additional study of toxicological properties, dermatological tolerance, skin sensitization and clinical efficacy of developed shampoo formulation with the 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), (-)-α-bisabolol (CAS 23089-26-1) with purity of at least 99.0% were purchased from Sigma-Aldrich (Sigma Chemical Co, St. Louis, MO, USA). The prepared 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. Shampoo formulations in this research work.

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
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 for several parameters by inspections in terms of physical appearance, pH-stability, viscosity,

density foam characteristics. Each formulation was assessed for organoleptic characteristics such as color, odor, appearance, homogeneity. Determination of density foam characteristics was determined using the cylinder shake method [12] in triplicate. pH stability of final formulations in concentration of 10% (*w*/*w*) was evaluated by using a pH meter at room temperature in triplicate. Viscosity measurement was performed by using a Brookfield Rheometer (Model DV1M, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with spindle 4 and 10 rpm at 23 °C in triplicate. The stability test of shampoo formulations was performed at heat and room cycle. The shampoo formulations were kept in a thermal cabinet at 42 °C for 14 days as approach for evaluation of accelerated stability. After that, the described above parameters such as appearance, pH stability, density foam characteristics, viscosity were evaluated in triplicate (n = 3). The data are presented as averages with standard deviations (SD).

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

The tested microorganisms such as bacteria *S. epidermidis* ATCC 14990, *S. aureus* ATCC 29213 and fungi *C. albicans* ATCC 10231 were selected from American Type Culture Collection (ATCC). The Mueller Hinton broth and tryptone soy agar (TSA) were used for initial cultivation of tested bacteria at 37 °C for 24 h. The fungal strain *C. albicans* was initially cultivated in selective Sabouraud dextrose agar (SDA) at 37 °C for 24 h and in a double concentrated Sabouraud dextrose broth (SDB) medium during the experiments. The broth microdilution method was chosen for antimicrobial susceptibility testing in accordance with EUCAST rules [13]. The Log10CFU reduction of 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. Antifungal drugs such as climbazole at a concentration 1.0%, piroctone olamine at a concentration of 1.0% and ketoconazole at a concentration 2.0% in formulation C were used as controls. Then, 1 mL of each microorganism at a 1.0×10^6 CFU/mL concentration is cultured with 9 mL of each shampoo formulation with substance, prepared in Mueller Hinton Broth. The incubation of sample with microorganisms at 37 °C for 24 h was performed. After incubation period, the viable bacteria and fungi population was monitored by counting the viable cells on agar plate and CHROMagar Malassezia respectively. The suspension of the microorganisms without formulation and substances was used as a negative control. The experiments were performed in triplicate. The result was expressed in a decimal logarithm of reduction of colony forming units per mL (Log10CFU/mL). The Log10CFU reduction by more than 1 and 2 values corresponded to an antimicrobial activity of more than 90.0% and 99.0% respectively.

4. Conclusions

The natural medical shampoo with the investigated plant-based substance for the treatment of SD was developed. The natural surfactants such as sodium coco-sulfate, cocamidopropyl betaine, lauryl glucoside and coco glucoside were used to achieve proper physical appearance, pH-stability, and foam characteristics. The optimal formulation passed the accelerated stability test and retained their viscosity and pH values for 14 days. Additionally, the developed formulation with the plant-based substance at a concentration of 0.75% demonstrated a high antimicrobial activity against *S. epidermidis*, *S. aureus*, *C. albicans* involved in the pathogenesis of SD. The antimicrobial activity of the tested formulation was comparable to the piroctone olamine, climbazole and ketoconazole. Thus, the developed shampoo formulation is promising for the treatment of SD after additional research.

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