

Scalp microbiome dynamics can contribute to the clinical effect of a novel anti-seborrheic dermatitis shampoo containing patented antifungal actives: a randomized controlled study

Running head: A novel anti-dandruff shampoo for seborrheic dermatitis

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Supplementary Material

Supplementary Methods

Participant criteria

Women of childbearing potential had to use an effective method of contraception during the whole study duration. Pregnant or breastfeeding women, subjects with frizzy hair, an history of allergy or intolerance to any of the study products ingredients, or SD requiring a medical treatment were not included. Other exclusion criteria comprised:

- Having any other condition which may interfere with the study assessments, whether a dermatologic disorder on the scalp (e.g., alopecia on the upper part of the scalp, alopecia areata, alopecia totalis, contact dermatitis, psoriasis, severe excoriations, severe sunburn, cutaneous bacterial superinfection, etc.) or an acute or chronic disease.
- A systemic treatment with nonsteroidal anti-inflammatory drugs, corticosteroids, antibiotics, or antihistamines taken for five consecutive days within the two weeks before inclusion or planned during the study and likely to interfere with the study assessments.
- Any other systemic treatment (such as cardiovascular, endocrine, antidepressant, antipsychotic, antifungal medications) initiated or modified within four weeks before inclusion, planned during the study or modified during the study and likely to interfere with the study assessments, as determined by the investigator's judgment.
- Topical products applied on the scalp which may interfere with the efficacy of the study product (anti-inflammatory, anti-dandruff, anti-fungal agents), as well as dyeing, bleaching, permanent, smoothing / straightening procedures were prohibited within two weeks before the inclusion and during the course of the study.

- Subjects unable to understand the information given, including study procedures or unable to give their written consent, subjects under administrative or legal award or under guardianship.
- Subject with a history of allergy or intolerance to any material used for the research (PBS triton X100-buffer used for swab samples).

In addition, subjects committed to avoid sun exposure of the scalp without protection.

All the subjects receive compensation for the total duration of the study.

Supplementary Tables

Table S1. Strain references and primers for normalization and quantification by droplet digital PCR (ddPCR)

	Analyzed strain	Strain reference	Primers
Bacteria	<i>Staphylococcus epidermidis</i>	ATCC 12228_PCI 1200	5'TTTAGAAGCTAAATCAATCGAAGAAA 3' 5'GGTGACCACCGCCATTATTA 3'
	<i>Cutibacterium acnes</i>	ATCC6919_NCTC 737	5'GTGTCGAGGTCGAAGTCGTT 3' 5'GTGAAGGCTGCTGTGCATAA 3'
Fungi	<i>Malassezia globosa</i>	ATCC MYA4612_CBS 7966	5'GGCCAAGCGCGCTCT 3' 5'CCACAACCAAATGCTCTCCTACAG 3'
	<i>Malassezia restricta</i>	ATCC MYA4611_CBS 7877	5'GGCGGCCAAGCAGTGTTT 3' 5'AACCAAACATTCTCCTTTAGGTGA 3'

Table S2. P-values for the intra-group and inter-group analyses of the dandruff, erythema and pruritus severity scores

		Intra-group		Inter-group
		Test group	Control group	
Dandruff score	W10-W02	0.0027	<0.0001	<0.0001
	W06-W02	0.0055	0.0002	<0.0001
	W02-W0	<0.0001	<0.0001	0.4058
	W06-W0	<0.0001	<0.0001	<0.0001
	W10-W0	<0.0001	<0.0001	<0.0001
Erythema score	W10-W02	0.0497	0.0051	0.0008
	W06-W02	0.1031	0.1621	0.0333
	W02-W0	<0.0001	<0.0001	0.0596
	W06-W0	<0.0001	0.0436	0.0035
	W10-W0	<0.0001	0.5111	0.0001
Pruritus score	W10-W02	<0.0001	<0.0001	<0.0001
	W06-W02	0.0004	0.0029	<0.0001
	W02-W0	<0.0001	<0.0001	0.1386
	W06-W0	<0.0001	0.0007	<0.0001
	W10-W0	<0.0001	0.0129	<0.0001

Table S3. Squamous state and scalp discomfort assessment at each time point.

Percentage of subjects	W0	W2		W6		W10	
		Test group	Control group	Test group	Control group	Test group	Control group
Squamous state							
Absent	0.0%	14.3%	14.3%	38.1%	20.0%	55.0%	10.0%
Mild	9.5%	61.9%	61.9%	61.9%	40.0%	40.0%	30.0%
Moderate	71.4%	23.8%	23.8%	0.0%	25.0%	5.0%	45.0%
Severe	19.1%	0.0%	0.0%	0.0%	15.0%	0.0%	15.0%
Scalp discomfort							
Absent	0.0%	38.1%	9.5%	47.6%	15.0%	60.0%	5.0%
Mild	28.6%	52.4%	85.7%	47.6%	40.0%	30.0%	35.0%
Moderate	61.9%	9.5%	4.8%	4.8%	35.0%	10.0%	50.0%
Severe	9.5%	0.0%	0.0%	0.0%	10.0%	0.0%	10.0%

Table S4. *P-values* for the intra-group and inter-group analyses of the squamous state and scalp discomfort

		Intra-group		Inter-group
		Test group	Control group	
Squamous state	W02-W0	<0.0001	<0.0001	0.4984
	W06-W0	<0.0001	0.0473	0.0037
	W10-W0	<0.0001	0.3232	0.0003
Scalp discomfort	W02-W0	<0.0001	<0.0001	0.3433
	W06-W0	<0.0001	0.1431	0.0059
	W10-W0	<0.0001	1.0000	0.0008

Fungal microbiota analyses

Table S5. Differential analysis of fungi in the whole study population–Intensive phase

Phylum	Genera	Mean relative abundances (N=31)		Adjusted <i>p</i> -value
		W0	W2	
<i>Basidiomycota</i>	<i>Malassezia</i>	0.75444766	0.54823047	0.002296
<i>Ascomycota</i>	<i>Penicillium</i>	0.01267435	0.04381241	0.005785
<i>Ascomycota</i>	<i>Cladosporium</i>	0.00454220	0.03509232	0.000069
<i>Ascomycota</i>	<i>Debaryomyces</i>	0.00223320	0.04586500	0.032797
<i>Basidiomycota</i>	<i>Itersonilia</i>	0.00046040	0.00664858	0.005785
<i>Basidiomycota</i>	<i>Inonotus</i>	0.00005265	0.00100853	0.012945
<i>Basidiomycota</i>	<i>Sistotrema</i>	0.00002197	0.00139755	0.016567
<i>Ascomycota</i>	<i>Saccharomyces</i>	0.00001226	0.00179336	0.016567

Table S6. Differential analysis of fungi in the study groups–Maintenance phase

Phylum	Genera	Mean relative abundances					
		Test group (N=15)			Control group (N=16)		
		W2	W10	Adjusted <i>p</i> -value	W2	W10	Adjusted <i>p</i> -value
<i>Basidiomycota</i>	<i>Malassezia</i>	0.5827911	0.8052620	0.050415	0.5158298	0.7981592	0.001800
<i>Ascomycota</i>	<i>Debaryomyces</i>	0.0789211	0.0060732	0.713700	0.0148749	0.0125929	0.296443
<i>Ascomycota</i>	<i>Cladosporium</i>	0.0428290	0.0258545	1	0.0278392	0.0012068	0.012603
<i>Basidiomycota</i>	<i>Itersonilia</i>	0.0067086	0.0013890	0.713700	0.0065923	0.0002518	0.136187
<i>Basidiomycota</i>	<i>Sistotrema</i>	0.0012218	0.0006517	0.976499	0.0015623	0.0000467	0.136187
<i>Basidiomycota</i>	<i>Inonotus</i>	0.0004787	0.0001104	0.713700	0.0015052	0.0000280	0.204191
<i>Ascomycota</i>	<i>Saccharomyces</i>	0.0002951	0.0003724	1	0.0031979	0.0000022	0.199742
<i>Ascomycota</i>	<i>Penicillium</i>	-	-	-	0.0322716	0.0036467	0.042012

Table S7. Alpha-diversity indices for the scalp fungal population–Intensive phase

Alpha-diversity indices (median [min-max])	Study group (N = 31)		
	W0	W2	P-value
Observed	15 [5-122]	37 [7-101]	<0.0001
Chao1	17 [5-169]	38 [7-127]	0.004
Shannon	0.73 [0.26-2.04]	1.57 [0.55-2.73]	<0.0001
Inv-Simpson	1.92 [1.10-5.43]	2.78 [1.35-8.71]	<0.0001

Table S8. Alpha-diversity indices for the scalp fungal population–Maintenance phase

Alpha diversity indices (median [min-max])	Test group (N = 15)			Control group (N = 16)			Test group vs Control group
	W2	W10	P-value	W2	W10	P-value	P-value
Observed	41 [9-101]	39 [7-99]	0.28	27 [7-99]	13 [5-67]	0.0001	0.024
Chao1	46 [9-112]	39 [7-104]	0.41	28 [7-127]	17 [5-143]	0.053	0.433
Shannon	1.45 [0.69-2.01]	1.42 [0.52-2.32]	0.27	1.63 [0.55-2.73]	0.86 [0.06-1.32]	<0.0001	0.0001
Inv-Simpson	2.49 [1.43-4.38]	2.51 [1.33-5.59]	0.22	3.31 [1.35-8.71]	1.97 [1.02-3.05]	<0.0001	0.0078

Table S9. Absolute quantification of *M. globosa* and *M. restricta* by ddPCR in the whole study population–Intensive phase

Targeted species	Mean abundances [log]		<i>p</i> -value
	W0	W2	
<i>M. globosa</i>	5.61	4.53	<0.001
<i>M. restricta</i>	6.74	5.09	<0.001

Table S10. Absolute quantification of *M. globosa* and *M. restricta* by ddPCR–Maintenance phase

	Mean abundances [log]						
	Test group			Control group			Test group vs Control group
Targeted species	W2	W10	<i>p</i> -value	W2	W10	<i>p</i> -value	<i>p</i> -value
<i>M. globosa</i>	4.49	4.94	0.3492	4.58	5.42	<0.001	0.2285
<i>M. restricta</i>	4.99	5.59	0.0028	5.19	7.11	<0.001	<0.001

Bacterial microbiota analyses

Table S11. Differential analysis of bacteria in the whole study population–Intensive phase

Phylum	Genera	Mean relative abundances (N=31)		Adjusted <i>p</i> -value
		W0	W2	
<i>Actinobacteria</i>	<i>Cutibacterium</i>	0.4838125	0.6436609	0.000045
<i>Firmicutes</i>	<i>Staphylococcus</i>	0.3685052	0.1947288	0.002957
<i>Proteobacteria</i>	<i>Stenotrophomonas</i>	0.0272233	0.0183159	0.036060
<i>Firmicutes</i>	<i>Anaerococcus</i>	0.0013601	0.0029203	0.018502
<i>Firmicutes</i>	<i>Peptoniphilus</i>	0.0006476	0.0020202	0.036060

Table S12. Differential analysis of bacteria in the study groups–Maintenance phase

Phylum	Genera	Mean relative abundances (N=31)					
		Test group			Control group		
		W2	W10	Adjusted <i>p</i> -value	W2	W10	Adjusted <i>p</i> -value
<i>Actinobacteria</i>	<i>Cutibacterium</i>	0.7393776	0.6885253	0.582330	0.5539265	0.4771090	0.304865
<i>Firmicutes</i>	<i>Staphylococcus</i>	0.1641542	0.2548862	0.415533	0.2233926	0.4314772	0.029297
<i>Proteobacteria</i>	<i>Stenotrophomonas</i>	0.0040259	0.0003366	0.570502	0.0317129	0.0002253	0.104439
<i>Firmicutes</i>	<i>Anaerococcus</i>	0.0008593	0.0007137	0.823760	0.0048524	0.0025789	0.304865
<i>Firmicutes</i>	<i>Peptoniphilus</i>	0.0003456	0.0003894	0.975375	0.0035901	0.0013290	0.104439

Table S13. Alpha-diversity indices for the scalp bacterial population–Intensive phase

Alpha diversity indices (median [min-max])	Study group (N = 31)		
	W0	W2	P-value
Observed	54 [9-223]	79 [13-288]	0.023
Chao1	67 [9-362]	94 [13-337]	0.018
Shannon	1.02 [0.39-3.10]	1.07 [0.08-3.57]	0.054
Inv-Simpson	2.14 [1.19-5.37]	2.04 [1.02-10.34]	0.075

Table S14. Alpha-diversity indices for the scalp bacterial population–Maintenance phase

Alpha diversity indices (median [min-max])	Test group (N = 15)			Control group (N = 16)			Test group vs Control group
	W2	W10	P-value	W2	W10	P-value	P-value
Observed	51 [17-288]	43 [10-207]	0.007	90 [13-258]	42 [17-271]	0.020	0.726
Chao1	72 [21-337]	51 [10-245]	0.003	115 [13-300]	70 [24-302]	0.051	0.348
Shannon	0.93 [0.08-2.38]	1.08 [0.30-1.78]	0.09	1.41 [0.58-3.57]	0.93 [0.49-3.14]	0.014	0.599
Inv-Simpson	1.83 [1.02-5.17]	2.33 [1.18-3.19]	0.06	2.33 [1.49-10.34]	2.10 [1.31-6.42]	0.016	0.711

Table S15. Absolute quantification of *C. acnes* and *S. epidermidis* by ddPCR in the whole study population–Intensive phase

Targeted species	Mean abundances [log]		<i>p</i> -value
	W0	W2	
<i>C. acnes</i>	6.54	6.24	0.0904
<i>S. epidermidis</i>	4.54	4.06	<0.001

Table S16. Absolute quantification of *C. acnes* and *S. epidermidis* by ddPCR–Maintenance phase

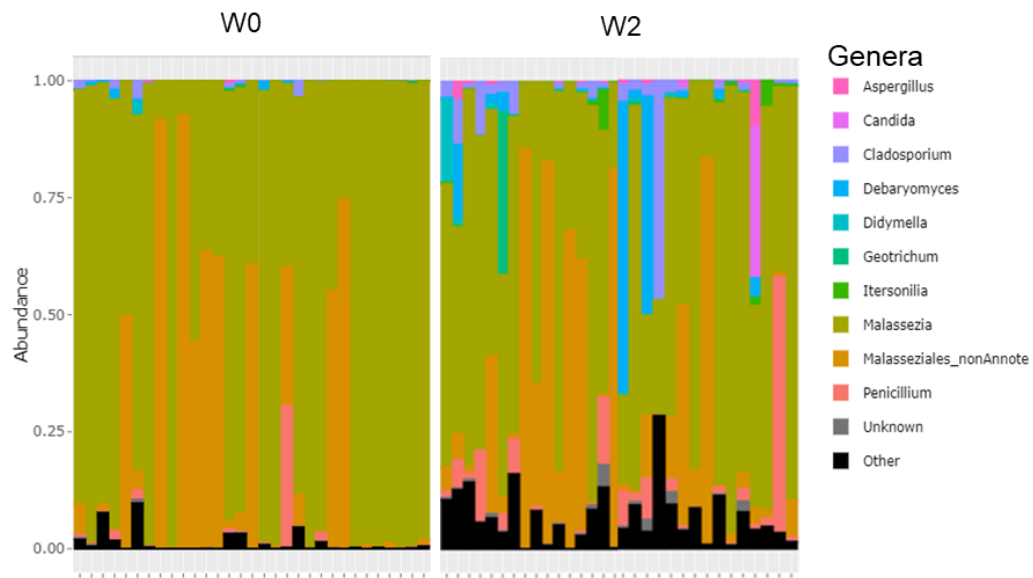
	Mean abundances [log]						
	Test group			Control group			Test group vs Control group
Targeted species	W2	W10	<i>p-value</i>	W2	W10	<i>p-value</i>	<i>p-value</i>
<i>C. acnes</i>	6.13	6.84	0.0113	6.35	6.65	0.2222	0.3267
<i>S. epidermidis</i>	3.85	4.57	0.0120	4.27	4.46	0.6653	0.1384

Supplementary Figures

Fig. S1. Relative abundances of the top ten fungal and bacterial genera in scalp samples at baseline and after the intensive phase of the study product use, presented by subject (N = 31).

Relative abundances of the top ten fungal (A) and bacterial (B) genera at baseline (W0) and at the end of the 2-week intensive phase of the product use (W2). Each bar corresponds to one subject.

A. Fungi



B. Bacteria

