Effectiveness of Targeted Antidandruff Shampoos: Clinical, Instrumental and Metagenomic Analysis

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The differences existing between oily and dry dandruff conditions seem to justify the formulation of products specifically adapted to each type of dandruff.
We wanted to assess the effectiveness of 2 shampoos targeting oily or dry dandruff and the persistence of this effect 4 weeks after stopping use.

33 subjects with mild to moderate dandruff scalp completed this 2-steps study: 4-weeks treatment phase followed by a for 4 weeks persistence phase, shampoos being used thrice a week.
Efficacy was assessed from clinical and instrumental measurements done during the 2 steps, and from microbiological assessments done during treatment phase.
Statistical analysis was done by ANCOVA, paired Student or Wilcoxon tests, according to the nature of data. Metagenomic statistical analysis of Alpha and Beta diversity allowed to identify genus or species that changed during the treatment.

During the treatment phase, both shampoos showed a significant improvement in clinical signs. Biophysical parameters changed slightly, mainly with the Oily Dandruff Shampoo and the ratio *Malassezia restricta* to *Cutibacterium acnes* decreased.
With the Dry Dandruff Shampoo, richness and equitability increased, reflecting a rebalance of fungi and bacterial populations.
During the persistence phase, clinical improvements were maintained for both shampoos.

These shampoos allowed to improve the dandruff condition and to rebalance the skin scalp microflora. The clinical improvements remained for at least 4 weeks.
A formulation specially adapted to the dandruff state (greasy/dry) makes it possible to target the effectiveness and to limit the undesirable effects which could occur with a less specific product.

Keywords:

Oily dandruff; dry dandruff, clinical assessment, biophysical data, microbiota

Introduction

It is accepted that dandruff (D) states are associated with an imbalance of the fungal and bacterial flora, a change in scalp hydration, pH and cutaneous barrier function, and various discomfort sensations compared to a non-dandruff scalp [1,2,3].
In addition, different kinds of dandruff are described: Clinically, Oily dandruff (OD) are characterized by large, yellowish, greasy flakes, that stick to the head and the hair, while Dry dandruff (DD) are rather small, white and they fall off the head and out of the hair. They develop on a very dry scalp that tends to be itchy.
In addition to these known clinical differences, previous work found differences between the 2 kinds of dandruff in terms of lipid, hydration levels and microbiota, justifying the formulation of products specifically adapted to each type of dandruff, for a targeted effectiveness and skin scalp acceptability [4].
We wanted to assess the effectiveness of 2 shampoos targeting oily or dry dandruff and the persistence of this effect 4 weeks after stopping use with clinical and auto-assessments scoring, instrumental and microbiological methods, to check if targeted formulations are justified [5].

Materials and Methods.

33 subjects (16 OD and 17 DD) with mild to moderate dandruff scalp (clinical score of dandruff ≥ 4 on a 10 points scale) completed this 2-steps study: 4-weeks (W0 to W4) anti dandruff shampoo adapted to each kind of dandruff (oily or dry), followed by a 4-weeks (W4 to W8) neutral shampoo use, shampoos being used thrice a week.
These anti-dandruff shampoos were formulated with natural active ingredients, among them a common anti *Malassezia* ginger extract. Other ingredients were selected regarding the type of scalp dandruff (oily or dry).
Efficacy was assessed from measurements done at W0, W2, W4, and W8:

- ✓ Clinically: Overall Clinical Dandruff Score, global efficacy, itching and discomfort sensations, satisfaction rated by investigator and/or subject using clinical 10 -point-scale
- ✓ Instrumentally: scalp hydration with Dermalab® (HI), pH with pHmeter®, barrier function (Trans Epidermal Water loss: TEWL) with Aquaflux®, lipids with Sebumeter® (LI).

In addition, during treatment phase (W0, W2, W4), microbiologic assessments were done from swab samples analysed by:

- ✓ droplet digital Polymerase Chain Reaction (ddPCR) for 4 microbial major species in OD and DD scalp: *Malassezia restricta (M. restricta*), *Malassezia globosa* (*M. globosa*), *Staphylococcus epidermidis* (*S. epidermidis*) and *Cutibacterium acnes* (*C. acnes*)
- ✓ Metagenomic analysis by NGS sequencing methods in DD scalp only, as follows:
    - Bacteria population: Taxonomic identification, based on the sequencing of the ribosomal RNA gene, present in all microbial genomes. V1-V3 region of 16S ribosomal RNA genes (prokaryotes) was amplified by PCR using oligonucleotides targeting conserved regions common to all bacteria.

- Fungi population: Taxonomic identification based on the sequencing of the ITS1 region of the ribosomal RNA gene, present in all fungi genomes. ITS1 region of the ribosomal RNA genes (Eukaryotes) was amplified by PCR using oligonucleotides targeting conserved regions common to all fungi.

The sequence of each DNA fragment duplicated was carried out by high-speed sequencing (MiSeq Illumina). The analysis of the sequences obtained and their comparison with international databases allowed the phylogenetic identification of the microorganisms present in each sample in relation to already known organisms.

After a cleaning process, the sequences with 100% homology between them were grouped into unique sequences and then into OTUs (operational taxonomic units: 99% threshold) which will be identified later.

The bioinformatics analysis of the sequencing data allows the identification of microorganisms present at different taxonomic levels (phylum to genus). The analysis allowing phylogenetic affiliation up to the genus level was carried out using bioinformatics tools for the processing of large amounts of sequence data (Mothur). The identification was carried out on the Findley (2013) DATA BASE taxonomy for fungi [8] and Greengenes DATA BASE taxonomy for bacteria.

Statistical analysis was done by ANCOVA, paired Student or Wilcoxon tests, according to the nature of data.
For metagenomic analysis, Alpha and Beta diversity were compared after normalization by rarefaction. Observed, Chao1, Shannon and invSimpson indices were compared by an ANOVA analysis for Alpha diversity evaluation. Beta diversity was evaluated with Jaccard, Bray Curtis, Unifrac and weighted Unifrac indices. The *pValue* of the comparison was carry out by PERMANOVA analysis.

Results.

Both shampoos showed a significant improvement in clinical signs during the treatment phase, with decrease in dandruff, itching, discomfort scores observed from W2. These changes were maintained during the persistence phase. (fig. 1, 2)

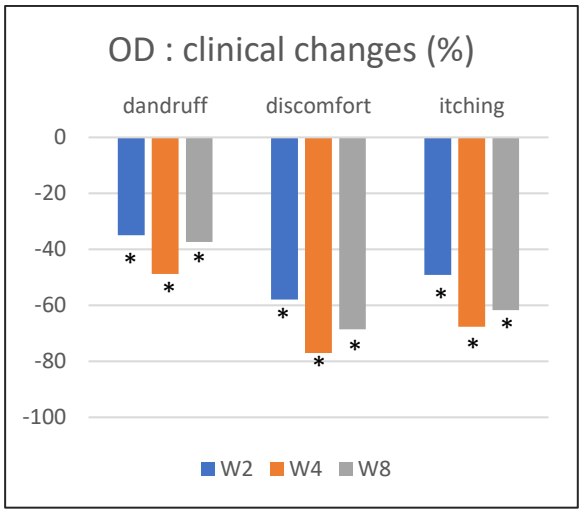

fig 1 *: p<0.05

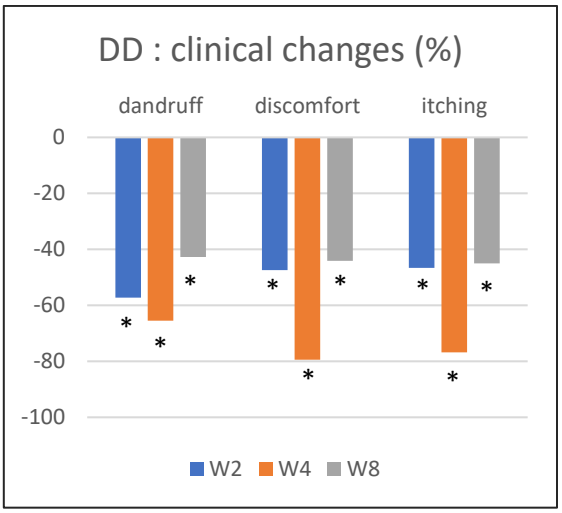

fig 2 *: p<0.05

Clinical improvement assessed by the investigator (IGA) and perceived by the subjects (PGA) confirmed these results (fig. 3, 4) :

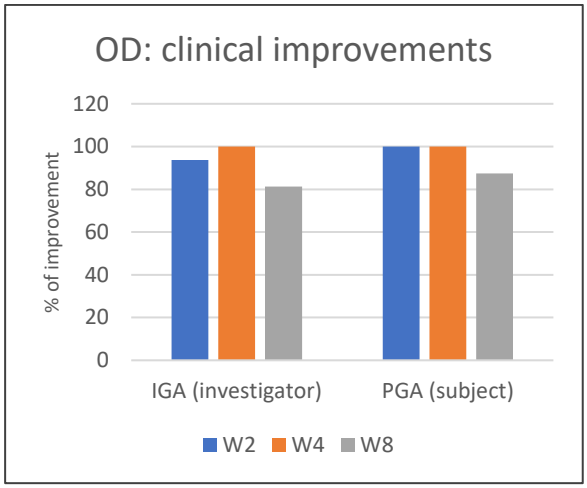

fig 3

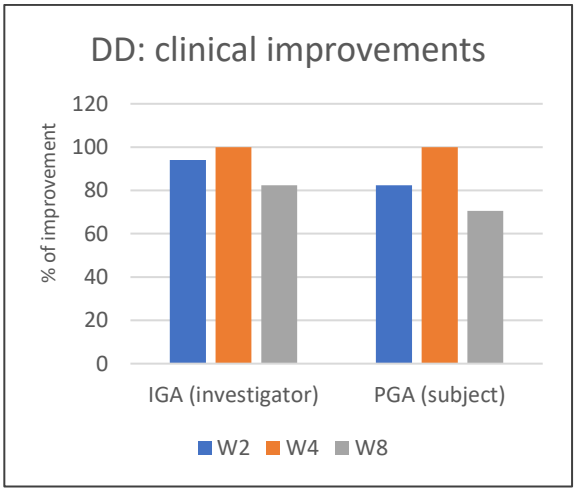

fig 4

Regarding the <u>biophysical changes</u>: HI, LI, TEWL increased, and pH decreased during treatment phase with the ODS. These changes, while significant, were slight. A possible explanation could be that the decrease in the physical barrier constituted by the dandruff would allow lipids and hydration to be more accessible to the measurements, hence these increases (same for TEWL). Only the increase of HI remained 4 weeks after stopping shampoo, other parameters returning to baseline. (fig. 5)

With the DDS, HI increased only at W2, then returned to baseline, LI, pH and TEWL remained stable. (fig. 6)

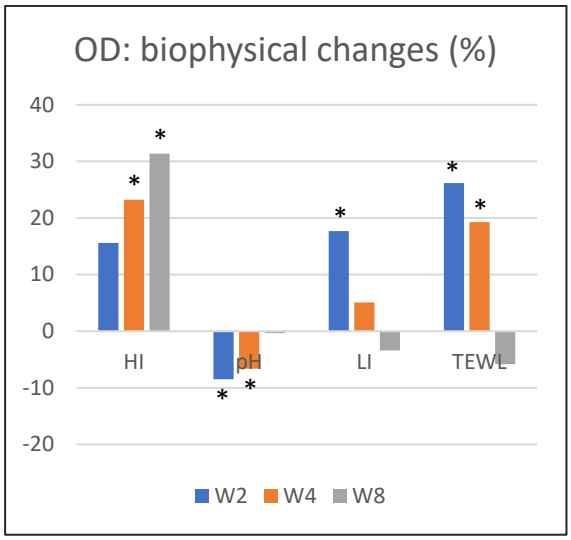

fig 5 *: p<0.05

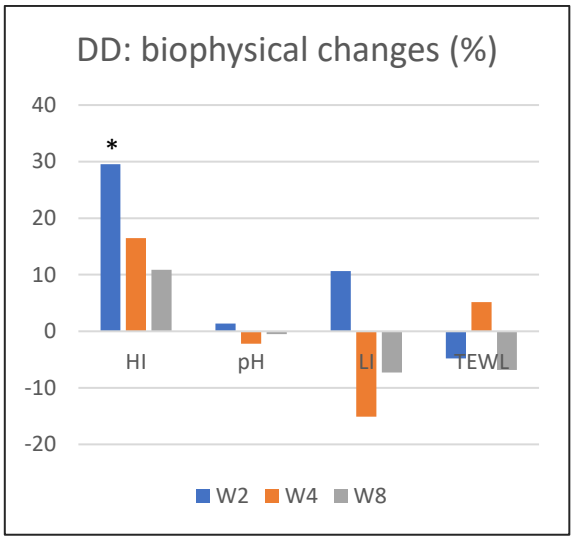

fig 6 *: p<0.05

Regarding the microbiota:

ddPCR analysis: During treatment phase (W0-W4), a decrease of *Malassezia restricta* and *globosa* species, and an increase of the proportion of *C. acnes* are observed. With the ODS, the ratio *Malassezia restricta* to *Cutibacterium acnes* decreased significantly in W4 (fig.7A), and with the DDS, significant changes from W2 were observed for the ratios *C. acnes*/Total that increased and *M.restricta*/Total that decreased, as a result of a rebalancing of the microflora. (fig.7B)

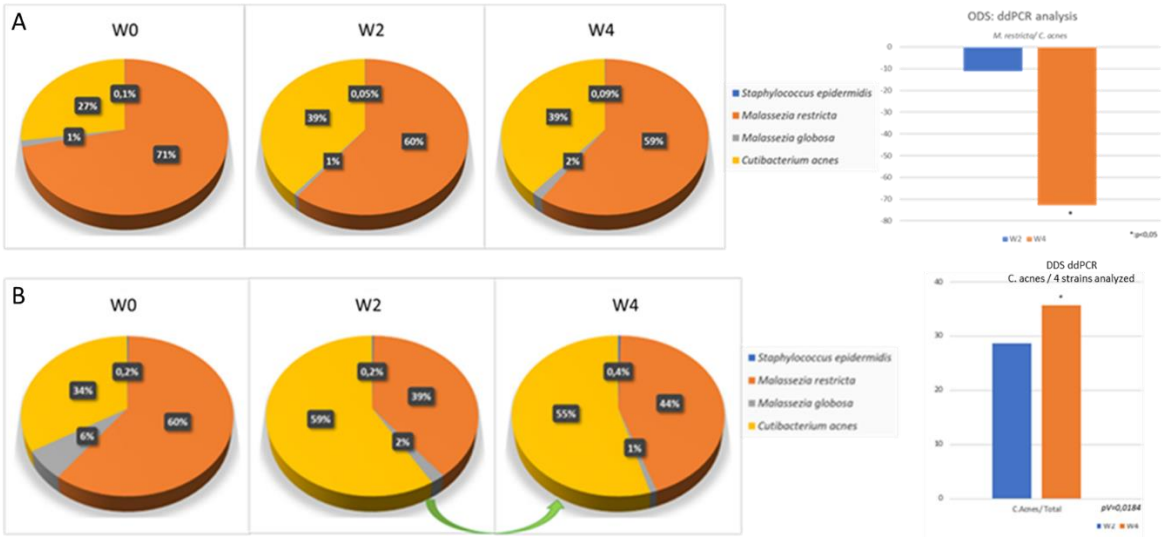

fig 7: (**A**) **Oily dandruff**. Evolution of proportions of *C. acnes*, *S. epidermidis*, *Malassezia restricta* and *globosa* quantified by ddPCR. Ratio *M. restricta/ C. acnes*. (**B**) **Dry dandruff**. Evolution of proportions of *C. acnes*, *S. epidermidis*, *Malassezia restricta* and *globosa* quantified by ddPCR. The proportions of *C. acnes* significantly increase after two weeks of using anti-dry dandruff shampoo.

Metagenomic analysis: The microbiota composition from W0 to W4 changed significantly during treatment phase:

Descriptive analysis of the fungal population showed a predominance of the *Malasseziaceae* family of the phylum *Basidiomicota*. During treatment, the number of genus from the phylum *Ascomycota* increased in correlation with a positive clinical evolution of the scalp condition. As illustration, differential analysis identified *Penicillium* and *Cladosporium*, two genus of *Ascomycota* phylum. There abundance increased with using anti-dry dandruff shampoo (fig.8).

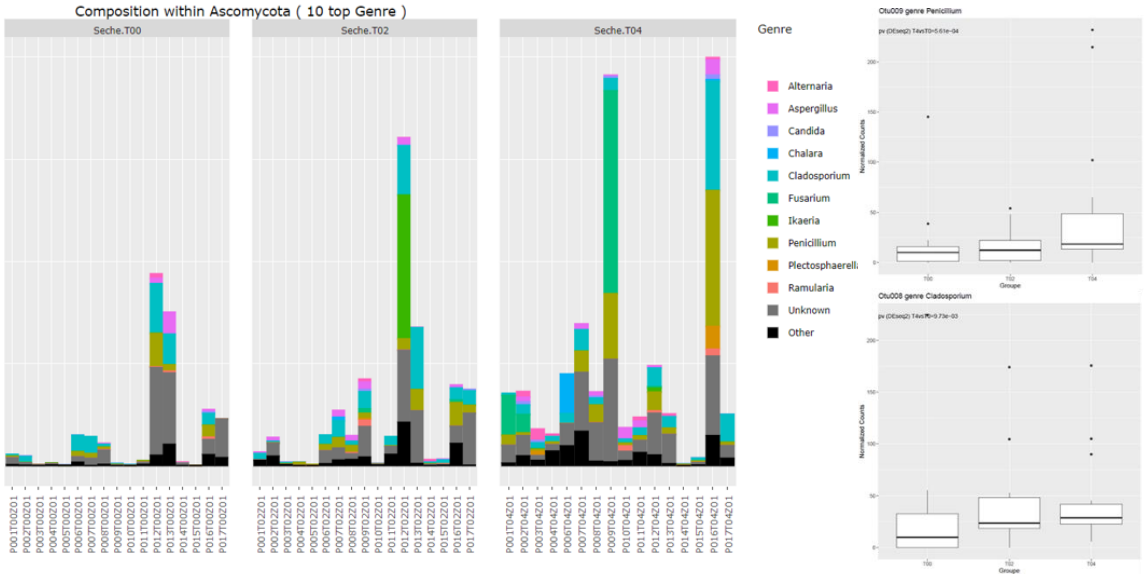

fig 8: Histograms of abundance of genus of *Ascomycota phylum* by subject. boxplot of *Penicillium* and *Cladosporium* genus evolution with Anti-dry dandruff shampoo.

After 4 weeks of using anti-dry dandruff shampoo,

- Alpha diversity of fungi population**,** reflecting the richness of fungi population, significatively increased with clinical improvement (significant change of the following indexes: Observed (p.value = 0,01) and Chao1(p.value=0,03)).
- Beta diversity of fungi population significantly increased, reflecting a change in the fungi population (significant change of Jaccard (pV=0,01), Bray Curtis (pV=0,01), unifrac (p=0,011) and weighted Unifrac (p=0,002) indexes).

Descriptive analysis of bacteria microbiota showed predominance of *Staphylococcus* and *Cutibacterium* genus on the scalp. During treatment, a decrease of *Staphylococcus genus* abundance while *Cutibacterium* genus increased (fig. 9), in parallel to the improvement of dry dandruff condition.

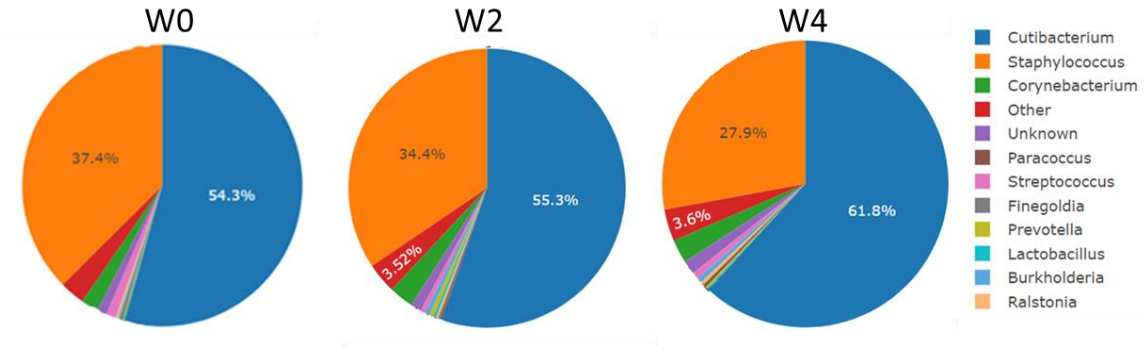

fig 9: Mean of the relative abundances of the top ten bacteria genus before and after 2 and 4 weeks anti-dry dandruff shampoo.

As highlighted by differential analysis, *Staphylococcus* genus significantly decreased while *Lactococcus*, *Leuconostoc* and *Burkholderia* genus abundance increased with clinical improvement (fig. 10).

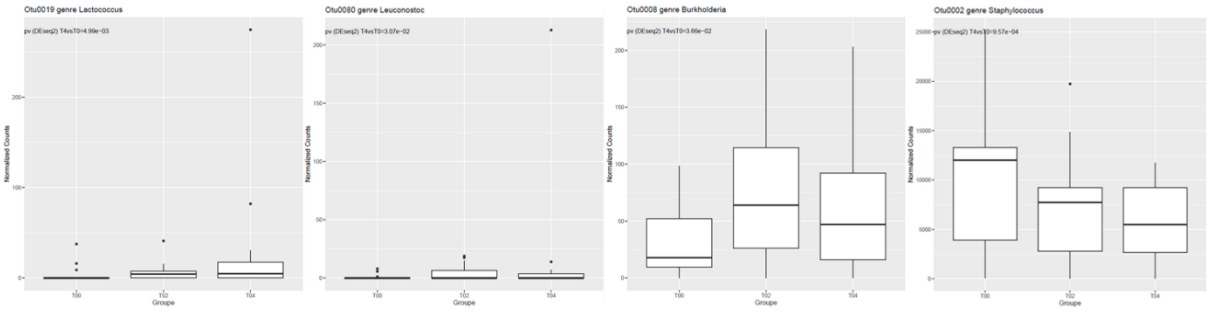

fig 10: Boxplot of the evolution of bacteria genus before and after 2 and 4 weeks of use of anti-dry dandruff shampoo.

Alpha diversity of the bacterial population was not significantly different, meaning that the richness and equitability of the microbiota were not changed.
Beta diversity analysis showed significant modifications of abundance of bacteria scalp composition, Bray Curtis (*pValue = 0,034*) and weighted-unifrac (*pValue = 0,008*).

Discussion.

These results show the efficacy on clinical signs and the soothing effect of both anti-dandruff shampoos during the treatment period, confirmed by the perception of the investigator and the subject, and the persistence of the clinical effects for at least 4 weeks after stopping treatment.
These shampoos also helped to rebalance significantly the skin scalp microflora, with an increase of *C. acnes* at the expense of *M. restricta,* species widely involved in the occurrence of dandruff states (specially in oily scalp).

The metagenomic study of the microbiota in relation to shampoo use and dandruff reduction highlights an enrichment of genus of the phylum *Ascomycota*. This result is in line with the

work of Hee Kuk Park et al [6], where the metagenomic study between healthy and dandruff scalp highlights that the phylum *Basidiomycota* is associated with the presence of dandruff and the phylum *Ascomycota* is more abundant on healthy scalps.
The use of anti-dry dandruff shampoo significantly increased and changed the diversity of the scalp fungal population. Bacteria microbiota was also modified as *Staphylococcus* genus decreased with clinical improvement. These results are in line with Rituja Saxena et al. [7] that showed that the genus *Staphylococcus* is significantly more abundant on dandruff scalp than on healthy scalp.
These results reflect a rebalance of fungi and bacterial populations.

Conclusion.

The use of non-specific anti-dandruff shampoo could aggravate the condition, such as dryness/tugging or discomfort for dry scalps frequently encountered when using aggressive shampoo or more oily dandruff using too mild shampoo that may not remove sebum enough.

A formulation specially adapted to the dandruff state (greasy/dry) makes it possible to target the effectiveness and to limit the undesirable effects which could occur with a less specific product.

Conflict of Interest Statement. NONE.

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