

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

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## 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]. Oily and Dry dandruff (OD/DD) differ clinically, and recent work has highlighted objective differences between them in scalp lipids/hydration/microbiota, that could justify 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 & Methods:

**Population** : 33 subjects (16 OD and 17 DD) with mild to moderate dandruff scalp



**Products**: anti-dandruff shampoos formulated with natural active ingredients, among them a common anti *Malassezia* ginger extract. Other ingredients selected regarding the type of scalp dandruff (oily or dry).

ASSESSMENT METHODS	W0	W2	W4	W8
<b>Clinically</b> : (investigator and/or subject ratings using clinical 10 -point-scale) Overall Clinical Dandruff Score, global efficacy (IGA, PGA), itching and discomfort sensations, satisfaction		•	•	•
<b>Instrumentally</b> : scalp hydration (Dermalab® -HI), pH (pHmeter®), barrier function (Trans Epidermal Water loss: TEWL-Aquaflux®), lipids (Sebumeter® -LI).		•	•	•
<b>Microbiologically</b> : Digital droplet PCR quantification of <i>M. restricta</i> , <i>M. globosa</i> , <i>C. acnes</i> and <i>S. epidermidis</i>		•	•	•
<b>Metagenomic analysis</b> by NGS sequencing methods in DD scalp only: Taxonomic identification of Bacteria and Fungi populations		•	•	•

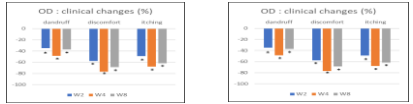
Statistical analysis: ANCOVA, paired Student or Wilcoxon tests, according to the nature of data.

## References:

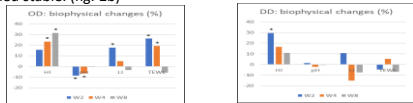
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## Results & Discussion:

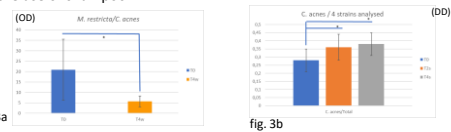
**Clinically** : OD and DD shampoos: significant improvement during the treatment phase, (decrease in dandruff, itching, discomfort scores observed from W2 and maintained during the persistence phase. (fig. 1a,b)- Results confirmed by investigator and subject assessments (IGA, PGA).



**Instrumentally**: OD: slight and significant changes of HI, LI, TEWL (increase), and pH (decrease) during treatment phase. Only the increase of HI remained 4 weeks after stopping anti-dandruff shampoo, other parameters returning to baseline. (fig. 2a)  
DD: HI increased only at W2, then returned to baseline, LI, pH and TEWL remained stable. (fig. 2b)

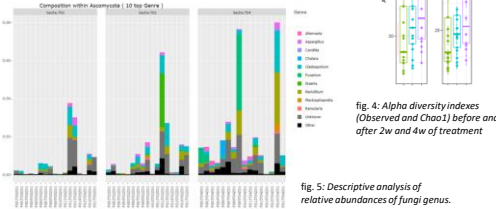


**Microbiologically**: **ddPCR analysis**:  
OD: significant decrease in 4 weeks of the ratio *Malassezia restricta* to *Cutibacterium acnes* (fig.3A).  
DD: significant changes from 2 weeks of the ratios *C. acnes*/Total of the four strains analysis (*S. epidermidis*, *M. restricta*, *M. globosa* and *C. acnes*). In accordance with improvement of clinical signs, the proportion of *Cutibacterium acnes*, associated with a healthy scalp [7], increased with the use of shampoo.



## Metagenomic analysis of dry dandruff Scalp microbiota:

**Alpha diversity** → Increase of Richness of Fungi population with clinical improvement (Observed index  $p=0,0101$  and Chao1 index  $p=0,03438$ ) (fig.4).  
**Beta diversity** → significant change of the composition of Fungi population (Jaccard index  $p=0,01$ , Bray Curtis index  $p=0,01$ , Unifrac index  $p=0,011$  and weighted Unifrac index  $p=0,002$ ). And increase of Genus of *Ascomycota* phylum (fig. 5) like *Penicillium* and *Cladosporium* Genus associated with healthy scalp [6].



**Bacterial population** is also modified, correlated with the clinical effect of the shampoo. **Beta diversity** indexes (Bray Curtis  $p=0,034$ , and Weighted unifrac  $p=0,008$ ) are significantly modified after 4 weeks of treatment. **Lactococcus**, **Leuconostoc** and **Burkholderia** genus population increase while **Staphylococcus** genus decrease with shampoo using.

## Conclusions:

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*

The metagenomic study showed a significant rebalance of the fungi and bacterial populations of the skin scalp microflora, in line with previous findings.

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.