International Journal of Cosmetic Science

International Journal of Cosmetic Science, 2011, **33**, 44–52 doi: 10.1111/j.1468-2494.2010.00581.x

On the effects of a plant extract of *Orthosiphon stamineus* on sebum-related skin imperfections

B. Vogelgesang, N. Abdul-Malak, C. Reymermier, C. Altobelli and J. Saget
BASF Beauty Care Solutions France SAS, 32 rue Saint-Jean-de-Dieu, 69007 Lyon, France

Received 3 September 2009, Accepted 14 January 2010
Keywords: 5-α reductase type 1, *in vivo* efficacy, *Orthosiphon stamineus* leaf extract, sebum, squalene

Synopsis

Overproduction of sebum is very common and results in an undesirable oily, shiny complexion with enlarged pores. Sebum secretion is basically under the control of 5-α reductase, and more particularly under that of type 1 isozyme. But it is also highly sensitive to environmental factors such as temperature, humidity and food. Moreover, in Asia, the edicts of a flawless facial skin turn oily skin into a major concern for Asian women. We identified *Orthosiphon stamineus* leaf extract as an interesting ingredient for reducing the oily appearance of skin thanks to its ability to reduce 5-α reductase type 1 expression in normal human epidermal keratinocytes *in vitro*. This was confirmed *ex vivo*, where *Orthosiphon stamineus* leaf extract was shown to reduce 5-α reductase activity as well as the production of squalene, one of the main components of sebum that was used as a tracer of sebum. To evaluate the efficacy of *Orthosiphon stamineus* leaf extract at reducing sebum-related skin imperfections *in vivo*, we performed two different clinical studies, one in France on a panel of Caucasian volunteers and the other one in Thailand on a panel of Asian volunteers. Using instrumental techniques as well as clinical evaluation and self-evaluation, we could highlight that an O/W cosmetic formula containing 2% of *Orthosiphon stamineus* leaf extract could visibly reduce the oily appearance of skin as well as the size of pores, thus leading to a significant improvement of complexion evenness and radiance. Overall, the results obtained were better than those observed with the same formula containing 1% of zinc gluconate, an ingredient frequently used in oily skin care products.

Résumé

La surproduction de sébum est très courante et se traduit par une peau à l'aspect gras, brillant et des pores élargis. La sécrétion de sébum est au départ sous le contrôle de la 5-α réductase, et plus particulièrement sous celui de l'isozyme de type 1. Mais elle est également très sensible aux facteurs environnementaux tels que la température, l'humidité ou l'alimentation. Par ailleurs, en Asie, où l'idéal d'une peau immaculée fait loi, la peau grasse est une préoccupation majeure pour les femmes. Nous avons identifié *Orthosiphon stamineus leaf extract* comme un ingrédient intéressant en vue de réduire l'aspect gras de la peau grâce à sa capacité à réduire in vitro l'expression de la 5-α reductase de type 1 dans des kératinocytes humains normaux. Cet effet a été confirmé *ex vivo* où nous avons pu montrer que *Orthosiphon stamineus leaf extract* permettait de réduire l'activité 5-α reductase ainsi que la production de squalène, l'un des principaux composants du sébum utilisé ici comme traceur du sébum. Pour évaluer la capacité d'*Orthosiphon stamineus leaf extract* à réduire *in vivo* les imperfections liées à la production excessive de sébum, nous avons réalisé deux études cliniques, l'une en France sur un panel de type Caucasien, l'autre en Thaïlande sur un panel de type asiatique. Par des mesures instrumentales ainsi que par une évaluation clinique et une auto-évaluation, nous avons pu montrer qu'une formule cosmétique H/E contenant *Orthosiphon stamineus leaf extract* à 2% permettait de réduire visiblement l'apparence brillante de la peau ainsi que la taille des pores, menant ainsi à un teint plus homogène et éclatant. D'une façon générale, les résultats obtenus avec une telle formule étaient meilleurs que ceux observés avec la même formule contenant 1% de gluconate de zinc, un ingrédient fréquemment utilisé dans les produits cosmétiques pour peau grasse.

Correspondence: Boris Vogelgesang, BASF Beauty Care Solutions France SAS, 32 rue Saint-Jean-de-Dieu, 69007 Lyon, France. Tel.: +33 (0)4 72 76 70 11; fax: +33 (0)4 78 58 09 71; e-mail: boris.vogelgesang@basf.com

Introduction

Oily skin is a widespread condition that is difficult to manage. The oily appearance of the skin results from an overproduction of sebum also referred to as hyperseborrhea. But regulating sebum production is complex because it is actually highly sensitive to numerous more or less identified environmental factors. Climatic conditions such as the temperature and humidity dramatically affect seborrhea [1]. Likewise, some types of food favour sebum overproduction [2]. It has also been hypothesized that all skin types may not have the same sebum excretion rate. For instance, it is often said that Asian skin may be oilier than Caucasian skin. However, no study could evidence any difference between Asian and Caucasian skin regarding the basal

sebum excretion rate [3–6]. Actually, it is likely that sebum excretion is higher in Asia because of the climatic features and living habits but not in Asian skin itself. Moreover, the Asian ideal of a flawless, clear and pure facial skin is hardly compatible with oily skin and turns it into a major concern.

Independent from the environmental factors, the major factor contributing to excess sebum is 5-α reductase. In the skin, this enzyme converts testosterone into dihydrotestosterone (DHT) having a much greater potency to increase the activity of sebocytes, the sebaceous gland cells responsible for sebum synthesis [7–10]. Two 5-α reductase isoforms exist, namely type 1 and type 2 [11]. In the skin, although type 2 is mainly expressed in the hair follicle and involved in hair loss, it seems that type 1 is more specific to the sebaceous glands and primarily associated with excess sebum as shown in whole human skin explants by immunohistochemical stainings [12] and indirectly *in vivo* where type 2 inhibitors appeared to have no effect on sebum excretion [7].

In our attempt to develop a cosmetic ingredient able to improve the shiny appearance of skin and other sebum-related skin imperfections, we first screened several compounds for their ability to reduce 5-α reductase type 1 expression *in vitro* in normal human epidermal keratinocytes (NHEK). Using RT-PCR methods, we could identify a water extract of the aerial parts of *Orthosiphon stamineus* (INCI name: *Orthosiphon stamineus* leaf extract) as a potent plant extract. Given that NHEK constitutively express 5-α reductase type 1 [13, 14] but do not produce sebum, the efficacy of the selected extract was then confirmed *ex vivo* in full-thickness human skin biopsies. For this, we performed a double-radiolabelling to measure both DHT and squalene syntheses in the same experiment. On one hand, a decreased DHT synthesis would indicate a reduction in 5-α reductase activity. On the other hand, a decreased squalene synthesis would predict a decrease in sebum production. Indeed, squalene has been proposed as a marker of human sebum [15]. Moreover, squalene is highly sensitive to UV-A and quickly becomes oxidized into squalene monohydroperoxyde [16], whose reported effects are increasing skin roughness, contributing to wrinkle formation, favouring comedo formation and even possibly participating in bacterial colonization [17]. Decreasing squalene would thus be of major interest to improve sebum related imperfections others than skin's oily appearance. Finally, the ability of *Orthosiphon stamineus* leaf extract to help reduce the oily appearance of skin *in vivo* was assessed in double study involving two panels of volunteers, one comprising Caucasian subjects recruited in France, the other one Asian subjects in Asia.

Materials and methods

Screening study

NHEK obtained from a facial biopsy of a 52-year-old female donor were cultured in 24-well plates in defined FCS-free medium until total confluence. The NHEK were then incubated for 24 h in FCS free culture medium and the candidate to test. NHEK cultured in the medium alone were used as untreated/negative control and NHEK cultured with zinc gluconate at 4 μg mL^{-1} (0.4×10^{-3}%) were used as positive control [18, 19]. The treatments were stopped by rinsing with PBS and discarding the medium. Samples were frozen dry before extraction. Each condition was tested in quadruplicate ($n = 4$).

Total RNA was then extracted using the 'SV 96 Total RNA Isolation System' (Promega, Charbonnières-les-bains, France) according to supplier's instructions. Briefly, the samples were diluted in RNAse-free water to obtain a final RNA concentration of 5 ng mL^{-1} and then distributed in a 96-well plate and frozen dry before PCR analysis. After thawing, the reaction mixture was added, containing SYBR Green 2× buffer, reverse transcriptase and DNA polymerase, as well as sense and anti-sense primers of actin (reporter gene) or of 5α-reductase type 1 (test) genes. Fifty amplification cycles in an Opticon thermocycler (MJ Research, Waltham, MA, U.S.A.) were performed. The results were first normalized to the expression of the actin gene and then expressed as percentage of the untreated control.

Assay of zinc

As zinc gluconate was used all along the study as positive control, zinc was quantified in *Orthosiphon stamineus* leaf extract. Results revealed that the extract contained less than 1 ppm of zinc, a concentration that could not influence the results.

Double labelling of normal human skin explants

Two normal human skin explants were used in this experiment: one facial skin biopsy of a 52-year-old female donor, one abdominal skin biopsy of a 34-year-old female. Each skin explant was first incubated for 24 h in RPMI culture medium (Invitrogen, Cergy-Pontoise, France) containing *Orthosiphon stamineus* leaf extract at 2% (test, $n = 3$) or 1% zinc gluconate (10 mg mL^{-1}, 2.2×10^{-2} mol L^{-1}) (positive control, $n = 2$) or in the culture medium alone (untreated control, $n = 2$). The products to test were then re-incubated for 24 h in the same conditions in the presence of 1, 2, 6, 7-^{3}[H] testosterone (American Radiochemical, St Louis, MO, U.S.A.) and mevalonate 4-^{14}[C] (a natural precursor of squalene [20–22]) before extraction of DHT and squalene in isopropyl ether. The explants were then dried and weighed. The dry residues were diluted in chloroform and deposited on high-performance thin-layer chromatographic plates (Merck Chemicals, Fontenay-sous-Bois, France) with reference samples of testosterone, DHT, mevalonate and squalene (Sigma-Aldrich, St Quentin-Fallavier, France). For testosterone and DHT measurements, the mobile phase used was heptane toluene acetone-chloroform and the spots were visualized using ammonium molybdate/sulphuric acid. Each spot was then scraped off the plate and assayed by liquid

scintillation spectrometry (Tri-Carb 1500, Packard Instruments, IL, U.S.A.). For mevalonate and squalene, the mobile phase was heptane. Squalene spots were scratched off and assayed by liquid scintillation spectrometry after staining with iodine vapours. Results of DHT assay are expressed as percentage of ^{3}[H]-DHT compared with ^{3}[H]-testosterone measured in dpm ^{3}[H]. Results of squalene assay are expressed as dpm ^{14}[C]/g dry tissue weight.

Clinical study

Two groups of 25 subjects each were recruited in France and two groups of 20 subjects each in Thailand. All subjects were women aged 18–40 years having oily skin (sebum > 140 μg cm^{-2} or score > 4 – normal to oily skin – on the EEMCO reference scale 'Guidance for the in vivo Assessment of Skin Greasiness', 6 scores possible) and enlarged pores. Volunteers having used sebo-regulating agents such as vitamin A derivatives within the 2 months preceding the study were excluded. The products to test were light O/W cosmetic emulsions containing either *Orthosiphon stamineus* leaf extract *at* 2% or zinc gluconate at 1% (positive reference). Products were applied twice-daily for 28 days on the whole face in normal conditions of use. The products were distributed after randomization and the study was conducted double-blind. All measurements were performed in the laboratory after 20 minutes of acclimatization in controlled temperature and humidity conditions (20–22°C; relative humidity 40–60%).

France

Instrumental measurements: The return of the oily appearance ('re-greasing') was measured 4 hours after a standardized cleansing using a quantitative polarimetric imaging system (SAMBA Advanced Vision System, Bossa Nova Technologies, Venice, CA, U.S.A.). The principle of this technique is the following: when light is projected on the skin, there are two components of reflected light that can be analysed, the diffusion component (light waves diffusing through the skin, giving its colour) and the specular component (light waves reflected by the skin). Analyzing the polarization of the reflected light (orientation and direction of different light waves reflected) provides information on skin shininess ('mirror-like' effect).

Clinical evaluation: A clinical expert scored visible 'shiny appearance', 'pore size', 'complexion evenness' and 'complexion radiance' by visual evaluation using a 10-point ordinal scale (from 0 = minimum pore size to 10 = maximum pore size) on the first day of the study, before the first application (D0) and after 28 days of treatment (D28).

Self-evaluation: After 28 days of treatment, subjects filled out a satisfaction questionnaire to gather their opinion and their judgment of the products tested. The results were then pooled and expressed in percentage of volunteers responding with a 4-point ordinal scale (agree totally, agree somewhat, disagree somewhat, disagree totally). For each item, the frequencies of positive responses (agree totally and agree somewhat) were added.

Digital photos: Illustrative pictures of all volunteers were also taken under parallel-polarized light using SAMBA Advanced Vision System.

Thailand

Clinical evaluation: The clinical evaluation of the items 'shiny appearance of the skin' and 'visible pore size' was conducted visually in the laboratory on D0, D14, D28 and D30 by a clinical expert using a 10-point ordinal scale. Evaluations on D30 were used to determine the rebound effect of shininess 2 days after treatment was stopped (last application in the evening of D27).

Self-evaluation: The volunteers filled out a satisfaction questionnaire on D14, D28 and D30 and the results were analysed with the same procedure used for the study performed in France.

Digital photos: Digital pictures of the entire face were taken on the same time-points using a high resolution Canon EOS 5D camera equipped with a 12 million pixel CMOS detector. Illumination was provided by two Elinchrom D-lite 4 2 digital flashes (Elinca SA, Renens, Switzerland). Illustrative pictures were taken with polarizing filters to select light reflected by the skin.

Statistics

Depending on whether the distribution was normal or not, the statistical comparison between products for the *ex vivo* experiments was conducted running either one-way ANOVA followed by Bonferroni *t*-test or ANOVA on Ranks followed by Dunn's method.

The statistical comparison between products for the *in vivo* tests was made running Tukey's test for paired samples. For the satisfaction questionnaire, the statistical significance of the frequencies of positive responses was assessed running chi-squared test.

For all tests, the significance threshold set at 5% ($P < 0.05$).

Results

Orthosiphon stamineus leaf extract reduces 5α-reductase type 1 mRNA expression in cultured NHEK

Figure 1 is an extract of the screening results. A 50% inhibition was considered significant with this technique. The positive control zinc gluconate provided a 58% inhibition of 5α-reductase type 1. Among more than 100 compounds tested, *Orthosiphon stamineus* leaf extract, a water extract of the aerial parts of *Orthosiphon stamineus* (common names 'Java tea' or 'Cat's whiskers'), was found to be the most potent plant extract at reducing 5α-reductase type 1 mRNA expression with a 64% inhibition, and was thus retained for further investigations.

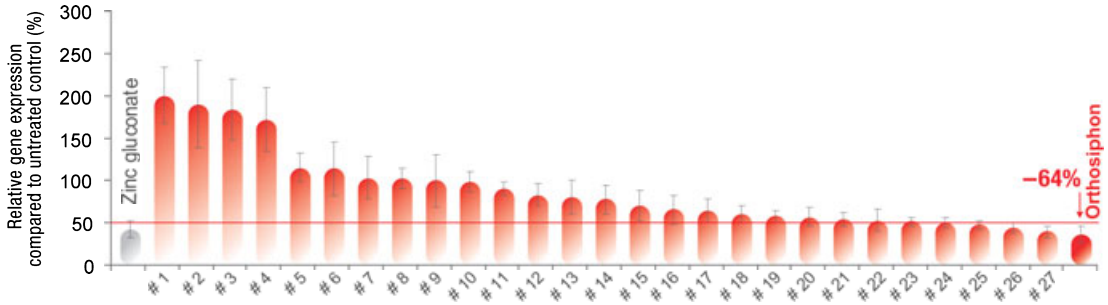


Figure 1 RT-PCR screening results. Results are expressed as relative 5-α reductase type (SRD5A1) mRNA level compared with untreated control.

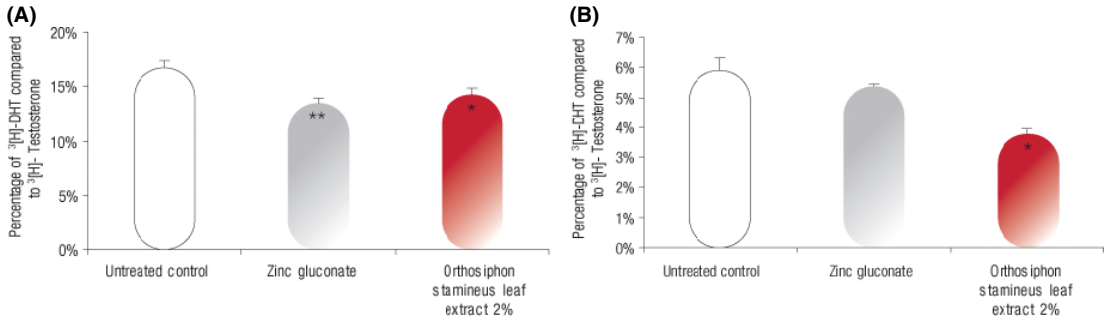


Figure 2 Measurement of DHT synthesis in two different skin biopsies. (A) Abdominal skin biopsy from a 34-year-old female donor. (B) Facial skin biopsy from a 52-year-old female donor. Statistically significant vs. untreated control, $^{*}P < 0.05$, $^{**}P < 0.01$ respectively.

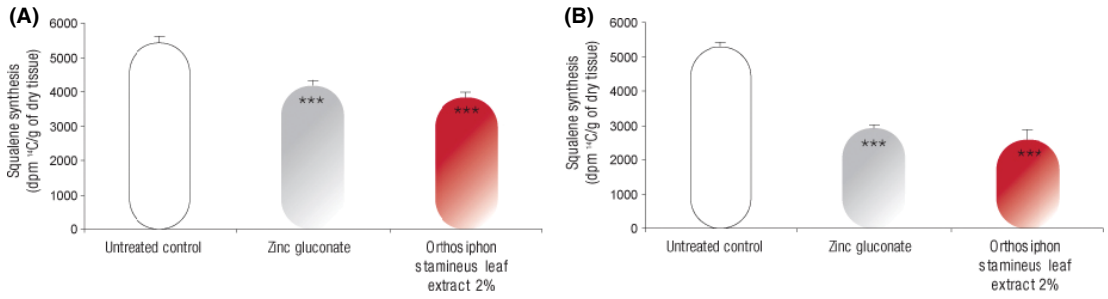


Figure 3 Measurement of squalene synthesis in two different skin biopsies. (A) Abdominal skin biopsy from a 34-year-old female donor. (B) Facial skin biopsy from a 52-year-old female donor. Statistically significant vs. untreated control, $^{***}P < 0.001$. An instrumental measurement of the return of oily appearance in Caucasian volunteers after 28 days of treatment. Statistically significant vs. baseline, $^{*}P < 0.05$.

Measurement of DHT and squalene productions *ex vivo*

In the two different skin samples analysed, DHT production was significantly reduced after treatment with *Orthosiphon stamineus* leaf extract at 2% (−14.8% and −36%, respectively, $P < 0.05$; Fig. 2). Although zinc gluconate reduced DHT synthesis in both samples, a statistically significant reduction was only observed in the abdominal skin biopsy of the 34-year-old female donor ($P < 0.01$).

Regarding squalene, both treated skin biopsies exhibited a significantly reduced synthesis (Fig. 3). In the abdominal skin biopsy of the 34 year old female donor, zinc gluconate and

Orthosiphon stamineus leaf extract reduced squalene synthesis by 23.4% and 29.2%, respectively ($P < 0.001$). Likewise, in the facial skin biopsy of the 52-year-old donor, zinc gluconate and *Orthosiphon stamineus* leaf extract reduced squalene synthesis by 45.1% and 51.3%, respectively.

These results seem to confirm those previously obtained by PCR for which we have seen that *Orthosiphon stamineus* leaf extract could reduce the mRNA expression of 5α-reductase type 1 in skin cells. The reduction of squalene synthesis is also a good indication that sebum production was reduced in the skin biopsies treated with *Orthosiphon stamineus* leaf extract.

Clinical study

France

Instrumental results: The results obtained using SAMBA® Advanced Vision System show that the shiny appearance measured in Caucasian volunteers 4 h after a standardized cleansing was significantly reduced by 42% ($P < 0.05$) after 28 days of application of the formula containing 2% of *Orthosiphon stamineus* leaf extract (Fig. 4). No significant improvement was observed in the volunteers who applied zinc gluconate (−4%).

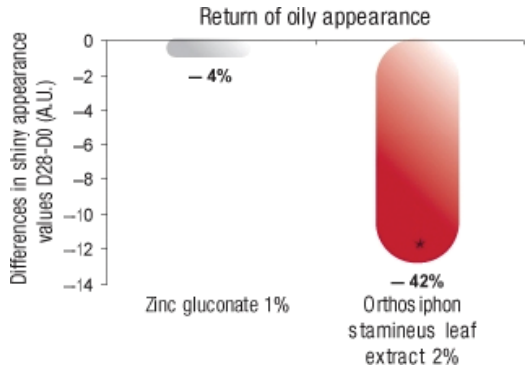


Figure 4 Instrumental measurement of the return of oily appearance in Caucasian volunteers after 28 days of treatment. *: statistically significant vs. baseline, $P < 0.05$.

Clinical evaluation: The results of the clinical evaluation of the Caucasian volunteers show that after 28 days of application of *Orthosiphon stamineus* leaf extract at 2%, the shiny appearance was significantly reduced by −17.5% ($P < 0.01$) and the size of pores significantly reduced by 30% ($P < 0.001$). As far as zinc gluconate is concerned, no improvement of the shiny appearance was observed and a significant but lower improvement of pore size was observed (−17.5, $P < 0.01$).

Complexion evenness and radiance were also significantly improved by 28% and 35%, respectively ($P < 0.001$), in the volunteers who applied *Orthosiphon stamineus* leaf extract, Once again, the use of zinc gluconate 1% led to significant but lower improvements of these items(+22% and +28%, respectively, $P < 0.01$; Fig. 5).

Interestingly, most of the volunteers appeared to confirm these results: after 28 days of treatment, 80% of them found that their complexion was more even, 100% their skin more radiant and 92% their skin texture improved (Fig. 6). These results were statistically significant unlike those obtained in the zinc gluconate group (64%; 68% and 68% of satisfaction, respectively).

Picture 1 hereunder is an example of results obtained in Caucasian volunteers who applied the formula containing *Orthosiphon stamineus* leaf extract at 2% for 28 days.

Thailand

The clinical evaluation of the Asian volunteers provided the following results (Fig 7):

From 14 days of use, the shiny appearance of the skin was significantly reduced by −14% in the volunteers who applied the formula containing *Orthosiphon stamineus* leaf extract. These results were increasingly better after 28 days with a highly significant 25% reduction of the shiny appearance. Finally, 2 days after the end of the treatment, and still compared with baseline, the skin appeared 31% less shiny ($P < 0.001$). Zinc gluconate only provided a significant reduction of the shiny appearance from 28 days (−13%,

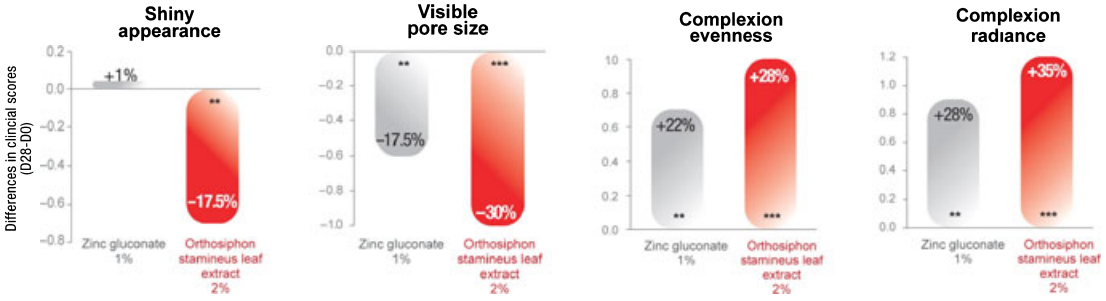


Figure 5 Figure 5 Clinical evaluation of shiny appearance, visible pore size, complexion evenness and radiance in Caucasian volunteers after 28 days of product application. **, ***: statistically significant vs. baseline, $P < 0.01$ and $P < 0.001$ respectively.

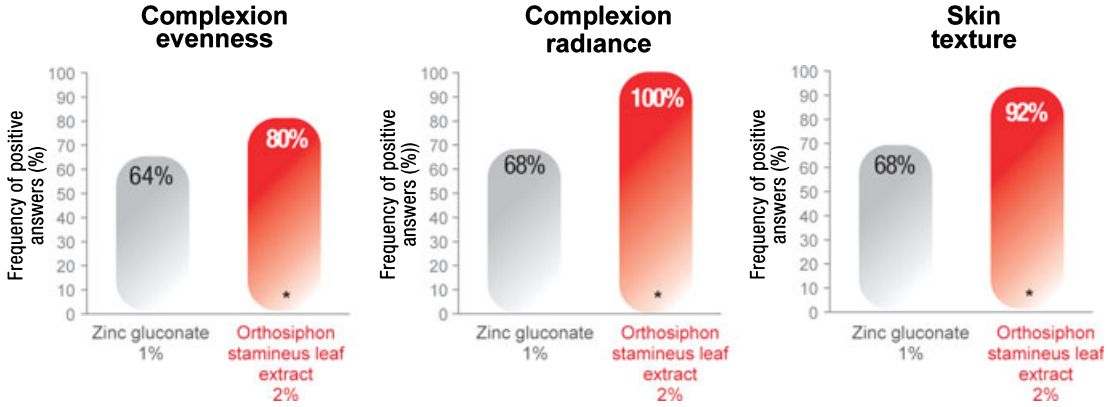
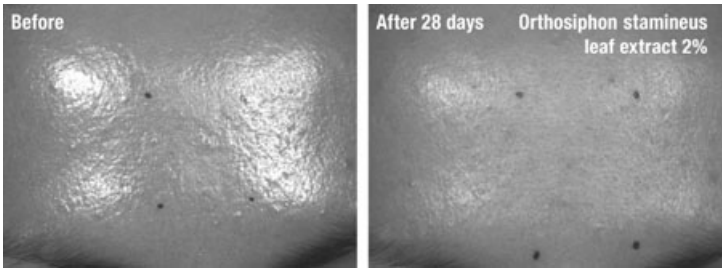


Figure 6 Caucasian volunteers self-evaluation of complexion evenness, complexion radiance and skin texture after 28 days of product application. *: frequency of positive answers statistically significant, $P < 0.05$



Picture 1 Illustration of the results obtained in Caucasian volunteers before and after 28 days of application of Orthosiphon stamineus leaf extract.

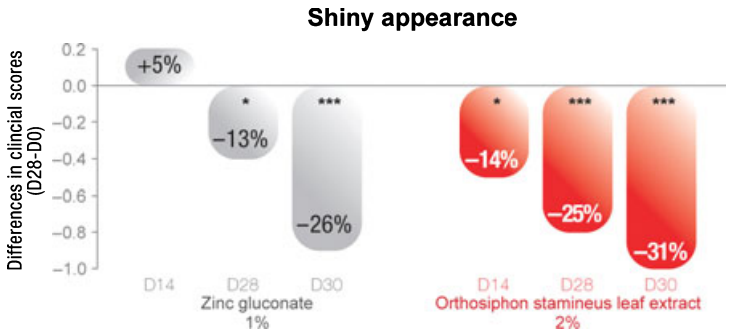


Figure 7 Clinical evaluation of skin's shiny appearance in Asian volunteers after 14, 28 and 30 days of application. *, ***: statistically significant vs. baseline, $P < 0.05$ and $P < 0.001$ respectively.

$P < 0.05$) which reached a maximum significant −26% improvement after 30 days ($P < 0.001$).

The clinical results of pore size were quite consistent with those of the shiny appearance. From 28 days of application, volunteers who applied *Orthosiphon stamineus* leaf extract exhibited a significant −20% reduction in pore size ($P < 0.001$) compared with a lower but nonetheless significant −8% decrease in the zinc gluconate group ($P < 0.05$; Fig. 8). Two days after the end of the treatment and compared with baseline, pore size was still significantly reduced by 16% ($P < 0.001$), a result comparable to that of zinc gluconate (−21%, $P < 0.001$).

Self-evaluation: The volunteers themselves confirmed these results: after 14 days of use, 81% of those who used the formula containing *Orthosiphon stamineus* leaf extract at 2% declared that their skin was less oily; after 28 days, 71% noted that their skin texture was improved and 2 days after the end of the treatment (D30), 76% still found their imperfections less visible (Fig. 9). For the same items and at the same time-points, the volunteers who applied zinc gluconate

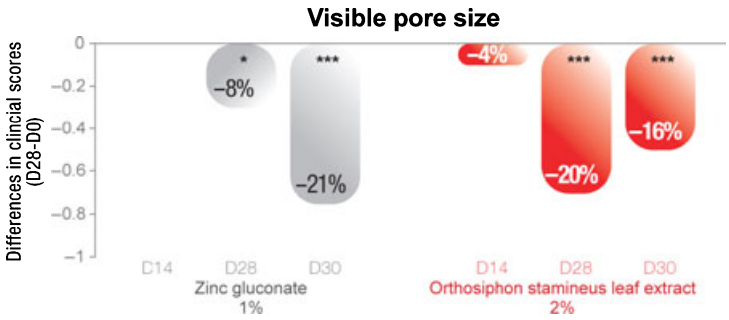


Figure 8 Clinical evaluation of visible pore size in Asian volunteers after 14, 28 and 30 days of application. *, ***: statistically significant vs. baseline, $P < 0.05$ and $P < 0.001$ respectively.

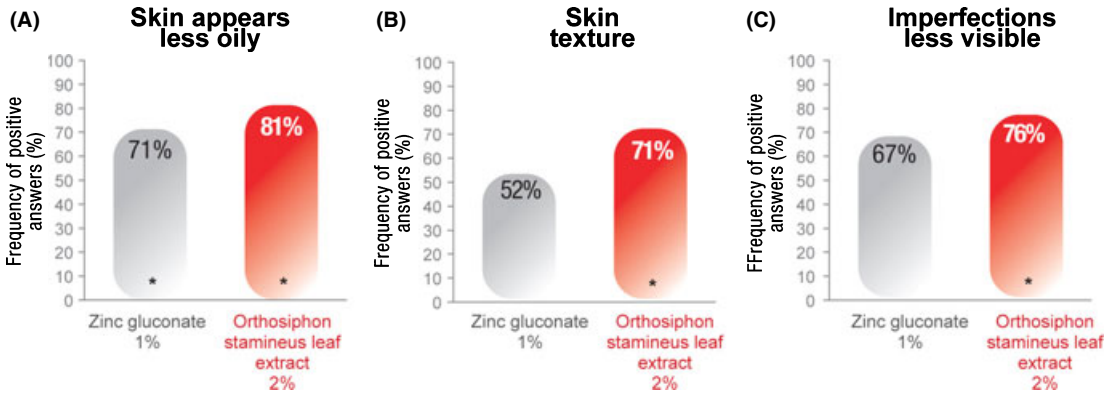
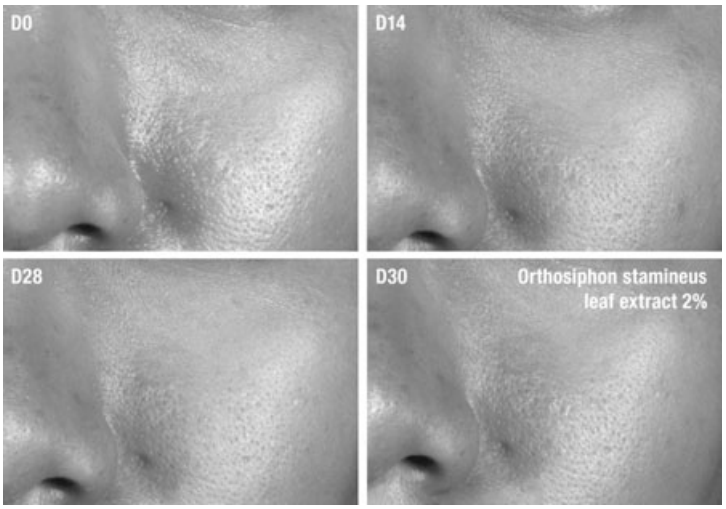


Figure 9 Self-evaluation of skin's oily appearance, skin texture and appearance of imperfections in Asian volunteers after 14, 28 and 30 days (A, B and C respectively). *: frequency of positive answers statistically significant, $P < 0.05$.



Picture 2 Illustrative example of the clinical results obtained in Asian volunteers who applied the cosmetic formula containing *Orthosiphon stamineus* leaf extract for 28 days.

were satisfied at the rate of 71% ($P < 0.05$), 52% (NS) and 67% (NS), respectively.

Picture 2 shows an illustration of the evolution of the shiny appearance over time observed in an Asian volunteer treated for 28 days with the formula containing *Orthosiphon stamineus* leaf extract.

Discussion

Orthosiphon stamineus (syn. *O. aristatus*, *O. spicatus*, *O. grandiflorus*) is a plant from the lamiaceae family native to South-East Asia. It is used as decoction in traditional herbal medicine to treat rheumatisms, hypertension, liver and

kidney pains, and more generally to ease excretory functions. Mainly grown in Java, hence its common name of 'Java tea', it is also referred to as 'cat's whiskers' because of its long stamina.

As a result of a screening study performed to evaluate the ability of various compounds to reduce the mRNA expression of 5α-reductase type 1 in NHEK, an aqueous extract of *O. stamineus* (INCI name: *Orthosiphon stamineus* leaf extract) appeared as the best candidate to develop a cosmetic ingredient having anti-seborrhea properties.

Results were later confirmed by measuring DHT and squalene productions *ex vivo* in full-thickness human skin samples, one being biopsy an abdominal of a 34-year-old donor and the other one a facial biopsy of a 52-year-old donor. To our knowledge, this is the first time that such a double-labelling was performed. Although interindividual variations were clearly observed, it appeared that *Orthosiphon stamineus* leaf extract used at 2% in the culture medium significantly reduced DHT production in both samples, thus providing further evidence on the ability of *Orthosiphon stamineus* leaf extract to decrease 5-α reductase activity in human skin. Likewise, we observed that squalene synthesis was also significantly reduced in both skin samples indicating that *ex vivo* sebum production may decrease in the presence of *Orthosiphon stamineus* leaf extract.

Assuming that sebum production is highly dependent on climatic conditions and living habits, we performed a double *in vivo* study in which the efficacy of a cosmetic formula containing *Orthosiphon stamineus* leaf extract at 2% was assessed in France on Caucasian subjects on one side and in Thailand on Asian subjects on the other side.

As measured instrumentally using an instrumental imaging system (SAMBA®) in Caucasian subjects, we first observed that the return of the oily appearance measured after 28 days of treatment was significantly reduced.

As measured by clinical evaluation, the shiny appearance of skin and the visible pore size were reduced in both groups. Interestingly, in Asian subjects, results were increasingly better after 14 and 28 days of use as well as 2 days after the end of the treatment.

As declared by the Caucasian volunteers themselves skin texture, complexion radiance and complexion evenness were also significantly improved. The Asian volunteers also declared that their skin texture was improved from 14 days of use, their skin less shiny from 28 days and the imperfections less visible even 2 days after the end of the treatment.

The reference molecule zinc gluconate used *in vivo* at 1% also provided significant improvements for most of the items evaluated. Nonetheless, for a large majority of the items evaluated whether instrumentally or by clinical evaluation or by self-assessment, results obtained with *Orthosiphon stamineus* leaf extract were directionally better.

In conclusion, we demonstrated the *in vitro* and *in vivo* efficacy of *Orthosiphon stamineus* leaf extract as a cosmetic ingredient intended to reduce the oily appearance of skin and improving skin imperfections related to excess sebum.

Acknowledgements

We thank Prof. J. Portoukalian at the Laboratory of Dermatological Research (University of Lyon-1 and Edouard Herriot Hospital, Lyon, France) for his help on the radio-labelling experiments.

This work was funded by BASF Beauty Care Solutions France S.A.S.

References

1. Cunliffe, W.J., Burton, J.L. and Shuster, S. The effect of local temperature on the sebum excretion rate. *Br. J. Dermatol.* **83**(6), 650–654 (1970).
2. Nouveau-Richard, S., Zhu, W., Li, Y.H. *et al.* Oily skin: specific features in Chinese women. *Skin Res. Technol.* **13**(1), 43–48 (2007).
3. Yosipovitch, G. and Theno, C.T.S. Asian skin: its architecture, function and differences from Caucasian skin. *Cosmet. Toiletries* **117**(9), 57–62 (2002).
4. Berardesca, E. and Maibach, H.I. Ethnic skin: overview of structure and function. *J. Am. Acad. Dermatol.* **48**, 139–142 (2003).
5. Taylor, S.C. Skin of color: biology, structure, function, and implications for dermatologic disease. *J. Am. Acad. Dermatol.* **46**, S41–S62 (2002).
6. Rawlings, A.V. Ethnic skin types: are there differences in skin structure and function. *Int. J. Cosmet. Sci.* **28**, 79–93 (2006).
7. Chen, W.C., Thiboutot, D. and Zouboulis, C.C. Cutaneous androgen metabolism: basic research and clinical perspectives. *J. Invest. Dermatol.* **119**, 992–1007 (2002).
8. Imperato-McGinley, J., Gautier, T., Cai, L.Q., Yee, B., Epstein, J. and Pochi, P. The androgen control of sebum production. Studies of subjects with dihydrotestosterone deficiency and complete androgen insensitivity. *J. Clin. Endocrinol. Metab.* **76**(2), 524–528 (1993).
9. Deplewski, D. and Rosenfield, R.L. Role of hormones in pilosebaceous unit development. *Endocr. Rev.* **21**, 363–392 (2000).
10. Pochi, P. and Strauss, J.S. Endocrinologic control of the development and activity of human sebaceous gland. *J. Invest. Dermatol.* **62**(3), 191–201 (1974).
11. Jenkins, E.P., Andersson, S., Imperato-McGinley, J., Wilson, J.D. and Russell, D.W. Genetic and pharmacological evidence for more than one human steroid 5α-reductase. *J. Clin. Invest.* **89**, 293–300 (1992).
12. Thiboutot, D., Bayne, E., Thorne, J. *et al.* Immunolocalization of 5α-reductase isozymes in acne lesions and normal skin. *Arch. Dermatol.* **136**, 1125–1129 (2000).
13. Milewich, L., Kaimal, V., Shaw, C.B. and Sontheimer, R.D. Epidermal keratinocytes: a source of 5 alpha-dihydrotestosterone production in human skin. *J. Clin. Endocrinol. Metab.* **62**, 739–746 (1986).
14. Milewich, L., Shaw, C.B. and Sontheimer, R.D. Steroid metabolism by epidermal keratinocytes. *Ann. N Y Acad. Sci.* **548**, 66–89 (1988).
15. Downing, D.T., Stewart, M.E. and Strauss, J.S. Estimation of sebum production rates in man by measurement of the squalene

content of skin biopsies. *J. Invest. Dermatol.* **77**(4), 358–360 (1981).
16. Mudiyanselage, S.E., Hamburger, M., Elsner, P. and Thiele, J.J. Ultraviolet A induces generation of squalene monohydroperoxide isomers in human sebum and skin surface lipids in vitro and in vivo. *J. Invest. Dermatol.* **120**, 915–922 (2003).
17. Wertz, P.W. and Michniak, B.B. Sebum. In: *Cosmeceuticals, Drugs vs. Cosmetics* (Elsner, P. and Maibach, H.I., ed.), pp. 45–56. Marcel Dekker Inc, New York (2000).
18. Sugirnoto, Y., Lopez-Solache, I., Labrie, F. and Luu-The, V. Cations inhibit specifically type 1 5α-reductase found in human skin. *J. Invest. Dermatol.* **104**, 775–778 (1995).
19. Piérard, G.E. and Piérard-Franchimont, C. Effect of a topical erythromycin-zinc formulation on sebum delivery. Evaluation by combined photometric-multi-step samplings with Sebutape®. *Clin. Exp. Dermatol.* **18**(5), 410–413 (1993).
20. Witting, L.A. and Porter, J.W. Intermediates in the conversion of mevalonic acid squalene by a rat liver enzyme system. *J. Biol. Chem.* **234**(11), 2841–2846 (1959).
21. Rilling, H.C. and Bloch, K. On the mechanism of squalene biogenesis from mevalonic acid. *J. Biol. Chem.* **234**(6), 1424–1432 (1959).
22. Popják, G. and Cornforth, J.W. Substrate stereochemistry in squalene biosynthesis: the first Ciba medal lecture. *Biochem. J.* **101**(3), 553–568 (1966).