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"Application of Norway Spruce Leaf Extract in Oily Skin Care: a Mechanism and Clinical Study"

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1. Introduction

Oily skin is one of the most common dermatologic concerns reported by all types of patients. It is characterized by enlarged facial pores and an "unclean" or "greasy" appearance [1]. Excessive sebum can easily lead to diseases such as acne and seborrheic dermatitis [2]. Research indicates that individuals with oily skin are five times more prone to acne vulgaris compared to those without oily skin [3]. In a study of 1,787 Chinese women, oily skin was observed in 25.6 percent [4]. Another study of 60 subjects (30 males and 30 females) reported that the male casual sebum secretion was significantly higher than that of females, as measured at all facial sites [5].

Currently, there are various methods to treat excessive sebum secretion, such as botulinum toxin injections, photodynamic therapy, and laser treatments. However, these methods are expensive and need to be administered by medical professionals [6]. Therefore, it is essential to develop a non-prescription, easily accessible, and cost-effective solution for controlling sebum production.

Norway spruce (*Picea abies*), a coniferous tree native to Europe, is known for its various phytochemical properties and potential therapeutic benefits [7]. However, there are limited studies on the specific application of Norway spruce leaf extract (NSLE) in managing oily skin. Research indicates that the leaves and bark of Norway spruce are rich in polysaccharides, flavonoids, polyphenols, lignin, and so on [8]. This study evaluated the inhibitory effects of NSLE on sebum secretion and inflammation in vitro. Subsequently, an emulsion containing 2.5% NSLE and a 2-week trial involving 30 subjects with oily skin were conducted to evaluate the clinical efficacy of NSLE on controlling sebum production and improving skin glossiness and lightness.

2. Materials and Methods

2.1. Materials

The SZ95 human sebaceous gland cells were purchased from Qingqi (Shanghai) Biotechnology Development Co. Ltd. Mouse macrophages (RAW264.7) cells were purchased from the Chinese Academy Medical Sciences. The Norway spruce leaf extract (NSLE) was provided by Norture. Fetal Bovine Serum (FBS), Dulbecco's Modified Eagle Medium (DMEM), and pancreatic enzyme were purchased from Gibco. Dimethyl sulfoxide (DMSO), methylthiazolyldiphenyl-tetrazolium bromide (MTT), and linoleic acid were purchased from SIGMA (St. Louis, MO, USA). Phosphate-buffered saline (PBS) was purchased from Wuhan Servicebio Technology Co., Ltd. Nile red dye and isotretinic acid were purchased from Shanghai Yuanye Biotechnology Co., Ltd. NO assay kit was purchased from Beyotime Biotechnology Co., Ltd.

2.2. Instruments

SpectraMax 190 Microplate Reader was purchased from Molecular. The CLM-170B-8-NF CO2 incubator was purchased from ESCO. Primovert was purchased from Zeiss. DW-86L338J ultra-low temperature refrigerator was purchased from Haier. The sebumeter SM815 instrument and Glossmeter instrument were purchased from Courage & Khazaka Electronic GmbH (CK). The chroma meter CM-700D was purchased from Konica Minolta Sensing.

2.3. Cell viability assay

The cells were seeded (1.0×10^5 cells/mL) on 96-well cell culture plates and incubated for 24 hours until the cells were grown to approximately 80% confluence. After incubation, the medium was removed, and 100 µL of the test samples were added to each well. After 48 h (for SZ95 sebaceous) or 24 h (for RAW264.7 cells) incubation with the test samples, 20 µL of MTT solution was added to the wells. After 4 h incubation, the medium was removed, and 100 µL of DMSO was added to the wells. The absorbance of each well was measured at 570 nm using a microplate reader to calculate the cell viability. The cells were shaken for 10 to 15 minutes before measurement. Cell viability for each extract concentration was calculated and expressed as a percentage of the mean negative control value (set at 100% viability).

2.4. Determination of neutral lipids

SZ95 cells in the logarithmic growth phase were digested and counted, and they were then seeded (2.0×10^4 cells/well) on 96-well cell culture plates and incubated for 18 to 24 hours until the cells were grown to approximately 80% confluence. To induce the differentiation of the sebocytes, linoleic acid was added to the culture medium, and the cells were then treated with various concentrations of NSLE (as treatment group, T) as well as isotretinoin (as positive control, PC). The cells treated with the vehicle served as the control (Ctrl) and treated with linoleic acid as the model group (M). After incubating for 48 h, the medium was removed, and the cells were washed twice with PBS before the addition of 10 µg/mL Nile red dye. The released fluorescence was read on a multifunctional microplate reader using 485 nm excitation and 565 nm emission filters for neutral lipids. The inhibition rate of neutral lipids was calculated from the following formula:

$$\text{Inhibition rate} = (A_T - A_M)/A_M \times 100\%$$

where A_T and A_M were the fluorescence values of cells in the treatment group and the model group, respectively.

2.5. Measurement of NO Production

The RAW264.7 cells were seeded in the wells of 48-well plates and grown for 24 hours. The cells were pre-incubated for 24 h and were then induced with LPS at 37°C in a 5% CO_2 incubator for 24 h according to the following conditions: DMSO (0.1%)/LPS (1 μg/mL) (negative control, NC), dexamethasone (0.001%)/LPS (1 μg/mL) (positive control, PC), and LPS (1 μg/mL)/NSLE (2.5%, 5.0%, 10.0%). The NO produced in the culture supernatant was measured according to the instructions provided with the assay kit.

2.6. Clinical trial

2.6.1. Subjects

The measurements were performed in Guangzhou, China, from July to August. Thirty male subjects with oily skin enrolled in this study, and all of them signed the informed consent. The subjects were evaluated as follows: (a) those aged 18 to 45 with oily skin (sebum content exceeding 120 μg/cm^2 within 8 hours before product application); (b) those not using any skincare products on the day of testing; (c) those without excessive sun exposure during the experimental period; (d) those who voluntarily participated and signed the informed consent; (e) those who completed the specified content according to the experimental requirements. Exclusion criteria included: (a) those with systemic diseases, immune deficiency, or autoimmune diseases; (b) those with allergic diseases or who had experienced cosmetic allergies within the past 1-2 years; (c) those with dermatological diseases in the test area or undergoing drug treatment; (d) those deemed unsuitable by a dermatologist; (e) those receiving dermatological treatment or who had undergone skin treatment, beauty treatments, or other clinical tests that could affect the results within the past month in the test area; (f) those with wounds, abrasions, tattoos, acne, papules, erythema, or other conditions in the test area that could affect the determination of experimental results; (g) those who had participated in other project tests in the test area within the current or previous month.

2.6.2 Test samples and treatments

The test emulsion contained only 2.5 wt% of the NSLE as the sebum control ingredient. All subjects applied the test emulsion all over the face twice a day, morning and evening, after washing their face for 2 weeks. The formulation of the test emulsion was detailed in Table 1, and the prepared emulsion was subsequently packaged and sealed for storage.

Table 1. The formulation of the test sample

Ingredients	wt%
Water	To 100
1,3-Butanediol	7
Aristoflex AVC	0.5
NIKKOL LECINOL S-10	0.08
Polydimethylsiloxane	5
MONTANOV 202	0.8
NSLE	2.5
4-Acetylphenol	0.5
Hexanediol	0.5

2.6.3 Clinical assessment

Clinical assessment was conducted at baseline (Week 0), Weeks 1, and 2. When subjects arrived at the research site, they had to wash their faces with the provided facial cleanser, then wait in a closed room with a temperature of 20 ± 2°C and relative humidity of 50 ± 5% for 30 min. The subject's skins were then examined. The sebum level was measured with Sebumeter® SM815 (Courage & Khazaka) on the forehead, and the skin lightness (L* value) and glossiness were measured with Chromameter CM-700D and Glossmeter (Courage & Khazaka), respectively.

2.7. Statistical analysis

SPSS 25 software was used for data analysis, and a normal distribution test was conducted on the test data. If the test data were normally distributed, the *t*-test was used for statistical analysis; if the test data was not normally distributed, the rank sum test was used for statistical analysis. Data were measured at least three times and expressed as mean ± standard deviation. A *P*-value of less than 0.05 was considered statistically significant. All graphical representations of the data were performed by the GraphPad Prism 8.0 software.

3. Results

3.1. Effects of NSLE on lipid droplet accumulation in SZ95 cells

Sebocytes, the major cell type in sebaceous glands, are differentiated epithelial cells that gradually accumulate lipids and eventually disrupt, releasing their content (sebum) in a secretory process known as holocrine secretion [9]. To investigate the effects of NSLE on the secretion of lipids in SZ95 sebocytes, the cells were induced with the lipid inducer linoleic acid and stained with Nile Red to assess their intracellular lipid levels.

The effect of NSLE on the viability of SZ95 cells was determined by MTT reduction assay, and the results are presented in Figure 1. The results indicated that the cell viability was nearly 90% when the concentration of NSLE was 4.8% (v/v). However, the cell viability decreased to 85.8% when the concentration of NSLE was increased to 6% (Figure 1A). Therefore, 5% of NSLE was considered to have little effect on cell viability. Concentrations of 1.25%, 2.5%, and 5% were selected to evaluate the inhibitory effect of NSLE on neutral lipids in SZ95 sebocytes, indicating that the NSLE significantly inhibits the secretion of neutral lipids in a dose-dependent manner. The neutral lipid inhibition rates were 17.10%, 23.4%, and 23.73% when the concentrations of NSLE were 1.25%, 2.5%, and 5%, respectively, and all were significantly different from the control ($P < 0.05$, Figure 1B). Additionally, the secretion of neutral lipids in SZ95 sebocytes significantly decreased by treatment with NSLE (Figure 2), as evidenced by the results of microscopic visualization after Nile red staining. The above results suggested that NSLE could inhibit the secretion of sebum.

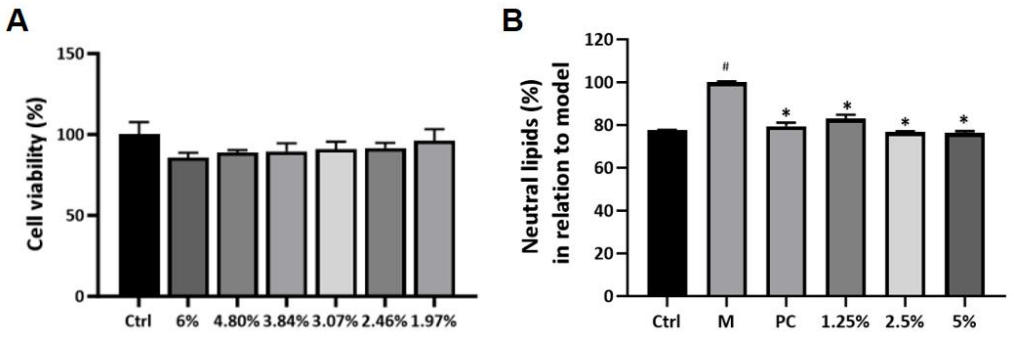

Figure 1. The results of the neutral lipid inhibitory effect on SZ95 by different concentrations of NSLE. (A) The effects of NSLE on the viability of SZ95 cells. (B) The content of neutral lipids in SZ95 cells was treated with various concentrations of NSLE. Compared with the model group, * $P < 0.05$; Compared with the control, # $P < 0.05$.

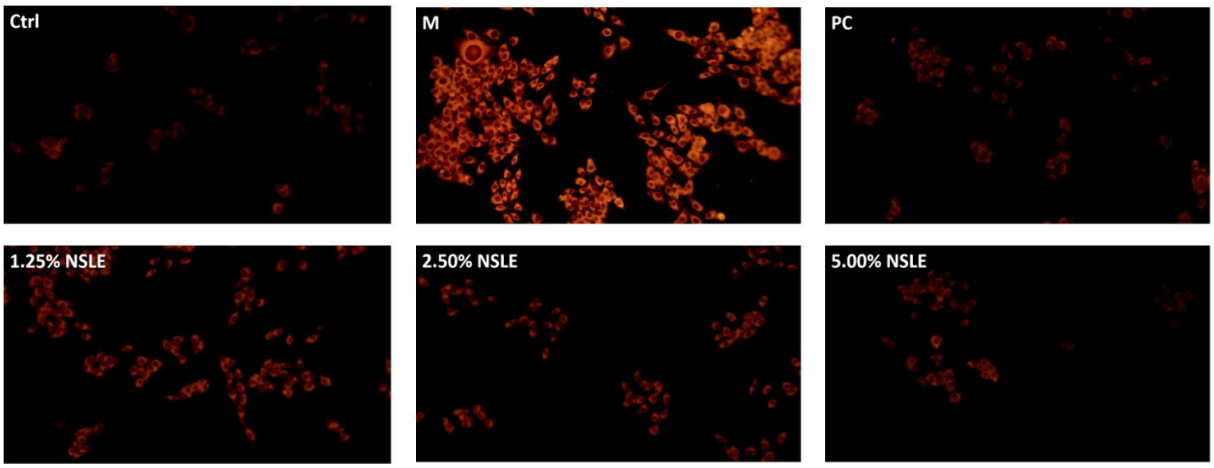

Figure 2. The sebum-controlling efficacy of the NSLE.

In macrophages, the sebum lipids, such as oleic and linoleic acids, activate signaling pathways and stimulate or inhibit the release of inflammatory mediators [10]. Therefore, the production of pro-inflammatory cytokines can be inhibited by reducing sebum secretion. The effects of NSLE on the viability of RAW264.7 cells were determined by MTT reduction assay, and the result showed that there was no cytotoxicity up to a concentration of 20%. The inhibitory effects of 2.5%, 5%, and 10% NSLE on NO production were evaluated in LPS-induced macrophage RAW264.7 cells, as shown in Figure 3. The results indicated that a 10% concentration of NSLE significantly inhibits NO production by 20.25% ($P < 0.05$).

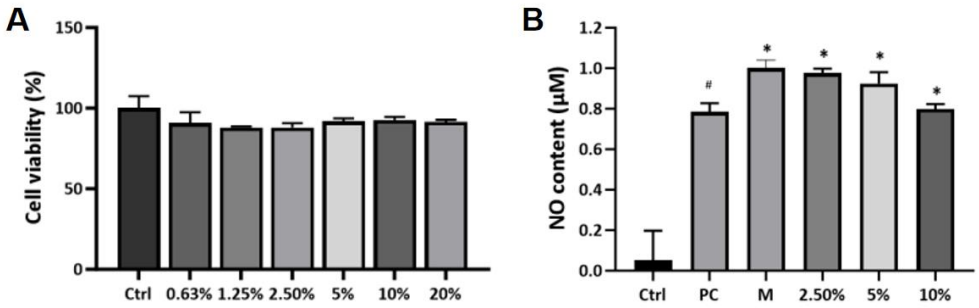

Figure 3. Inhibition of NO content in RAW264.7 cells by different concentrations of NSLE. (A) The effects of NSLE on the viability of RAW264.7 cells. (B) The NO content of RAW264.7 cells was treated with various concentrations of NSLE. Compared with the model group, * $P < 0.05$; Compared with the control, # $P < 0.05$.

3.2. The sebum-controlling and brightening effects of NSLE on human skin

The activity of sebaceous glands is influenced by the secretion of androgens. Studies have shown that sebum production in males is approximately double that of women between the ages of 20 and 40 [11]. It is common knowledge that the central forehead represents areas of high sebum secretion. Therefore, the forehead, which has greater sebum secretion in men, was chosen as the test area. Studies indicate that the relationship between skin temperature and sebum excretion rate was highly significant, and heating and cooling produced changes of the orders of 10% per 1°C. Additionally, sebum secretion is also affected by environmental humidity [12,13]. Thus, this study was performed in Guangzhou, a representative city with high summer air temperatures and humidity (28.2 ± 5.6°C and 76.9 ± 11.8% RH) [14].

The forehead sebum was reduced by 29.3% after one week and 20.2% after two weeks of application, both of which were significantly different from baseline ($P < 0.05$; Figure 4). The subjects also showed significant improvements in skin glossiness and lightness, with the glossiness increasing by 8.4% and 11.3% ($P < 0.05$), and the skin lightness increasing by 1.1% and 1.9% ($P < 0.05$). These results suggested that the NSLE could reduce skin sebum content without compromising the skin's natural lightness (L* value) and glossiness, thereby indicating its efficacy in controlling sebum levels.

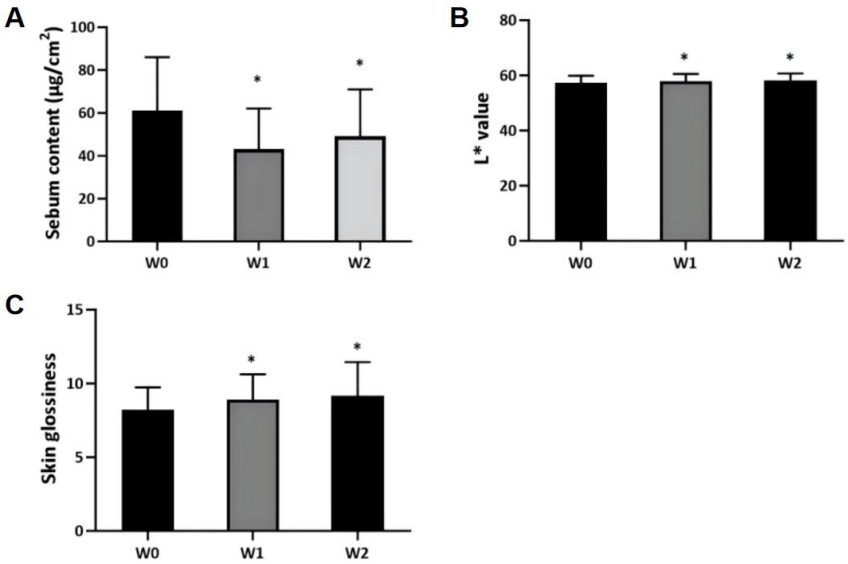

Figure 4. Clinical efficacy test of emulsion containing NSLE. The effects of 2.5 wt% NSLE emulsion on the facial sebum content (A), L* value (B), and skin glossiness (C) at weeks 0, 1, and 2. Compared with baseline, * $P < 0.05$.

4. Discussion

In recent years, oily skin has become a prevalent dermatological issue, with excessive sebum secretion being linked to conditions such as acne and seborrheic dermatitis. Despite the availability of treatments like botulinum toxin injections and photodynamic therapy, these

options are often costly and require professional administration, highlighting the need for accessible and cost-effective alternatives. Norway spruce leaf extract (NSLE), a natural compound known for its rich phytochemical composition, offers promising potential for addressing this concern. However, limited research exists on the specific application of NSLE for controlling sebum production.

This study aimed to evaluate the efficacy of NSLE in reducing sebum secretion and improving skin appearance. The research consisted of two main components: an in vitro investigation on SZ95 sebocytes and an in vivo clinical trial involving human participants with oily skin. The results from the cell-based experiments demonstrated that NSLE significantly inhibited lipid secretion in a dose-dependent manner, with the 2.5% and 5% concentrations showing notable reductions in neutral lipids. Additionally, NSLE was found to reduce inflammatory mediator production in macrophages, further supporting its potential anti-inflammatory properties.

In the clinical trial, the application of an emulsion containing 2.5% NSLE for two weeks resulted in a significant reduction of sebum on the forehead, with a decrease of 29.3% after one week and 20.2% after two weeks. Moreover, improvements in skin glossiness and lightness were observed, highlighting NSLE's ability to control sebum production while enhancing overall skin appearance.

The findings of this study underscore the potential of NSLE as a non-invasive, cost-effective solution for managing oily skin. Its ability to reduce sebum production and improve skin characteristics makes it a promising alternative to traditional treatments, with further research needed to explore its broader applications in dermatological care.

5. Conclusion

The NSLE effectively enhanced the skin health of individuals with oily skin by reducing sebum production in SZ95 sebocytes, with an inhibition rate of 23.73%, and also inhibiting the levels of the inflammatory marker NO in RAW264.7 cells. An emulsion containing 2.5% NSLE was subjected to a two-week trial involving 30 individuals with oily skin. The results indicated a significant reduction in sebum content by 29.3% after one week and 20.2% after two weeks of application. Additionally, there were notable improvements in skin glossiness, increasing by 8.4% after the first week and 11.3% after the second week, as well as skin lightness, which increased by 1.1% and 1.9%, respectively. In conclusion, the findings of this study indicated that the NSLE effectively enhanced the skin health of oily skin by reducing sebum production and inhibiting inflammation, suggesting a potential effect as a cosmetic ingredient in alleviating critical conditions related to acne-prone skin.

6. References

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