Romanian Biotechnological Letters *Vol. 22, No. 6, 2016*

ORIGINAL PAPER

The valorification of *Linum usitatissimum* oil as sebum-reducing agent

Received for publication, June 06, 2015
Accepted for publication, December 23, 2015

ANCA DRAGOMIRESCU[1], FELICIA ANDREI[1], GEORGETA POP[2], DORICA BOTAU[2*], ERSILIA ALEXA[2]
[1]University of Medicine and Pharmacy "Victor Babes" Timisoara, 2Eftimie Murgu, 300041Timisoara, Romania;
[2]Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, 119 Calea Aradului, 300645 Timisoara, Romania;
**** Address correspondence to:** Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, 119 Calea Aradului, 300645 Timisoara, Romania; e-mail: dbotau@yahoo.com*

Abstract

The study aims to: (1) determine fatty acids in Linum usitatissimum oil, in order to be, at least, protective and nutritive for human skin, in topical application, (2) evidencing the sebum-reducing capacity of linum oil, compared to a olive oil as control (without expected activity on seborrhea), (3) assessment of the linseed oil tolerance of the skin and (4) observation of a possible improvement of skin texture (a result of skin hydration), for the cases of seborrhea sicca (dry). Linseed oil was obtained from the seeds of the Alexin cultivar grown in the Didactic Station Timisoara of Banat's University of Agricultural Sciences and Veterinary Medicine Timisoara in 2013. The cutaneous study benefited from the enrollment for testing of 24 healthy female volunteers, aged between 18 and 46, with oily, seborrheic complexion, and the sebaceous glands were assessed in terms of numbers and dimensions, by using the apparatus Proderm Analyser (NU SKIN, Provo, UT, USA). We concluded that linseed oil is substantially a sebaceous secretion-reducing agent, for carefully selected cases.

Keywords: linseed oil, skin protection, sebum-reducing effect.

1. Introduction

The use of oil and fiber *Linum usitatissimum* dates back over 6000 years (some archaeological data confirms this assertion), both for benefits related to the textile industry as well as for the nutritional, pharmaceutical benefits, and all its directions of use have currently developed new bearings of research **(**JHALA & HALL [1]). There are also numerous records in the literature regarding the utility of linseed oil on the skin, either by topical application or as a consequence of the diet with this oil. It is worth to mention here the experimentally confirmed effects, such as increased skin hydration and the ability to reduce irritation after introducing in the diet *Linum usitatissimum* oil (DE SPIRT & al.[2]). The linoleic and alpha-linolenic essential fatty acids are involved in restoring the cutaneous barrier, indispensable in the endogenous synthesis of epidermal ceramide and produces skin hydration by reducing transepidermal water loss. Other authors have noted the favorable effect of the *Linum usitatissimum* oil on wound healing, on an animal model, a process for which scarring is the key (DE SOUZA FRANCO & al.[3]). This study is a confirmation of a previously proposed effect, achieved by a team specializing in veterinary surgery (FARAHPOUR & al., [4]), which starts from the premise that the restoration of the surgical wound is often the secret of favorable postoperative progress. Early closure of the surgical incision eliminates the risk of infection and other complications from surgery. There is also experimental evidence regarding the favorable effect on skin excoriations (SAXENA & al. [5]), for which all the

healing and anti-inflammatory effect was invoked. The inflammatory effect is also extended to edema and lesion area erythema, (O'NEILL & al. [6]) as evidenced by the authors for atopic dermatitis, caused by allergies, in horses. This study also proposes as hypothesis the skin barrier recovery effect, in the case of nutritional supplementation with *Linum usitatissimum* oil, thanks to the essential fatty acids constituting the seeds of this species. The anti-inflammatory and even antibacterial effect leads some authors to cite flax as having a positive effect on treating ringworm (LEPORATTI & al. [7]). A small part of the studies, all starting from the idea that the oil of this species restores the nutrition of the skin, also launch the idea of hair follicle stimulating effect after ingestion of linseed, on an animal model (BEROUAL & al. [8]). In one of our previous studies (POP & al. [9]), we have demonstrated on volunteers a cosmetic - dermatological effect of *Linum usitatissimum* oil, namely the anti-photoaging effect. In that study, as a working hypothesis, we started not only from the richness in essential fatty acids, but also from the phytoestrogen content of flax. The presence of lignans as phytoestrogens was launched by manufacturer Lucas Mayer Cosmetics [10]. Theoretically, under the effect of lignans, (*secoisolariciresinol diglucoside* - *SDG* - the most abundant flax lignan) with estrogen-mimetic action, the oil of this species should have effects similar to other phytoestrogens: i) *non-comedogenic and sebum-reducing action,* as *secoisolariciresinol diglucoside (SDG)* is an inhibitor of the enzyme 5 alpha-reductase (the key enzyme of androgenization in the target cell), implicitly a reducing agent of sebum in sebocytes; ii) *chronoaging action at epidermal level,* a level at which estrogens are stimulators of cellular restoration. While the second effect was already pointed out by us previously on a group of volunteers, the first effect is currently worth an evaluation. For this reason, the present study tries to quantify the sebum-regulating effectiveness of linseed oil, on volunteers with clear signs of seborrhea, using *Proderm Analyser*. The test objectives were: (1) fatty acids determination in *linum* oil, in order to be, at least, protective and nutritive for human skin, in topical application (2) evidencing the sebum-reducing capacity of *linum* oil, by diminishing the number or size of the oversized sebaceous glands marked by the device software in green, compared to a control oil (without expected activity on seborrhea): olive oil (3) assessment of the linseed oil tolerance of the skin and (4) observation of a possible improvement of skin texture (a result of skin hydration), for the cases of seborrhea *sicca* (dry). Finding natural anti-seborrheic products is beneficial because: i) seborrhea is not just an aesthetic damage, it is also the starting point for the development of acne and seborrheic dermatitis (erythematous and scaling dermatitis), ii) dermo-cosmetic consumers consider acceptable and compliant active substances of plant origin, a fact which is easily exploited by the marketing campaigns in this field.

2. Material and method

2.1. Obtaining and characterization of linseed oil

2.1.1. The obtaining of linseed oil

Linseed oil was obtained from the seeds of the Alexin cultivar grown in the Didactic Station Timisoara (longitude 21013`E, 45045` N latitude) of Banat's University of Agricultural Sciences and Veterinary Medicine Timisoara in 2013. The oil was extracted with Soxtherm system using petroleum ether as solvent. Seed oil content was 40%.

2.1.2. Fatty acids determination

Fatty acid composition was determined by GC-MS using SCAN mode. Gradient oven temperature: 100°C for 5 minutes followed by a rate of 10°C/min up to 250°C and held for 12 min. Quadrupole temperature was 150°C, MS source temperature was 200°C, the volume of sample injected into the column was 1 μL. Split mode was used with a split ratio of 50:1,

carrier gas was He. The mass spectrometer was set to the ionization energy of 70 eV with a scan range from 33-400 (m/z). For identification and quantification of fatty acids standard compounds were used: oleic acid, linoleic acid and linolenic acid (Sigma-Aldrich Germany). Calibration curves were performed in the range 0.06-2.25 mg/ml.

2.2. Skin tests

Volunteers. The study benefited from the enrollment for testing of 24 healthy female volunteers, aged between 18 and 46, with oily, seborrheic complexion, at the cosmetology – dermatopharmacy laboratory of the Faculty of Pharmacy of Timisoara, during the period October 2014 - December 2014. Each volunteer agreed to apply linseed oil in the facial seborrheic regions, with a frequency of 1 / per day for a maximum of 28 days. The test spread over a maximum period of one month because: (1) it aimed to highlight the effectiveness of the oil for a period to be perfectly acceptable and compliant for consumers of dermocosmetic products and (2) the major season changes such as temperature decreases may change the sebaceous secretion (diminishing it), which would generate a false positive.

The criteria for including the volunteers in the study were*:* written consent of the volunteers after they understood the test procedure, diagnostic of seborrhea / above-normal oily complexion, based on clinical criteria, lack of any pathological complications (eg. acne, seborrheic scaling dermatitis) situations in which the oil is potentially harmful (obstructive, comedogenic, or irritating), elimination of intolerance, after a preliminary test of the oil at a topical skin application. A similar group, selected on the same type criteria was introduced in the study, in order to test the effect of olive oil (control group).

Evaluation. The sebaceous glands were assessed in terms of numbers and dimensions. The inhibition of sebaceous secretion by limiting the hormonal secretion mechanism entails the reduction of the number of oversized glands. These glands were investigated using the apparatus *Proderm Analyser* (NU SKIN, Provo, UT, USA). The apparatus takes digital photos of an area of 1 cm^2 of skin, and - through its software - marks in green the oversized sebaceous glands. Simply counting those colored dots, in their dynamic, during the testing, provides quantifiable information on the function of sebaceous secretion. In addition, for the 4 cases diagnosed to have *seboreea sicca* (dry seborrhea) skin texture was monitored with the same device, set to *skin texture* function. The device monitored the potential loss of the "scaly", desquamating appearance of the skin. The assessments with *Proderm Analyzer* were recorded after 7 days of therapy, at the times: 7, 14, 21 and 28 days respectively. For each volunteer, at each time point, were counted the colored points / skin field explored (number of oversized sebaceous glands per cm^2) and the average was calculated for each time unit, separately for *seboreea oleossa* and for *seboreea sicca.*

2.3. Statistical analysis

Results are presented as means ± standard deviation (SD) of triplicate measurements. Statistically significant differences are marked (*) and indicate a p value <0.05. Statistically highly significant differences are marked (**) and indicate a p value <0.01. Statistically extremely significant differences are marked (***) and indicate a p value <0.001. Statistical processing of data was performed using the Statistical Analysis System - SAS (Software Version 8.1. SAS Institute, Inc., Cary, NC).

3. Results and Conclusions

3.1. Linseed oil fatty acids composition

GC/MS analysis of linseed oil revealed the presence of oleic, linoleic and linolenic acids, as presented in Figure 1. The average composition is presented in table 1.

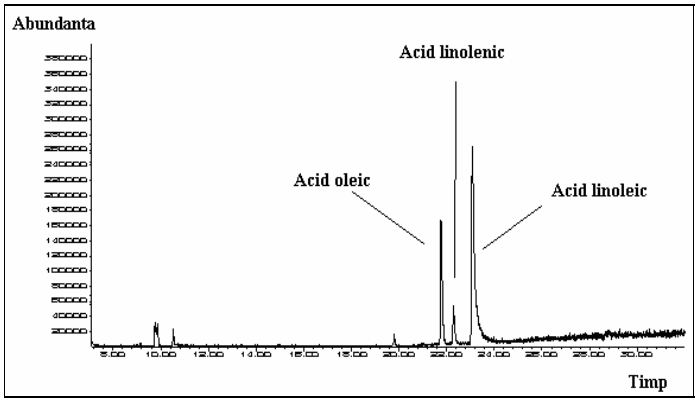

Figure 1. Cromatogramm of fatty acids from linseed oil

Table 1. Fatty acids composition of linseed oil

Fatty acid	mg/mL
oleic acid	0.195
linolenic acid	0.214
linoleic acid	0.313

Linseed oil is characterized by a high content of linoleic acid (0.313 mg / ml) and linolenic acid (0.214 mg / ml). The results are consistent with literature data mentioning a high concentration of linoleic acid in oil from linseed seeds cultivated in Romania (POPA & al. [11]) but also in other countries (BAYRAK & al. [12]).

3.2. Effect of linseed oil on reducing the number of oversized sebaceous glands

For group cases that were carefully selected, based on firm criteria for inclusion in the study, including by eliminating the causes of allergy and intolerance (there was a pre-assessment of the volunteers), there was no adverse effect of the oil on the skin. Moreover, there is ample evidence of cutaneous use of this oil for other purposes, presented in the introduction. For all the volunteers in the study group were counted the green points generated by the software of the device (Figure 2) and their arithmetic average was calculated separately for *seborrea oleossa* and *sicca*, for different times of testing. The values are presented in **Table 2**.

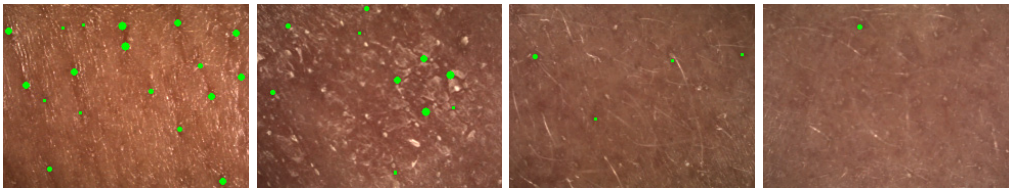

Figure 2. Oversized sebaceous gland involution in a volunteer aged 33, at the times: day 0, day 14, day 21, day 28.

Table 2. Size of sebaceous glands

Type of seborrhea	*Size of sebaceous glands (mm)*[a]				
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
			Control group		
Seborea oleossa	20.10±4.32	20.05±4.12	19.15±4.00	19.45±4.20	19±4.22
Seborea sicca	22.00±4.55	21.00±2.94	20.75±2.50	21.25±3.59	20.75±3.30
			Linseed oil group		
Seborea oleossa	19.80±4.69^{ns}	10.30±3.8**	8.20±3.69***	4.60±2.23***	1.20±0.89***
Seborea sicca	18.25±3.00^{ns}	9.75±2.63**	7.75±3.30***	3.00±1.83***	0.75±0.5***

[a] *means ± standard deviation (n=3)*

Statistical differences are indicated between linseed and control groups: P>0.05= ns (non-significant), P<0.05= (significant), P<0.01=** (highly significant) and P<0.001=*** (extremely significant).*

By comparing the two groups (linseed oil treated and control) we observed a statistically significant reduction of number of oversized sebaceous glands per cm^2, for the linseed oil treated group. In figure 3 are presented the reduction of the number of oversized sebaceous glands after linseed oil application.

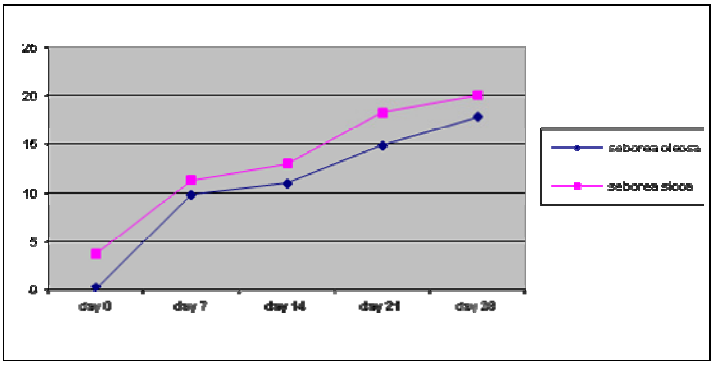

Figure 3. The reduction size (mm) of the sebaceous glands

The analysis of this progress demonstrates certain important aspects: (1). Linseed oil is substantially a sebaceous secretion-reducing agent, for carefully selected cases, which have no signs of microcysts (early stage of acne), where oil could aggravate lesions by the possible obstructive effect. Since the reduction of this function implicitly entails the decrease of the size and number of the glands, on the images captured by the camera there is a drop in the green points. We can launch the assumption that the lignans in the composition of linseeds, known in the literature as phytoestrogens, implicitly have an anti-androgenic activity. However, androgens are the physiological stimulator of sebaceous secretion inside the sebocyte; (2). There are no obvious differences between the evolution of the seborrhea *oleossa* and the *sicca* during the test for the linum oil treated group, meaning that this oil has efficiency in both types of disorders ($p>0.05$); (3). 14 days after the treatment, the *number of oversized sebaceous glands* reaches approximately half of the original value. The 14 days also represent the turnover of the sebocyte. In other words, after two weeks there are other new sebocytes holding secretory activity under another hormonal stimulation, modified in the meantime. As a matter of fact, the sebocyte is a cell with a prompt response to other pharmaceutical treatments as well, for example after the treatment with topical retinoids the sebaceous secretion decreases dramatically, sometimes after only 7-10 days. The problem with retinoids, however, is their adversity to the epidermis, dryness and the erythema generated.

3.3. The evolution of skin texture, for the cases with seboreea sicca

Seborrhea sicca (dry seborrhea) is known by the slight superficial epidermal desquamations, without actually dealing with seborrheic dermatitis (*Malassezia furfur* agent, producing primarily erythema of the nasal wings). In the case of seborrhea sicca, considered a quasi-normal state, there are desquamations in the cheeks. The daily use of linseed oil also produced an improvement in the appearance of the skin, reducing the appearance of scaly skin to complete fade-out (Figure 4). This evolution suggests an improvement of the skin hydration, an effect for which we put forward two possible hypotheses: (1) a decrease in transepidermal water loss observed by other authors[2] as well and (2) an improvement in the epidermal lipids (ceramides, fatty acids), involved in water preservation in the extracellular environment of the epidermis, due to their dual polarity: hydrophilic pole, hydrophobic pole.

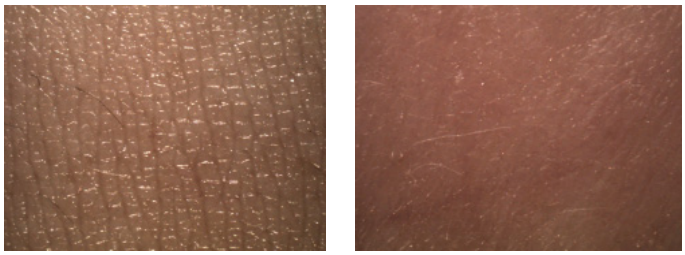

Figure 4. Skin texture images before and at the end of the treatment in a patient aged 42. We can notice the disappearance of scaly-type skin squaring.

Linseed oil is one of the oldest natural remedies for skin nutrition. A previous study of our team[9] revealed the anti-photoaging role of this oil, and now, this study brings again *linum usitatissimum* oil in the foreground for its sebum-reducing effect. There are no obvious differences between the evolution of the seborrhea *oleossa* and the *sicca* during the test for the linum oil treated group, meaning that this oil has efficiency in both types of disorders. The daily use of linseed oil also produced an improvement in the appearance of the skin, reducing the appearance of scaly skin to complete fade-out.

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