REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS, INTERNATIONAL
EDITION 36 (2-3): 31-36 (2022)
PUBLISHED BY PHARMAKON-Press

Open Access Article ***Research Article***

Study of the cosmetic properties of microalgae extracts – The case of *Nannochloropsis oculata*

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Key words: microalgae; *Nannochloropsis oculata*; cosmetics; UV filters; transepidermal water loss, skin hydration.

Citation: A. Liakopoulou, S. Mountsaki, V. Andriopoulos, M. Gkioni. Study of the cosmetic properties of microalgae extracts – The case of Nannochloropsis oculata. Rev. Clin. Pharmacol. Pharmacokinet., Int. Ed. 2022, 36, (2-3), 31-36.

https://doi.org/10.5281/zenodo.10211529

Received: 27 July 2022
Accepted: 04 August 2022
Republished: 27 November 2023

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S u m m a r y: *Microalgae are an exceptional source of bioactive compounds which during the recent years have found applications as active ingredients in cosmetic products. The aim of this study was to investigate the possibility of using microalgae extracts in cosmetic formulations. For this purpose, the sun protection factor (SPF) of Tisochrysis lutea, Nannochloropsis oculata, Isochrysis galbana, Dunaliella salina, and Chlorella minutissima extracts were evaluated in vitro. Additionally, the effect of Nannocloropsis oculata (NO) extract on skin barrier and hydration was also assessed on healthy volunteers.*
The results suggested that the maximum SPF (~30) was achieved by Tisochrysis lutea and Chlorella minutissima extracts. Medium SPF (~20-25) was calculated for Isochrysis galbana and Dunaliella salina ex-tracts, while Nannochloropsis oculata extract did not exceed SPF 12,5.
The in vivo study showed that the incorporation of Nannochloropsis oculata extract in a cream did not contribute to the improvement of the disturbed skin barrier, however it caused a significant increase of skin hydration at least one hour after its application.
In conclusion, these findings suggest the ability of NO extract to affect some basic features of skin properties, offering new insights into the beneficial role of bioactive microalgae compounds in cosmetic formulations.

INTRODUCTION

Sustainable raw materials derived from natural resources are gaining considerable attention, especially in the cosmetic industry [1]. One of the most promising sources for exploration as novel ingredients for cosmetics is marine environment [2]. Its wealth of plants, animals and

microorganisms, may provide ingredients with potent activities, for the treatment of the skin [3,4]. Microalgae are photosynthetic microorganisms that can grow rapidly and survive in different environmental conditions of either fresh or sea water [5]. Microalgae have emerged as an alternative source of novel bio-compounds such as pigments, proteins, lipids, carbohydrates, minerals, vitamins, among others, that can be employed in pharmaceuticals, cosmetics and dietary supplements [1-3,6,7].

Among other interesting species, *Chlorella, Spirulina, Nannochloropsis, Nostoc*, and *Dunaliella* are the most studied microalgae that have been used in cosmetics [3,8]. Due to their composition that comprises polysaccharides, carotenoids, phycocyanobilin, chlorophyll, vitamin E, among others, they have found various applications such as UV filters, tanning products, anti-aging agents, moisturizers, whitening agents, hair care agents, natural colorants, deodorant products, toothpastes, or hygiene products [1,3,4,8-10]. Moreover, they have been employed in prevention or repair of dermatologic emergencies that relate with the disruption of the skin barrier, seborrhea, inflammation, or wounds [3,11].

In compliance with the European Regulation (EC) No. 1223/2009, the formalization and marketing of a relevant cosmetic product require conducting stability studies as well as toxicological studies to ensure the safety and efficacy [4]. Efficacy claims of cosmetic products usually arise from various investigation types (*in vivo, ex vivo*, *in vitro)*, aiming to measure and assess the efficacy, the formulation, and their basic characteristics [12].

The aim of this study was to investigate the potential of extracts of *Nannocloropsis oculata* (*NO*) as ingredient of cosmetic formulations. For this purpose, its ability to act as a UV-filter was assessed *in vitro* and compared to the performance of other microalgae extracts. Additionally, a cream containing NO was prepared and its effect on skin parameters such as skin hydration and skin barrier, was also assessed *in vivo* using non-invasive methods on healthy volunteers.

PREPARATION OF MICROALGAE EXTRACTS

The biomass of microalgae *Tisochrysis lutea, Nannochloropsis oculata, Isochrysis galbana, Dunaliella salina*, and *Chlorella minutissima* was cultivated and extracted as previously described [13, 14].

IN VITRO EVALUATION OF SUN PROTECTION FACTOR OF EXTRACTS (SPF) OF MICROALGAE EXTRACTS

The sun protection factor (SPF) indicates the effectiveness of a sunscreen cream against UVB rays [15]. The SPF of microalgae extracts were estimated *in vitro* by measuring the absorption of microalgae extract solutions (6%, 2%, 1.5%, 1%, and 0.5%), using UV-Vis Spectrophotometry. Then, the SPF of each sample was calculated using the Mansur equation (1):

$$\mathrm{SPFspectrophotometer} = \mathrm{CF} \times \sum_{290}^{320} \mathrm{EE}\,(\lambda) \times \mathrm{I}(\lambda) \times \mathrm{Abs}\,(\lambda) \quad (1)$$

Where, CF is a correction factor equal to 10, EE (λ) is erythematogenic effect of radiation with wavelength λ and Abs (λ) is spectrophotometric absorbance values at wavelength λ. The value of EE (λ) × I (λ) is constant. The higher value of the SPF indicates the higher efficacy of a product in preventing sunburn [15].

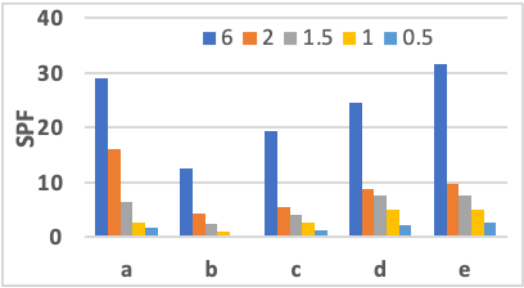

Figure 1: SPF of extracts of Tisochrysis lutea (a), Nannochloropsis oculata (b), Isochrysis galbana (c), Dunaliella salina (d) and Chlorella minutissima (e) at 6% (—), 2% (—), 1.5% (—), 1% (—) and 0.5% (—) concentration.

The SPF was proportional to the extract concentration (Figure 1). The highest value (~30) was achieved by *Tisochrysis lutea* and *Chlorella minutissima* extracts. Medium SPF value (~20-25) was observed for *Isochrysis galbana* and *Dunaliella salina* extracts, while *Nannochloropsis*

oculata extract did not exceed SPF 12,5. At the lowest tested concentration, the SPF value did not exceed SPF 3 regardless the extract.

FORMULATION OF THE FINAL COSMETIC PRODUCT

Nannochloropsis oculata (NO) extract was formulated in an o/w emulsion (ACNO 2%) resulting in a homogeneous cream of light green color comprising 2% NO. The same emulsion without the extract was prepared as control (AC). The stability of both creams as tested by centrifugation (@ 6000 rpm, 20 min) and storage at room temperature was found adequate.

EFFECT OF THE FINAL COSMETIC PRODUCT ON SKIN PARAMETERS

Non-invasive methods were used to study the effect of ACNO 2% or AC on skin characteristics after the application of each cream on the volar forearm of 10 healthy volunteers (Figure 2 a, b). Skin barrier function and skin hydration were assessed by measuring the Transepidermal Water Loss (TEWL) and the water content of stratum corneum respectively. The study design followed the *in vivo* model proposed by Wolf et al., with modifications [16]. Ten randomly chosen healthy women between 24 and 54 years of age participated in the study after giving their informed consent. Criteria for inclusion were a healthy skin state and age between 18 and 55; exclusion criteria were a skin model with burns, wounds or thick hair growth, history of skin diseases or allergies, and recent use of medicines or confirmed pregnancy. The participants were consulted not to use skin care products, especially those containing moisturizing ingredients and to avoid water-contact in the test areas before measurements.
A control (untreated) and three treated sites were attributed at random to each volunteer.

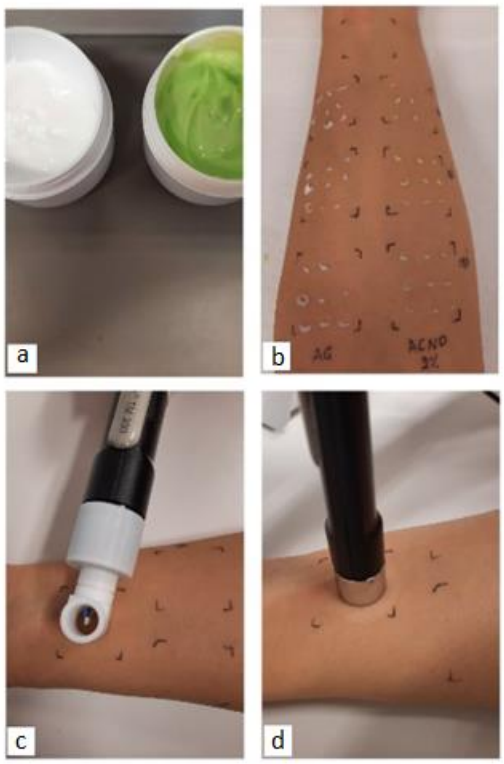

Figure 2: Illustration of the measuring process on healthy volunteer's skin: (a) the tested formulations, AC (left) and ACNO 2% (right), (b) application of the samples to the specified areas of the skin, (c) measuring TEWL and (d) skin hydration measurements.

Before each measurement the volunteers were allowed to acclimatize for 20 minutes under ambient conditions (temperature: 22 °C ± 1 °C and relative humidity 50-55%). The base line measurements of transepidermal water loss (TEWL) and stratum corneum (SC) hydration were assessed using a Tewameter TM 300 (Courage & Khazaka, Germany) and a Corneometer CM 825 (Courage & Khazaka, Germany), respectively (Figure 2 c, d). Immediately after, the skin barrier was disrupted by rubbing the skin with a cotton bud impregnated with acetone and the skin characteristics were measured to confirm the disruption.
Then, the creams were spread out in a uniform way on the marked areas gently rubbing with a fingertip wearing rubber glove, in a dose of 2 mg / cm^2.

Measurements were conducted on intact skin - before the sample application (Tb), after

disrupting the skin barrier with acetone (Ta), and at predefined time points (T30min, T60min and T120min) after the application. The untreated skin area was also monitored. The TEWL was measured, and skin hydration measurement followed, after removing the hydrolipidic film from the surface of the skin by tapping gently a dry and soft paper on the site. The results of each time point were obtained after calculating the change from the initial value (ΔTEWL, ΔHydration) and subtracting the results of the untreated from the results of the treated areas (normalized values) to eliminate the biorhythm fluctuations.

TEWL value increased by the acetone application, confirming the disruption of the skin barrier. Both samples presented a significant increase of TEWL at 60 min ($p < 0.01$ for ACNO 2% and $p < 0.05$ for AC, respectively), while the incorporated NO extract contributed to the maintenance of that increase for at least 120 min ($p < 0.05$) (Figure 3a).

Skin hydration decreased by the acetone application, which confirm an inverse relationship between TEWL and skin capacitance [16-18]. Normalized hydration values, indicate that the incorporated NO extract increased the skin hydration by 35.5% at the first 30 minutes ($p < 0.05$). However, this effect did not maintain at the same levels after 120 minutes of application (Figure 3b).

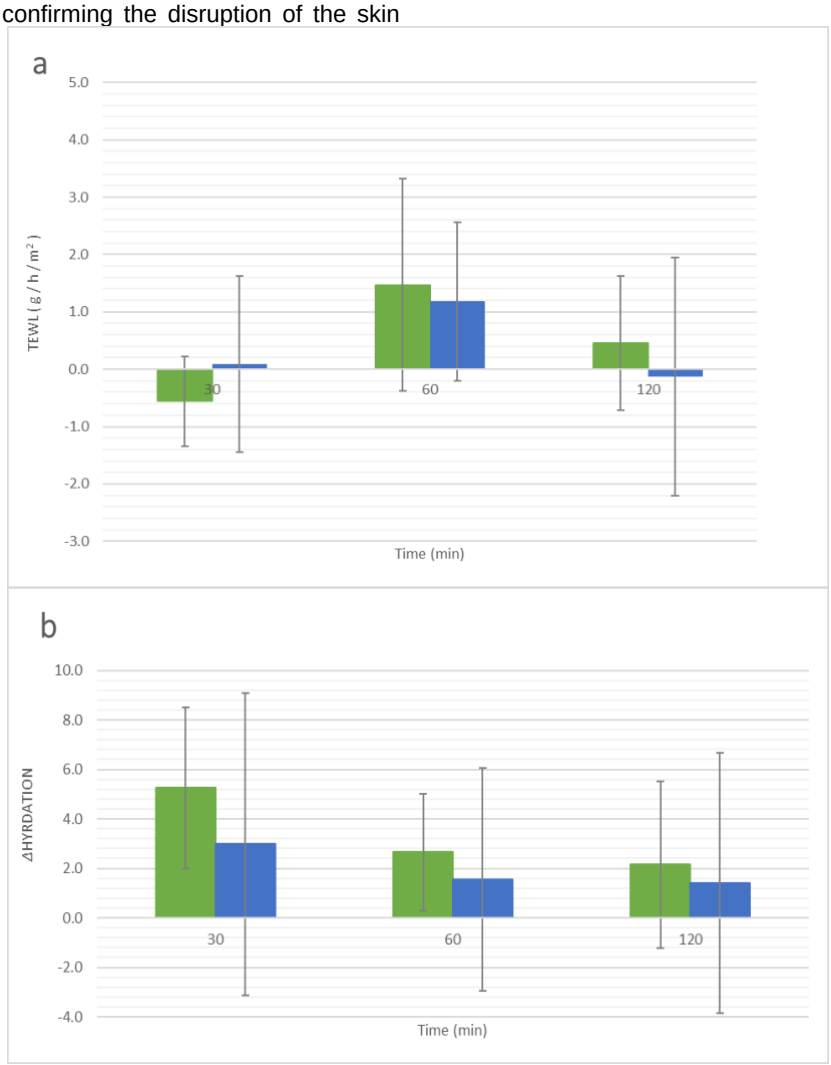

*Figure 3: Normalized values of TEWL (**a**) and skin hydration (**b**) after AC ( ) or ACNO 2% ( ) application over time.*

DISCUSSION

Microalgal-derived ingredients for cosmetics have gained significant attention due to their interesting composition. Although multiple reports can describe various microalgal bioactivities, this field can still be considered as underexplored especially for cosmetic purposes [3,17-20].

As reported in the literature, there is a wide range of microalgae extracts with promising *in vitro* results of their photoprotective activity. The main ingredients of these extracts that contribute to this action are carotenoids (astaxanthin, β-carotene, lutein), pigments such as chlorophylls, phenolics and mycosporine-like amino acids (MAAs) [3,21,23]. Our results support that SPF is concentration-dependent, as confirmed by the research of He and the co-workers [24]. Ariede and co-workers, in their review article reported that the *Nannochloropsis* algae was effective against both UVA and UVB radiation [10]. However, our results indicated low protection against UVB radiation since *in vitro* calculated SPF of NO extract at the max tested concentration, was below 15. This result may be justified by the chemical composition of this extract as reported by Gkioni and the co-workers [14].

The design of commercial skin care products (creams, gels, sunscreens etc.) is always based on “claim support” tests, including skin protection, skin hydration, reduction of fine lines and wrinkles, or repairing / relieving signs of sun damage, among others. Thus, it is important that topically applied products maintain the skin’s epidermal barrier properties.

The main function of skin barrier is the regulation of the flux of various endogenous and exogenous agents. Healthy skin prevents excessive water loss to the external environment, which in turn correlates with a low TEWL measurement.

High level of SC hydration is also necessary for the effective skin barrier function. Low skin hydration may lead to skin irritation, and this automatically leads to the product exclusion from the market [25].

In this small study, skin on the forearm of healthy human volunteers was treated by cream containing an ethanolic extract of the strain *Nannochloropsis oculata*. Non-invasive techniques were used to evaluate basic markers of epidermal barrier function, including TEWL, and skin hydration.

According to our results, we confirmed that the TEWL measured in the sites treated with the control cream and the microalgae cream were significant different from those obtained in the untreated control. However, the literature supports that those changes of TEWL are not indicative of negative effect on skin’s barrier function as it may act as a penetration enhancer facilitating the penetration of actives ingredients in the skin [25]. On the other hand, the SC hydration measurement revealed that there was no difference between the controls while the microalgae cream offered a significant skin hydration boost. The lipid content of NO ethanolic extract may be responsible for the moisturizing effect on skin [14, 26]. Thus, the cosmetic product containing the NO extract could be further investigated, providing a good insight into the biocompatibility of this material.

CONCLUSION

The effects of microalgae in health and nutrition products are worldwide studied, while there is a need to further explore their cosmetic applications.

Tisochrysis lutea, Chlorella minutissima, Isochrysis galbana & *Dunaliella salina* extracts could be further studied for their utilization as potent raw materials for the development of sunscreen formulations. On the other hand, *Nannochloropsis oculata* extract could be utilized as an ingredient of a tanning product due to its low SPF value. Our research also revealed the impact of the incorporated NO extract on skin properties, such as hydration, offering new insights into the utilization of microalgae in the cosmetics industry.

In conclusion, taking into consideration the increased demand, worldwide, for natural skin care products, microalgae may be a significant source of ingredients with beneficial role for skin health. Therefore, further investigation of these ingredients and their uses should be promoted.

FUNDING

This research has been co-financed by the European Union and Greek national funds

through the Operational Program "Competitiveness, Entrepreneurship, and Innovation 2014–2020 (EPAnEK)" in the context of the project entitled "Production of phenolic compounds from microalgae and characterization of biomass residue for utilization in fish farming facilities (AlgaPHESH, MIS 5045802)".

Conflicts of Interest: The author declares no conflicts of interest regarding the publication of this paper.

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