Communication

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Exploiting the Potential of *Ankistrodesmus braunii* Polysaccharides: A Focus on Cutaneous Biocompatibility

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Abstract: Current demands for sustainable cosmetic sources containing naturally derived compounds have led to extensive research for promising microalgae strains for large-scale cultivation. Given the enormous variety of microalgae strains, as well as their diverse cosmetic potential, here, we cultivated *Ankistrodesmus braunii* in tubular photobioreactors to extract polysaccharides (PSs) aiming their incorporation into a dermocosmetic prototype gel. In vitro antioxidant activity was determined by the DPPH• (2,2-diphenyl-1-picrylhydrazyl) assay, and the in vivo experiments were performed on participants., being the skin superficial (SK) hydration and transepidermal water loss (TEWL) measured to determine cutaneous biocompatibility, i.e., the PS safety. *A. braunii* aqueous PS had superior performance through the DPPH• test, thus, it was incorporated in a gel prototype, and its safety profile was established by the cutaneous biocompatibility assay. The biocompatibility of the samples was confirmed by the SK hydration and TEWL results, which were not affected after the application of the gels. Our results suggested not only the biocompatibility of the microalgae PS dermocosmetic, but also the in vitro antioxidant potential, confirming this material's conceivable as a new ingredient in cosmetic formulations.

Keywords: skin biocompatibility; in vitro antioxidant activity; gel; corneometer; tewameter

1. Introduction

Consumers increasingly demand highly effective cosmetic products. Aging-related skin concerns further necessitate the regular use of dermocosmetics tailored to specific skin needs. Moreover, recent advancements in cosmetic science have highlighted the importance of incorporating novel, sustainable ingredients derived from natural sources. Our research group has identified several such compounds from natural origins with potential applications in dermocosmetic formulations [1–3]. The diversity of microalgae offers opportunities to obtain several kinds of compounds with high potential for application in dermocosmetics. For instance, pigments were well-described due to their antioxidant potential [4–6]. Furthermore, lipophilic and hydrophilic compounds with high value-added, such as structural polysaccharides (PS) extracted from microalgae biomass and extracellular polymeric substances, have revealed practical applications due to the rheological properties, as well as to many biological activities, for instance, protecting cells from oxidative damage [7–9].

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A few species from the *Ankistrodesmus* genus (Chlorophyceae) were successfully cultivated for biodiesel production due to the lipid content [10–12]. However, it should be noted that some nutrient and environmental conditions, such as pH, CO_2 level, temperature, and light intensity, are the main factors behind changes in biomass composition during photosynthesis. For instance, carbohydrates and lipids are the central competing pathways for the biosynthesis of storage products in microalgae species. Thus, carbohydrates are an important raw material in fermentative biofuel production [13]. Additionally, phototrophic, mixotrophic, and heterotrophic were tested as mode cultivations and monoculture or mix-culture of *Ankistrodesmus* sp. with *Chlorella sp.* [14]. Moreover, the presence of extracellular polymeric substances from *Ankistrodesmus falcatus* var. *acicularis* [9].

A. braunii has been successfully cultivated in tubular photobioreactors [15], and has been probed to produce carbohydrates for dermocosmetic applications [16]. This species of microalgae seems to be a proper candidate to extract compounds to be incorporated into innovative formulations to enhance cosmetic attributes. Thus, we aimed to cultivate *A. braunii* to extract polysaccharides to be incorporated into a dermocosmetic prototype. In vitro assay of the antioxidant activity was conducted, and the in vivo cutaneous biocompatibility was performed to investigate the safety of a prototype gel containing the *A. braunii* polysaccharides.

2. Materials and Methods

2.1. A. braunii Cultivation, Biomass Composition, and Polysaccharide Extraction

Ankistrodesmus braunii UTEX 245 sourced from the University of Texas Culture Collection (UTEX), was maintained at 25±1°C in agar medium. A small aliquot of this culture was used to inoculate 10 units of 10 mL glass tubes, each containing 5 mL Bold medium [17] securely sealed. Fluorescent lamps provided light at the intensity of 60 µmol photons $m^{-2}.s^{-1}$. A rotatory shaker (Multitron, Inforts Ht, Switzerland), at 25±1°C with the inoculum tubes, was maintained at 110 rpm for 25 days. Subsequently, a portion of this biomass was exposed for 30 minutes to artificial UVC radiation in Petri dishes. Thus, 10% of these two different biomasses were used to inoculate *A. braunii* in 500 mL Erlenmeyer flasks containing approximately 200 mL Bold medium at the same conditions in the batch mode. An airlift system was employed to ensure circulation of the culture, maintained by an air pump with a flow rate of 40±1 $L.h^{-1}$. The culture pH was maintained at 7.0±0.2 and continuous light intensity was regulated at 60 µmol photons $m^{-2}.s^{-1}$. Cultivation temperature was fixed at 25±1°C. Batch mode with 20 mM $NaNO_3$ was used for the cultivation experiments. Biomass was harvested at the maximum biomass concentration (Xm) by centrifugation. Dried biomass was lyophilized and then frozen for further analysis.

The total amounts of lipids (%), proteins (%), and carbohydrates (%) were determined in the dried biomass from each cultivation, as already described [18,19]. A ratio of freeze-dried biomass/water (1:45) was maintained for 120 min at 90 °C and under constant agitation at 400 rpm. The mixture was filtered using 0.45µm pore size. The filtrate was mixed in a separator funnel with absolute ethanol (1:3) and left in a cold chamber (approx. 4 °C). After the complete precipitation of PS, as white crystals, the mixture was centrifuged. The supernatant was stored for later recovery of the ethanol and the PS solid sample was redissolved in sterile water. After filtration, it was recrystallized using the same initial proportion of ethanol. The suspension was centrifuged, and the crystals were placed in an oven with air circulation at 55°C for 12h [20].

2.2. In Vitro Antioxidant Activity

Antioxidant activity was assessed in vitro via the free radical neutralization assay using the stable free radical DPPH• (2,2-diphenyl-1-picrylhydrazyl) [21]. Concisely, 10 µL of each extract and PS was incorporated into a 990 µL methanol solution of DPPH•. This mixture was incubated for 30 min at room temperature, followed by absorbance readings at 517 nm against a corresponding blank. The in vitro antioxidant activity, SA (%), was calculated for each extract and PS.

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$$SA\ \% = \frac{\left(A_{DPPH} - A_{sample}\right)}{A_{DPPH}} \times 100$$

Equation (1)

Where: SA % is the scavenging activity of the free radical; A_{DPPH} is the absorption of DPPH against the blank, and A_{sample} is the absorption of the extract or the control against the blank. Butylated hydroxytoluene (BHT) was used as the reference substrance. All experiments were performed in triplicate.

2.3. Prototype Formulation

Ammonium acryloyldimethyltaurate/VP copolymer was used as the gelling agent. Two 1.0% *w/w* ammonium acryloyldimethyltaurate/VP copolymer dermocosmetics were prepared: 1) blank gel, and 2) gel with 5.0% (*w/w*) PS from the *A. braunii*.

2.4. In Vivo Trial - Cutaneous Biocompatibility

The study was conducted according to the Declaration of Helsinki, and approved by the Ethics Committee of Universidade Lusófona (protocol code 01/2016).

The evaluation of cutaneous biocompatibility was performed on 14 healthy male and female subjects, with an average age of 31.5±12.6 years. All participants provided oral and written informed consent prior to the study.

The assessment was carried out on the inner forearm, with each participant randomly assigned to a control site (untreated) and two treated sites: one for the blank sample and one for the 5.0% *A. braunii* PS gel. Randomized parameters included the selection of either the left or right forearm and either the upper or lower section of the forearm. A prerequisite washout period of one day was enforced, during which the participants refrained from applying any cosmetic products with moisturizers to the designated area.

Environment conditions were controlled at 21±2 °C with relative humidity between 40–60%. Baseline measurements of superficial skin (SK) hydration and transepidermal water loss (TEWL) were conducted using the Corneometer® CM825 and the Tewameter® TM 300 (CK Electronics GmbH, Köln, Germany). The test formulations were applied under occlusion for 24 hours using Finn Chambers® epicutaneous patches (Epitest Ltd., Oy, Finland). SK hydration and TEWL were assessed again two hours after removing the patches. Results were expressed as ratios of post-application to baseline values to account for individual variability [22,23].

2.5. Statistical Analyses

All the measurements were made in triplicate. The overall results were evaluated by ANOVA. The means were considered statistically significant when $p < 0.05$ (confidence level of 95 %). Tukey's Test was used to compare the means using Minitab® 19 software.

3. Results

3.1. A. braunii Cultivation, Biomass and Polysaccharide Extraction

The composition of the biomass was characterized by a predominance of proteins, comprising 61% of the total mass, followed by lipids at 32%, and carbohydrates making up 6%. The extraction methodology detailed in this study successfully yielded white crystalline substances, achieving an extraction efficiency of approximately 4%.

3.2. In Vitro Antioxidant Activity

Three distinct extraction solvents—methanol, acetone, and water—were utilized to evaluate the radical scavenging activity (SA, %) in both biomass and polysaccharides from *A. braunii*. As illustrated in Figure 1, the aqueous extracts exhibited the highest SA values, followed by acetone

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extracts, and lastly, methanol extracts. Notably, the SA values for the polysaccharides consistently exceeded those obtained for the whole biomass, though they were lower than the control using BHT.

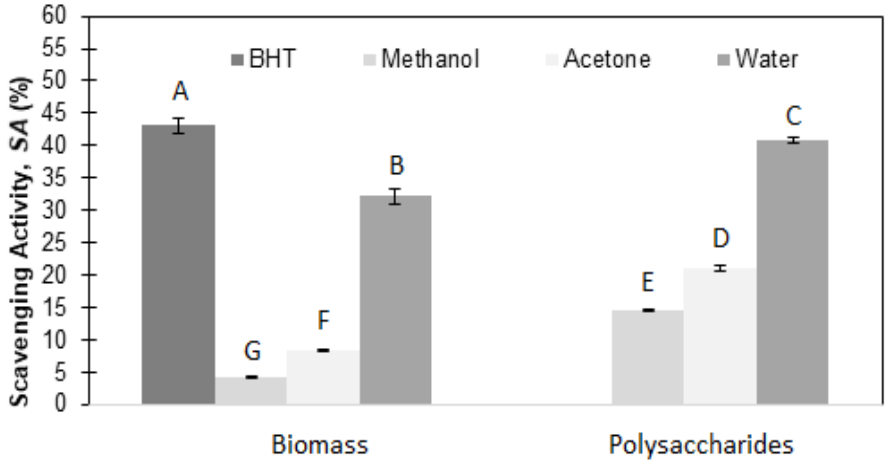
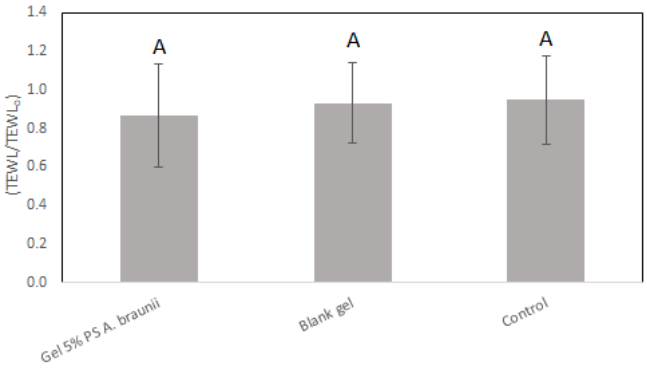


Figure 1. Scavenging activity (SA, %) of biomass and polysaccharides from *A. braunii*. The error bars correspond to the SD (n=3). One-way ANOVA followed by the Tukey test was used for comparison between groups. *P*-values were considered statistically significant when <0.05, being different letters distinct results.

Considering the *A. braunii* aqueous PS superior performance through the DPPH• test, this sample was incorporated in a gel formulation prototype, and its safety profile was established by the cutaneous biocompatibility assay.

3.3. Cutaneous Biocompatibility of A. braunii Gel

Assessment of cutaneous biocompatibility for the *A. braunii* gel was rigorously performed, and the results affirm the gel's suitability for topical application. Upon application of both the blank gel and the gel containing 5.0% polysaccharides from *A. braunii*, there was a sustained maintenance of stratum corneum hydration across all subjects, showcasing the gels' adeptness in preserving skin moisture. Notably, the TEWL measurements depicted in Figure 2(a) further substantiate that neither the blank gel nor the polysaccharide-enriched gel adversely affected the skin's barrier function, as there was no significant increase in water loss compared to the untreated control. This uniformity in hydration and barrier function preservation across different formulations underscores the potential of *A. braunii* polysaccharides in skin care products.



(a)

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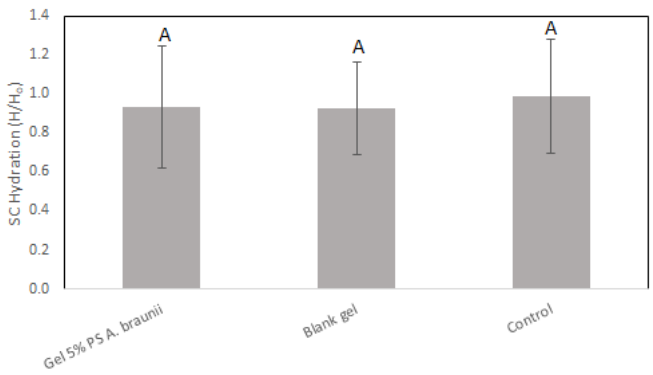

(**b**)

Figure 2. Variation in (**a**) transepidermal water loss (TEWL); and (**b**) stratum corneum hydration after application of hydrogel with 5.0% PS from *A. braunii*. The error bars correspond to the SD (n=14). One-way ANOVA followed by the Tukey test was used for comparison between groups. *P*-values were considered statistically significant when <0.05, being different letters distinct results. PS = polysaccharides.

4. Discussion

Microalgae cultivation in closed system photobioreactors offers more controlled environmental conditions for microalgae development compared to cultivation in open systems, such as open ponds [18]. Polysaccharides from some microalgae species are well described in the literature. However, there are no studies detailing the PS composition of *Ankistrodesmus* sp. [24]. Polar compounds such as PS can be extracted from natural resources using the proper solvent or a mixture of them. Many factors influence extraction yields, such as time, pH, type of solvent, and sample chemical composition, among others [25]. Since most microalgae species contains high level of polar compounds, such as polysaccharides and proteins, this justifies why we reached the highest antioxidant activity using water as a solvent.

Moreover, a study revealed that the role of carotenoids, as antioxidants, is not the unique responsible for the antioxidant activity of microalgae samples. The presence of phenolic compounds can also increase the antioxidant activity values in some microalgae extracts [26,27], and here, we demonstrated the antioxidant activity of the water-soluble PS from microalgae [28]. This protocol is particularly crucial for pinpointing cosmetic ingredients that offer antioxidant benefits. The insights gained from these results critically support the development of dermocosmetic products aimed at enhancing skin defense mechanisms against oxidative stress, thereby ensuring cellular health and integrity. Furthermore, the higher antioxidant activity may be quite relevant when considering incorporating this extract in dermocosmetics, namely for antiaging products, since this strategy may help slow the skin aging process and signs by including compounds that may act as damage defenders.

In vivo skin biocompatibility assays were conducted using non-invasive bioengineering approaches. This methodological approach is crucial for identifying dermocosmetic products that are compatible with human skin, highlighting the importance of such studies in ensuring product safety. These tests not only confirm the mildness of formulations but also their suitability for prolonged contact with skin, thereby supporting the potential of new ingredients for everyday cosmetic use. Superficial skin hydration and TEWL are biological variables that depend not only on the skin condition but also on the aging context [29,30]. Statistical analysis revealed no significant differences in SK hydration and TEWL between control sites and those treated with *A. braunii*-derived PS gels, affirming the dermatological compatibility of the samples. The consistent preservation of SK hydration and TEWL post-application, mirroring control site values, underscores the efficacy of the compounds in maintaining skin homeostasis. These results indicate that *A. braunii* polysaccharides, when applied topically, effectively support the skin's barrier functions without disrupting normal

skin physiology, which is crucial for protecting against pathogens and preventing moisture loss [31][32].

Furthermore, the lack of adverse effects on SK hydration and TEWL parameters strengthens the safety profile of these compounds, making them ideal candidates for inclusion in dermocosmetic formulations. This not only ensures that the final products are safe for extended use but also contributes to the sustainability of the formulations, using natural ingredients that are effective without being invasive to the skin [33].

Therefore, the collected data provide a solid foundation for recommending these microalgae extracts in the development of new skin care products, particularly those aimed at hydration and barrier protection [34].

5. Conclusions

Herein, the *Ankistrodesmus braunii* species was cultivated in a closed photobioreactor system and polysaccharides were extracted and incorporated into a dermocosmetic prototype. The in vitro antioxidant scavenging activity indicated that the aqueous PS showed the highest SA values, even compared to biomass, unveiling the relevance of the water-soluble compounds. Consequently, a gel containing 5.0% *w/w* PS from *A. braunii* was prepared, and the cutaneous biocompatibility of this sample was demonstrated through in vivo skin superficial hydration and transepidermal water loss experiments performed in participants.

Overall, there has been an increasing interest in using microalgae as sustainable products to be included in dermocosmetics due to the valuable properties that arise from the multiple compounds that can be extracted from these species. Nonetheless, much is still to be done due to the vast variety of microalgae strains. This investigation encourages the development of dermocosmetics derived from microalgae, considering, for example, the safe profile of the polysaccharides from *A. braunii*, and their antioxidant potential.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Universidade Lusófona's Ethics Committee of School of Health Sciences and Technologies (protocol code 1/2016, 7 December 2016).

Informed Consent Statement: Written informed consent has been obtained from the participants of this investigation.

Data Availability Statement: Dataset available on request from the authors.

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Conflicts of Interest: The authors declare no conflicts of interest.

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