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Exploring the antioxidant potential of lavender and grape in cosmetics

Bachelor's Project in Biotechnology

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Grado: Biotecnología

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Abstract

It has been shown that skin exposure to oxidative stressors overwhelms the tissue defense and damages the cells that compose it. For this reason, the cosmetic industry has been focusing on the search for active compounds that counteract this harmful effect on our health, thus discovering polyphenols. Polyphenols, as secondary metabolites, shield plants that produce them, like lavender or grapevine, from oxidative stress. Additionally, there are other reasons why these two components arouse promising interest, in the case of lavender the relaxing properties. Moreover, the sustainability of obtaining antioxidants from grapes using by-products of wine production aligns with the Sustainable Development Goals related to economic growth and responsible consumption. This study aims, first, to evaluate the antioxidant capacity of lavender and grapes through various methodologies by analyzing their effectiveness in cosmetics. Furthermore, as a second objective, it aims to formulate a light emulsion incorporating different extracts of the active ingredients and study how its composition affects the sensory perception of the final product.

Keywords

Antioxidants; Lavender; Grapes; Polyphenols; Liposomes; Emulsion

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Investigación del potencial antioxidante de la lavanda y las uvas en cosmética

Resumen

Se ha demostrado que la alta exposición de la piel a agentes oxidantes satura la defensa del tejido y daña las células que lo componen. Por este motivo la industria cosmética se ha centrado en la búsqueda de compuestos que contrarresten este efecto perjudicial para nuestra salud, descubriendo así los polifenoles. Los polifenoles son metabolitos secundarios que protegen del estrés oxidativo a las plantas que los producen, como por ejemplo la lavanda o la vid. Además, existen otras razones por lo que estos dos componentes suscitan un interés prometedor como las propiedades relajantes en el caso de la lavanda. Asimismo, la sostenibilidad de obtener antioxidantes de las uvas a partir de subproductos de la producción vinícola se alinea con los Objetivos de Desarrollo Sostenible relacionados con el crecimiento económico y consumo responsable. En este estudio se pretende evaluar la capacidad antioxidante de la lavanda y las uvas a través de diversas metodologías para analizar su efectividad en cosmética. Por otra parte, como segundo objetivo, se pretende formular una emulsión ligera incorporando diferentes extractos de los principios activos y estudiar como afecta su composición a la sensorialidad del producto final.

Palabras clave

Antioxidantes; Lavanda; Uvas; Polifenoles; Liposomas; Emulsión

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Abbreviations

UV: Ultraviolet

SC: Stratum corneum

PGE-2: Prostaglandin E-2

ROS: Reactive oxygen species

SASP: Senescence-associated secretory phenotype

NF-kB: Nuclear Factor kappa-light-chain-enhancer of activated B cells

Nrf2: Nuclear erythroid 2-related factor

GST: Glutathione S-transferase

HO-1: Heme oxygenase-1

NQO1: Quinone reductase NAD(P)H

ARE: Antioxidant-response element

IL-1β: Interleukin 1-beta

TNF-α: Tumor necrosis factor

RESV: 3,5,4'-trihydroxy-stilbene, resveratrol

TEWL: Trans-epidermal water loss

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid

TEAC: Trolox Equivalent Antioxidant Capacity

TE: Trolox equivalents

TPC: Total polyphenol content

GAE: Gallic acid equivalents

EE: Encapsulation efficiency

PTFE: Polytetrafluoroethylene

Introduction

Skin

The skin is the largest organ of the human body. It is a complex organ covering the entire body surface, protecting the body from the environment by acting as a physical barrier. It performs several indispensable functions for our system's correct operation, such as preventing losses of water and electrolytes, reducing the penetration by chemicals, protecting against microorganisms, and regulating the body temperature.

Skin is organized into three compartments: the epidermis, the dermis, and the subcutaneous fat tissue (Papaccio et al., 2022). Providing a protective barrier against microbes, environmental pollution, and ultraviolet (UV) radiation is the main function of the epidermis. The epidermis is a stratified squamous epithelium that contains mostly keratinocytes. As the keratinocytes rise to the skin surface they differentiate progressively to form the corneocytes, which form the superficial part of the epidermis, the stratum corneum (SC) (A.Puillot et al., 2008). Melanocytes are another type of cell located at the basal layer of the epidermis. They are derived from neural crest cells and are responsible for the production and distribution of melanin. This pigment ensures the absorption of a broad spectrum of solar irradiation and consequent protection from UV radiation.

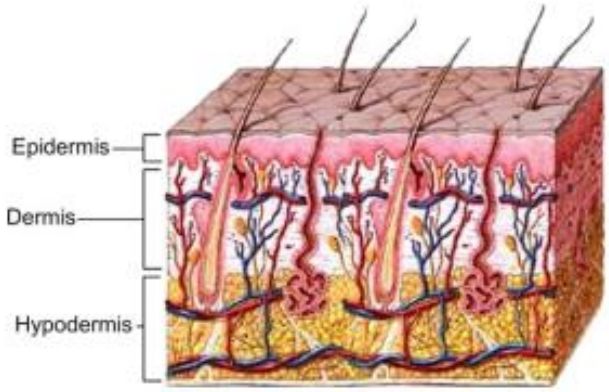

*Figure 1. The 3 compartments of the skin: the epidermis, the dermis, and the subcutaneous fat tissue (*Khavkin & Ellis, 2011).

Aging and UV radiation

Skin aging occurs by extrinsic and intrinsic processes. Intrinsic aging is an inevitable process in the skin by genetic determination. During this process the skin experiments with laxity and expression lines. Contrariwise, extrinsic aging is generated by external factors. As the most harmful factor is sun exposure, this process can also be called photoaging. Photoaged skin is characterized by elastosis, which means the elastin material accumulation below the

dermal-epidermal junction. Also associated are epidermal atrophy and fragmentation of collagen and elastic fibers (Khavkin & Ellis, 2011).

Due to its critical location, the SC is a major interface between the body and the environment, which entails constant exposure to air pollutants, chemical products, and most important, chronic exposure to solar ultraviolet (UV) radiation. The solar spectrum is formed by three segments according to the wave length of the radiation: short wave (UVC; 200-290 nm), middle wave (UVB; 290-320 nm), and long wave radiation (UVA; 320-400 nm).

UVC radiation normally does not reach the earth's surface, is the most energetic radiation, and is mostly absorbed by the atmospheric ozone layer. Despite of this, the radiation that is able to penetrate the atmosphere can penetrate the skin to a depth of 60 to 80 μm and damage DNA molecules. UVB radiation is mainly responsible for some skin diseases, for instance, non-melanoma and melanoma skin cancers. This middle range of radiation can penetrate the skin to a depth of 160 to 180 μm, crossing the whole epidermis and penetrating the dermis. Lastly, UVA radiation is considered aging radiation, since penetrates deeper into the skin, to a depth of 1000 μm, inducing the generation of singlet oxygen and free radicals. Contrariwise to UVC or UVB, this largest spectrum of solar UV radiation barely can excite the DNA molecules directly, the mutagenic action is mediated through the reactive oxygen species (ROS) that it generates (Nichols & Katiyar, 2009). These last years the danger related to the UV radiation has increased since the stratospheric ozone depletion, that enhance the risk of photooxidative skin damage (Biesalski et al., 1996).

From the total amount of oxygen present in the atmosphere, 5% produces these ROS metabolites, which are extremely reactive (The increase in the production of ROS and other oxidants that exceed the antioxidant capacity is known as oxidative stress. This oxidative stress produced by exposure to UV light can accelerate the acquisition of the aged phenotype. Thickness of the epidermis, irregular pigmentation, and dermal connective tissue damage are some effects of premature photoaged skin. Skin UVB exposure activates some signaling pathways responsible for the secretion of inflammatory mediators (interleukins, leukotrienes, and prostaglandins). Some inflammatory molecules, such as Prostaglandin E-2 (PGE-2), are highly related to the development of skin damage associated with aging. Additionally, UVA rays can penetrate deeply into the dermis, generating ROS that increase the mutagenic risk caused by UVB exposure, potentially leading to precancerous lesions and skin cancers Nakai et al., 2021).

As explained above, the interaction of UVA radiation with the skin induces the production of ROS. These metabolites, also called activated oxygen, are usually superoxide, hydrogen peroxide, hydroxyl radical, and singlet oxygen. The impact of ROS on skin cells has been deeply investigated at a molecular level by Wenk et al. (2000). To understand their conclusions it is necessary to clarify the concept of photoaging in physiologic terms. Photoaging skin shows an increase in the deposition of glycosaminoglycans and residual elastotic material in the deep dermis which is translated into the accumulation of tropoelastin and its associated microfibrillar component fibrillin (Werth et al., 1996). Fibrillin has recently been reported to be a target for the matrix metalloprotease proteolytic attack (Ashworth et al., 1999). ROS have been reported to enhance tropoelastin mRNA levels and also to impact collagen metabolism. They destroy interstitial collagen by gene expression

changes, inactivate the tissue inhibitors of metalloproteases, and the ROS UVA generated produces a common deletion mutation of mitochondrial DNA, activating the transcription of matrix-metalloproteases-encoding genes. This is an interesting aspect to know because it can provide useful information that allows the design of target molecules that can avoid these reactions.

Cellular senescence is an important process that contributes to age-related tissue dysfunction to take into account. Senescent cells secrete a diverse group of molecules such as pro-inflammatory cytokines, chemokines, growth factors, lipids, and proteases in a phenomenon called the senescence-associated secretory phenotype (SASP). During aging, the accumulation of senescent cells takes place in the dermis and epidermis, and cellular perturbations such as DNA damage by external factors , UV radiation for example, can induce and accelerate this accumulation. One cellular interaction that is altered during aging is the binding of NF-kB to nuclear DNA. NF-kB is a transcription factor with a main role in the production of SASP, so an improvement of NF-kB-DNA interaction is related to age-related inflammatory disorders.

Antioxidant mechanism of the skin

Under normal conditions, the skin is protected against oxidative stressors by different mechanisms. Melanin is a skin pigment that acts as the first defense against UV radiation. As mentioned before, this pigment is synthesized in the melanocytes, which transports them in granular form. Melanin blocks UV radiation and dissipates it as harmless heat. Moreover, under the proper stimulus of UV radiation, the rate of pigment formation increases in the cells, and already existing pigment appears to darken, providing additional protection. Another mechanism is the keratinocytes apoptosis, that usually happens in the majority of keratinocytes in sunburn, preventing malignant transformation (Mohania et al., 2017).

On the other hand, the skin has some natural antioxidant mechanisms which could be enzymatic and non-enzymatic. Non-enzymatic antioxidants include vitamin C, vitamin E, and β-carotene. Meanwhile, one of the crucial antioxidant enzymes is catalase, which decomposes cellular H_2O_2 into water, mitigating oxidative stress. Another way of protection is the cellular pathways, one of the main players involved in those is the transcription factor Nrf2 (nuclear erythroid 2-related factor). Indeed, Nrf2 is involved not only in the transcription of defensive enzymes such as glutathione S-transferase (GST), heme oxygenase-1 (HO-1) or quinone reductase NAD(P)H (NQO1), but also in keratinocyte differentiation, melanocyte maturation, and fibroblast cell cycle (Kannan & Jaiswal, 2006).

Polyphenols and aging

Despite the various self-defense mechanisms of the body against UV radiation and ROS species, quantities of free radicals can overwhelm the tissue antioxidants, so the cells lose their antioxidant defense and lipid peroxidation occurs, resulting in skin barrier damage, collagen fiber breakdown, promoting chronic inflammation, and finally, skin disorders (Valacchi et al., 2001). Polyphenols are known as a class of natural phytochemicals characterized by their multiple phenol units and aromatic structure, and they can be classified as flavonoids, phenolic acids, stilbenes, and lignans (Matić et al., 2017). Produced by plants as secondary metabolites to protect them from photosynthetic stress, polyphenols possess antioxidant properties, acting as ROS scavengers due to their ability to easily donate one electron to compounds with higher redox potentials. Furthermore, these molecules are strong

chelators of metal ions such as Fe^{2+}, Fe^{3+}, Cu^{2+}, $Zn^{2+,}$ and Mn^{2+}. These ions contribute to redox imbalance also called oxidative stress, which can damage the cell.

Flavonoids, a subgroup mentioned before, are a diverse group of phytonutrients present in fruits with variable phenolic structures containing 15 carbon atoms. They are divided into several subtypes: flavones, flavonols, isoflavones, flavanones, anthoxanthins, anthocyanins, and chalcones. Flavonoids are particularly notable for their strong antioxidant and chelating abilities, which depend on the arrangement of hydroxyl groups in their structure (Sharififar et al., 2009). Moreover, polyphenols can induce many antioxidant and phase II detoxifying enzymes (GST, HO-1, NQO1), by transcriptional activation mediated by Nrf2 through its interaction with the antioxidant-response element (ARE) (Chedea et al., 2017). Another interesting pathway where flavonoids take part is the NF-kB transcription factor metabolism was described by Lim et al. (2015). Some flavonoids have proven to disrupt the activation of NF-kB in vitro. As explained before, the NF-kB transcriptional factor is related to the production of SASP. The disruption of the activation of NF-kB avoids this signaling responsible for SASP production and reduces the inflammatory response. Moreover, flavonoids prevent the increased production of IL-1β and tumor necrosis factor (TNF-α), protecting animals of age-related disorders.

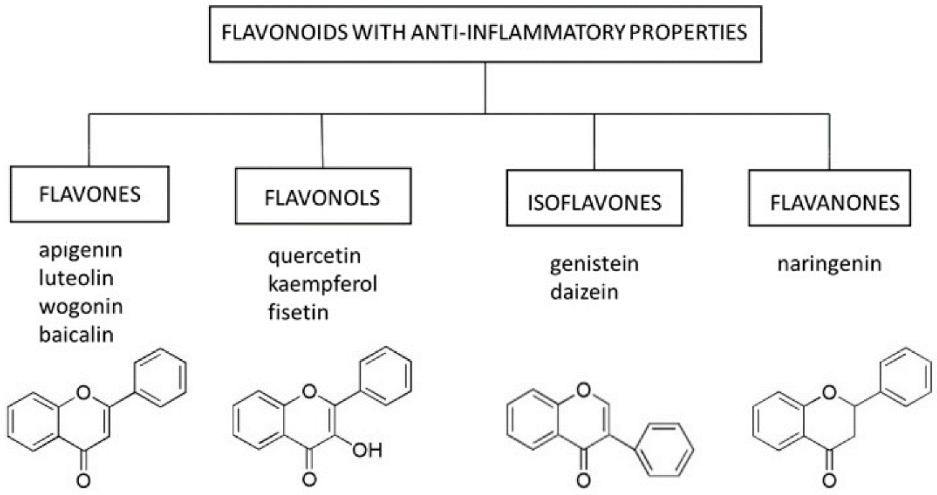

Figure 2. Select flavonoids with anti-inflammatory activity (Domaszewska-Szostek et al., 2021).

Lavender as an antioxidant source

Lavender (*Lavandula angustifolia, Lavandula officialis, Lavandula vera)* is a well-known Mediterranean aromatic plant native to France, Spain, Andorra, and Italy. It belongs to the Lamiaceae family and contains linalool and linalyl acetate as the major oil constituents. It was also reported to contain phenolics, however, there is still a lack of detailed data neither on the polyphenolic content and pharmacological activities (Miliauskas et al., 2004). There is some controversy surrounding the antioxidant properties attributed to Lavender. Some studies indicate a modest antioxidant activity, even less potent than different members within the Labiatae family (Oboh & Henle, 2009). However, Sakurai et al. (2005) propose that since Lavender can inhibit the formation of singlet oxygen, which is responsible for significant

damage induced by UVA/UVB radiation, using Lavender oil regularly on the skin could be advantageous in mitigating the sun-induced aging effects.

Besides, some volatile oils components of this plant as linalool, and terpinol, have remarkable properties related to the relaxation of the central nervous system. That is why lavender is also employed in aromatherapy and massage, the therapeutic inhalation of essential oils can reduce anxiety in humans and animals, and help them to sleep. During sleep, some important processes as the repair of damaged tissue take place (Pandey & Kar, 2018). It has been shown that ROS are generated due to sleep deprivation, which alters body physiology. The nervous system effects of lavender oils could positively enhance antioxidant activity because it can counteract the lack of sleep and anxiety oxidative stress increase (Buchbauer et al., 1991).

Grapes as an antioxidant source

One interesting polyphenol is the stilbene derivative resveratrol (3,5,4'-trihydroxy-stilbene; RESV). Both, *cis* and *trans* configurations can be found in nature, but is *trans*-resveratrol configuration the most interesting in terms of antioxidant protection due to its stability and biologic activity. When sunlight, heat, or UV radiation strikes *trans*-resveratrol it undergoes isomerization to the *cis*-resveratrol form. RESV is synthesized in the leaf epidermis and the grape skin (Creasy & Coffee, 1988).

The initial discovery of RESV was reported by Renaud and Lorgeril (1992), who described the "French Paradox" phenomenon: despite French people having a high consumption of saturated fats, they present a lower incidence of heart diseases compared to the rest of Europe populations, which has been attributed to their increased red wine intake. Furthermore, RESV is also an active ingredient of interest from an environmental sustainability standpoint due to its association with the production of wine from *Vitis vinifera*. This process generates substantial waste and the accumulation of two by-products, generating a high volume of the main source of RESV, particularly from the skins, seeds, and lees of grapes (Spatafora, 2012).

From the cosmetic point of view, this active principle is stimulating too. Recent studies by Alonso et al. (2017) have thoroughly assessed both the "*in vitro"* and "*in vivo"* permeation of topical application of RESV. The results revealed that not only that RESV topically applied penetrate the skin in a gradient manner, but it also after its penetration was able to maintain its antioxidant efficiency. This makes RESV an appealing ingredient for cosmetic formulations, as it offers potential benefits for skin health and real anti-aging properties.

Skin barrier and natural oils

Skin aging also affects the skin barrier function. Because of this, the loss of hydration due to the loss of water accumulation occurs in the SC, allowing the entrance of exogenous and endogenous irritants and allergens. The penetration of this non desired molecules can damage barrier-structural proteins and alter lipid-based structures, exacerbating skin-barrier dysfunction.

Restoring the barrier function significantly prevents trans epidermal water loss (TEWL) and maintains moisture. Moisturizers help to repair the skin barrier by two mechanisms: occluding the skin with hydrophobic constituents to prevent TEWL and using hydrophilic constituents such as glycerol to provide hydration. According to Van Logtestijn et al. (2015) investigation, of topical oil application enhances hydration through occlusion and produces a depth-dependent rise in hydration through the SC. Some moisturizer products may contain allergenic and irritating chemicals, such as fragrances and preservatives. The use of natural alternatives is an alluring alternative for barrier repair.

Plant-based oils are substances constituted mainly of glycerides that are liquid at room temperature due to the high polyunsaturated and monounsaturated fats. Olive oil it has been used for skin care for centuries. González-Acedo et al. (2023) studied the effects of some olive oil compounds on the proliferation of cultured human fibroblasts, that contribute to maintaining tissue integrity and homeostasis in the skin barrier. The conclusion was that the treatment had a beneficial effect, increasing the human fibroblast proliferation, migratory capacity, and expression of key markers in the wound healing process without altering their cell cycle.

Almond oil is also widely used in cosmetic applications and is very well considered by the industry as an excellent carrier oil in skin preparations(Blaak & Staib, 2022). In a study on old skin, an electron microscopy, and SC lipid analysis study showed that the treatment with almond oil has a noticeable increase in lipid density and an enhancement in the intercellular space of the SC (Daehnhardt-Pfeiffer et al., 2012). In other studies, for instance, the Saeed, A.K. (2019) investigation, almond oil has been reported to prevent UV-induced photo-damage in the skins of mice.

The incorporation of these natural oils into the cosmetic formulation as emulsifiers will enhance the moisturizing capacity of the product, enhancing the skin-barrier repairment and helping the antioxidant active ingredients to penetrate and maintain in the skin cells.

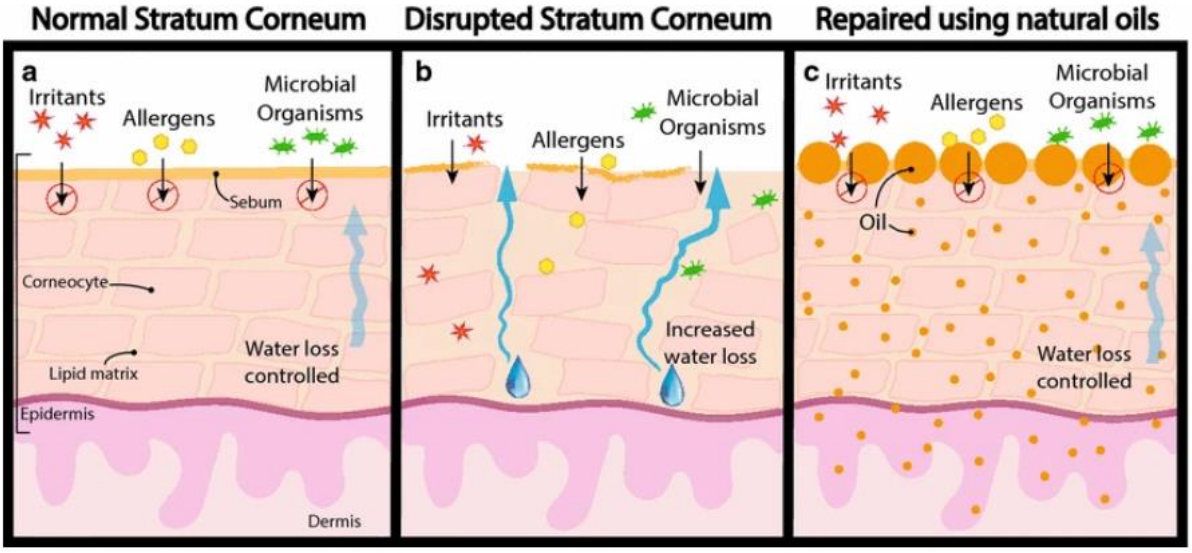

*Figure 3. Skin barrier repair using natural oils. **a** Healthy skin barrier. **B** SC disruption with TEWL. **C** Natural oils repairment.* (Vaughn et al., 2017)

Objectives

In this study, the bibliographic controversy about Lavender will be resolved, by conducting a comprehensive exploration of different extraction methods. The goal is to identify the optimal conditions not only preserve but enhance its exceptional antioxidant activities. Moreover, the analysis of certain variety of grapes will offer new perspectives about the importance of some decisions regarding the polyphenol content and antioxidant activity. Both, lavender and grapes, will be carefully extracted and analysed in the laboratory, to obtain some conclusions from the results.

On the other side, we aim to formulate a cosmetic product that capitalizes on the synergistic benefits of both Lavender and grapes' active principles. The process implies strategically utilizing their distinct characteristics to develop a product that excels in effectiveness and appeal. The use of natural sources as active ingredients has inspired the main goal, profiting from what nature offers to us to take care of our skin, revert the aging process, and avoid diseases.

Finally, an essential component of the study will be the sensory analysis of the final product. This critical evaluation will validate that the active principles derived from lavender and grapes are compatible and integrated into a formulation that not only delivers tangible skincare benefits but also offers an enjoyable user experience.

Materials and methods

Lavender

Lavender extraction

Different amounts of Lavender were considered, resulting in 5g of Lavender with 100 mL of extraction solvent, which were carefully measured in two 100 mL Erlenmeyer flasks. To determine the best extract method, two distinct solvents were employed: hot water and ethanol 60%. Each solvent was chosen for its ability to extract different types of compounds form the lavender plant material.

The flask containing the ethanol sample was brought into the agitator to enhance the extraction process, ensuring mixing and contact between the plant material and the solvent. Meanwhile, the flask with the water sample was subjected to the gentle agitation at a controlled temperature of 38 ºC for 24 hours. This temperature and duration were selected to maximize the extraction of water-soluble compounds from the lavender. Following the extraction period, both solutions were filtrated with cheesecloth filters to separate the liquid extract from the solid plant material residues. Each filtered extract was collected into two separate containers, that were sealed with paraffin to prevent evaporation and contamination.

Antioxidant activity evaluation

Determination of the total antioxidant activity using ABTS.*

This method evaluates the antioxidant sample's ability by measuring its capacity to quench the ABTS cation radical. It is also called the TEAC method (Trolox Equivalent Antioxidant Capacity), as the anti-radical activity of the sample and Trolox are compared. Trolox or 6-hydroxy-2,4,7,8-tetramethylchroman-2-carboxylic acid is a derivative of vitamin E and is used as a synthetic standard substance for comparison, due to its known antioxidant properties. The extinction of the ABTS radical by antioxidants, acting as hydrogen donors, is spectrophotometrically monitored by changes in the absorption spectrum of ABTS.

To initiate the assay, ABTS was dissolved in distilled water at a concentration of 7 mM. The ABTS radical was then obtained by reacting this solution with potassium peroxydisulfate at a concentration of 2.45 mM. The resulting ABTS solution is allowed to stand in the dark at room temperature for at least 12 hours to ensure consistent radical formation. Before use, the stock solution of ABTS was diluted with ethanol until reaching an absorbance of 0,7±0,02 at 734 nm, using a UV/VIS Helios spectrophotometer. Then, only this diluted ABTS solution was used for the subsequent antioxidant capacity measurements.

For the assay, 1 mL of diluted ABTS solution was pipetted into a cuvette; and 10 µL of distilled water was also added as a control. The initial absorbance of this mixture was immediately measured at time 0 using the spectrophotometer. Simultaneously, in another cuvette, 1 mL of ABTS solution and 10 µL of the sample extract being tested were pipetted. This solution is mixed thoroughly, and the timer was started at the exact moment of sample

adding. Both cuvettes were stored in the darkness to prevent further reaction with light. After exactly 10 minutes, the absorbance of both cuvettes is measured again at 734 nm. The decrease in absorbance is measured and recorded at 10 minutes, it is directly proportional to the antioxidant capacity of the sample The results are typically expressed in Trolox equivalents (TE) per gram or milliliter of sample, providing a standardized measure of antioxidant activity that allows for comparisons across different samples and experimental conditions (Peč P. a kol., 2000) (Mikeš V., 1997).

Spectrophotometric determination of total polyphenols

The determination was carried out by spectrophotometric method with the Folin-Ciocalteu reagent. The Folin-Ciocalteu test is used to determine the total phenolic content (TPC). It was originally designed to analyze proteins and later adopted by Singleton et al. (1999) to analyze the phenolic content in wine. The Folin-Ciocalteu reagent, which is believed to contain a complex of phosphomolybdic acid, reacts with the phenolic compounds and is reduced by them in an alkaline environment, resulting in the obtaining of a blue chromophore with maximum absorption at 765 nm.

To begin the procedure, a saturated solution of sodium carbonate was prepared by dissolving 7,5g of $NaCO_3$ in 95 mL of water. Additionally, a diluted solution of Folin-Ciocalteu reagent was made by mixing the reagent with distilled water in a 1: 9. For the assay, 1 mL of diluted Folin-Ciocalteu reagent, 1 mL of distilled water, and 100 µL of extract of the sample extract were pipetted into a graduated cylinder. For the blank sample, 100 µL of distilled water was used instead of the sample extract. Then, the solutions were thoroughly mixed and left to stand for five minutes to allow the initial reaction to occur. Once the time had passed, 1 mL of a saturated solution of sodium carbonate was added to each cylinder, and the solutions were mixed again. The addition of sodium carbonate ensures an alkaline environment for the reaction, which is necessary for the formation of the blue chromophore. The solutions were than allowed to stand for an additional fifteen minutes to complete the reaction. Following this incubation period, the absorbance of each sample was measured at 750 nm using a spectrophotometer, with the blank sample serving as a reference to correct for any background absorption (Lucía Dzurická, 2024).

The absorbance values obtained were used to quantify the total phenolic content of the samples. To do this, a standard curve is needed, which in this case was constructed using gallic acid as the known phenolic compound. Using this curve, the absorbance readings were converted into gallic acid equivalents (GAE). This method provides a reliable measure of the phenolic content in various samples, enabling the comparison of antioxidant potential across different extracts.

Estimation of total flavonoids.

The determination of the total amount of flavonoids in natural extracts was determined by a spectrophotometric method, which involves the reaction between aluminum salt and sodium

nitrite. This method was explained by Kalita et al. (2013), it is based on the formation of acid-stable complexes. These complexes form when the C-4 keto group of flavonoids interact with the C-3 or C-5 hydroxyl group and aluminum chloride. This reaction with aluminum can serve as a method in spectrophotometry analysis.

In each tube, 0,5 mL of sample extract (besides the blank sample, where the extract is replaced with water) was mixed with 1,5 mL of distilled water, and 0,2 mL of 5% sodium nitrite solution was pipetted. The content of the tubes was mixed and allowed to stand for five minutes. Then, 0,2 mL of 10% aluminum chloride solution was added to the tubes, were mixed again, and allowed to stand for another five minutes. Following the five-minute incubation, 1,5 mL of a 1 M sodium hydroxide solution and 1 mL of distilled water were added to each tube. The contents were mixed again, and allowed to settle for fifteen minutes. After the time had elapsed the resulting solutions were analyzed in the spectrophotometer at 510 nm[1718]. The absorbance values obtained were used to quantify the total flavonoid content of the samples. As in the previous procedure, a standard curve was constructed with a known flavonoid compound, specifically with catechin in a concentration range from 5 to 50 ug/ml. This method allows the conversion of absorbance readings into flavonoid concentration equivalents, providing a reliable measure of the flavonoid content in various natural extracts, and facilitating the comparison of antioxidant potential across different samples.

Lavender encapsulation

Lavender encapsulation procedure

Liposomes are colloidal, artificial, and vesicular systems that self-assemble from phospholipids, which form bilayers that surround an aqueous core. These structures can entrail bioactive compounds in their protective matrix. Phospholipids impulsively form closed structures when they are hydrated in aqueous solutions. Encapsulation of bioactive substances can increase the bioavailability of the substances, prevent the risk of accelerated clearance, and avoid allergic problems due to more progressive releasement (Huang et al., 2019)

In this study, two beakers were used for weighing 10 mg of Cholesterol and 90 mg of phosphatidylcholine in each one. To one beaker, 2 mL of lavender water extract was added to one beaker, while the other received 2 mL of distilled water. Subsequently, the beakers were subjected to sonication in a cold bath for one minute, divided into four intervals of fifteen seconds each. This step stimulates the liposome formation and prevents them from disassembling due to high temperatures.

Following sonication, the mixtures underwent two centrifugation rounds. The first one was conducted at 6000 rpm for five minutes to clean the undesirable small substances from the liquid, keeping the liquid phase. The second round of centrifugation was performed at 11000 rpm for one hour. After the expiration of the time, the supernatant was carefully transferred to another tube and stored, with the solid phase one, in the fridge.

This is an efficient method to ensure the encapsulation of bioactive compounds from the plant water extract into the liposomes, enhancing their stability and bioavailability for subsequent cosmetic applications. With the sonication steps and centrifugation rounds, the integrity of the liposomes is maintained.

Evaluation of polyphenol encapsulation

The supernatant obtained after the encapsulation rescued from the second centrifugation, following the encapsulation process, was used to determine its total polyphenol content with the spectrophotometric method previously described. This analysis aimed to evaluate the polyphenol encapsulation efficiency. The encapsulation efficiency (EE%) was determined as follows:

$$EE\ (\%) = \frac{Total\ polyphenol - Free\ polyphenol}{Total\ polyphenol} x\ 100$$

This formula was used to calculate the proportion of polyphenols successfully encapsulated within the liposomes relative to the initial amount of the polyphenols after the extraction of the lavender. The *Free polyphenol* value is the content of polyphenols present in the supernatant after the second round of centrifugation. The higher the *Free polyphenol* value the lower EE%, indicating a less efficient encapsulation process. By obtaining this value, it is possible to optimize the liposome preparation process to maximize the benefits of bioactive compound delivery.

Grapes

Ultrasonic grape's extraction

Resveratrol is well-known for its great antioxidant capacity, and its concentration can influence the overall properties of the grapes. *Vitis vinifera* has been proven to be a highly concentrated source of resveratrol, for this reason, the extraction of this variety could have been interesting to improve the antioxidant potential cosmetic application. However, the *Vitis vinifera* variety also has drawbacks, mainly its limited availability. The market presence of this type of grape is highly limited to the harvest season, so without careful planning, experimenting and working with it can be challenging.

It was not possible to procure wine lees grapes due to the conclusion of the vintage period. Consequently, seedless dark grapes, variety "Ampulia Rosè Variety, Arra19, Flame, Crimson, Alison, Timco, Summer Royal, Autumn Royal, Melody, 1st grade" were employed as substitutes.

Following the crushing of the grapes in a mortar, they were carefully packed into plastic bottles, ensuring only a minimal sample quantity per container. Subsequently, these samples were lyophilized for a week. However, owing to the elevated sugar content of this grape variety, it became necessary to extend the waiting period beyond initial projections. Given the impracticality of crushing in the mortar, the decision was made to proceed with the

extraction process and incorporate additional filtration into the extraction procedure of Dujmić et al. (2020). This ensured that the final extracts were free of any residual compounds that could interfere with the intended applications. Despite the initial setbacks, we decided to continue with the experiment using the seedless dark grape variety to study the impact of the type of grape on the polyphenol content.

Samples were prepared by mixing 200 mL of 50% ethanol solution with 1,5% formic acid. The ratio of dry matter to solvent was maintained at 1:60 in the sample tubes. The containers were then placed into an ultrasonic device. The optimal extraction conditions identified during the study of Dujmić et al. (2020) were replicated, specifically using 60% of amplitude, 1500 seconds, and a 13 mm diameter probe, with the inclusion of a cold bath to prevent solvent evaporation due to excessive heat.

After the designated extraction time had passed, the extract was transferred to 50 mL tubes and centrifuged at 4000 rpm for 15 minutes. Following centrifugation, the supernatant was filtered into new tubes to remove any remaining particulate matter. An essential step in the process involved the removal of formic acid, particularly significant considering the intended use in cosmetic products. To achieve this, vacuum evaporation was employed to eliminate formic acid from the extract. After the formic acid was successfully removed, the remaining extract in the bottle was diluted in ethanol to ensure proper distribution of the active ingredients and stability.

Finally, an additional filtration step was performed using a 0,05 μm PTFE hydrophobic filter to ensure the highest purity of the extract. This final filtration was added, as mentioned before, to compensate for the lack of crushing before the extraction, ensuring the extract's suitability for cosmetic applications.

Antioxidant activity evaluation

To assess the antioxidant capacity of the extraction, the determination of total antioxidant activity was carried out using the ABTS* method. This method, as explained before, involves generating the ABTS* radical cation through the reaction of ABTS with potassium persulfate, which is reduced in the presence of antioxidants. The extent of decolorization was measured spectrophotometrically and is directly correlated to the total polyphenol content. The standard curve employed in the determination of Trolox equivalents from the absorbance values was the same as in the analysis of lavender.

Additionally, the spectrophotometric determination of the total polyphenol content was conducted following the previous procedure, using the Folin-Ciocalteu reagent and measuring the absorption at 765 nm. The standard curve used for the conversion of absorbance values into polyphenol content was the same as in the evaluation of lavender.

Lighter emulsion elaboration with active ingredients

The emulsion base was initially prepared with the final product's quantity set at 50 grams. The emulsifier component was "Hydrogenated Sunflower & Seed Oil Polyglyceryl-3 &

Esters (and) Hydrogenated & Sunflower Seed Oil & Glyceryl Esters & Cetearyl Alcohol & Sodium Stearoyl Lactylate" comprising 6% of the total weight. The vegetable oil selection was: grape seed oil (Vitis Vinifera), olive oil, and refined almond oil (100%) in a proportion of 7, 3,5, and 3,5 % respectively. These oils act as emollient components and were weighted with the emulsion component in one beaker (phase A).

The selection of vegetable oils as emulsifying agents for the cream was made to develop a product suitable for all skin types, including those with sensitive tendencies, to maximize the efficacy of the active principles. Olive oil has some well-known ingredients in the dermo-cosmetic industry such as oleic acid and squalene that contain moisturizing and nourishing properties. Oleic acid is known for its ability to strengthen the skin's natural lipid barrier, keeping it hydrated and protecting it from environmental damage. Additionally, oleic acid has been shown to have antimicrobial activity. Squalene is also a natural antioxidant that further enhances the skin's barrier function and provides additional moisturizing benefits. Moreover, olive oil is rich in other antioxidants, such as vitamin E, which contributes to its anti-inflammatory and anti-aging properties, making it an excellent choice for maintaining skin health and combating oxidative stress (Gorini et al., 2019).

On the other hand, almond oil is notable for its nourishing and emollient properties. As olive oil, it is rich in vitamins E and A, as well as fatty acids like omega-6. These components make almond oil highly moisturizing, and ideal for dry and sensitive skin. This is a non-comedogenic oil, which means that it is lightweight, easily absorbed, and does not clog pores, making it suitable for acne-prone skin. Furthermore, the inclusion of almond oil in the formulation ensures that the cream remains suitable for a wide range of skin types, enhancing its versatility and appeal (Ahmad,2010).

In another three beakers, the three different active principles with water were balanced (phase B), ensuring it was sufficient to reach 100% of the final product, excluding phase A and the preservative to be added later. The three ingredients that were considered active components were lavender water extract, lavender ethanol extract, and lavender encapsulated with liposomes. The ratio of water to the active component was tested in each container, beginning with the 15% concentration of lavender water extract in phase B, 8% for the ethanol extract, and utilizing all the liposomes present in the tube after encapsulation, which were resuspended in water. One phase A per phase B was needed. The lavender water extract sample was also tested at concentrations of 10% and 5%.

Phase A and Phase B beakers were carefully placed within a water bath at an approximate temperature of 80ºC. The oil phase was stirred until the complete dissolution of all the components. Once the temperature was reached, the containers were removed from the water bath. While ensuring continuous agitation with an electric stirrer, the water phase was gradually introduced into the oil phase. Following the addition of the entire volume of the water phase, a cooling water bath was positioned beneath the container, and stirring persisted until the emulsion cooled down (35-25ºC). In the case of the emulsion derived from liposome extraction, to prevent particle disassembly, the active ingredient was directly incorporated into the agitator, avoiding the step of heating to the solvent used for particle resuspension.

After cooling, the product was immediately preserved by incorporating 0,1% of the total weight of Euxyl K 703 as a preservative (phase C). Finally, the final product was stored in small containers sealed with paraffin and preserved it at a refrigerated temperature (Lucía Dzurická, 2024).

Table 1. Ingredients, phases, and proportions required for preparing a lighter emulsion, where "x" represents 8% for the ethanol extract sample; or 15%, 10%, and 5% for water samples. In the case of the emulsion containing liposomes, phase B is composed solely of water, which is utilized for the resuspension of the particles.

Phase	Name	INCI	Function	%
A	Emulsion	Hydrogenated Sunflower & Seed Oil Polyglyceryl-3 & Esters (and) Hydrogenated & Sunflower Seed Oil & Glyceryl Esters & Cetearyl Alcohol & Sodium Stearoyl Lactylate	Emulsifier	6
A	Vegetable oil	Grape seed oil (Vitis Vinifera) Olive oil Refined almond oil (100%)	Emollient	7 3,5 3,5
C	Preservative	Phenoxyethanol/Euxyl	Preservative	0,1
B	Water	Aqua	Solvent	To 100
B	Herbal extract	Aqua, XY Extract	Active component	x

Analysis of the product.

Measurement of the hydration capacity

The measurement of the hydration capacity was performed with CK electronic microscope, using the camera and the Coreofix F20.To begin with, different surfaces in the forearm were delimited with a marker, ensuring the accuracy of the image. A sticky foil was used to collect the dry skin scales (desquamation) of the surface, and the sticking part of the foil was pressed, in the first place, to the dry and clean skin. After five seconds, the foil was carefully removed by peeling it away using the tab.

Then, the side of the foil that had been in contact with the skin was attached to the camera opening, which was held in front of a dark background. The image was freed using the parallel polarized light. This method allowed for precise measurement and evaluation of the skin's hydration levels, so it is a great tool to evaluate the hydration impact that a product have in the skin.

Sensory analysis of the final cosmetic product

The questionnaire for Sensory Analysis of Cosmetic Products was transferred into a Google form, to facilitate the testing evaluation and the results processing. Using this form, the volunteers gave information regarding their attitude toward cosmetic products before proceeding to evaluate three different samples tested in their forearms.

In the first place, the volunteers answered questions regarding its gender, age, skin type, irritation skin tendencies, dehydration problems, and the regularity they use hydrating products. Once their personal information is collected, they proceed to answer the questions related to the samples. First, they evaluate the various emulsions based on standard cosmetic product characteristics such as consistency, shine, spreadability, adsorption, stickiness, hydration, and perfumery. Following, the participants arranged the samples from best to worst according to their preferences. Finally, they indicated the aspects they would improve or change about each tested product. The data was collected and translated into graphs to obtain clear and visual results.

Results and discussion

Lavender evaluation

Lavender extraction antioxidant activity

*Determination by ABTS**

The total antioxidant activity is calculated by substituting the absorbance obtained into the calibration equation, which was prepared by dissolving in 60% ethanol the Trolox standard in a concentration range from 50-400 ug/mL ($A = 0{,}0013913\ x\ C$). The results were expressed om terms of the amount equivalent to Trolox, TEAC unit (mg/g).

In spite of the fact that Table 2 shows that the antioxidant capacity is higher in the water extraction compared to ethanol, the ABTS* extinction shows minimal difference between both extraction methods, as illustrated in Figure 1.

Table 2. Absorbance results of TEAC method of water and ethanol extraction samples. Amount [mg] of antioxidant activity per gram of sample was calculated.

Samples	TEAC
Ethanol	11,27 ± 0,03
Water	11,42 ± 0,03

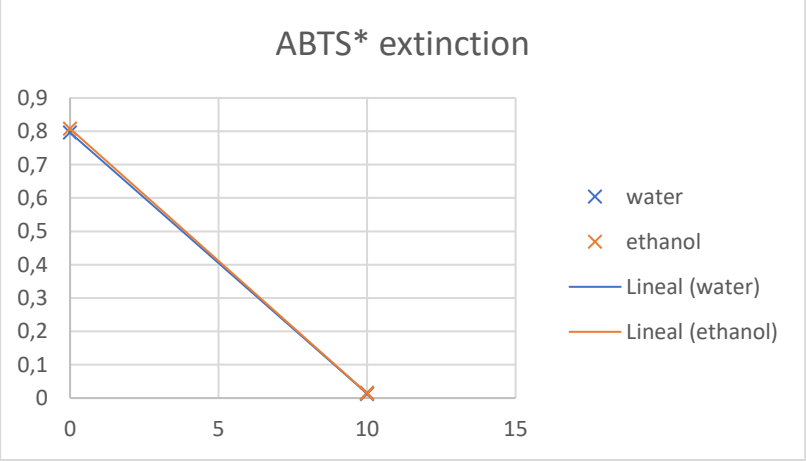

Figure 4. Graph illustrating the ABTS extinction after 10 minutes in lavender samples extracted using both ethanol and water.*

Total polyphenol content

The total polyphenol content in the sample was calculated by substituting the absorbance obtained into the calibration equation, which was constructed for the gallic acid solution in a concentration range of 0,1 mg/ml to 0,5 mg/mL($A = 1{,}2921\ \times C$) The total polyphenol

concentration was then calculated as the polyphenol content in mg per g of dry matter. Due to the high values of absorbance, the dilution 1:9 of samples was needed to achieve a range of absorption that allows an accurate measurement.

Comparing the results indicated in Table 3 it is noticeable that the effectiveness of polyphenol extraction is higher in water than in ethanol. However, in both extractions, the polyphenol content is notable enough to consider the extracts' great active principles.

Table 3. Total Polyphenol Content in mg of polyphenol per gram of sample determined by Folin-Ciocalteu method of ethanol lavender extraction and water lavender extraction.

Samples	TPC
Ethanol	35,75 ± 3,32
Water	42,97 ± 1,28

Total flavonoid content

The total flavonoid content was calculated by substituting the absorbance obtained into the calibration equation, which was prepared with catechin in a concentration range from 5 to 50 µg/mL ($A = 1{,}4424 \times C$). The concentration of total flavonoids was then recalculated to express the flavonoid content in mg per gram of dry weight.

The quantity of flavonoids present in both samples is not significant, as evidenced in Table 4, thus indicating that the potential antioxidant effect of lavender may not be greatly influenced by its flavonoid content.

Table 4. Total Flavonoid Content in mg of polyphenol per gram of sample

Samples	TFC
Ethanol	0,0377 ± 0,0019
Water	0,0496 ± 0,0019

Encapsulation efficiency

The Folin-Ciocalteu method was employed with the supernatant obtained after centrifugating the liposome encapsulation tubes. The aims were to determine the total polyphenol that had not been encapsulated and to be able to elucidate the effectiveness of lipids with active component formation. Similarly to the first TPC determination, a dilution 1: 9 was necessary.

Results shown in Table 5 indicate that the encapsulation effectiveness is 12%. This result is lower than in other studies on polyphenol encapsulation. For instance, Huang et al. (2019) achieved encapsulation efficiencies ranging from 59% to 80% for curcumin and resveratrol. This highlights the necessity of optimizing the encapsulation protocol to attain satisfactory production outcomes. Nevertheless, given that this is an experimental trial conducted on a small scale, the encapsulation of 12% of polyphenols will be acceptable for the production of a final product that is effective in its intended purpose.

Table 5. Total Polyphenol Content in mg of polyphenol per gram of sample determined by Folin-Ciocalteu method of ethanol lavender extraction before and after the encapsulation, and calculation of encapsulated polyphenols.

	TPC
Extract	35,75 ± 3,32
Supernatant	31,57 ± 1,35
Encapsulated	4,18 ± 3,58

Grapes' evaluation

*Determination by ABTS**

The data presented in Table 6 revealed that the antioxidant capacity of the extraction of grapes is irrelevant and insufficient to provide antioxidant benefits in cosmetics. This is probably attributed to a low polyphenol content of the grape variety or inadequate extraction thereof. The polyphenol content plays a crucial role in determining the antioxidant potential of grape extracts, so any deficiencies in the extraction process coul significantly impact the results. Moreover, other conducted trials (Shi et al.,2021) have demonstrated that the antioxidant activity values of resveratrol, the principal polyphenol found in grapes, are around 50 mg/l.

Table 6. Absorbance results of TEAC method of grapes extraction in methanol. Amount [mg] of antioxidant activity per gram of sample was calculated.

Samples	TEAC
Ethanol	0,31 ± 0,18

Total polyphenol content

The results displayed in Table 6 were less than 50% of the polyphenols extracted in the article on which the protocol is based (Dujmić F et al. 2020). The most probable reason was the seedless grape variety used in the present determination, revealing that the most greatest polyphenol content will be in the seed. Another difference between *Vitis vinifera* and the used grape type is the sugar content, which has affected the extraction process since the crushing was not possible due to the sticky texture, possibly leading to homogenization

problems. Furthermore, the lack of crushing and sieving induced the necessity to incorporate additional filtration steps, raising the risk of losses and experimental error odds. Due to this explanation, the grapes' extraction was not used in the final product preparation. Instead, the cream incorporated grape seed oil as part of its vegetable oil component, serving the dual purpose of emollient and ensuring the preservation of the beneficial properties originally sought in this study.

Table 7. Total Polyphenol Content in mg of polyphenol per gram of sample determined by Folin-Ciocalteu method of grapes extraction.

Samples	TPC
Ethanol	23,26 ± 0,19

Emulsion evaluation

The standard base showed excellent texture and consistency, which is a demonstrating efficacy proof of the oil combination. Regarding the samples containing the active principle, the ethanol formulation also appeared to possess favorable texture and consistency. However, the water-based sample with a concentration of 15% exhibited excessive liquidity, displaying non-uniform emulsification, prompting a decision to diminish the active component.

Subsequently, the water sample with a concentration of 10% proved to be superior to the 15% variant in terms of liquidity and viscosity; nevertheless, it still exhibited excessive fluidity, requiring another reduction of the lavender extract. At last, the water-based sample with a concentration of 5% demonstrated the most refined texture among the water lavender extraction samples but still had a higher degree of liquidity than the ethanol sample.

Meanwhile, the liposome sample featured a texture that was more fluid compared to the ethanol-based sample but superior to the water-based sample. These observations highlight the complex balance required in formulation development to achieve optimal texture and efficacy in cosmetic products.

Hydration capacity

The number and thickness of the flakes on the foil indicate the dryness of the skin. Sufficiently moist skin shows a thin and even film of corneocytes.

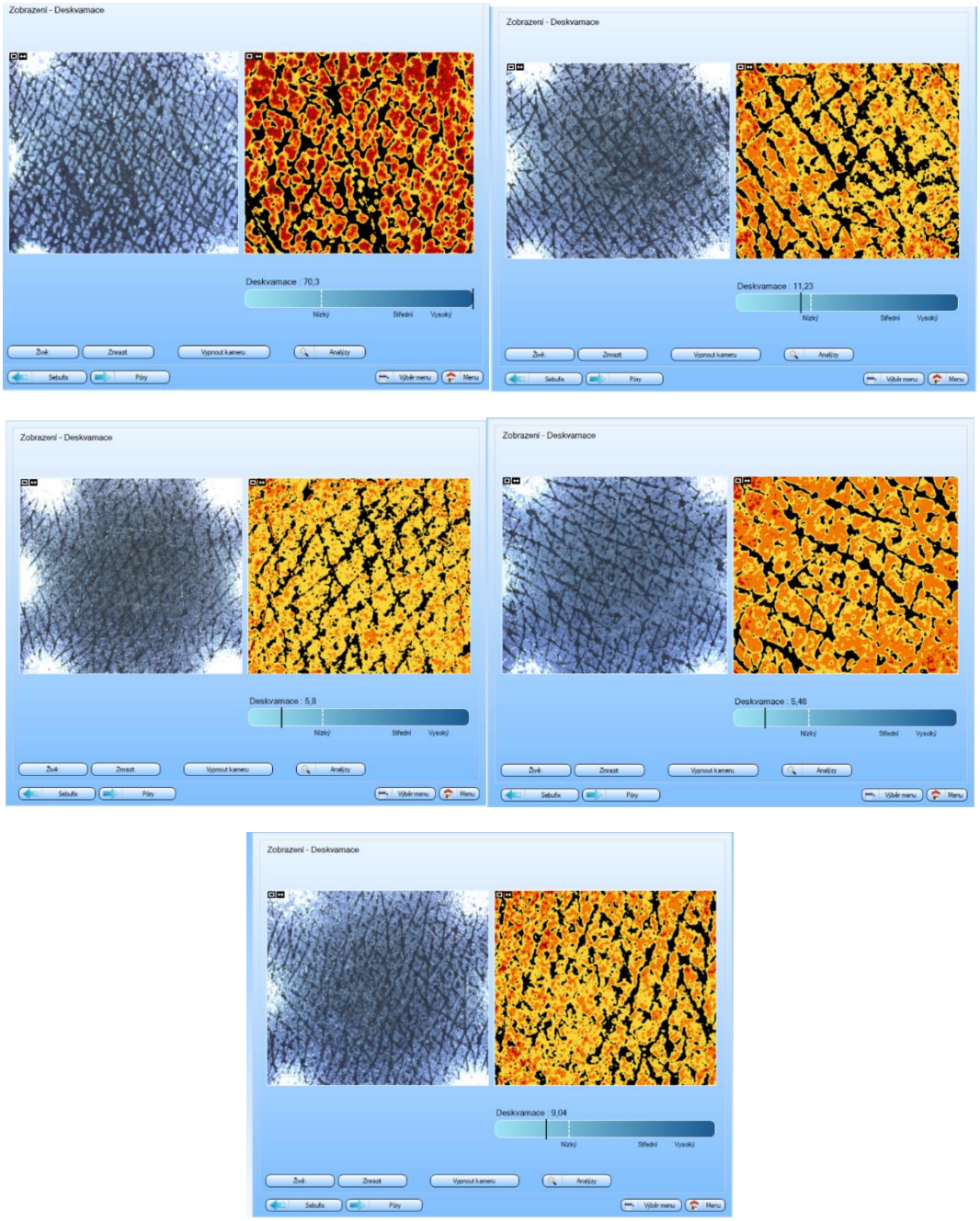

Figure 5. Clean dry skin, standard base cream, ethanol cream, liposome cream, and water cream freeze images of the measurement of hydration capacity with CK electronic microscope (Camera and Croreofix with parallel polarized light)

The desquamation degree difference before and after the application of all the samples is considerable, as evidenced in Table 8. Firstly, the distinction between clean skin and after standard cream measurement is mainly an indicator of the remarkable hydration properties of the vegetable oil content combination. Secondly, the minimization after using the other products is related to the hydration properties of the active ingredient itself. Finally, the comparison between water, liposome, and ethanol cream shows that the preferable extraction for preserving the hydration properties of lavender is liposome extraction, followed by ethanol, which maintains a significant lead over the water extraction method.

Table 8. Desquamation degree values were obtained with a CK electronic microscope.

Measurement	Desquamation degree
Clean skin	70,3
Standard cream	11,23
Water cream	9,04
Liposome cream	5,48
Ethanol cream	5,8

Sensory analysis of the final cosmetic product

The sensory analysis of the three final products (Ethanol lavender extract 18%, Water lavender extract 5%, and Liposome Lavender extract) was conducted through a test comprising four parts. The first one was related to the identification details of each participant, inquiring about age, skin type, prevailing skin conditions, and frequency of moisturizer utilization. Based on this data, it was determined that the samples were evaluated and compared among a group of 22 volunteers, comprising 40,9% male and 59,1% female participants. Notably, 81,1% fell within the age bracket of 19 to 25 years, 9,1% aged between 26 to 30, and an older minority. Predominant skin types identified were dry and a combination of dry-oily-normal skin, characterized by moderate dehydration issues and slight irritation tendencies. Additionally, most participants reported regular use of hydrating products, so the comparison with frequently used market products is accounted for in the results.

The second part of the test includes questions about some sensory parameters which are key for the sensory evaluation. In general, the trend in the scores for consistency, shine, spreadability, and absorption indicates a preference for the ethanol sample, followed by the liposome sample, with the water sample receiving the lowest rating. Regarding stickiness and perfumery, the ethanol sample continues to receive the highest score, while the evaluation of the other two samples is evenly matched. Ultimately, stickiness is assessed more evenly across all three samples.

The third part deals with an arrangement among the three products from the best to worst, taking into account collectively the aspects previously evaluated and their preferences. The volunteers had assigned 3 points to the best sample, 2 points to the second-best one, and 1 point to the worst sample. Despite the closely aligned scores, the rankings positioned the liposome sample in the lead, followed by the ethanol sample, and finally the water sample.

To conclude, there was a last time where participants could express what they would improve or change about the tested products. The sample requiring the most improvement according to the volunteers would be the one derived from the extraction with water (5%), as can be shown in Figure 3. Among participants, 54,4% noted the need for enhancements in

appearance, while 45,5% mentioned both spreadability and scent. Regarding the evaluation of the liposome sample, 40,9% of the volunteers highlighted the mildness of its aroma, thus suggesting it as an area for improvement. Lastly, 40,9% of the subjects expressed satisfaction with the ethanol extraction sample, indicating no need for improvement, and only 27,3% suggested enhancing absorbability.

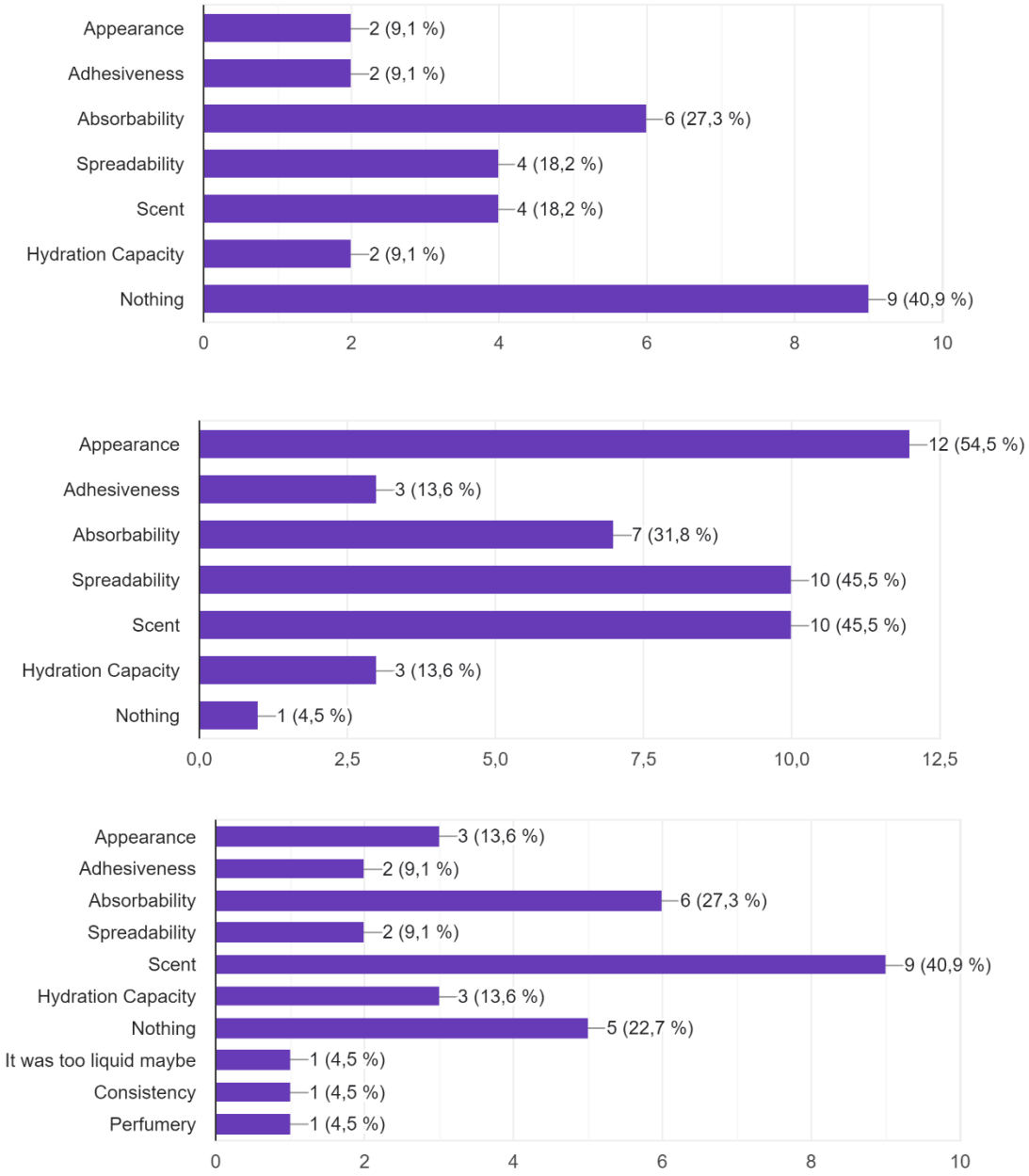

Figure 6. Graph illustrating the aspects for enhancement in the products derived from extraction with ethanol, water, and liposomes, respectively.

Conclusions

In this study, the entire process from investigation to the obtainment of the final cosmetic product has taken place. Lavender has demonstrated great antioxidant activity, high polyphenol content, and other properties that can enhance its profits in cosmetics. Moreover, it has also been found that the extraction method does not significantly affect the antioxidant capacities maintenance, since the ABTS results of the lavender extract are the similar in the water-based and the ethanol-based extraction. Nevertheless, the results of the polyphenol content differ between the extraction methods, this should be studied deeply and more replicates before making any determinant conclusion. Furthermore, the extraction method has an impact on the final product texture, probably influenced by the volatility of the ethanol solvent, which affects the concentration after the heating.

Based on the comprehensive findings, the optimal product resulting from this preliminary research phase, considering all the antioxidant activity, the polyphenol content, the hydration capacity, and the sensory perception would be the derivative obtained through ethanol extraction (8%). However, considering the hydration ability of the liposome sample, this sample should also be considered as a potential high efficient product after optimizing the obtention method.

Subsequent research steps along this trajectory should involve primarily optimizing the process of lavender extraction with liposomes, given its superior hydrating capacity and favorable sensory attributes. Then, another analysis that should be taken into account would be the replication of the total polyphenol content determination with the water and ethanol extraction, to verify the result obtained in this experiment and report an accurate result with precise conclusions.

On the other hand, despite the promising literature during the research stage, it was not possible to achieve a significant antioxidant capacity of the grape extract due to the grape variety employed. However, these results serve to emphasize the impact of grape variety on polyphenol content. The following research phases would include repeating the study with *Vitis vinifera* variety, and meticulously planning the sample collection step at the appropriate time and conditions to obtain the maximum antioxidant potential and the highest possible amount of resveratrol. After duplicating the analysis of ABTS and TPC determination, and considering enough the results obtained, the next stage would be the incorporation into a cosmetic product. For this purpose, would be necessary to study again the optimal formulation proportions of the active ingredient incorporation, ensuring the cosmetic product preserves a texture and sensory perception appealing to the consumer.

References

- A.Pouillot, N. Dayan, A. S. Polla, L. L. Polla and B. S. Polla, The stratum corneum: a double paradox, J. Cosmet. Dermatol., 2008, 7, 143.

- Ahmad, Z. (2010). The uses and properties of almond oil. *Complementary Therapies In Clinical Practice*, *16*(1), 10-12.

- Alonso, C., Martı, M., Barba, C., Carrer, V., Rubió, L., & Coderch, L. (2017). Skin permeation and antioxidant efficacy of topically applied resveratrol. *Archives Of Dermatological Research*, *309*(6), 423-431.

- Ashworth, J. L., Murphy, G., Rock, M. J., Sherratt, M. J., Shapiro, S. D., Shuttleworth, C. A., & Kielty, C. M. (1999). Fibrillin degradation by matrix metalloproteinases: implications for connective tissue remodelling. Biochemical Journal, 340(1), 171-181.

- Biesalski, H. K., Hemmes, C., Hopfenmuller, W., Schmid, C., & Gollnick, H. P. (1996). Effects of Controlled Exposure of Sunlight on Plasma and Skin Levels of β-Carotene. Free Radical Research, 24(3), 215-224.

- Blaak, J., & Staib, P. (2022). An updated review on efficacy and benefits of sweet almond, evening primrose and jojoba oils in skin care applications. International Journal Of Cosmetic Science, 44(1), 1-9.

- Buchbauer, G., Jirovetz, L., Jäger, W., Dietrich, H., & Plank, C. (1991). Aromatherapy: Evidence for Sedative Effects of the Essential Oil of Lavender after Inhalation. *Zeitschrift Für Naturforschung. C, A Journal Of Biosciences*, *46*(11-12), 1067-1072.

- Chedea, V. S., Vicaș, S. I., Sticozzi, C., Pessina, F., Frosini, M., Maioli, E., & Valacchi, G. (2017). Resveratrol: from diet to topical usage. *Food & Function*, *8*(11), 3879-3892.

- Creasy, L. L., & Coffee, M. (1988). Phytoalexin Production Potential of Grape Berries. *Journal Of The American Society For Horticultural Science*, *113*(2), 230-234.

- Daehnhardt-Pfeiffer, S., Surber, C., Wilhelm, K., Daehnhardt, D., Springmann, G., Boettcher, M., & Foelster-Holst, R. (2012). Noninvasive Stratum Corneum Sampling and Electron Microscopical Examination of Skin Barrier Integrity: Pilot Study with a Topical Glycerin Formulation for Atopic Dermatitis. Skin Pharmacology And Physiology, 25(3), 155-161.

- Danby, S. G., AlEnezi, T., Sultan, A., Lavender, T., Chittock, J., Brown, K., & Cork, M. J. (2012). Effect of Olive and Sunflower Seed Oil on the Adult Skin Barrier: Implications for Neonatal Skin Care. Pediatric Dermatology, 30(1), 42-50.

- Domaszewska-Szostek, A., Puzianowska-Kuźnicka, M., & Kuryłowicz, A. (2021). Flavonoids in Skin Senescence Prevention and Treatment. International Journal Of Molecular Sciences, 22(13), 6814.

- Dujmić, F., Ganić, K. K., Ćurić, D., Karlović, S., Bosiljkov, T., Ježek, D., Vidrih, R., Hribar, J., Zlatić, E., Prusina, T., Khubber, S., Barba, F. J., & Brnčić, M. (2020). Non-Thermal Ultrasonic Extraction of Polyphenolic Compounds from Red Wine Lees. *Foods*, *9*(4), 472.

- González-Acedo, A., Ramos-Torrecillas, J., Illescas-Montes, R., Costela-Ruiz, V. J., Ruiz, C., Melguizo-Rodríguez, L., & García-Martínez, O. (2023). The Benefits of Olive Oil for Skin Health: Study on the Effect of Hydroxytyrosol, Tyrosol, and Oleocanthal on Human Fibroblasts. Nutrients, 15(9), 2077.

- Gorini, I., Iorio, S., Ciliberti, R., Licata, M., & Armocida, G. (2019). Olive oil in pharmacological and cosmetic traditions. *Journal Of Cosmetic Dermatology*, *18*(5), 1575-1579.

- Huang, M., Liang, C., Tan, C., Huang, S., Ying, R., Wang, Y., Wang, Z., & Zhang, Y. (2019). Liposome co-encapsulation as a strategy for the delivery of curcumin and resveratrol. *Food & Function*, *10*(10), 6447-6458.

- Kalita, P., Tapan, B. K., Pal, T., & Kalita, R. (2013). ESTIMATION OF TOTAL FLAVONOIDS CONTENT (TFC) AND ANTI OXIDANT ACTIVITIES OF METHANOLIC WHOLE PLANT EXTRACT OF BIOPHYTUM SENSITIVUM LINN. *Journal Of Drug Delivery And Therapeutics*, *3*(4).

- Kannan, S., & Jaiswal, A. K. (2006). Low and High Dose UVB Regulation of Transcription Factor NF-E2-Related Factor 2. *Cancer Research*, *66*(17), 8421-8429.

- Khavkin, J., & Ellis, D. A. (2011). Aging Skin: Histology, Physiology, and Pathology. Facial Plastic Surgery Clinics Of North America, 19(2), 229-234.

- Lim, H., Park, H., & Kim, H. P. (2015). Effects of flavonoids on senescence-associated secretory phenotype formation from bleomycin-induced senescence in BJ fibroblasts. Biochemical Pharmacology, 96(4), 337-348.

- Lucía Dzurická (2024).*Laboratory procedure of emulsion synthesis with active ingredients.* VUT Brno.

- Matić, P., Sabljić, M., & Jakobek, L. (2017). Validation of Spectrophotometric Methods for the Determination of Total Polyphenol and Total Flavonoid Content. *Journal Of AOAC International*, *100*(6), 1795-1803.
- Mikeš V.: *Základní biochemické praktikum.* Skriptum, MU Brno, 1997

- Miliauskas, G., Venskutonis, P. R., & Van Beek, T. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry*, *85*(2), 231-237.

- Mohania, D., Chandel, S., Kumar, P., Verma, V., Digvijay, K., Tripathi, D., Choudhury, K., Mitten, S. K., & Shah, D. (2017). Ultraviolet radiations: Skin Defense-Damage Mechanism. En Advances in experimental medicine and biology (pp. 71-87).

- Nakai, K.; Tsuruta, D. What Are Reactive Oxygen Species, Free Radicals, and Oxidative Stress in Skin Diseases? Int. J. Mol. Sci. 2021, 22, 10799.

- Nichols, J. A., & Katiyar, S. K. (2009). Skin photoprotection by natural polyphenols: anti-inflammatory, antioxidant and DNA repair mechanisms. Archives Of Dermatological Research, 302(2), 71-83.

- Oboh, G., & Henle, T. (2009). Antioxidant and Inhibitory Effects of Aqueous Extracts of Salvia officinalis Leaves on Pro-Oxidant-Induced Lipid Peroxidation in Brain and Liver In Vitro. *Journal Of Medicinal Food*, *12*(1), 77-84.

- Pandey, A., & Kar, S. K. (2018). Rapid Eye Movement sleep deprivation of rat generates ROS in the hepatocytes and makes them more susceptible to oxidative stress. *Sleep Science*, *11*(04), 245-253.

- Papaccio, F., D'Arino, A., Caputo, S., & Bellei, B. (2022). Focus on the Contribution of Oxidative Stress in Skin Aging. *Antioxidants*, *11*(6), 1121.
- Peč P. a kol.(2000). *Laboratorní cvičení z biochemie.* Skriptum, UP Olomouc.

- Renaud, S., & De Lorgeril, M. (1992). Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*, *339*(8808), 1523-1526.

- Saeed, A. K. (2019). Photo-protective Measurements of Almond Oil on UVB-Irradiated Mouse's Skin and Cyclin D1 Expression. Jordan Journal of Biological Sciences, 12(4).

- Sakurai, H., Yasui, H., Yamada, Y., Nishimura, H., & Shigemoto, M. (2005). Detection of reactive oxygen species in the skin of live mice and rats exposed to UVA light: a research review on chemiluminescence and trials for UVA protection. *Photochemical & Photobiological Sciences*, *4*(9), 715-720.
- Sharififar, F., Dehghn-Nudeh, G., & Mirtajaldini, M. (2009). Major flavonoids with antioxidant activity from Teucrium polium L. *Food Chemistry*, *112*(4), 885-888.

- Shi, Q., Wang, X., Tang, X., Zhen, N., Wang, Y., Luo, Z., Zhang, H., Liu, J., Zhou, D., & Huang, K. (2021b). In vitro antioxidant and antitumor study of zein/SHA nanoparticles loaded with resveratrol. *Food Science & Nutrition*, *9*(7), 3530-3537.

- Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. En *Methods in enzymology on CD-ROM/Methods in enzymology* (pp. 152-178).
- Spatafora, C. (2012). Valorization of Vegetable Waste: Identification of Bioactive Compounds and Their Chemo-Enzymatic Optimization. □*The* □*Open Agriculture Journal*, *6*(1), 9-16.

- Valacchi, G., Rimbach, G., Saliou, C., Weber, S., & Packer, L. (2001). Effect of benzoyl peroxide on antioxidant status, NF-κB activity and interleukin-1α gene expression in human keratinocytes. *Toxicology*, *165*(2-3), 225-234.

- Van Logtestijn, M. D. A., Domínguez-Hüttinger, E., Stamatas, G. N., & Tanaka, R. J. (2015). Resistance to Water Diffusion in the Stratum Corneum Is Depth-Dependent. PloS One, 10(2), e0117292.

- Vaughn, A. R., Clark, A. K., Sivamani, R. K., & Shi, V. Y. (2017). Natural Oils for Skin-Barrier Repair: Ancient Compounds Now Backed by Modern Science. American Journal Of Clinical Dermatology, 19(1), 103-117. https://doi.org/10.1007/s40257-017-0301-1

- Wenk, J., Brenneisen, P., Meewes, C., Wlaschek, M., Peters, T., Blaudschun, R., Ma, W., Kuhr, L., Schneider, L., & Scharffetter-Kochanek, K. (2000). UV-Induced Oxidative Stress and Photoaging. En KARGER eBooks (pp. 83-94).

- Werth, V. P., Shi, X., Kalathil, E., & Jaworsky, C. (1996). Elastic Fiber-Associated Proteins of Skin in Development and Photoaging. Photochemistry And Photobiology, 63(3), 308-313. https://doi.org/10.1111/j.1751-1097.1996.tb03032.x

Anexo

A. Indicar el grado de relación del trabajo con los Objetivos de Desarrollo Sostenible (ODS).

	Alto	Medio	Bajo	No procede
ODS 1. Fin de la pobreza				**x**
ODS 2. Hambre cero				**x**
ODS 3. Salud y bienestar	**x**			
ODS 4. Educación de calidad				**x**
ODS 5. Igualdad de género				**x**
ODS 6. Agua limpia y saneamiento				**x**
ODS 7. Energía asequible y no contaminante	**x**			
ODS 8. Trabajo decente y crecimiento económico	**x**			
ODS 9. Industria, innovación e infraestructuras				**x**
ODS 10. Reducción de las desigualdades				**x**
ODS 11. Ciudades y comunidades sostenibles				**x**
ODS 12. Producción y consumo responsables	**x**			
ODS 13. Acción por el clima				**x**
ODS 14. Vida submarina				**x**
ODS 15. Vida de ecosistemas terrestres				**x**
ODS 16. Paz, justicia e instituciones sólidas				**x**
ODS 17. Alianzas para lograr objetivos.				**x**

B. Describir brevemente la alineación del TFG con los ODS, marcados en la tabla anterior, con un grado alto.

***Sea conciso, pero utilice el número de páginas necesarias.

ODS 3: Los antioxidantes provenientes de fuentes naturales ayudan a proteger la piel del daño producido por la radiación ultravioleta, importante en la prevención del envejecimiento prematuro y el desarrollo de enfermedades como el cáncer.

ODS 7: El uso de sustancias naturales como ingredientes activos de productos cosméticos evita el empleo de otras sustancias químicas cuya producción pueda ser perjudicial para el medio ambiente.

ODS 8: La obtención de ingredientes activos a partir de recursos naturales es una alternativa económicamente favorable para la industria cosmética.

ODS 12: Los subproductos de la industria vinífera pueden ser empleados para la obtención de Resveratrol, un potente antioxidante ampliamente utilizado en la industria cosmética, por lo que es un recurso sostenible tanto a nivel económico como medio ambiental.

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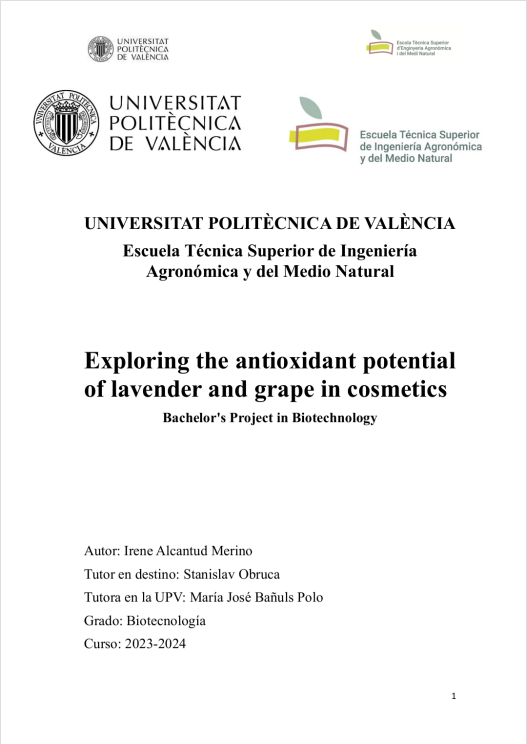

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Exploring the antioxidant potential of lavender and grape in cosmetics

Bachelor's Project in Biotechnology

Autor: Irene Alcantud Merino

Tutor en destino: Stanislav Obruca

Tutora en la UPV: María José Bañuls Polo

Grado: Biotecnología

Curso: 2023-2024

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