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Strychnos Madagascariensis fruit components as promising ingredients for skin care products

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Strychnos Madagascariensis fruit components as promising ingredients for skin care products

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Abstract

Xerosis is a common skin condition characterized by impaired barrier function, dehydration, oxidative stress, and altered tissue repair, creating a demand for sustainable dermocosmetic ingredients with multifunctional biological properties. *Strychnos madagascariensis* is an underutilized African fruit whose pulp has previously been recognized for its high lipid content and abundance of bioactive lipophilic compounds. However, the chemical composition and biological potential of its inner white pericarp remains unexplored. This study aimed to characterize the phenolic profile and evaluate the dermocosmetic potential of extracts obtained from the pulp and inner white pericarp of *Strychnos madagascariensis*.

Total phenolic content, antioxidant capacity, and phenolic composition were determined using spectrophotometric assays and HPLC-DAD/MS. Biological activity was assessed in human dermal fibroblasts through cellular viability, scratch wound healing, and alkaline phosphatase activity under hyperosmotic conditions.

The inner white pericarp exhibited a substantially higher total phenolic content (27.60 ± 0.12 mg GAE/g freeze-dried material) and antioxidant capacity (30.95 ± 0.30 mg TE/g by DPPH and 88.85 ± 2.14 mg TE/g by ABTS) than the pulp (6.43 ± 0.51 mg GAE/g, 5.98 ± 0.76 mg TE/g DPPH, and 12.31 ± 0.73 mg TE/g ABTS). It was characterized predominantly by hydroxycinnamic acid derivatives, mainly caffeoylquinic and dicaffeoylquinic acids, whereas the pulp contained considerably lower amounts of phenolic compounds, represented mainly by glucosylated dihydroxybenzoic acid derivatives. Both extracts were biocompatible with fibroblasts, promoted cell migration, and modulated alkaline phosphatase activity under hyperosmotic conditions, suggesting an influence on cellular responses associated with tissue repair and extracellular matrix remodelling.

Overall, this work demonstrates that the pulp and the previously unexplored inner white pericarp possess complementary chemical compositions and biological properties. While the pulp derives its value mainly from its lipid-rich composition, the inner white pericarp emerges as a phenolic-rich and antioxidant fraction with promising dermocosmetic potential, supporting the sustainable valorization of different *S. madagascariensis* fruit tissues and providing a scientific basis for their future incorporation into multifunctional dermocosmetic formulations.

Resumo

A xerose cutânea é uma condição frequente caracterizada pela alteração da função barreira da pele, desidratação, stress oxidativo e comprometimento dos processos de reparação tecidular, tornando necessária a identificação de ingredientes dermocosméticos sustentáveis com propriedades multifuncionais. *Strychnos madagascariensis* é um fruto africano subvalorizado cuja polpa se destaca pelo elevado teor lipídico e pela presença de diversos compostos bioativos lipofílicos. No entanto, a composição química e o potencial biológico da fração correspondente ao pericarpo branco interno permaneciam inexplorados. Assim, este trabalho teve como objetivo caracterizar a composição fenólica e avaliar o potencial dermocosmético de extratos obtidos da polpa e do pericarpo branco interno de *Strychnos madagascariensis*.

O teor de compostos fenólicos totais, a capacidade antioxidante e o perfil fenólico foram avaliados através de métodos espectrofotométricos e por HPLC-DAD/MS. A atividade biológica foi estudada em fibroblastos dérmicos humanos através de ensaios de viabilidade celular, migração celular (scratch assay) e atividade da fosfatase alcalina em condições de stress hiperosmótico.

O pericarpo branco interno apresentou um teor significativamente superior de compostos fenólicos totais (27,60 ± 0,12 mg EAG/g de material liofilizado) e maior capacidade antioxidante (30,95 ± 0,30 mg ET/g pelo método DPPH e 88,85 ± 2,14 mg ET/g pelo método ABTS) comparativamente à polpa (6,43 ± 0,51 mg EAG/g, 5,98 ± 0,76 mg ET/g e 12,31 ± 0,73 mg ET/g, respetivamente). O perfil fenólico do pericarpo branco interno foi dominado por derivados de ácidos hidroxicinâmicos, principalmente ácidos cafeoilquínicos e dicafeoilquínicos, enquanto a polpa apresentou menores quantidades de compostos fenólicos, representados sobretudo por derivados glicosilados do ácido di-hidroxibenzóico. Ambas as frações revelaram ser biocompatíveis com fibroblastos, promoveram a migração celular e modularam a atividade da fosfatase alcalina em condições de stress hiperosmótico, sugerindo um potencial efeito na resposta celular associada à reparação tecidular e à remodelação da matriz extracelular.

Em conjunto, os resultados demonstram que a polpa e o pericarpo branco interno apresentam composições químicas e propriedades biológicas complementares. Enquanto a polpa parece dever o seu potencial dermocosmético sobretudo ao elevado teor lipídico e aos compostos bioativos lipofílicos, o pericarpo branco interno destaca-

se como uma fração rica em compostos fenólicos e com elevada capacidade antioxidante. Estes resultados reforçam o potencial da valorização integral de diferentes frações de *Strychnos madagascariensis* como fonte sustentável de ingredientes multifuncionais para futuras formulações dermocosméticas.

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List abbreviations

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

AGEs Final products of advanced glication

ALP Alkaline Phosphatase

ANOVA Analysis of variance

API Atmospheric Pressure Ionization

DMEM Dulbecco's modified eagle medium

DMSO Dimethyl sulfoxide

DNA Deoxyribonucleic acid

DPPH 2,2-diphenyl-1-picrylhydrazyl

EDTA Ethylene diaminetetraacetic acid

Eos Phytosphingosine

ESI ElectroSpray Ionization

FBS Fetal Bovine Serum

HaCat Human Adult Low-Calcium High-Temperature keratinocytes

HDF Human dermal fibroblasts

HPLC/DAD-MS High Performance Liquid Chromatography coupled with Diode Array Detection and Mass Spectrometry

ISO International Organization Standardization

LPP Long-period lamellar phase

MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide

MUFA Mono-unsaturated fatty acids

Nds N-acyl-dihydrosphingosine

NHFs Non-hydrating factors

NS N-acyl-sphingosine

NST Non-specific tissue

OECD Organization for Economic Cooperation and Development

PBS Phosphate Buffered Saline

pNPP para-nitrophenylphosphate

PUFA Poly-unsaturated fatty acids

RHE Human epidermis model

ROS Reactive oxygen species

SFA Short-chained fatty acids

TCC Total carotenoid content

TEWL Transepidermal water loss

TNSALP Tissue-nonspecific isoenzyme

TPC Total phenolic content

Introduction

1.1. Importance of skin and cutaneous barrier

The skin is the largest organ of the human body representing about 16% of a human's body weight. (McKnight et al., 2022) It acts as a protective barrier between the internal and external medium, and its vital functions focus on protection, thermic regulation, sensorial perception, photometabolism and intercellular signalling. (Mohamed & Hargest, 2022)

The skin has three main layers, as shown in Figures 1A and 1B:

- Epidermis: It's the most external and visible layer, mainly composed of keratinocytes that produce keratin, essential for resistance and impermeability. It's subdivided into five strata, as seen in Figure 1B: *basale, spinosum, granulosum*, *lucidum* and *corneum* which represent the continuum process of keratinization and cellular renovation. (Osseiran et al., 2018) It also contains melanocytes responsible for pigmentation and Langerhans and Merkel cells responsible for immunity and tactile perception.
- Dermis: It's located right below the epidermis and formed by conjunctive tissue rich in collagen and elastin that confers elasticity and resistance to the skin. It can be divided into papillary dermis and reticular dermis. (Zorina et al., 2022) Blood vessels, nerves, hair follicles and sebaceous sweat glands responsible for nutrition, thermoregulation and sensibility, can be found.
- Hypodermis: It's the deepest layer composed essentially of adipose and conjunctive tissue. It functions as thermic insulation, energy reservation and shock absorbers keeping the skin connection with muscles and underlying structure. (Zorina et al., 2022)

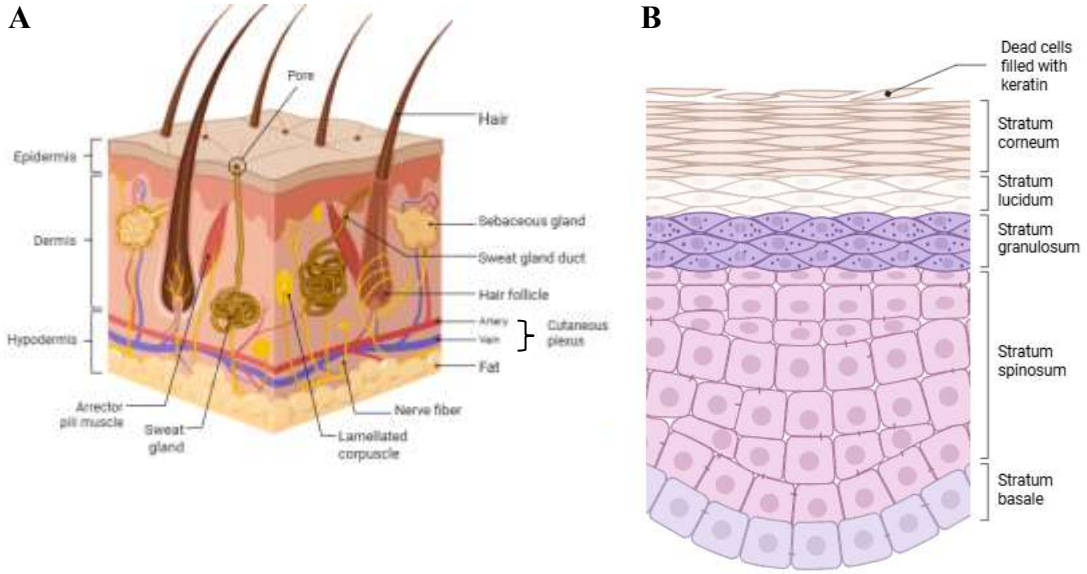


Figure 1- A- Anatomy of the skin (Epidermis - where mainly keratinocytes can be found; Dermis – where mainly fibroblasts can be found; Hipodermis). B - Structure of the epidermis, divided in its five strata. Created with Biorender.com (2026/02/17).

The skin microbiome has a main role in cutaneous barrier maintenance, hydration and lipid matrix regulation. (Lee & Kim, 2022) The interaction between resident microorganisms and epidermal cells contributes to the physical and immunological integrity of the skin, protecting against pathogens and modulating inflammatory responses. (Swaney & Kalan, 2021)

In this context, the microbiome participates in enzyme and metabolites production that influence the synthesis and organization of lipids of the *stratum corneum*. (Li et al., 2024) The microbial diversity and harmony between commensal species are fundamental to avoid dysbiosis, which can compromise the lipid matrix and increase transepidermal water loss (TEWL) leading to dehydration and inflammation. (Kengmo Tchoupa et al., 2023)

The skin microbiome influences cutaneous hydration by modulating the pH and the production of amino acids and other components of the NMF, impacting the capacity of hydration retention in the corneocytes. (Roux et al., 2022) Alterations in microbial composition are associated with changes in the lipid matrix and in hydration, avoiding the interdependence between microbiome, lipids and barrier function. (Siqueira et al., 2025) (Li et al., 2024)

The *stratum corneum* is composed of corneocytes that are enveloped in a highly organized lipid matrix, formed by ceramides, cholesterol and free fatty acids. (Baroni et al., 2012) This structure assures great protection against excessive water loss,

pathogens, ultraviolet radiation and more besides maintaining the organism homeostasis. (Bouwstra et al., 2023) Its integrity depends on coordinated processes of keratinocyte differentiation, cornification and desquamation, regulated by pH and calcium gradients and specific proteases. (Fukuda et al., 2024) Alterations in lipid constitution or corneocyte maturation may compromise the cutaneous barrier.

The hydration of the *stratum corneum* depends on three main factors: organization of intercellular lipids (that form a physical barrier to water flow); the presence of the natural hydration factors (NHFs) (that retain water inside the corneocytes); and keratin structure, that by interacting with water and NHFs, modulates the hydration retention capacity and plasticity of the *stratum corneum*. (Choe et al., 2017) The NHFs, mainly derived of filaggrin dehydration, is highly hygroscopic and essential to maintain elasticity and hydration of the *stratum corneum*, especially in intermediary layers. (Kubo et al., 2013)

The pH modulation in the *stratum corneum* exerts direct influence in hydration and barrier restauration in patients with cutaneous xerosis, especially considering the alterations in lipid composition. (Proksch, 2018) The pH is acid, between 4.1 and 5.8, fundamental to the activity of enzymes involved in synthesis, organization and ceramide remodeling. When there is an increase in the pH, there's a reduction in the activity of those enzymes, harming the formation and organization of lipid lamellae, which results in a decrease of ceramides, destruction of lipid matrix and increase of TEWL. (Novackova et al., 2021) Topical acidification by emollients with pH close to 4 demonstrated to accelerate barrier improvement, increase hydration, improve compaction and length of lipid lamellae while elevating ceramide levels even without exogeneous reposition of those lipids. (Kilic et al., 2019) An acid pH favors the antimicrobial function and reduces inflammation by promoting ideal conditions to the cutaneous microbiota and epidermal homeostasis.

The lipid matrix of the skin, localized mainly in the *stratum corneum,* is composed of ceramides, cholesterol and free fatty acids organized in highly organized layers. (Bouwstra et al., 2023) This matrix is the main determinant of the cutaneous barrier function, limiting TEWL and protecting against external agents like microorganisms and chemical substances. (Shamaprasad et al., 2022) (Wertz, 2021) The integrity and organization of the lipid matrix are essential to skin hydration because lipids create a hydrophobic barrier that stops excessive water evaporation, therefore qualitative and quantitative alterations in these lipids (as is the case in cutaneous inflammatory

diseases) lead to barrier disfunction and cutaneous dryness. (Mijaljica et al., 2024) Besides, lipid matrix influences the plasticity and cohesion of *stratum corneum,* directly impacting hydration retention and skin elasticity. (Shamaprasad et al., 2022)

The disorganization of lipids or NHFs deficiency results in an increase of TEWL leading to dry skin and higher susceptibility to irritants and pathogens. The topical applications of emollients and humectants can restore hydration, promoting lipid reorganization and NHF reposition. (Kubo et al., 2013)

Biosynthesis and lipid metabolism are regulated processes and depend on the secretion of lamellar bodies by the keratinocytes, that in return release lipid precursors to the extracellular space where they are converted in the final lipids of the matrix. (Feingold, 2007) The topical application of physiological lipids can restore barrier function in deficiency situations, being a therapeutic strategy based on evidence. (Feingold, 2007)

1.2. Cutaneous xerosis

Cutaneous xerosis is a dry skin condition characterized by the loss of water from the epidermis, resulting in itchy, flaky, and rough skin. (Görög et al., 2022) It is considered a natural part of the skin's aging process, being more prevalent in elderly people, but it can also be exacerbated by several other pathologies such as atopical dermatitis, diabetes, hypothyroidism and more. (Vestergaard et al., 2025) The main environmental factors affecting cutaneous xerosis include cold and dry weather, wind exposure, low humidity, frequent use of hot water, detergents and aggressive soaps and atmospheric pollution. (Görög et al., 2022) It may cause embarrassment; emotional distress and it can interfere with daily routines jeopardizing a person's quality of life.

Cutaneous xerosis is also characterized by a reduction in lipids and moisturizing agents, such as ceramides and free fatty acids, leaving the skin barrier compromised due to water loss and increasing the risk of infections and inflammation.(Vyumvuhore et al., 2018) Ceramides can be divided in three subclasses: N-Acyl-Sphingosine (NS) and N-acyl-Dihydrosphingosine (Nds) are non-hydroxylated ceramides that contribute to the formation of lipid lamellae, promoting compaction and and barrier permeability, limiting water loss; the esterified with linoleic acid, based on phytosphingosine (Eos) ceramide is an omega-acylated ceramide, indispensable to formation of the long-period lamellar phase (LPP) which is the structure responsible for maximum resistance to water loss and entry of external agents. (Zorina et al., 2022) (Nobumoto et al., 2026)

(Opalka et al., 2020) There is an increase in short chain ceramides, specially Nds ceramides with minor acyl chains, which is associated with the decrease of lipid packing density and higher permeability to the cutaneous barrier. (Uche et al., 2021)

The hydration mechanisms involve three main components: humectants, that attract and retain water in the *stratum corneum*; occlusives that reduce the TEWL; and physiological lipids that restore the intercellular lipid matrix promoting the lamellae reorganization and improving barrier function. (Augustin & Brignone, 2025) The drier the skin, the higher the content in lipids of the emollient should be, preferred water in oil formulations. (Augustin & Brignone, 2025)

To make an informed decision on which hydrating formulas are best suited to different patients, it is necessary to understand that the hydrating properties come, mainly, from three active agents, as shown in Figure 2. Occlusive agents, such as liquid paraffin, create a layer on top of skin's surface, reducing TEWL and promoting supporting hydration. (Fluhr et al., 2025) They are indicated mainly for very dry skin and should be applied in a liberal and frequent way. Humectant agents, such as glycerol, attract and retain water in the upper layers of the epidermis, increasing the hydration capacity of the *stratum corneum*. (Fluhr et al., 2025) They are frequently combined with occlusives to maximize the hydrating effect and improve skin texture. Lastly, emollients, such as vegetable oils, smooth and lubricate the skin, filling the spaces between the corneocytes and improving cutaneous flexibility. (Augustin & Brignone, 2025)

Basic emollients are considered the golden standard to handle cutaneous xerosis in patients with or without atopical dermatitis. (Eichenfield et al., 2014)

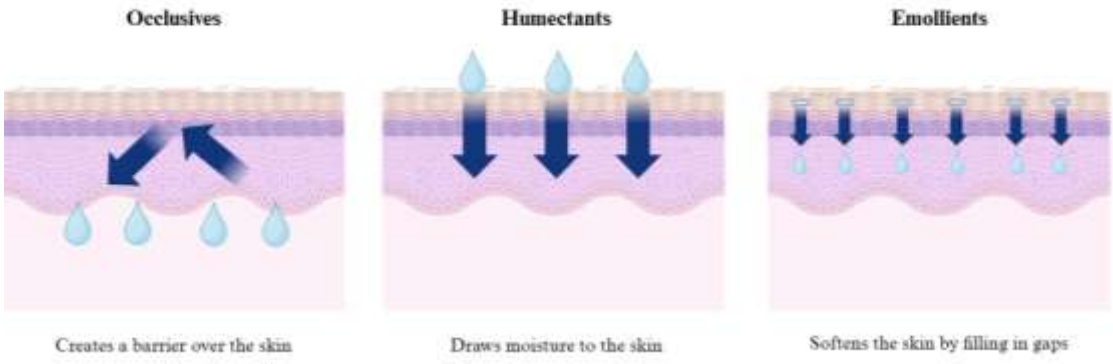


Figure 2. Mechanism of action of occlusives, humectants and emollients. Created with Biorender (2026/02/17).

1.3. Therapeutic approaches

The recommended treatment for this condition (cutaneous xerosis) includes avoiding fragrances, mindful showers and, especially, hydration alongside the use of corticosteroids in the worst cases. The recommendation to hydrate the skin in cutaneous xerosis, considering the alteration in lipid composition, emphasizes the use of emollients with re-hydrating properties and lipid reposition. (Fluhr et al., 2025) The choice of the product should be guided by the severity of xerosis and the need to restore the lipid barrier of the *stratum corneum*, that is compromised. (Augustin et al., 2019)

The emollients should be applied multiple times a day with the amount adjusted to the severity of the xerosis, preferring formulations rich in lipids for very dry skin and hydrophilic lotions or creams to less severe cases. (Augustin & Brignone, 2025) The choice of the emollient should consider specific symptoms: urea for flakiness or fissures, licochalcone A for erythema and polidocanol for itching. (Szepietowski & Tadini, 2025) The combination of ingredients with complementary properties such as humectants and occlusives maximizes the effects. (Fluhr et al., 2025) The use of emollients with ceramides is recommended specially in xerosis associated with diabetes. Patient education, choice of product personalization and regular follow-up are key strategies to promote adherence to treatment and optimize clinical trials. (Augustin & Brignone, 2025)

In more severe cases, it can be advised the use of corticosteroids. They are used in situations where xerosis is associated with inflammation, like in atopical dermatitis or eczema, and especially when there is itching, erythema or lesions. (Guttman-Yassky et al., 2025) In these cases, they are prescribed to control inflammations and alleviate symptoms, always being used alongside emollients that remain the base of the treatment. (Eichenfield et al., 2014) (Sidbury et al., 2023) However, their use should be limited in duration to minimize risks.

The main risks of these substances include cutaneous atrophy, stretch marks, hypopigmentation, secondary infections and, if in prolonged use, suppression of the hypothalamus-hypophysis-adrenal axis, especially in the elderly and infants. (Stacey & McEleney, 2021) The use in areas where the skin is thinner and under occlusion increases the risk of side effects.

The mineral based formulations include ingredients such as mineral oil, *petrolatum* and dimethicone, that act mainly as occlusive agents. They are indicated as first line to treat

cutaneous xerosis, especially in cases of very dry skin, the elderly and patients with chronic diseases associated with xerosis. (Szepietowski & Tadini, 2025) The efficacy of these formulations is well established: they promote the improvement of hydration, reduce symptoms such as itching and fissures, and partially restore the skin's barrier function. (Shim et al., 2016)

These formulations, though, present some limitations such as low acceptance sensory wise (they can feel too oily), potential to obstruct the pores and environmental sustainability issues, as they are petrol derived and present low biodegradability. (Fluhr et al., 2025) Besides, they don't fully restore the physiological lipids of the *stratum corneum* being less effective in promoting epidermal renewal when compared to formulations with ceramides or urea. (Gold et al., 2024)

1.4. Sustainability in dermocosmetics

Despite the wide range of hydrating creams available on the market, current formulations don't adequately address specific needs, such as rapid absorption or compatibility with sensitive skin. A lot of these products appeal to the use of ingredients of mineral origin, such as *petrolatum*, whose efficacy is recognized, but that raise environmental concerns and rejection by more sustainable buyers. (Barnes et al., 2021) Nowadays, it is known that conventional hydrating formulas containing mineral derived ingredients and sustainable alternatives, like vegetable oils, are both effective in improving skin hydration and barrier function. (Poljsak & Kocevar Glavac, 2022) (Shim et al., 2016) Although *petrolatum* has a stronger occlusive effect and thus reduces TEWL, it is known that vegetable oils improve the hydration of the *stratum corneum* and promote the renovation of the epidermis. (Rubio-Santoyo et al., 2025)

Also, hydrating formulas containing synthetic ceramides demonstrated better improvements in skin hydration, skin pH and barrier function when compared to hydrophilic formulas. (Lueangarun et al., 2019) Current literature states that the majority of basic emollient formulations are safe and effective when treating these conditions and that as sustainability concerns are increasing by the day, vegetable oils and ceramides offer a better alternative, environmental wise. (Poljsak & Kocevar Glavac, 2022)

Sustainability in dermo cosmetics involves the adoption of practices that minimize environmental impact throughout the product life cycle, from the selection of ingredients until the disposal of the package. (Nirmal et al., 2024)

In this context, the development of new creams to treat cutaneous xerosis of vegetal origin, biodegradable and with the same efficacy as *petrolatum* represents an answer to an unmet need but also an opportunity to bring sustainability to the cosmetic field.

The valorization of agricultural and food-industries by-products is a crucial step when we talk about sustainability, circular economy and resource optimization. The concept involves giving a new destination to materials like shells, pulps and seeds that would otherwise be wasted for not being fitted to be used on the market in their natural form. It reduces waste by minimizing the environmental impact and generates new products that can be used later in the market, providing a new source of income. In this line, the dermocosmetic industry has increasingly focused on the valorization of underutilized fruit fractions and agro-food by-products as sustainable sources of high-value bioactive ingredients. These materials are particularly attractive due to their richness in polyphenols and other phytochemicals with antioxidant, anti-inflammatory, photoprotective, and skin-regenerative properties, supporting their potential incorporation into innovative dermocosmetic formulations (Ghada Alhamdani, 2026; Machado, 2024)The transition to green dermo-cosmetics and the use of biodegradable ingredients is one of the biggest innovation opportunities in cosmetic industry, driven by an increasing demand of consumers to have ethical, sustainable and proved efficacy products. The main focus resides in the substitution of synthetic ingredients, many with petrochemical and non-biodegradable origin, for safe and environment friendly options. The use of biotechnology allows us to produce biocosmetics with ingredients obtained from biological processes, like fermentation, and may result in more sustainable plant extracts, that can be more effective due to its purity and controlled concentration.

1.5. Skin models

The International Organization for Standardization (ISO) norms applicable to cosmetics include, mainly, the 12787 ISO, that establishes validation criteria to analytical methods using chromatography techniques in analyzing cosmetics. This norm is widely used to assure the quality and security of cosmetic ingredients through validated analytical methods. (Gimeno et al., 2012) The ISO 12787:2011, entitled

“Cosmetics – Analytical Methods – Validation Criteria for analytical results using chromatographic technics” gives specific guidelines to the identification and quantification of ingredients in cosmetic products. This norm has been applied in diverse contexts, including phthalates, kojic acid and parabens analysis, among other ingredients. (Qadir et al., 2016) The main applications of this ISO are validation of chromatographic methods to analysis of cosmetic ingredients; establishment of quantification limits and precision criteria; guaranteeing conformity and European and international regulations and analysis of prohibited or restricted substances in cosmetics.

There is an array of skin models used in dermatological investigation, pharmaceutical development and security tests, that vary from simple cell cultures to complex tridimensional systems. (Lakeh & Shafiee, 2025) These models evolved significantly in the last decades, driven by ethical considerations and the alternative necessities to animal testing. (Costa Gagosian et al., 2025)

In vitro models represent the most advanced category and include several approaches. The bidimensional (2D) models consist of monolayers of cultured keratinocytes and fibroblasts, useful to basic studies of cellular biology. The tridimensional (3D) models represent the tissue microenvironment and include reconstructed human epidermis, equivalents of complete skin with dermis and epidermis, and cutaneous organoids derived from pluripotent stem cells. These 3D models replicate keratinocyte differentiation, barrier function and allow studies of cell-cell interaction.

Animal models are still being used, mainly mice, rabbits and pigs. (Saeed & Martins-Green, 2023) Miniature pigs are particularly valued for structural and functional resemblance with human skin. (Alex et al., 2020) Xenograft models, where reconstructed human skin is grafted in immunodeficient mice, combine advantages of both approaches. (Salgado et al., 2017)

Applications of these models include toxicity tests, topical pharmaceuticals development, cutaneous aging studies, disease modulation and cosmetic products evaluation. (Zhao et al., 2023) Commercial standardized models have regulatory acceptance to diverse security assays.

1.5.1. Irritation skin models

Irritation skin models are systems used to evaluate the irritative and corrosive potential of chemical substances, cosmetic ingredients and pharmaceutical products, having significantly evolved since traditional animal testing to validated *in vitro* alternatives. (Liao et al., 2021)

The reconstructed human epidermis models (RHE) are the current standard to cutaneous irritation tests and are formally validated by the Organization for Economic Co-operation and Development (OECD). (Akagi et al., 2023) These tridimensional models, like EpiDerm®, EpiSkin®, SkinEthic® and EpiTRI®, have morphological, biochemical and physiological properties like human epidermis, with well differentiated viable multilayers of cells and barrier function. (Liao et al., 2021) The typical protocol involves topical exposure of the sample for 60 minutes, followed by extensive rinsing and a 42-hour post incubation. (Kandarova et al., 2009) Cellular viability is measured through an 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay, with a limit of 50 % to deem the substance as irritative. (Kandarova et al., 2009)

Organotypic cultures that include dermic and epidermic components offer a more realistic system than models based only in keratinocytes, allowing to evaluate toxicologic responses that imitate the *in vivo* environment better. (Gaviria Agudelo et al., 2020) These models can incorporate measurements of cytokines liberation and transepithelial electrical resistance and additional endpoints to viability. (Wei et al., 2020)

The Draize test in rabbits was the standard method to evaluate cutaneous irritation, basing itself on the erythema and edema observation, translated to semi-quantitative scores. (Charmeau-Genevois et al., 2021) However, this method has been criticized by its subjectivity, overestimation of human responses, significative variability and ethical concerns. (Rooney et al., 2021) Reproducibility analysis shows that substances classified as mild irritative are re-classified as light irritative or non-irritative in at least 40% of the repeated tests. (Rooney et al., 2021)

Besides cellular viability, complementary endpoints include cytology, histology, liberation of pro-inflammatory cytokines and barrier function measurements. (Hwang et al., 2020) Cytology demonstrated higher precision than isolated viability in some studies, being particularly useful for borderline results or color interference. (Hwang et

al., 2020) Validated RHE models are accepted as a complete substitution for animal testing for hazard identification and chemical substances labelling in the European Union and other countries. (Eskes et al., 2012) However, some specific chemical classes, like organosilicon based substances, may present predictability challenges, requiring adapted test strategies. (Forlini et al., 2025) Voluntary human tests, patch tests, are also used as ethical alternatives to acute irritation evaluation. (Robinson et al., 2000)

1.6. Interstitial skin biochemical marker

Alkaline phosphatase (ALP), by the International Union of Biochemistry and Molecular Biology, has the number EC 3.1.3.1; it is a phosphomonoesterase that catalyses the hydrolysis of phosphate monoesters under alkaline conditions; in humans, the tissue-nonspecific isoenzyme, (TNSALP). (Guerreiro et al., 2007; Guerreiro et al., 2021) It catalyses the hydrolysis of organic phosphate esters in the extracellular space, with zinc and magnesium being essential cofactors.(Guerreiro et al., 2007; Guerreiro et al., 2021) Although it is present in several body tissues, more than 80% in ALP serum comes from the liver and bone. Its exact physiological function in several tissues remains partially unknown. (Makris et al., 2023) In the skin, soluble ALP is located almost in the dermis being both present in a soluble form or connected to the cell membrane. (Mier & van Rennes, 1982) Its main function is dephosphorylation for the absorption and transportation of chemical substances necessary for the growth, maintenance and differentiation of the cells (Conroy & Green, 1975). Studies in cutaneous fibroblasts demonstrated that TNSALP acts as an ectophosphatase hydrolysing phosphoethanolamine and pyridoxal-5'-phosphate, natural substrates important to cellular metabolism. (Fedde & Whyte, 1990)

The extracellular matrix regulates the induction of ALP expression by ascorbic acid in fibroblasts. (Abe et al., 2001) Fibronectin facilitates the positive regulation of ALP expression through the interaction with α5β1 integrin, while type I collagen fibrils suppress ALP expression. (Abe et al., 2001) This differential regulation by the extracellular matrix may explain why fibroblast express high ALP during healing and inflammation, when they are not in contact with collagen fibrils in the granulation tissue rich in fibronectin. (Abe et al., 2001) In the study by (Guerreiro et al., 2021) our group identified regions of activated fibroblasts with high ALP activity in fibroblast–

endothelial cell cocultures. Molecular docking analyses further revealed two putative collagen-binding sites on ALP, supporting a previously unrecognized role for ALP–collagen interactions in collagen organization, cell migration, and microvessel-like structure assembly during angiogenesis.

1.7. *Strychnos Madagascariensis*

Strychnos madagascariensis is a tropical fruit found in the South of Africa (Marracuene), whose pulp has been gaining recognition for its nutritional characteristics as it is rich in β-carotene and vitamin E, and has a slight antioxidant power. (Chemane, Casal, et al., 2022; Chemane, Ribeiro, et al., 2022) It's harvested during the months of October through December, and it's characterized by a round shape and a woody, hard exterior that goes from green to yellow when it's ripe, as shown in Figure 3A. Inside, there are seeds surrounded by a fleshy pulp, as seen in Figures 3B and 3C, as well as a white pericarp that surrounds the seeds, as seen in Figure 3D. It can be consumed in its natural form when ripe or used to make flour rich in fat (~30 %), to be consumed in times of drought. The flour is produced by cracking the fruit to separate the pulp; then the seeds (that are enveloped by the pulp) are removed, letting the pulp sun dry and roast on a metallic plate heated over a low heat to obtain the flour (Chemane, Ribeiro, et al., 2022).

The reason why it is used as a food is because of its energetic value and macronutrient composition, mainly in fatty acids, as is shown in Tables 1 and 2.



Figure 3: A - *Strychnos madagascariensis*. B – Inside of the fruit. C – Pulp of the fruit. D – White pericarp of the fruit. A and B were retrieved from Chemane 2022 and C and D are original pictures.

Table 1: Nutritional composition and energetic value of the pulp fruit *Strychnos Madagascariensis* from Marracuene. Data from Chemane 2022.

Components	g/100 g
Moisture	76.01 ± 0.2
Protein	1.51 ± 0.0
Fat	4.01 ± 0.1
Ash	1.50 ± 0.34
Carbohydrates	10.41 ± 0.1
Fiber	6.72 ± 0.06
Energy (kJ)	406.7 ± 8
Energy (kcal)	97.2 ± 2

Table 2: Main fatty acids, vitamins, total phenolic content and antioxidant capacity of the *Strychnos Madagascariensis* pulp from Marracuene. Adapted from Chemane 2022.

Compound	Representation	mg/100 g
Lauric Acid	C12:0	3.4 ± 0.2
Myrstic Acid	C14:0	34.1 ± 1.9
Palmitic Acid	C16:0	5229.9 ± 118.3
Palmitoleic Acid	C16:1-ω9	421.4 ± 13.5
Stearic Acid	C18:0	1198.7 ± 13.2
Oleic Acid	C18:1-ω9	16464.1 ± 298.7
Linoleic Acid	C18:2-ω6	1791.6 ± 55.4
Arachidic Acid	C20:0	161.0 ± 2.8
Alpha-Linolenic Acid	C18:3-ω3	457.5 ± 21.1
Gondoic Acid	C20:1-ω9	78.1 ± 1.4
SFA		6926 ± 138
MUFA		17013 ± 313
PUFA		78.1 ± 1.4
Vitamin E		3.31 ± 0.01
Vitamin A		0.61 ± 0.01
β-carotene		1.48 ± 0.01
Total Carotenoid Content (TCC)		5.86 ± 0.12
Total Phenolic Content (TPC, espressed as gallic acid equivalents)		208.24 ± 3.27
Antioxidant capacity (expressed as Trolox equivalents): DPPH		46.00 ± 1.34
ABTS		5.74 ± 0.31

SFA– Short-chain fatty acids, MUFA – Mono-unsaturated fatty acids, PUFA – Polyunsaturated fatty acids, DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

In brief, *Strychnos madagascariensis* pulp presents mild antioxidant power, presence of carotenes and vitamin E, allowing for high skin hydration and maintenance of cellular viability. Beyond the fat-soluble vitamins, the fatty acid profile of this fruit (with ~30% of fat, expressed in dry weigh basis) reveals a high content of monounsaturated fatty acids, particularly oleic acid (62–63%), followed by palmitic acid (20%) and a smaller proportion of linoleic acid (7%). (Chemane, Ribeiro, et al., 2022) This composition suggests beneficial emollient and skin-conditioning properties and deserves to be studied. Furthermore, the oil fraction of the pulp contains substantial amounts of other bioactive compounds, including sterols and triterpenic alcohols, which, together with minor components such as tocopherols and carotenes, may contribute to its antioxidant and anti-inflammatory potential.

Aims

Preliminary evidence from a previous study suggested that the pulp of *Strychnos madagascariensis* may have potential for skin-related applications. However, its phenolic composition had not been comprehensively characterized, and, to the best of our knowledge, the inner white pericarp fraction adhering to the outer shell had not previously been investigated. The present study therefore builds on this preliminary work by comparing the pulp with this previously unexplored and underutilized fruit fraction, which would otherwise be discarded.

Accordingly, the primary aim of this study was to investigate the biocompatibility and potential skin-regenerative effects of different preparations obtained from the pulp and inner white pericarp fraction of *Strychnos madagascariensis*, including extracts, decoctions, and lyophilized whole matrices. Their biological effects were evaluated using complementary two- and three-dimensional *in vitro* skin cell models, comprising human fibroblasts and keratinocytes. Particular emphasis was placed on cell viability, fibroblast migration, spheroid formation and growth, and biochemical and enzymatic marker related to cellular function and tissue regeneration. The three-dimensional spheroid model was included to more closely reproduce cell–cell interactions and the spatial organization of native tissue, thereby providing a more physiologically relevant assessment than conventional two-dimensional cultures alone. The nutritional composition, phenolic content and profile, and antioxidant capacity of the two fruit fractions were additionally assessed to support the interpretation of the biological responses and explore the potential contribution of their bioactive constituents.

Materials and methods

1. Sample origin and harvest

Strychnos madagascariensis is a tropical fruit native to southern Africa and is typically harvested between October and December. The fruit samples used in this study were collected in the Marracuene community, Mozambique, and kindly provided by Dr Sandra Chemane from Eduardo Mondlane University. The samples were shipped by air to Portugal in November 2025.

2. Chemical characterization methods of analysis

2.1. Sample preparation

Fruit samples (both the pulp and white pericarp) were already separately stored at -80ºC and were then ground to a fine powder/paste in a food processer (Retsch GM 200, Verder), while frozen, and then stored at -20ºC until further analysis. The pulp was subjected to a new analysis, and it was obtained by being removed from the seeds using a knife or scalpel, while still frozen.

2.2. Decoction preparation for cell treatment

The decoction procedure was adapted from the preparation previously performed using fresh fruit pulp by the Mozambican research team. In the present study, the fruit pulp was freeze-dried and ground into a powder. Subsequently, 2 g of freeze-dried pulp were added to 200 mL of water and boiled for 3 min. After cooling, the decoction was stored at 4 °C and filtered before use.

2.3. Preparation of lyophilized samples for cell treatment

The lyophilized pulp and inner white pericarp fraction were separately dispersed in 0.9% NaCl solution at a concentration of 10 mg/mL, corresponding to 2 g of lyophilized material in 200 mL of solution. Additional preparations were made separately in dimethyl sulfoxide (DMSO). The mixtures were thoroughly homogenized to obtain uniform dispersions. All solutions obtained were then filtered with a 0.70 µm filter and a then with a 0.22 µm filter, before their application to the cell cultures.

2.4. *Strychnos Magascariensis* extracts preparation

Two different types of extracts were prepared:

- **For Total Phenolics and antioxidant activity (DPPH and ABTS):**
    - In a 15 mL centrifuge tube, approximately 0.1 g of freeze-dried pulp or white pericarp was weighed, to which later 4 mL of acidified methanol 50% (pH = 2) was added and homogenized. The tube, protected from light, was agitated for 1h at room temperature and was then centrifuged for 10 minutes at 5000 rpm. The supernatant was collected; the process was repeated and the pellet was re-extracted with acetone 70% and stored at -20ºC until further analysis. (Pérez-Jiménez et al., 2008)
- **For LC-MS/MS analysis:**
    - The extraction procedure combined previously described methodologies with minor modifications. An initial hexane-defatting step, applied only to the freeze-dried pulp because of its high lipid content, was adapted from Meyer et al. (2008): Approximately 1 g of freeze-dried pulp was weighed into a 50 mL polypropylene centrifuge tube. To remove the lipid fraction, 10 mL of hexane were added, and the mixture was thoroughly homogenized and agitated for 10–15 min at room temperature, protected from light. The sample was then centrifuged at 5000 rpm for 10 min, and the hexane supernatant was discarded. This defatting procedure was repeated once under the same conditions. The resulting defatted pellet was left to dry until complete evaporation of the residual hexane (Meyer & Terry, 2008). Approximately 0.5 g of the freeze-dried inner white pericarp fraction was weighed into a separate 50 mL polypropylene centrifuge tube. Owing to its low lipid content, this fraction was not subjected to the initial hexane-defatting step.
    - Both fruit fractions were subsequently extracted with 80% methanol acidified with 0.1% formic acid. For the pulp and inner white pericarp fraction, 10 and 20 mL of extraction solvent were added, respectively. The mixtures were homogenized and agitated for 1 h at room temperature, protected from light, and then centrifuged at 5000 rpm for

10 min. The supernatants were collected, and the extraction procedure was repeated once under the same conditions. For each fruit fraction, the supernatants obtained from the two extraction cycles were combined.

- The pooled methanolic extracts from each fruit fraction were separately evaporated to dryness under an argon stream at room temperature, while the samples were maintained on ice. The resulting dry extracts were stored under appropriate conditions until further analysis.

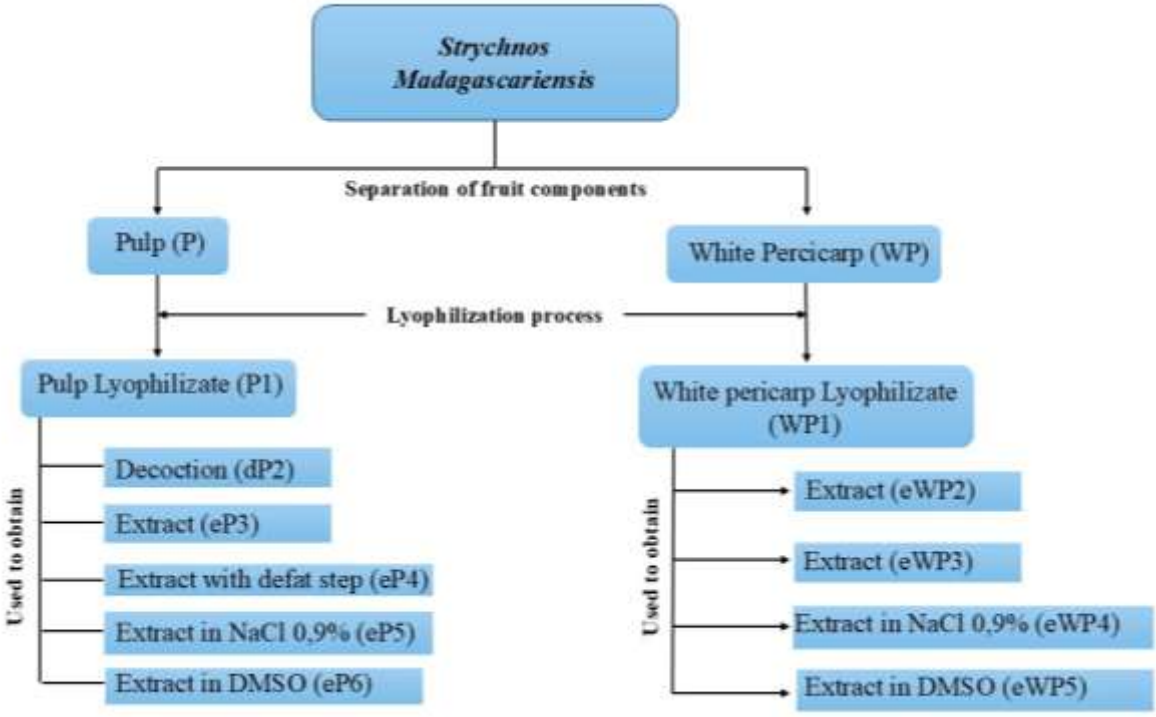


Figure 4: Flow chart of the preparation of the different samples, as well as their terminology.

2.5.Determination of ash content

Ash content was determined by dry combustion based on AOAC Official Method 923.03, with modifications. Approximately 5 g of previously thawed pulp or inner white pericarp fraction were accurately weighed into porcelain crucibles that had previously been incinerated at 500 °C, cooled in a desiccator, and weighed. The crucibles containing the samples were initially placed in a muffle furnace at approximately 120 °C, after which the temperature was gradually increased at a rate of 10 °C/h to 500 °C. Incineration was continued until a white or light-grey ash was

obtained. The crucibles were then cooled in a desiccator and weighed. Ash content was determined gravimetrically, and all analyses were performed in triplicate.

2.6. Determination of protein through nitrogen using the Kjeldahl method

Crude protein content was determined by the Kjeldahl method according to AOAC Official Method 920.152, with minor modifications. After digestion (Kjeldahl tablets from SigmaAldrich, Germany), the samples were allowed to cool and transferred to an automated distillation unit. Approximately 80 mL of distilled water and sufficient 30% NaOH solution were added to alkalize the digest. Distillation was performed for 4 min, and the distillate was collected in a flask containing 25 mL of 0.2 N H_2SO_4 and two drops of methyl red indicator. The excess acid was titrated with 0.2 N NaOH until a pale-yellow endpoint was reached. Total nitrogen was converted into crude protein using a nitrogen-to-protein conversion factor of 6.25. The analysis were performed in duplicate.

2.7. Determination of moisture

Moisture content was determined by infrared thermogravimetry using an infrared moisture analyzer (VWR). Approximately 2.5 g of previously thawed pulp or inner white pericarp fraction were placed on the aluminium weighing pan and dried at 105°C until a constant mass was reached. Moisture content (%) was recorded directly from the instrument. All analyses were performed in triplicate.

2.8. Determination of fat content by Soxhlet extraction

Crude fat content was determined by Soxhlet extraction based on AOAC Official Method 920.39, with modifications. Approximately 5 g of previously thawed pulp or inner white pericarp fraction were mixed with anhydrous sodium sulfate and sand and transferred to an extraction thimble. Lipids were extracted with petroleum ether using a Soxhlet apparatus (Minichiller 300, Huber). Following extraction, the solvent was removed, and the extraction flask containing the lipid fraction was dried at 105 °C until a constant mass was reached. Crude fat content was determined gravimetrically, and all analyses were performed in triplicate.

2.9. Folin-Ciocalteau method – quantification of total polyphenols

The method was applied based on (Magalhaes et al., 2012). The procedure was performed in a 96-well plate and measurements were made using a microplate reader by spectrophotometry (BioTek, Agillent). The used samples were the pulp extract and white pericarp extract (eP3and eWP2, respectively). In order to build the calibration curve it was used a stock solution of galic acid 1mM and samples were read at 765 nm. TPC was calculated using a gallic acid calibration curve and expressed as mean ± standard deviation in milligrams of gallic acid equivalents per gram of sample (mg GAE g^{-1}).

2.10. DPPH assay – antioxidant activity

The method was applied based on (Magalhaes et al., 2012) and modified by Viegas et al (2012). The procedure was performed in a 96-well plate and measurements were made using a microplate reader by spectrophotometry (BioTek, Agillent). The used samples were the pulp extract and white pericarp extract (eP3and eWP2, respectively). In order to build the calibration curve it was used a stock solution of Trollox 1mM and samples were read at 517 nm. For intrinsic absorption and blanks, methanolic solution was added instead of $DPPH^{\bullet}$ solution and samples, respectively. Antioxidant capacity was expressed as mean ± standard deviation in milligrams of Trolox equivalent antioxidant capacity per gram of sample (mg TE g^{-1})

2.11. ABTS assay – antioxidant capacity

The method was adapted from (Magalhaes et al., 2012). The procedure was performed in a 96-well plate and measurements were made using a microplate reader by spectrophotometry (BioTek, Agillent). The used samples were the pulp extract and white pericarp extract (eP3and eWP2, respectively). In order to build the calibration curve it was used a stock solution of Trollox 1mM and samples were read at 734 nm. For intrinsic absorption of samples, acetate buffer was used instead of $ABTS^{\bullet+}$ solution, and for blanks, ultrapure water was added instead of samples.. Antioxidant capacity was expressed as mean ± standard deviation in milligrams of Trolox equivalent antioxidant capacity per gram of sample (mg TE g^{-1})

2.12. High-liquid performance chromatography coupled with diode array detection and mass spectrometry (HPLC-DAD/MS)

Dried samples of pulp extract and white pericarp from 10 ml of methanolic extracts (eP4 and eWP3, respectively) were dissolved in 1 mL of deionized water and centrifuged before analysis. Samples were analyzed by MS analysis on a Finnigan Surveyor series liquid chromatograph, equipped with a 250 × 4.6 mm i.d., 2.7 µm Poroshell 120 reversed-phase C18 column (Agilent) at 25 °C. The eluents used were (A) 1% (v/v) formic acid in water and (B) 1% (v/v) formic acid and 30% (v/v) acetonitrile in water at a flow rate of 0.4 mL/min. The elution gradient was performed from 20 to 100% B for 70 min, then, the column was washed with 100 % B for 10 min and stabilized with the initial conditions for 10 min. The mass detection was carried out in the negative ion mode in a Finnigan LCQ DECA XP MAX (Finnigan Corp., San José, CA, USA) mass detector with an API (Atmospheric Pressure Ionization) source of ionization and an ESI (ElectroSpray Ionization) interface. Spectra were recorded in the negative ion mode between *m/z* 200 and 2000.

3. Biocompatibility assays: *in vitro* cell study

In our experimental design, cells were assigned to one of two groups: the control group (cells cultured only with complete medium) or treatment group (samples obtained from the pulp lyophilizate (P1) and white pericard lyophilizate (WP1)). In our experiments, cells were exposed to different treatments for 24h for prolonged exposure. Each experiment was repeated independently 2-3 times.

3.1. Cell culture: human dermal fibroblasts (HDFs) and human adult low-calcium high-temperature keratinocytes (HaCaTs)

HDFs were isolated from juvenile foreskin and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS; PAN-Biotech, Germany), penicillin G (100 U/mL; PAN-Biotech), and streptomycin (100 µg/mL; PAN-Biotech). HDFs were cultured in a humidified atmosphere at 37°C (5% CO_2). HDFs were used in experiments between passages 14-21.

2D cell culture: HDFs were maintained in T25 cm^2 flasks with 5mL of culture medium, in a concentration of $2x10^5$ cells/mL. Culture medium was exchanged 3 times per week,

and they were split after 10-12 days of growth (with approximately $2x10^6$ cells in the flask) in a 1:4 ratio. To perform the split, the medium was removed and the flask washed with 2-3mL of Phosphate Buffered Saline (PBS) to later incubate for 5 minutes with 500 µL of trypsin at 37ºC. After that time, medium was added to stop the action of trypsin/EDTA and to wash the flask.

Assays in 3D cell culture: Fibroblasts were detached from culture dishes by trypsin/EDTA, and a single cell-suspension (5×10^4 cells/mL) was prepared in a complete culture medium. To initiate spheroid formation, they were seeded into U-bottom 96-well plates (Costar, Cambridge, MA, USA), with each well containing 200 µL aliquots of cell suspension in a concentration of 1×10^4 cells/well. The dishes were incubated at 37°C in 5% CO_2 atmosphere. They were left to incubate for 5 days before applying treatments.

HaCaTs were in DMEM supplemented with 10% Fetal Bovine Serum (FBS; PAN-Biotech, Germany), penicillin G (100 U/mL; PAN-Biotech), and streptomycin (100 µg/mL; PAN-Biotech). HaCats were used in experiments between passages 70-75.

3.2. Cellular viability assays

HDFs were cultured in a 96 well plate ($1x10^4$ cells/well) and incubated for 24 hours in a humified atmosphere at 37ºC (5%CO_2). Treatments were applied after 24h and left to incubate for another 24h; then, 10% of resazurin solution (Invitrogen, PT)/ MTT solution were added to the wells and incubated for 3 hours in a humified atmosphere at 37ºC (5%CO_2). After that, samples were read in a spectrophotometer at 550 nm and 600 nm (resazurin) ; 570 nm (MTT).

3.3. Cellular viability by resazurin assay

This assay is based on the capacity of alive cells to reduce the resazurin (a dye, blue and little fluorescent) to resorufin (pink and highly fluorescent) by enzymes involved in cell metabolism, mainly those dependent in NADH and NADPH. This process occurs mainly in the cytoplasm and mitochondria of metabolic active cells (Chen et al., 2018). It was used to evaluate the cytotoxicity (in 2D cell culture) of the following treatments: different volumes (5 µL, 10 µL, 20 µL, 40 µL, 80 µL and 100 µL) of dP2; different concentrations (10 µg/mL, 50 µg/mL, 100 µg/mL, 250 µg/mL and 500 µg/mL) of eP5 and eWP4; different concentrations of (5 µg/mL, 10 µg/mL, 25 µg/mL and 50 µg/mL)

eP6 and eWP5 and to test different concentrations (20 mM, 35mM and 50mM) of manitol.

3.4. Cellular viability by MTT assay

This assay is based on the alive and metabolic active cells' capacity to reduce the MTT tetrazolium salt, which is yellow and water soluble, to formazan crystals, that are insoluble and violet colored. This reduction occurs mainly by NADPH dependent reductor' enzymes present in the mitochondria (even though other cellular components may contribute as well).

It was used to evaluate (both in 2D and 3D cell culture) cytotoxicity when adding 20 µL of pulp decoction (dP2).

3.5. Migration assay

This assay evaluates the capacity of cells to move (migrate) when exposed to external stimuli, which is a fundamental process in biological events like tissue healing. A mechanical damage is created in the monolayered culture, generating an area without cells, imitating a wound.

HDFs were cultured ($5x10^5$ cells/well) in a 24 well plate and incubated for 72 hours in a humified atmosphere at 37ºC (5%CO_2) , after which was performed a mechanical damage and treatments were then applied and left to incubate for 24h. Using the LEICA microscope, images of the scratch area were taken right after the scratch was made (our time 0h) and after 24h. The wound area was quantified using ImageJ (version 1.54g, National Institutes of Health, USA).

It was used to evaluate cell migration in low and high glucose media, as well as in our xerosis model, when treated with 50 µg/mL of pulp extract and white pericarp extract (eP5 and eWP4, respectively).

3.6. Measurement of spheroids

Using the LEICA microscope, pictures of the spheroids were taken at days 4, 6, 8 and 10 in order to follow their growth, and their diameter was measured using ImageJ (version 1.54g, National Institutes of Health, USA).

3.7. Bradford method – quantification of protein

This method is a colorimetric assay that measures the concentration of total protein in a solution. It involves the binding of the proteins to a dye (Coomassie Brilliant Blue G-250): when unbound it presents a brown color and when bound to a protein presents a blue color.

HDFs were cultured ($5x10^5$ cells/well) in two 6 well plates and incubated for 24 hours in a humified atmosphere at 37ºC (5%CO_2). Treatments were applied after 24h and left to incubate; then the wells were then treated with trypsin in order to obtain a pellet of cells that were stored in 1.5mL Eppendorf's vials and froze at -80ºC until ready for further use. The vials were put in ice and 200 µL of permeabilization solution that consists of 0.5% Triton - X 100 with PBS was added, until it was homogenized. To 50 µL of cell lysate were added 2.45 mL of Bradford reagent and the resulting solution was read in a spectrophotometer at 595 nm.

3.8. Alkaline phosphatase assays

HDFs were cultured ($5x10^5$ cells/well) in two 6 well plates and incubated for 24 hours in a humified atmosphere at 37ºC (5%CO_2). Treatments were applied after 24h and left to incubate; then the wells were treated with trypsin in order to obtain a pellet of cells that were stored in 1.5mL Eppendorf's vials and froze at -80ºC until ready for further use.

Dosimetry assay: The vials were put in ice and 200 µL of permeabilization solution that consists of 0.5% Triton-X 100 with PBS was added, until it was homogenized. In a different set of vials, 240 µL of deionized water was added, along with 100 µL of the homogenized cells and 100 µL of substrate (except for the control vials), para-nitrophenylphosphate (pNPP) 1mM. The vials were left to incubate at 37ºC for 1 hour. After, 1mL of sodium hydroxide (NaOH) 0.02mM was added and 100 µL of pNPP was added to the control vials. They were left for 10 minutes on ice, and the samples were read in a spectrophotometer at 410nm. This assay uses synthetic substrates, such as pNPP, that, when hydrolyzed by the alkaline phosphatase, release para-nitrophenol, a chromogenic compound quantifiable by spectrophotometry due to its yellow coloration with an alkaline pH (Akcakaya et al., 2007).

Staining assay: after fixation with paraformaldehyde (3.7%; Merck, Germany), ALP staining (after 14 days of culture) was performed using a Diagnostic Phosphatase Kit

(Sigma- Aldrich), as described by the manufacturer. ALP staining was examined by CLSM at λEx = 633 nm. (Guerreiro et al., 2021). This assay is based on this enzyme's capacity to catalyze the hydrolysis of phosphoric esters in alkaline medium, releasing inorganic phosphate and its corresponding alcohol. In cell culture it's frequently used as a cellular differentiation marker because its expression increases during osteogenic differentiation and pluripotent cells (Stefkova et al., 2015) It was used to visualize the distribution of ALP in HDFs 2D cell cultures.

3.9. Histochemical morphology analysis: Sirius Red Stainning

Cell morphology and extracellular matrix deposition were evaluated by cytochemical staining using Hematoxylin and Eosin, H&E, and Sirius Red. The assays were performed in 24-well culture plates. For staining, the culture medium was carefully removed from each well, and the cells were washed with PBS, to remove residual medium and non-adherent cells. Cells were then fixed with 3.7% paraformaldehyde for 15–20 minutes at room temperature. After fixation, the wells were washed again with PBS and prepared for staining.

For Sirius Red staining, fixed cells were incubated with Sirius Red solution to detect collagen-rich extracellular matrix deposition. After the staining period, excess dye was removed, and the wells were washed carefully, usually with acidified water to reduce background staining. The stained cultures were then observed under an optical microscope. Representative images were acquired from each experimental condition using an optical microscope. These images were used for qualitative morphological evaluation and comparison between control and experimental groups.

4. Statistical analysis

The results were analyzed by a one-way analysis of variance (ANOVA), that was followed by a Tukey post hoc test; a two-way ANOVA that was also followed by a Tukey post hoc test, t -tests and Brown-Forsyght and Welch's testes. Statistically significant differences were considered when a p-value < 0.05 was obtained; all statistical analysis was performed using Microsoft Excel and GraphPad Prism version 8.0.2 for Windows.

Results

1. Chemical characterization

1.1. Nutritional analysis

A bromatological analysis was performed in order to determine the percentage of the main macronutrients of the pulp and white pericarp (P and WP, respectively) of *Strychnos Madagascariensis*. Results are presented in Table 3 and expressed as mean ± standard deviation, n=3.

Table 3: Nutritional composition of the pulp and white pericarp of *Strychnos Madagascariensis* fruit.

Biological Sample	% Moisture	% Fat	% Protein	% Ash	%Carbohydrates*
Pulp	75.44 ± 0.01	8.24 ± 0.08	1.25 ± 0.06	4.55 ± 0.01	10.52
White pericarp	78.02 ± 0.01	0.16 ± 0.02	1.09 ± 0.41	5.11 ± 0.02	15.62

* Calculated by difference and includes dietary fiber

The reported values for the nutrition composition of Pulp were 76.01 % for moisture, 4.01 % for fat, 1.25 % for protein and 1.50 % for ash. As seen in Table 3, our values are similar for moisture and protein, considering the 75.44 % of moisture and 1.25 % for protein; however, we obtained higher values for fat and ash, those being of 8.24 % and 4.55 %, respectively. Expressed as dry weight, this percentage of fat corresponds to 33.56 % as described in nfuma (common name given to the pulp used to make flour) flours (Chemane, Ribeiro, et al., 2022). The percentage of carbohydrates and fiber comes to a total of 10.52 %.

As for the white pericarp, there are no reported values; as Table 3 states, the percentage of moisture is of 78.02 %, 0.16 % of fat (representing 0.74% in dry weight), 1.09 % of protein and 5.11 % of ash. The percentage of carbohydrates and fiber comes to a total of 15.62 %.

1.2. Phenolic content and antioxidant capacity

An analysis of bioactive compounds was performed in order to determine the phenolic content and antioxidant capacity of the pulp and white pericarp extracts (eP3

and eWP2, respectively) of *Strychnos Madagascariensis*. Results are presented in Table 4 and expressed as mean ± standard deviation (n=2).

Table 4: Phenolic content and antioxidant capacity of *Strychnos Madagascariensis*.

Biological Sample	TPC (mg GAe/g)	DPPH (mg TEe/g)	ABTS (mg TEe/g)
Pulp	6.43 ± 0.51	5.98 ± 0.76	12.31 ± 0.73
White pericarp	27.60 ± 0.12	30.95 ± 0.30	88.85 ± 2.14

GAe - gallic acid equivalent; Te - Trollox equivalent.

As shown in Table 4, the inner white pericarp fraction exhibited a signficantly higher total phenolic content and antioxidant capacity than the pulp, as demonstrated by both the DPPH and ABTS assays. These findings indicate that the inner white pericarp fraction represents a richer source of antioxidant compounds than the pulp. The differences observed between the two fruit fractions are further discussed in light of their distinct phenolic profiles in the following section.

1.3. Phenolic profile

In order to determine the main phenolic compounds of pulp and white pericarp extracts (eP4 and eWP3, respectively), a HPLC-DAD/MS analysis was performed (after said extracts were dried). The resulting peaks are shown in figures 5A and 5B and their identification is shown in tables 5A and 5B.

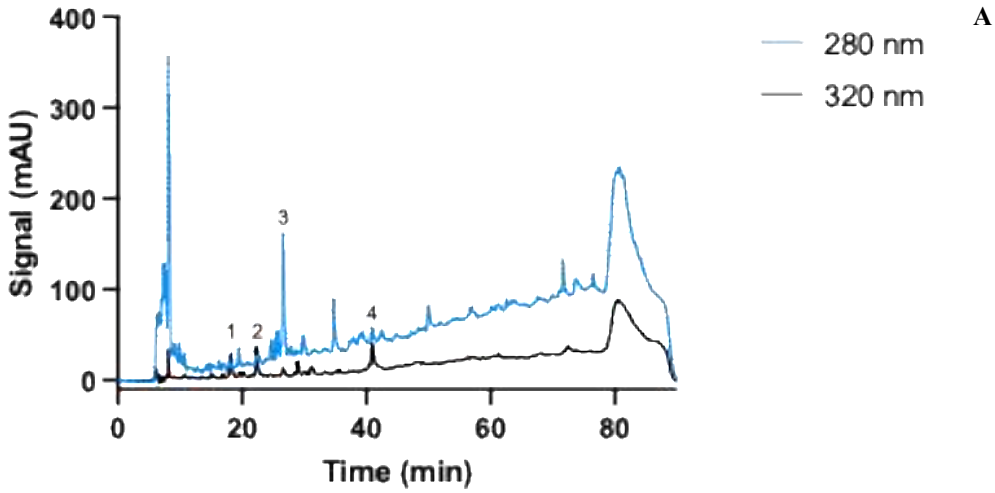


Figure 5A: MS spectrum of the extract of pulp of *Strychnos Madagascariensis.*

Table 5A: Phenolic compounds tentatively identified in the pulp of *Strychnos madagascariensis* by LC–MS/MS.

Peak	$[M-1]^-$	MS^2	Identity	λ_{max} (nm)
1	315	153	glucopyranosyl-dihydroxybenzoic acid	312
2	315	153	glucopyranosyl-dihydroxybenzoic acid	308
3	457	277; 411	not identified	264
4	633	409; 589	not identified	304

$[[M-1]^- \rightarrow [M-H]^-$ (m/z), deprotonated molecular ion; MS^2 fragments, product ions obtained by tandem mass spectrometry; λmax, wavelength of maximum UV–Vis absorption.

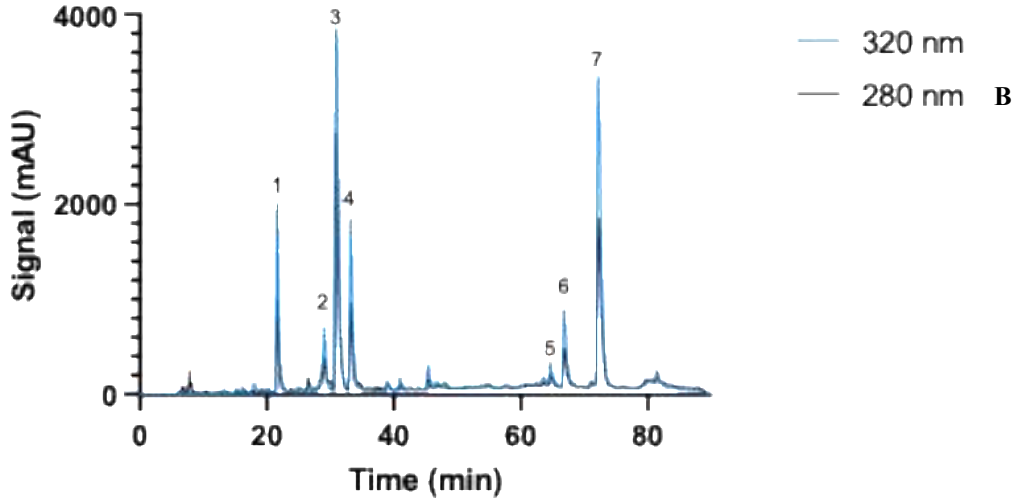


Figure 5B: MS spectrum of the white pericarp of *Strychnos Madagascariensis*.

Table 5B: Phenolic compounds tentatively identified in the white pericarp of *Strychnos madagascariensis* by LC–MS/MS white pericarp

Peak	$[M-1]^-$	MS^2	MS^3	Identity	l_{max} (nm)
1	353	191		caffeoylquinic acid	324
2	371	281	179	not identified	324
3	353	191		caffeoylquinic acid	324
4	353	173		caffeoylquinic acid	324
5	515	353	173	dicaffeoylquinic acid	324
6	571	373	193	not identified	328
7	515	353	173	dicaffeoylquinic acid	328

$[[M-1]^- \rightarrow [M-H]^-$ (m/z), deprotonated molecular ion; MS^2 fragments, product ions obtained by tandem mass spectrometry; λmax, wavelength of maximum UV–Vis absorption.

As seen mainly by tables 5A and 5B, in the white pericarp were identified mainly derivates of hydroxycinnamic acids, namely caffeoylquinic and di-caffeoylquinic acids, while the pulp mainly presented derivates of glucosylated dihydroxybenzoic acids. These results show that the observed differences in the antioxidant capacity of both fractions don't depend only on the total quantity of phenolics but also on the chemical nature of the same.

2. Biocompatibility assays: *in vitro* cell study

2.1. Cytotoxicity evaluation of the decoction

To evaluate cellular viability of HDFs when treated with the decotion made from pulp (dP2), we performed a viability assay using resazurin after 24h of treatment.

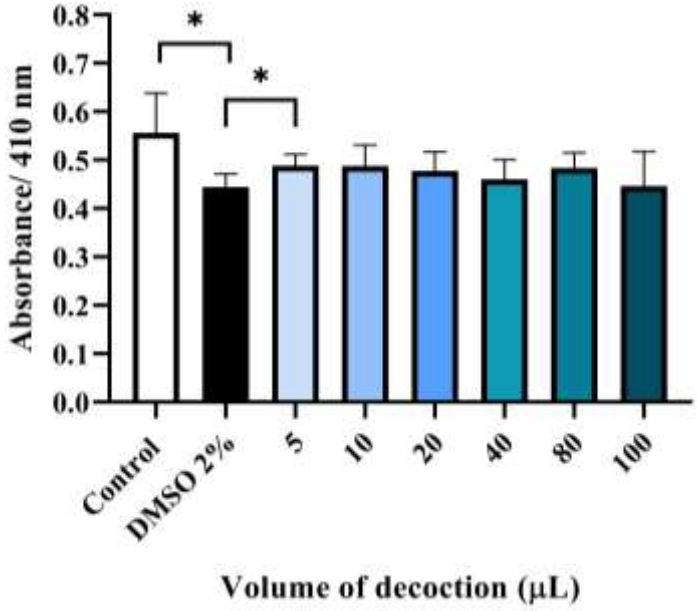


Figure 6: Cellular viability assay of HDF cell culture after 24h of treatment with different volumes of *Strychnos Madagascariensis* decoction (dP2). The results are expressed using mean ± standard deviation, for a n=5 and repeated 3 times. The values present statistically significant differences when *p<0.05, when using a one-way ANOVA.

In Figure 6 we observed a decrease in cell viability when treated with DMSO 2% (negative control) compared to cells without treatment (control, $p = 0.0263$). Regarding the treatment volumes, although cell viability slightly decreased, none of them showed statistically significant differences compared to the control group. When 5 µL of dP2 was used, the effect of DMSO was reversed ($p = 0.0289$). No statistically significant differences were obtained ($p > 0.05$) regarding the other volumes when compared to control group. It was deemed that the volume of 20 µL was to be used use in further experiments.

2.2. 2D vs 3D model

To evaluate cellular viability of the 2D and 3D cell cultures when treated with 20 µL of the decoction (dP2), we performed an MTT assay when the treatment reached 24h. In order to determine the optimal conditions for the use of spheroids, they were observed at the microscope on days 4, 6, 8 and 10 to register differences regarding their size. The results are shown in Figures 7 and 8. It was observed that the diameter of spheroids increased from day 4 to day 6, showing statistical significance ($p = 0.0002$), and from day 6 to day 10, their diameters decreased, also in a significant manner.

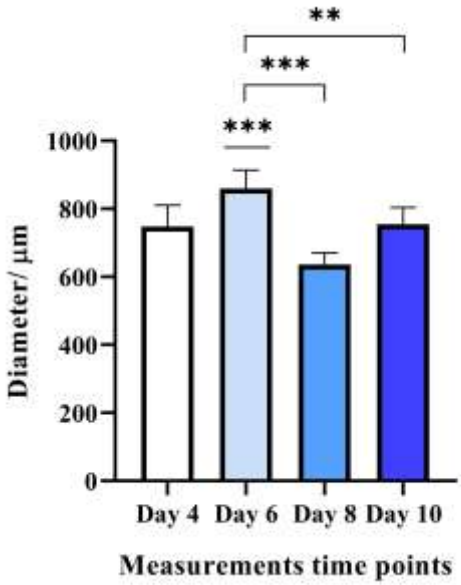


Figure 7: Measurement of spheroids after 4, 6, 8 and 10 days of culture. The results are expressed using mean ± standard deviation. The values present statistically significant differences when ***p<0.0005, (n=24) when using a Brown-Forsyhte and Welch's test.

A two-way ANOVA showed a significant effect in the cellular viability experimental model, while the treatment itself, as seen in Figure 8, did not present a significant effect ($p > 0.05$).

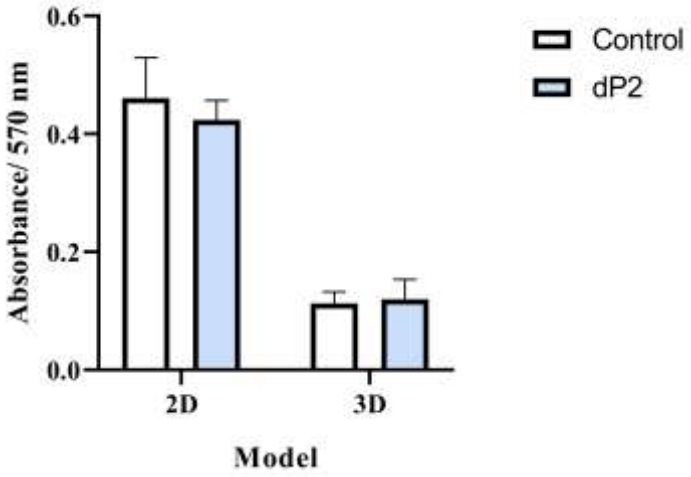
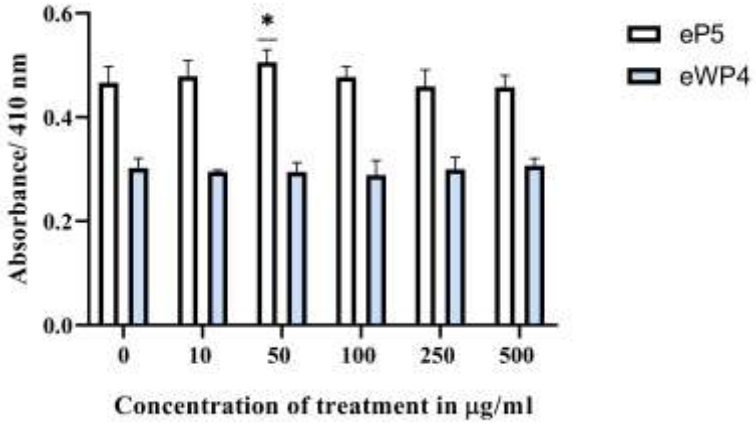


Figure 8: MTT assay on 2D and 3D model after 24h of treatment with *Strychnos Madagascariensis* decoction (dP2). The results are expressed using mean ± standard deviation (n=8). The values present statistically significant differences when *p<0.05, compared with control, using a two-way ANOVA.

2.3.Cytotoxicity of *Strychnos Madagascariensis* lyophilizates

To evaluate cellular viability of HDFs when treated with pulp and white pericarp extracts of NaCl 0.9 % (eP5 and eWP4, respectively) and with the pulp and white pericarp extracts of DMSO (eP6 and eWP5, respectively) we performed a viability assay using resazurin after 24h of treatment. The results are shown in Figures 9A and 9B.

A: NaCl



B: DMSO

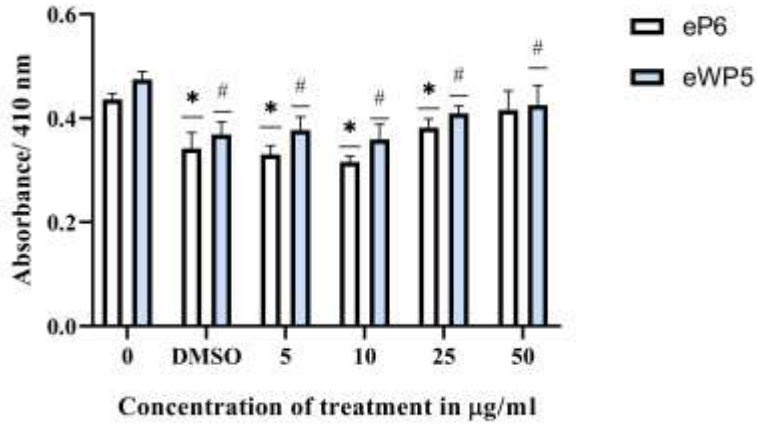


Figure 9: Cellular viability assay of HDF cell culture after 24h of treatment with different concentrations of pulp extracts (eP5 and ep6) and white pericarp extract (eWP4 and eWP5) in **A**- NaCl 0.9% and **B**- DMSO. The results are expressed using mean ± standard deviation, for a (n=5). The values present significant statistic differences when *$p<0.05$, when compared with the respective control, using a two-way ANOVA.

As seen by the previous Figures, a two-way ANOVA didn't show a significant interaction between eP5 and eWP4 vs concentration used. The main observed effect

was of eP5, that has higher viability than eWP4, and we could see that the concentration of 50 µg/mL of eP5 was statistically significant (p = 0.0457) when compared to its control.

Regarding Figure 9B, the two-way ANOVA revealed a significant effect of the concentration and type of compound (eP6 or eWP5), without significant interaction between both. We could also see that for eP6, all concentrations tested (with the exception of 50 µg/mL) presented statistic significancy regarding its control, while for WP5 that was the case for all concentrations tested.

2.4. HDFs viability in a xerosis *in vitro* model

To assess cellular viability of HDFs in an environment of dehydration, fibroblasts were treated with different concentrations of manitol, when cultured in DMEM with 25 mM of glucose (G_{25}) and DMEM with 5 mM of glucose (G_5) - to induce osmotic pressure - and evaluated through a viability assay using resazurin when treatment reached 24h. The results are shown in Figure 10.

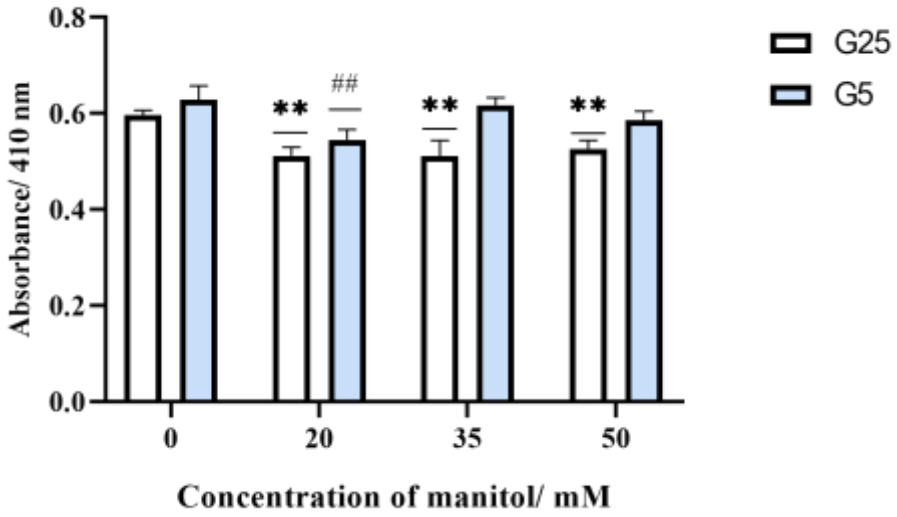


Figure 10: Cellular viability assay of HDF cell culture after 24h of treatment with different concentrations of manitol. The results are expressed using mean ± standard deviation (n=3). The values present statistically significant differences when *p<0.05, **p<0.01, when compared with control, using a two-way ANOVA.

By analysing Figure 10, we can see that manitol affects cellular viability, but that effect is modulated by the level of glucose in the medium. The two-way ANOVA revealed significant effects of the concentration of manitol, the glucose level and a significant interaction between both. In G_{25} we can see that all concentrations of manitol significantly reduced viability regarding its control, while in G_5 only the concentration of 20 mM manitol significantly differed from the control ($p<0.005$).

2.5. HDFs migration when treated with *Strychnos Madagascariensis* lyophilizates

To assess the occurrence of migration of fibroblasts when treated with of our lyophilizates, they were cultured in DMEM G_{25} and DMEM G_5, with and without manitol 50 mM and then treated with 50 µL/mL of pulp and white pericarp lyophilizates in NaCl 0.9 % (eP5 or eWP4). It was evaluated through a scratch assay when treatments were added (0 hours) and then 24 hours later. The results are shown in Figures 11, 12A and 12B.

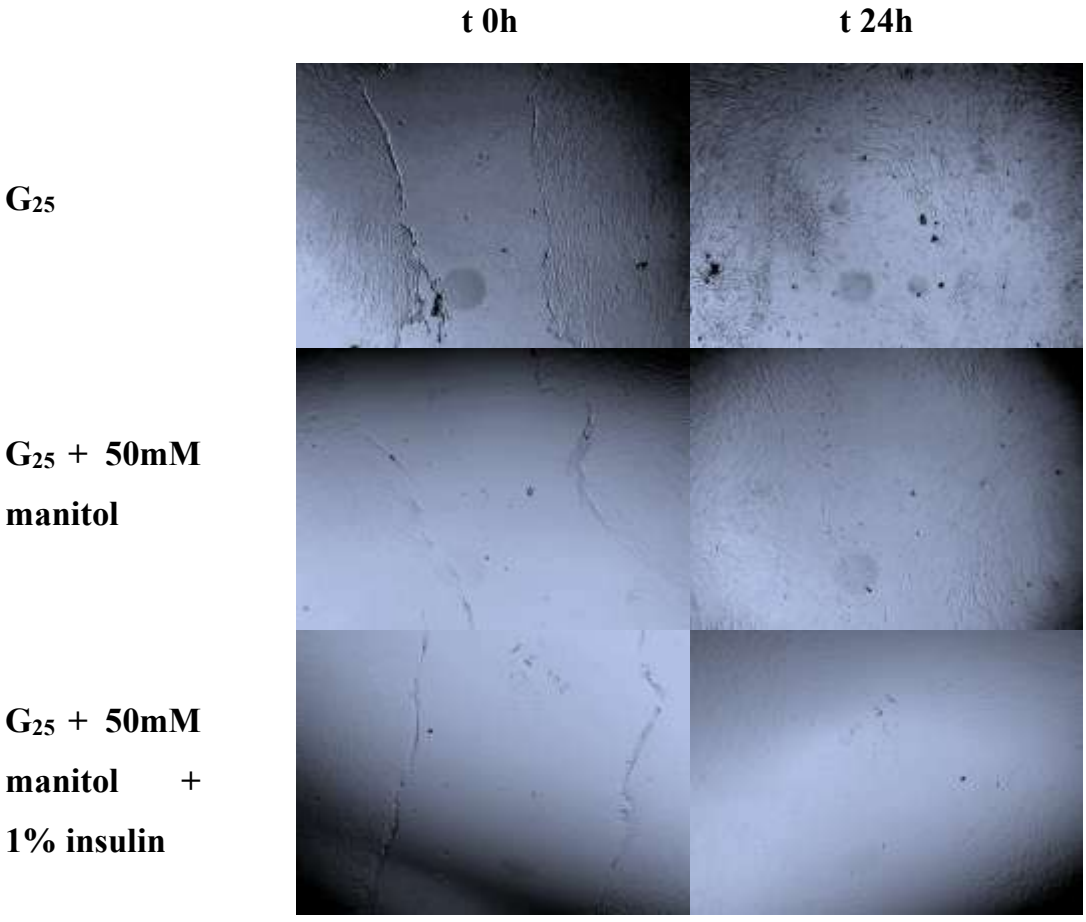
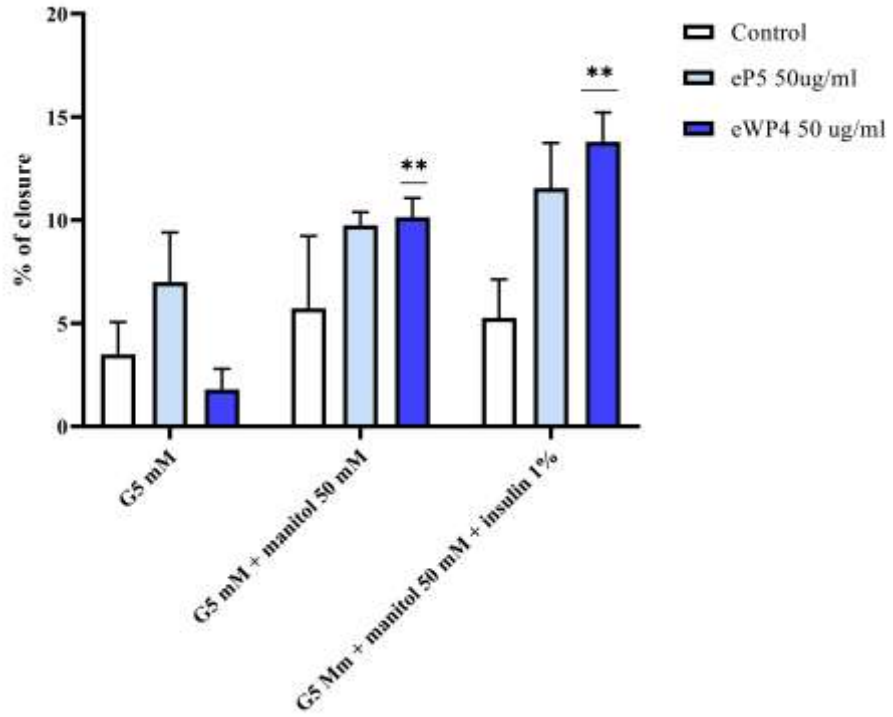


Figure 11: Illustrative image of the wound healing assay in HDFs when treated with insulin and/or manitol (n=4).

A: G5



B: G25

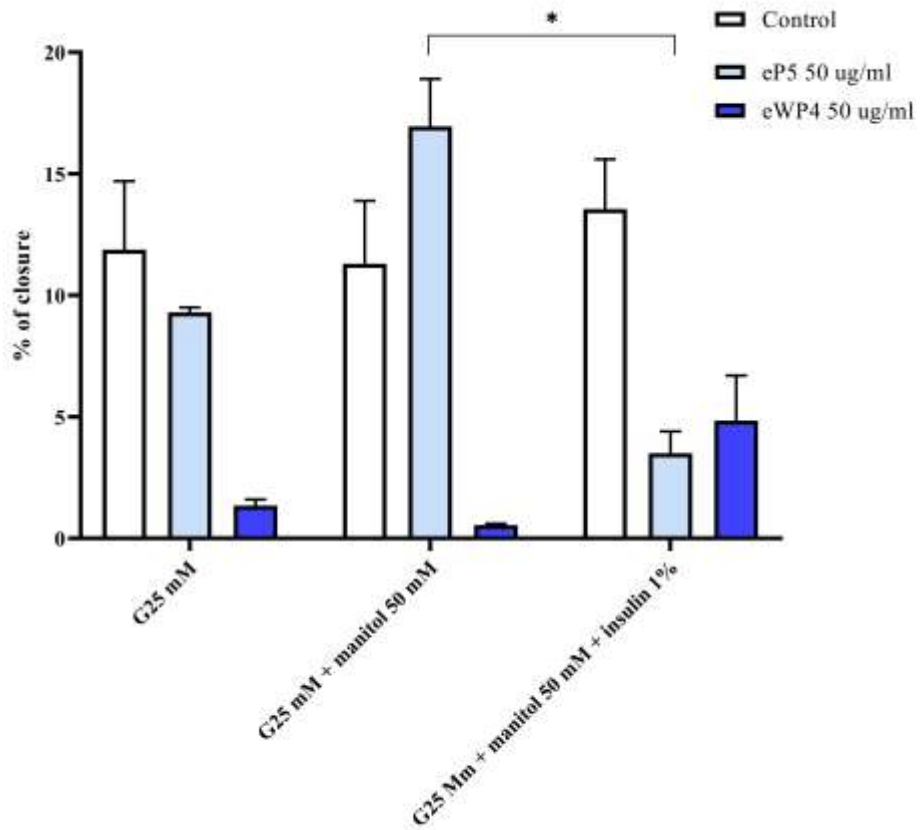


Figure 12: Effect of pulp extract (eP5) and white pericarp extract (eWP4) in NaCl 0.9% on cell migration (wound healing assay), in A – DMEM with 5 mM glucose and B – DMEM with glucose 25 mM. The results are expressed using mean ± standard deviation (n=4). The values present statistically significant differences when *p<0.05, **p<0.01, when compared with control, using a two-way ANOVA.

When analysing the results of Figure 12A, a two-way ANOVA showed a significant effect of the environment the cells were in and the treatment applied, as well as a significant interaction between both, indicating that the effect of the treatment depends on the experimental environment. When testing WP4 in the different environments, both presented significant relevance compared to the control ($p = 0.0042$ and $p = 0.0002$, respectively). Regarding the results of Figure 12B, the two-way ANOVA revealed a significative interaction between the different factors, the main one being the treatment applied. Contrary to Figure 12A, only when testing eP5 we obtained a significant difference between G_5 + manitol 50 Mm and G_5 + manitol 50 mM + 1% insulin ($p=0.0118$).

3. Alkaline phosphatase activity modulation by *Strychnos Madagascariensis* lyophilized extracts

ALP activity in the G_5 control condition was normalized to 100 %.

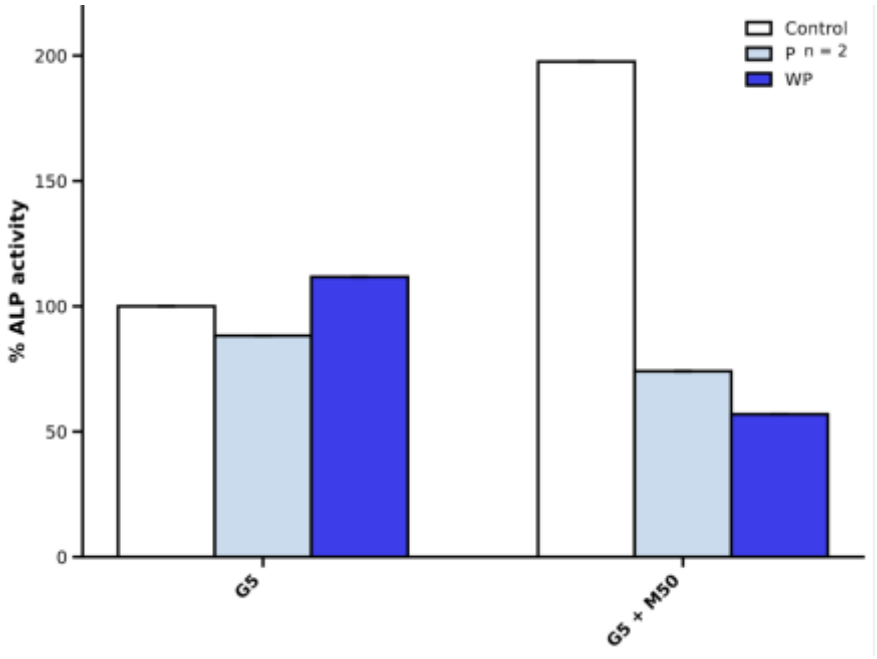


Figure 13: Effect of eP5, eWP4 and manitol 50 M on alkaline phosphatase activity. Alkaline phosphatase (ALP) activity was evaluated under G5 and G5 + manitol 50 mM conditions, in the absence or presence of eP5 or eWP4. Results are expressed as percentage of ALP activity relative to the G5 control condition, which was set to 100%. Bars represent the mean ± standard deviation of two independent measurements (n = 2).

Under G_5 conditions, treatment with eP5 reduced ALP activity to approximately 88 %. In contrast, eWP4 increased ALP activity to approximately 112 % under the same condition. The addition of manitol 50 mM markedly increased ALP activity to approximately 198 %. When eP5 was combined with manitol 50 mM, ALP activity decreased to approximately 74 %. The combination of WP with manitol 50 mM further reduced ALP activity to approximately 57 %. Thus, both eP5 and eWP4 reversed the increase in ALP activity observed under increased osmotic pressure. No statistical significance ($p > 0.05$) can therefore be established for this preleminary experiment.

Discussion

Cutaneous xerosis and its impact in dehydration and lipidic layer

Cutaneous xerosis is characterized by a decrease of hydric content of the *stratum corneum,* accompanied by qualitative and quantitative alterations of intercellular lipids that constitute the epidermic barrier. This condition results in a compromise of barrier function leading to TEWL, reduction in elasticity and higher susceptability to irritation and inflammation. The main lipids involved in barrier maintenance are ceramides, cholesterol and free fatty acids, whose lamelar organization is essencial to prevent excessive water loss.(Wertz, 2021)

In the present study, xerosis was modelated *in vitro* through exposing fibroblasts to different concentrations of manitol, an osmotic agent widely used to induce cellular dehydration. The results demonstrated a significative decrease in cellular viability induced by manitol, being this effect more pronunciated when DMEM had 25 mM of glucose. This data suggests that osmotic stress compromise fibroblast survival and that a hyperglicemic environment enhances the harmful effect. This behaviour is consistent with studies that demonstrate that hyperosmolarity promotes cellular retraction, increase in reactive oxygen species (ROS), mitochondrial dysfunction and activation of pro-apoptotic pathways. The obtained results corroborate this cellular response to osmotic stress, since cellular viability of fibroblasts significantly reduced after being exposed to manitol, particularly in conditions of high glucose levels.(Yu et al., 2011) This may compromise mechanisms of tissue regeneration and indirectly contribute to the deterioration of barrier function observed in xerosis.

The higher sensibility observed in cells cultivated in DMEM with high glucose concentration may also reflect similar alterations to the ones verified in diabetic skin. Hyperglicemia favours the formation of final products of advanced glication (AGEs), increases oxidative stress and promotes a chronic inflamatory state, factors that contribute to extracellular matrix alterations and barrier function. Clinically, these mechanisms are associated with a higher incidence of xerosis in individuals with diabetes mellitus.

Alterations in the lipidic layer observed in xerosis are equally relevant to interpret these results. The decrease in ceramide quantity and other structural lipids reduce the *stratum corneum* capacity to retain water, estabilishing a vicious cicle in which water loss

favours barrier desorganization, progressively agravating cutaneous dryness. (Bouwstra et al., 2023) Although the present experimental model doesn't allow to directly evaluate lipidic composition, the cellular viability reduction induced by osmotic stress supports the idea that dehydration constitutes a determinating factor in xerosis physiopathology and allows for a way to evaluate potential agents with hydrating and restoring properties.

Biocompatibility of *Strychnos Madagascariensis*

Biocompatibility evaluation constitutes and indispensable step in developing new ingredients for cutaneous application, since the compound destined to integrate cosmetic formulations should demonstrate an adequate security profile before its biological properties are evaluated. Besides the absence of citotoxicity, a dermocosmetic ingredient should preserve cellular viability and not compromise physiological processes involved in homeostasis maintenance and healing of the skin. (Charmeau-Genevois et al., 2021)

The cellular viability assays of this study demonstrated that dP2 didn't present cytotoxic effects in HDFs after 24 hours of exposure. Although a slight decrease in viability was observed in some conditions, none of them presented significant differences compared to the control, indicating a good cellular compatibility. Aditionally the volume of 20 µL was selected for following assays since it maintained cellular viability without evidence of toxicity. Similar results were obtained for eP5 and eWP4. These extracts maintained high cellular viability levels in the different evaluated concentrations, particularly eP5 in the concentration of 50 µg/mL. These results suggest that, besides being biocompatible, certain extracts may perform a benefic effect on the fibroblasts metabolic activity, although aditional studies are necessary to enlighten the involved cellular mechanisms.

The absence of cytotoxicity is particularly relevant in the context of the development of products destined to treat cutaneous xerosis. Xerotic skin presents a weakened epidermic barrier and higher susceptibility to irritation, so the used ingredients should present high tolerability. In this context, the good biocompatibility demonstrated by *Strychnos Madagascariensis* constitutes an advantage to its potential incorporation in dermocosmetic formulations destined to hydration and skin repair.

It’s important to note that biocompatibility evaluation represents an initial step in developing a new cosmetic ingredient. Although the assays performed in HDFs give important information on cellular security, the evaluation of cutaneous compatibility should be complemented with tridimensional models of reconstructed skin and skin irritation assays acording to the orientations of OECD, allowing for a closer responde of what can happen *in vivo*.(Akagi et al., 2023; Zhao et al., 2023)

The marked increase in ALP activity under elevated osmotic pressure suggests that ALP is sensitive to changes in the cellular microenvironment. Both eP5 and eWP4 counteracted this response, indicating that these treatments may interfere with osmotic stress-related regulation of ALP, with WP producing the strongest reduction. As ALP activity can reflect changes in cellular phenotype, differentiation and metabolic state, these results should be interpreted cautiously. Furthermore, additional experiments are required to determine whether the observed changes reflect direct modulation of ALP expression or enzymatic activity, alterations in cell viability or cell number, or broader changes in cellular differentiation and metabolic status.

All things considered, the obtained results demonstrate that *Strychnos Madagascariensis* present a favourable biocompatibility profile in the used experimental conditions, being an essential requirement to future exploration as an active ingredient in dermocosmetic formulations. Associating this cellular security with its lipidic profile, antioxidant capacity and potential in promoting cellular viability and migration, this fruit presents characteristics that justify continuing its investigation, aiming at the development of innovative products for skin application.

Sustainability and new products on the market

In the last years, the cosmetic industry has been directing its efforts to the development of more sustainable formulations, prioritizing natural origin ingredients, production processes envrionmentally friendly and the valorization of vegetal subproducts. At the same time, there is a growing demand for multifunctional ingredients capable of promoting, simultaneously, hydration, antioxidant protection and barrier regeneration, reducing the dependence on synthetic compounds. (Silva et al., 2025)

Nowadays, the used emollients to treat cutaneous xerosis are based majorily in ingredients like *petrolatum,* mineral oils, sillicones, glicerin, urea, hyaluronic acid, ceramides and essential fatty acids. Although these ingredients present a recognizable

efficacy in reducing TEWL and restoring barrier function, a lot of them have a predominant oclusive or hydrating property that does not give a simultaneous significant antioxidant capacity. (Augustin & Brignone, 2025)

Strychnos Madagascariensis vs products on the market

The obtained results, alongside with the studys of Dr. Sandra Chemane, demonstrate that *Strychnos Madagascariensis* present a particularly interesting composition for dermocosmetic applications. Chemical characterization revealed a high content of MUFA, especially, oleic acid as well as linoleic acid, α-linolenic acid, vitamin E, carotenoids and phenolic compounds. Aditionally, both the pulp and the white pericarp presented high antioxidant capacity, being evident the high phenolic content and antioxidant acitivity of the white pericarp. The total phenolic content of the inner white pericarp (27.60 mg GAE/g freeze-dried material) falls within the range reported for coffee silverskin extracts proposed for cosmetic applications and exceeds that reported for *Salicornia ramosissima* extracts evaluated in skin cells, although it remains lower than values described for optimized kiwiberry leaf extracts. (Rodrigues et al., 2015; Silva et al., 2022) These comparisons indicate that the previously unexplored white pericarp represents a relevant source of phenolic compounds with potential dermocosmetic interest, even if it cannot be considered among the richest plant sources described in the literature. A similar tissue-dependent distribution of phenolic compounds has been described in avocado, where the peel contains considerably higher phenolic levels than the lipid-rich pulp (Lyu et al., 2023). Likewise, the pulp of *Strychnos madagascariensis* exhibited a high lipid content but comparatively low phenolic content, whereas the inner white pericarp showed markedly higher total phenolic content and antioxidant capacity. This tissue-dependent distribution is consistent with the distinct physiological roles of the fruit tissues, with the pulp functioning primarily as a nutrient and lipid-rich storage tissue, while tissues associated with the outer fruit layers preferentially accumulate phenolic compounds involved in protection against oxidative, microbial, and environmental stress.

Although the determination of TPC demonstrated that the white pericarp presented a higher concentration in phenolic compounds than the pulp, the HPLC-DAD/MS characterization allowed to take a better look into their chemical composition. Caffeoylquinic acids belong to one of the main phenolic classes of vegetable origin and

are widely recognized by their high antioxidant capacity. It's chemical structure, as shown in Figure 13, rich in phenolic hydroxyl groups gives them a high capacity to scavenge ROS, quelate metallic ions and inhibit reactions of lipidic peroxidation. On the other hand, di-caffeoylquinic acids, as shown, present a higher antioxidant capacity compared to mono-caffeoylated analogues, due to the presence of two caffeoyl groups bound to the quinic acid molecule, enhancing its capacity to donate electrons and neutralize free radicals. (Rodrigues et al., 2023)

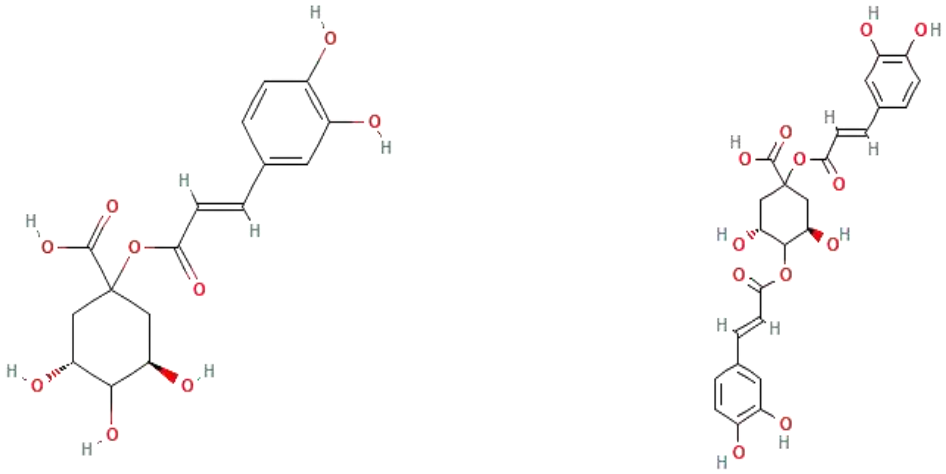


Figure 14: Chemical strucutres of caffeoylquinic (on the left) and di-caffeoylquinic (on the right) acids. Data obtained from PubChem.

In a cutaneous context, this aspect (higher antioxidant activity) takes a particular relevance, since oxidative stress plays a central role in xerosis physiopathology, cutaneous aging and delay in healing, promoting damage in lipids, proteins and cellular deoxyribonucleic acid (DNA).(Merecz-Sadowska et al., 2021) Thus, ingredients rich in caffeoylquinic acids can contribute to preserving cellular homeostasis and protect fibroblasts against oxidative damage.

The identification of di-hydroxybenzoic acids in the pulp also suggests a contribution to antioxidant activity in this fraction. Although these compounds present, generally, a lower antioxidant activity than the caffeoylquinic derivates, its glycosylation can increase chemical stability and solubility in aqueous solutions, which is relevant to future cosmetic applications.

These results suggest that both fractions present distinct phenolic profiles but potentially complementary. The identification of these phenolic compounds allows to establish a connection between the chemical composition and the biological results obtained. The capacity of the extracts to maintain cellular viability, favoring fibroblasts

migration and mitigate osmotic stress effects may be related, at least in some extent, to the presence of these bioactive metabolites, whose antioxidant action can contribute to limit oxidative damage and favor a cellular environment more prone to healing processes. (Melguizo-Rodríguez et al., 2021)

This composition distinguishes this fruit from a lot of ingredients used in comercial formulations; a comparation between well-known products in the portuguese market, such as Barral DermaProtect hydrating cream and Halibut Derma hydrating cream, allows to contextualize its potential. The formulation of these products are essentially destined to restoring cutaneous barrier and prevention of water loss, relying on products like *petrolatum*, liquid parafin, lanolin, etc. These products act by forming a protective film over the cutaneous surface reducing TEWL and promoting barrier function. In contrast, our fruit presents naturally diverse bioactive compounds relevant to the skin. This way, while conventional creams act mainly on physical mechanisms of hydration and occlusion, *Strychnos Madagascariensis* may offer a multifunctional approach, combining a favorable lipidic composition with antioxidant molecules naturally present on the fruit.This combination can represent an advantage in developing sustainable cosmetic formulations, since a single vegetable ingredint reunites both antioxidant and emollient properties.

Another important aspect is the potential to fully use the fruit. While most of cosmetic products uses only oils extracted from seeds or specific fruits, this study shows that both parts of the fruit present bioactive compounds with biological interest. This valorization of different parts is aligned with circular economy principles, promoting sustainable use of natural resources and reducing agri-food waste. Besides its chemical composition, the biological assays demonstrated that *Strychnos Madagascariensis* extratcts (eP5 and eWP4) didn't present citotoxicity in the tested concentrations and in some experimental conditions, promoted cellular viability and migration, suggesting a potential in cutaneous regeneration. These results reenforce the possibility to use this fruit as a functional ingredient in formulations destined to dry or damaged skin, combining antioxidant and indirect hydrating properties.

Strychnos Madagascariensis **as a potential new ingredient in dermacosmetics**

The efficacy of a dermocosmetic product doesn't depend only on the intrinsic properties of its bioactive compounds, but also on its concentration, stability, bioavailavility,

capacity to penetrate cutaneously and interaction with the remaining excipients in the formulation.

Although aditional studies, regarding its formulation, and in tridimensional models and clinical trials to confirm its efficacy and stability are needed, the obtained results suggest that *Strychnos Madagascariensis* gathers characteristics that make it a promising candidate to developing dermocosmetic sustainable products. The combination of a favourable lipidic profile, high antioxidant capacity and regenerative potential represents a diferentiating approach when compared to many conventional emollients, whose action focus on the hydration and occlusion of cutaneous surface.

Limitations and future perspectives

The use of 2D *in vitro* models, although providing valuable information, do not mimic the complexity of the human skin, whose vascularization, systemic interactions, immune responses, etc, all influence the biological response to our formulations. Further assays like extracelular matrix production (like collagen) and oxidative stress biomarkers, among others, should be carried out in order to provide a better understanding of the underlying mechanisms of the observed effects.

Regarding our extracts, a more thorough characterization is required, in order to identify the individual bioactive compounds responsible for the biological response obtained. Also, a broader number of concentrations and exposure times should be incorporated in future assays.

These limitations don't invalidate the findings of these studies; they are a first step to continue with these studies and incorporate new ones that incorporate more complex skin models, mechanistic analysis and *in vivo* validation.

The obtained results in this study support the potential of the fruit components of *Strychnos madagascariensis* as promising ingredients for skincare products. However there are additional studies to validate its efficacy and ease its transition to cosmetic applications.

A first step consists of evaluating the biological activity of the most promising extracts resorting to the skin-on-a-chip xerosis model, currently in development. This advanced microphysiological platform reproduce, in a more reliable way, the structural and functional characteristics of human skin, allowing to evaluate the extracts in conditions that mimic the pathological microenvironment of xerotic skin.(Mori et al., 2017; Wufuer et al., 2016) Additionally, future studies should centre themselves on understanding which underlying molecular mechanisms are being affected by the biological activity of the extracts. In particular, the exploration of the impact on the canonical and non-canonical pathways involved in cutaneous homeostasis, inflammation and skin healing processes will allow us to understand the mechanisms responsible for the observed biological effects. (Nusse & Clevers, 2017) Lastly, it would be important to investigate delivery systems capable of improving the stability, bioavailability and cutaneous penetration of the compounds. Among the different available approaches, liposomal vesicles represent a promising strategy due to their

high biocompatibility and their capacity to promote controlled release and higher permeation of active ingredients on the skin.(Pierre & Dos Santos Miranda Costa, 2011) The incorporation of the extracts in these formulations may potentiate its biological efficacy and increase its applicability in developing inovative dermocosmetic products destined to treat cutaneous xerosis.

These lines of investigation will contribute to bring closer the results obtained in this study and its future application in safe and effective dermocosmetic products.

Conclusion

The present study demonstrated that the pulp and the previously unexplored inner white pericarp fraction of *Strychnos madagascariensis* exhibit markedly distinct chemical compositions and biological potential, highlighting the complementary value of these two fruit fractions for dermocosmetic applications. The inner white pericarp fraction showed a substantially higher total phenolic content and antioxidant capacity than the pulp and was particularly rich in hydroxycinnamic acid derivatives, mainly caffeoylquinic and dicaffeoylquinic acids. These compounds have been widely reported to exhibit antioxidant, cytoprotective, anti-inflammatory, and skin-regenerative properties and may therefore contribute, at least in part, to the biological responses observed in the present study. By contrast, the pulp contained considerably lower amounts of phenolic compounds, represented mainly by glucosylated dihydroxybenzoic acid derivatives. The chemical characterization performed in this work complements previous studies on *S. madagascariensis*, which showed that the pulp is an unusual fruit matrix due to its high lipid content, accounting for more than 30 % of its dry weight, and its abundance of bioactive lipophilic constituents, including monounsaturated fatty acids, carotenoids, vitamin E, sterols, and triterpenic alcohols. Conversely, the present work identifies, for the first time, the inner white pericarp fraction as a phenolic-rich tissue with a markedly different chemical profile and a minor lipid fraction, suggesting that its biological potential may be associated predominantly with its phenolic composition. The *in vitro* assays demonstrated that the tested preparations were biocompatible with skin cell models and supported the potential of *Strychnos madagascariensis* fruit fractions as promising ingredients for dermocosmetic applications. In particular, the inner white pericarp fraction emerged as a promising and previously unexplored source of bioactive compounds that would otherwise be discarded, reinforcing the sustainable valorization of this underutilized fruit species. The *in vitro* assays demonstrated that the tested preparations were biocompatible with skin cell models, promoted fibroblast migration, and modulated alkaline phosphatase activity under hyperosmotic conditions, supporting the potential of *S. madagascariensis* fruit fractions as promising ingredients for dermocosmetic applications.Overall, this work provides new insights into the chemical composition and biological potential of *Strychnos madagascariensis* fruit tissues and establishes a

scientific basis for their future use in dermocosmetic formulations. Nevertheless, the tentative phenolic assignments should be confirmed and expanded by high-resolution LC–MS/MS, preferably using authentic reference standards. Further studies are also required to elucidate the mechanisms underlying the observed cellular responses, validate the biological effects in more physiologically relevant skin models, and optimize the incorporation and delivery of the most promising bioactive fractions in dermocosmetic formulations.

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Appendix A

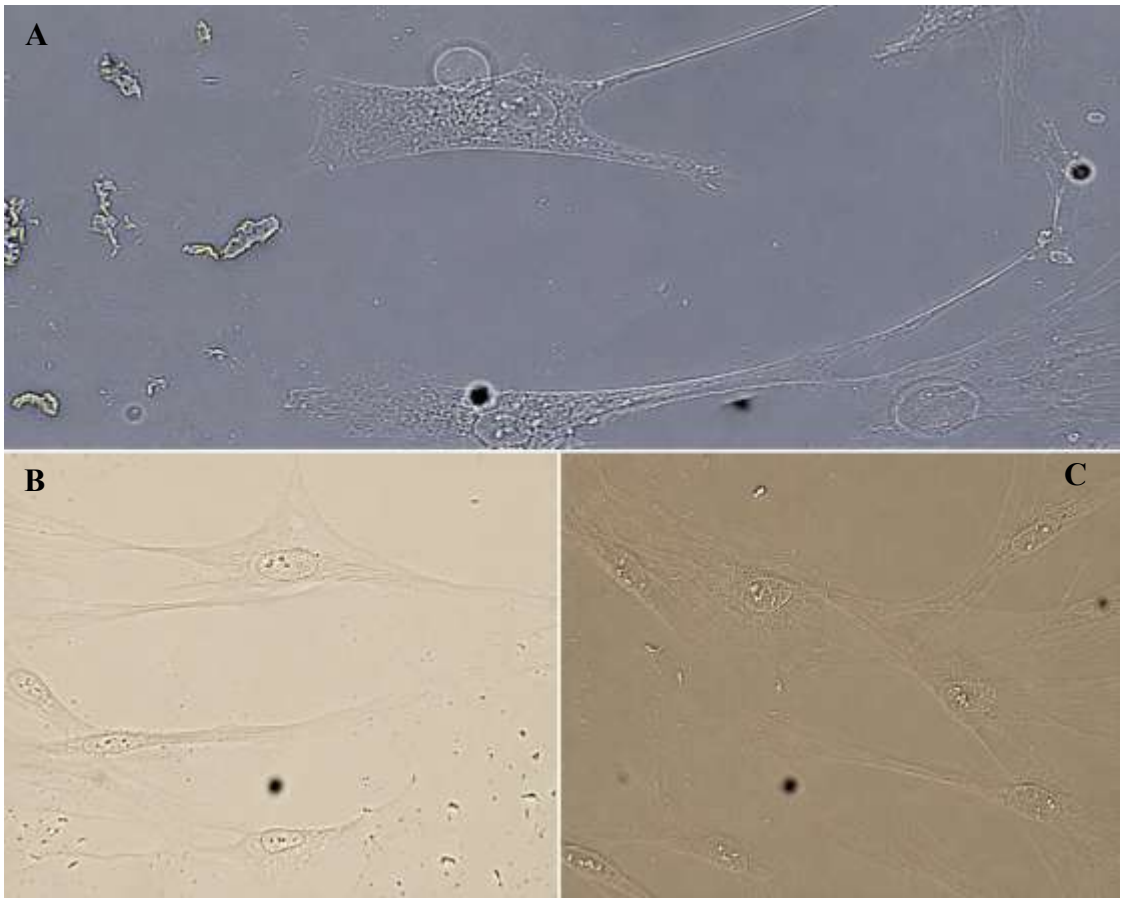


Figure 1: Fibroblasts after the wound healing assay in which were treated with **A -** 50 mM manitol and 1% insulin in high glucose medium; **B-** 50 mM manitol and 1% insulin in high glucose medium and 50 µg/mL of white pericarp extract (eEP4) and **C -** 50 mM manitol and 1% insulin in high glucose medium and 50 µg/mL of pulp extract (eP5).

Appendix B



Figure 1: Sirius red staining for collagen of fibroblasts, in low glucose medium, after wound healing assay - HDFs in medium; on the left; HDFs in medium with 50 mM manitol; in the middle, HDFs in medium with 50 mM manitol and 1% insulin, on the right.



Figure 2: Sirius red staining for collagen of fibroblasts, in low glucose medium treated with the white pericarp extract (eWM4), after wound healing assay - HDFs in medium; on the left, HDFs in medium with 50 mM manitol; in the middle, HDFs in medium with 50 mM manitol and 1% insulin, on the right.

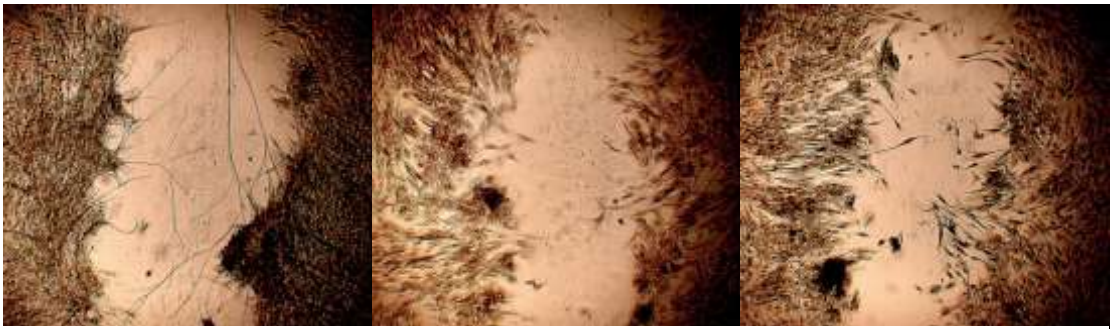


Figure 3: Sirius red staining for collagen of fibroblasts, in low glucose medium treated with the pulp extract (eP5), after wound healing assay - HDFs in medium; on the left, HDFs in medium with 50 mM manitol; in the middle, HDFs in medium with 50 mM manitol and 1% insulin, on the right.



Figure 4: Sirius red staining for collagen of fibroblasts, in HIGH glucose medium, after wound healing assay - HDFs in medium, on the left, HDFs in medium with 50 mM manitol; in the middle, HDFs in medium with 50 mM manitol and 1% insulin, on the right.



Figure 5: Sirius red staining for collagen of fibroblasts, in high glucose medium treated with the white pericarp extract (eWM4), after wound healing assay - HDFs in medium; on the left, HDFs in medium with 50 mM manitol; in the middle, HDFs in medium with 50 mM manitol and 1% insulin on the right.

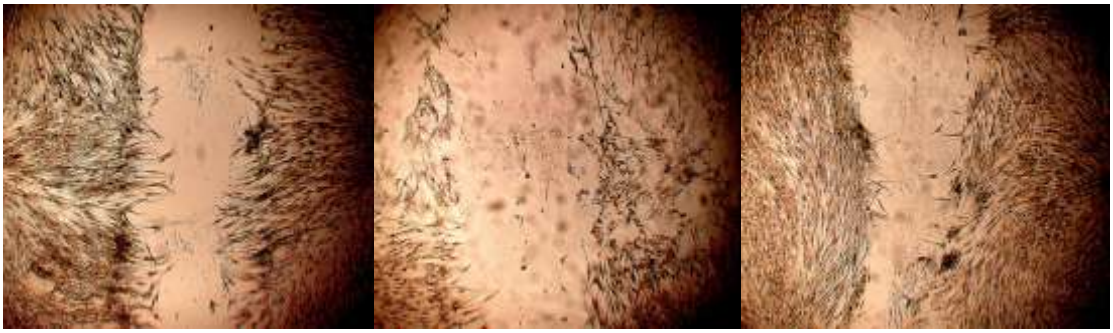


Figure 6: Sirius red staining for collagen of fibroblasts, in high glucose medium treated with the white pericarp extract (eP5), after wound healing assay -HDFs in medium on the left, HDFs in medium with 50 mM manitol in the middle, HDFs in medium with 50 mM manitol and 1% insulin, on the right.



Figure 7: Sirius red staining for collagen of keratinocytes, in low glucose medium, after wound healing assay. HDFs in medium, on the left; HDFs in medium with 50 mM manitol; in de middle, HDFs in medium with 50 mM manitol and 10% insulin, on the right.



Figure 8: Sirius red staining for collagen of keratinocytes+fibroblasts, in low glucose medium, after wound healing assay. HDFs in medium, on the left; HDFs in medium with 50 mM manitol; in de middle, HDFs in medium with 50 mM manitol and 10% insulin, on the right.



Figure 9: Sirius red staining for collagen of keratinocytes, in high glucose medium, after wound healing assay. HDFs in medium, on the left; HDFs in medium with 50 mM manitol and 10% insulin, on the right.



Figure 10: Sirius red staining for collagen of keratinocytes+fibroblasts, in high glucose medium, after wound healing assay. HDFs in medium, on the left; HDFs in medium with 50 mM manitol and 10% insulin, on the right.

Appendix C

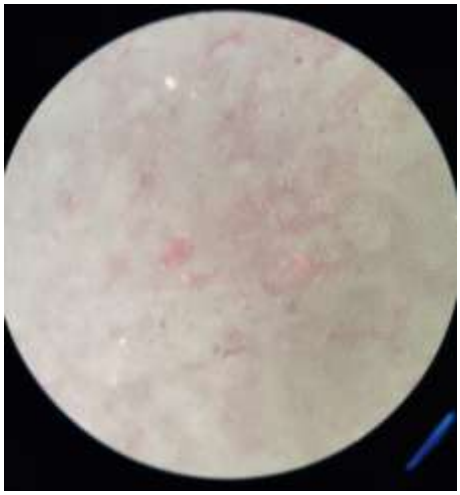


Figure 1: Alkaline Phosphatase stainning in HDFs in high glucose medium.

Appendix D

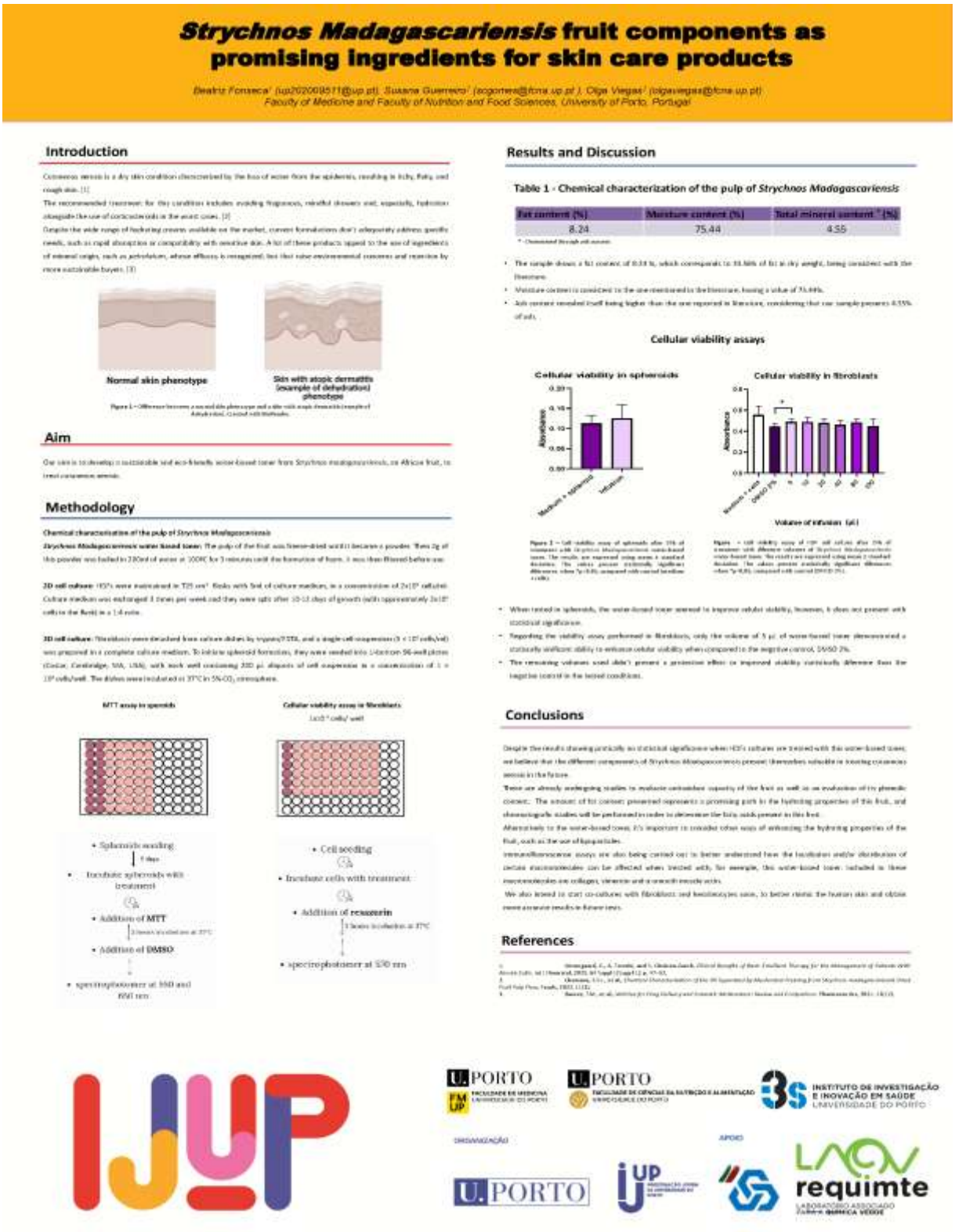


Figure 1: Poster presentation of Encontrro de Investigação Jovem da Universidade do Porto (IJUP), presented in 2026/05/08

Appendix E



Figure 1: Poster presentation of MetBalance "The effects of weight loss: friend or foe", presented in 2026/11/05

FACULDADE DE **MEDICINA**