UNIVERSIDADE D
COIMBRA

Carolina Ferreira Soares

Relatórios de Estágio sob orientação da Dra. Vanessa Ribeiro e da Dra. Dina Lopes e Monografia intitulada "Natural-derived pigments: focus on topical applications" sob orientação da Mestre Laura Victória Vieira Ferreira referentes à Unidade Curricular "Estágio", apresentados à Faculdade de Farmácia da Universidade de Coimbra para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas

Setembro de 2023

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Coimbra, 6 de setembro de 2023.

Carolina Ferreira Soares

(Carolina Ferreira Soares)

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Parte I

Relatório de Estágio em Farmácia Comunitária

FARMÁCIA BRANDÃO

Sob orientação da Dra. Vanessa Ribeiro

LISTA DE ABREVIATURAS

FB	Farmácia Brandão
IVA	Imposto sobre o Valor Acrescentado
MICF	Mestrado Integrado em Ciências Farmacêuticas
MNSRM	Medicamento não sujeito a receita médica
MNSRM-EF	Medicamento não sujeito a receita médica de dispensa exclusiva em farmácia
MSRM	Medicamento sujeito a receita médica
PVP	Preço de Venda ao Público
SWOT	*Strengths, Weaknesses, Opportunities and Threats*

1. Introdução

O Mestrado Integrado em Ciências Farmacêuticas (MICF) na Faculdade de Farmácia da Universidade de Coimbra contempla um plano de estudos abrangente e multidisciplinar com a duração de 5 anos letivos, culminando com a realização de um estágio curricular que funciona como elo de ligação entre os conhecimentos técnico-científicos adquiridos no curso e a prática profissional. Assim, o estágio em farmácia comunitária integra uma área obrigatória.

A atividade central do farmacêutico, e a formação académica que mais nos diferencia dos restantes profissionais de saúde, é a área do medicamento. Contudo, não se pode negligenciar a componente humana da nossa profissão, sendo fundamental que o desempenho do ato farmacêutico seja realizado não só com a maior diligência e competências técnicas e éticas, mas também com uma relação de proximidade com o utente. Por isso, considero o estágio como uma oportunidade para pôr à prova tanto os conhecimentos teóricos adquiridos no curso como as nossas capacidades pessoais e emocionais.

O presente relatório incide sobre o estágio curricular realizado na Farmácia Brandão, entre os dias 3 de janeiro até 13 de abril de 2023, sob a notável orientação da Diretora Técnica, Dra. Vanessa Ribeiro, e restante equipa. Este assume a forma de uma análise SWOT (do inglês, *Strenghts, Weaknesses, Opportunities, Threats*) na qual faço uma análise crítica à realidade profissional, relacionando os pontos fortes e fracos do ambiente interno da farmácia com as oportunidades e ameaças do ambiente externo à mesma.

2. Farmácia Brandão

A Farmácia Brandão (FB) é a mais antiga farmácia da Mealhada. Situada no centro da cidade, está à disposição para um primeiro contacto dos utentes com profissionais de saúde. Instalada num edifício de Arte Nova, inaugurado em 1912, a Farmácia Brandão tem este nome devido ao seu primitivo proprietário Augusto Brandão.

A FB disponibiliza aos seus utentes serviços de medição da glicémia, colesterol, peso e altura, índice de massa corporal, assim como os serviços de administração de vacinas fora do Plano Nacional de Vacinação e medicamentos injetáveis.

O horário de funcionamento nas semanas de não serviço é das 8h30 às 20h, trabalhando aos sábados das 9h às 13h; e nas semanas de serviço o horário é das 8h30 às 00h, sendo o horário de sábado das 8h30 às 13h e das 14h às 00h e o de domingo das 9h às 13h e das 15h às 00h.

3. ANÁLISE SWOT

3.1 Pontos Fortes

3.1.1 Plano de estágio bem delineado

A evolução do estágio foi realizada de forma contínua e gradual, o que permitiu uma adaptação progressiva e uma melhor integração nas atividades da farmácia.

Numa fase inicial comecei por desempenhar mais funções de *backoffice* através da receção de encomendas e arrumação das mesmas. A receção das encomendas foi realizada no Sifarma 2000® exigindo cautela uma vez que é necessário confirmar vários aspetos, tais como a quantidade de produtos recebidos, a validade, o estado dos produtos e os preços de venda ao público (PVP). Aquando da verificação de preços, devemos ter em conta se o produto é Medicamento Sujeito a Receita Médica (MSRM) ou Medicamento Não Sujeito a Receita Médica (MNSRM). Se for MSRM, o preço é fixo e regulado pelo INFARMED, I.P., no entanto, caso seja um MNSRM, o PVP não está estabelecido e é calculado tendo em conta a margem de lucro, o preço de custo e o Imposto de Valor Acrescentado (IVA) do produto. Para além disso, era também nesta fase que segregava os produtos que estavam reservados para os utentes, e que iriam ser armazenados em local próprio dos restantes produtos. Depois procedia à arrumação dos restantes produtos nos devidos locais, seguindo o princípio *first in first out*. Esta foi uma tarefa fulcral, uma vez que me ajudou a perceber como a farmácia está organizada, acabando, posteriormente por ter maior destreza e prontidão aquando do atendimento ao público.

Elaborei notas de devolução, as quais eram efetuadas quando os produtos que chegavam à farmácia não se encontravam dentro dos critérios pré-estabelecidos (embalagem danificada, prazo de validade demasiado pequeno, embalagem pedida por engano, entre outros). Auxiliei noutras atividades de gestão de *stocks* como verificação dos prazos de validade, contagens físicas, regularização de notas de crédito e reorganização dos produtos na zona de atendimento. Nos tempos livres, recomendaram-me estudar os fluxogramas de intervenção farmacêutica, os medicamentos não sujeitos a receita médica de dispensa exclusiva em farmácia (MNSRM-EF) e outros produtos de venda livre e cooperei na verificação do receituário.

Desde o início do estágio, em paralelo com as tarefas realizadas em *backoffice,* comecei a acompanhar os diferentes membros da equipa no atendimento ao público. Numa fase inicial apenas observava como procediam ao atendimento, prestando atenção à forma como abordavam os utentes, às questões que lhes eram colocadas e informações que eram dadas relativas ao modo de utilização ou toma de um produto ou medicamento. Para além disso, tentava acompanhar o modo de funcionamento do Sifarma. Progressivamente, à medida que

fui ganhando mais autonomia e confiança, fui começando a realizar atendimentos com supervisão dos colegas, tentando sempre adaptar o discurso ao tipo de utente e, sempre que pertinente, aconselhar medidas não farmacológicas. Dispensei tanto MSRM, explicando ao utente a posologia indicada, possíveis interações, como MNSRM, produtos de higiene oral, dermocosmética e suplementos alimentares, sempre sabendo que, caso tivesse alguma dúvida, a equipa estaria completamente disponível para me auxiliar. Durante o atendimento prestei ainda outros serviços tais como medição da pressão arterial.

3.1.2 Pedidos do lar

A Farmácia Brandão fornece medicamentos e outros produtos de saúde e bem-estar ao lar da Santa Casa da Misericórdia da Mealhada. Ao longo do meu estágio, tive a oportunidade de intervir na dispensa e entrega dos produtos solicitados semanalmente. Considero este aspeto um ponto forte para a farmácia no sentido em que aumenta a rotatividade dos produtos e o capital da farmácia. Pessoalmente também foi um ponto forte porque me permitiu manter ocupada com as atividades da farmácia e ao mesmo tempo contactar com os produtos, conhecendo melhor as embalagens e indicações terapêuticas.

3.1.3 Cartão Saúda

A Farmácia Brandão está integrada no programa das Farmácias Portuguesas, cujo cartão permite a acumulação e rebate de pontos em determinados produtos, o que ajuda a fidelizar os utentes. A equipa sempre me incentivou a informar os utentes da existência do cartão, enfatizando a sua gratuitidade e vantagens, de modo que os mesmos pudessem aderir e, desta forma, beneficiar do mesmo.

O contacto diário com este cartão permitiu-me criar uma relação mais próxima com alguns utentes pois era um facilitador de conversa durante o atendimento e fez-me perceber a importância que pequenas ofertas têm na fidelização dos utentes.

3.2 Pontos Fracos

3.2.1 Área de dermofarmácia e cosmética

Na gestão de uma farmácia é de extrema importância adaptar o *stock* à população, de forma a ir ao encontro das suas necessidades e preferências. A área da cosmética está em constante evolução e nos dias que correm há uma crescente preocupação com a imagem por parte da população.

Na FB existe uma fraca aposta nesta área, dispondo apenas de alguns produtos das seguintes gamas: Aveeno®, Barral®, Bioderma®, Caladryl®, Laboratórios Babé® e Oleoban®. O reduzido *stock* deste tipo de produtos e a competitividade com a outra farmácia e os supermercados faz com que estes produtos não sejam tão trabalhos, reduzindo as suas vendas. Quando há necessidade de adquirir um produto de uma categoria diferente é necessário encomendar aos fornecedores diários (Cooprofar e Empifarma), ficando o mesmo disponível apenas na tarde desse dia ou no dia útil seguinte, o que compromete o trabalho do farmacêutico. Assim, considero que esta área foi um dos pontos fracos do meu estágio, uma vez que não tive oportunidade de fazer um aconselhamento especializado relativamente a produtos que os utentes procuravam de outras gamas. Além disso não me foi permitido contactar com as marcas de referência e que são da preferência dos utentes.

3.2.2 Preparação de medicamentos manipulados

Segundo o Decreto-Lei n.º 95/2004, de 22 de abril, um medicamento manipulado é "qualquer fórmula magistral ou preparado oficinal preparado e dispensado sob a responsabilidade de um farmacêutico" [1].

Atualmente, devido ao crescimento da indústria farmacêutica, este tipo de medicamentos não tem a expressão que outrora tivera. Na Farmácia Brandão, em concreto, a procura por medicamentos manipulados é praticamente nula. Por esse motivo, a FB não dispõe de muitas matérias-primas necessárias à preparação destes medicamentos, devido ao facto de ser cada vez mais difícil a sua aquisição, mas também devido ao reduzido número de requisições. Portanto, quando surge um utente com uma prescrição de um medicamento manipulado cuja composição inclua matérias-primas que estão em falta na farmácia, esta solicita a preparação do manipulado a outra farmácia.

Assim sendo, ao longo do estágio apenas tive oportunidade de preparar uma solução alcoólica de ácido bórico à saturação, um desinfetante auricular. Este foi preparado de acordo com uma ficha de preparação que é preenchida logo no início, na qual consta a forma farmacêutica do manipulado, as matérias-primas, o seu lote e validade, os dados do utente e do médico prescritor, o cálculo do PVP (tendo em conta o valor das matérias-primas, do material da embalagem e dos honorários da manipulação) e os cuidados de armazenamento. Após total preenchimento da ficha e da preparação do manipulado, esta é datada e assinada pelo farmacêutico responsável pela preparação do manipulado e pelo supervisor (diretora técnica) e é arquivada em conjunto com uma cópia da receita, sendo todo o procedimento realizado de acordo com as Boas Práticas de Preparação de Medicamentos Manipulados.

3.2.3 Homogeneidade de utentes

Embora seja importante conhecer a população e realizar um atendimento de proximidade com os utentes quer a nível da terapêutica quer a nível pessoal, o conhecimento das suas necessidades farmacoterapêuticas faz com que os atendimentos sejam muitas vezes monótonos não apelando ao sentido crítico do farmacêutico na construção de um aconselhamento. O aconselhamento farmacêutico acaba por ser reservado aos utentes de faixas etárias mais jovens ou de passagem.

3.3 Oportunidades

3.3.1 Formações

Tendo em conta que a área da saúde está em constante evolução, o farmacêutico deve manter atualizadas as suas capacidades técnicas e científicas com o objetivo de aperfeiçoar constantemente o seu desempenho, permitindo prestar o melhor aconselhamento aos utentes.

Ao longo do estágio fui fazendo tanto formações *on-line* como assisti a gravações de *webinares* que abordam os mais variados temas. Efetivamente, estas formações são essenciais pois permitem consolidar e atualizar conhecimentos, mas também conhecer melhor alguns produtos, o que se mostra bastante vantajoso no momento de atendimento ao utente e respetivo aconselhamento farmacêutico.

Para além disso, alguns delegados que vinham à farmácia, acabavam por fazer breves sessões de formação sobre os produtos do laboratório que representam.

3.3.2 Medicamentos não sujeitos a receita médica de dispensa exclusiva em farmácia (MNSRM-EF)

Durante o meu estágio fui incentivada a inteirar-me sobre quais os MNSRM-EF existentes e a familiarizar-me com o seu protoloco de dispensa. Atualmente, devido aos numerosos espaços que existem de venda de MNSRM, é necessário que o farmacêutico possua conhecimento destes produtos de forma a valorizar as suas indicações, sendo também uma forma de fidelizar os utentes com produtos que apenas existem na farmácia e que são dispensados pelo farmacêutico, com o seu devido aconselhamento. Atualmente constam nesta lista 49 medicamentos [2].

3.4 Ameaças

3.4.1 Liberalização do mercado de MNSRM

Atualmente, a maioria dos MNSRM não são de venda exclusiva em farmácia, podendo ser comercializados noutros estabelecimentos que são devidamente registados no Infarmed. A existência destes espaços que, na sua maioria, se encontram em superfícies comerciais, constitui uma ameaça para as farmácias em dois níveis.

Por um lado, são uma grande concorrência a nível económico porque têm um grande poder de negociação com os laboratórios devido ao facto de comprarem grandes quantidades de cada vez, o que faz com que consigam colocar os produtos a preços muito competitivos. Por outro lado, devido à sua localização e à atual conjuntura económica, alguns utentes preferem abdicar do aconselhamento farmacêutico e utilizar estes espaços como primeiro recurso.

O farmacêutico, sendo o especialista do medicamento, tem um papel essencial na dispensa destes, mesmo os MNSRM, uma vez que estes, como todos os outros, podem conduzir a efeitos adversos ou interações quando tomados em conjunto com outros produtos. Neste sentido, e de forma a limitar o fácil acesso a alguns medicamentos com o objetivo de diminuir a automedicação irresponsável, o Infarmed criou o grupo de MNSRM-EF, lista esta que está em constante atualização e disponível no site.

Assim sendo a liberalização da venda de MNSRM foi uma ameaça ao meu estágio e uma possível ameaça ao futuro profissional uma vez que foram algumas as vezes em que existiu indignação por parte de alguns utentes, quando confrontados com preços mais elevados na farmácia e também porque contribuem para a visão de que os farmacêuticos são simples "vendedores de medicamentos".

3.4.2 Medicamentos esgotados

Durante o meu estágio, foram várias as vezes em que existiram medicamentos esgotados devido a rutura de *stocks*.

Caso não fosse possível resolver o problema e não fosse possível arranjar o medicamento, o utente era reencaminhado para outras farmácias. A maioria mostrou-se compreensível, no entanto, outros acabavam por achar que era a farmácia que não sabia fazer uma boa gestão de *stocks*, culpando a equipa por não poder levar o medicamento que necessitavam.

Estas situações constituíram uma ameaça uma vez que foram várias as vezes em que tive de lidar com utentes insatisfeitos, que tiveram de se dirigir a outras farmácias para conseguirem a medicação. A inviabilidade da dispensa de medicamentos ao utente prejudica-o de forma direta, principalmente quando se trata de medicamentos de uso crónico ou urgente, pois coloca em risco a sua saúde e bem-estar e, também, afeta a farmácia, uma vez que esta não consegue cumprir com duas das suas grandes finalidades, satisfazer a necessidade dos utentes e a dispensa de medicamentos.

Os casos mais relevantes de rutura de *stock* durante o meu estágio foram as do Rivotril®, Ovestin®, Inderal® 10 mg, Enstilar® e Ozempic®.

3.4.3 Prescrição por receita manual

Ao longo do estágio, contactei com os diferentes tipos de receituário, nomeadamente, prescrição eletrónica materializada, prescrição eletrónica desmaterializada e prescrição manual.

Fiquei surpreendida com a quantidade de receitas manuais que surgiam na farmácia, uma vez que estas atualmente são apenas permitidas em situações excecionais de acordo com a legislação em vigor. Senti que o meu atendimento aos utentes que tinham na sua posse receitas manuais era afetado devido à incerteza do que nelas estava redigido, acabando na maioria dos casos por recorrer aos restantes membros da equipa para tirar as dúvidas. Para além disso, estes tipos de prescrição têm de obedecer a regras específicas e bem-definidas, cujo cumprimento tinha de ser averiguado no ato de dispensa dos medicamentos. Assim, este tipo de prescrição tornava o atendimento mais moroso e menos fluído.

3.4.4 MSRM sem prescrição médica

O uso correto e racional dos medicamentos é um objetivo e responsabilidade dos farmacêuticos. Porém, atualmente verifica-se uma maior procura de medicamentos sujeitos a receita médica sem a devida receita.

Segundo o Decreto-Lei n.º 307/2007, de 31 de agosto, "os medicamentos sujeitos a receita médica só são dispensados aos utentes que a não apresentem em casos de força maior, devidamente justificados" [3]. A falta da receita poderá estar associada a uma prescrição em quantidade insuficiente para o intervalo entre consultas ou por cancelamentos consecutivos destas. Por outro lado, em alguns casos a sua falta passa também pelo descuido dos utentes que, como já tomam certos medicamentos há muito tempo, não vão pedir a renovação da receita. Esta situação requer especial atenção de modo a evitar a banalização da dispensa e a utilização abusiva dos medicamentos. O papel do farmacêutico passa, assim, pela educação da

população no sentido de evitar a automedicação e os respetivos efeitos adversos. Quando estamos perante doentes crónicos, devemos avaliar a situação uma vez que a falta da medicação pode afetar o tratamento e, consequentemente, o controlo da patologia.

3.4.5 Falta de serviços farmacêuticos

De acordo com a Portaria n.º 1429/2007 de 2 de novembro, alterado pela Portaria n.º 97/2018 de 9 de abril, as Farmácias podem prestar serviços farmacêuticos e outros serviços de promoção de saúde e bem-estar aos utentes. Desta forma, as farmácias são cada vez mais vistas como um local de prestação de cuidados de saúde e não apenas como um espaço para dispensa de medicamentos [4].

Na Farmácia Brandão apenas existem serviços de medição da glicémia, colesterol, peso e altura, índice de massa corporal, assim como serviços de administração de vacinas fora do Plano Nacional de Vacinação e medicamentos injetáveis. Para além disso, periodicamente recebe um profissional externo que executa rastreios auditivos.

Considero este aspeto uma ameaça, uma vez que as farmácias da zona já implementaram outros serviços, como consultas de nutrição, dermocosmética, entre outros, tirando partido desta lacuna.

3.4.6 Espaço de atendimento

No cenário de uma farmácia comunitária, o espaço disponível numa zona de atendimento, seja para a circulação dos utentes como para a disposição do mobiliário técnico são componentes que não podem ser desvalorizados. Este é um aspeto fulcral, uma vez que uma boa gestão irá criar valor e aprimorar a imagem da farmácia. Pontos estes que vão não só beneficiar a nível económico como acaba por conquistar novos clientes captando a sua atenção e prestando-lhes um atendimento súpero.

A zona de atendimento na Farmácia Brandão tem 4 postos de atendimento, 2 gôndolas centrais e 5 lineares. Uma vez que esta zona é reduzida e as gôndolas centrais são altas, retiram visibilidade ao utente dificultando de igual forma a divulgação de campanhas de *marketing* em vigor para produtos que a farmácia queira promover. Para além disso, acaba por criar zonas frias.

4. Casos Práticos

Caso Prático 1

Mulher, 74 anos, com mobilidade reduzida, queixa-se que está com dificuldade em defecar.

Questionei sobre os hábitos de defecação e referiu que tem costuma ir à casa de banho diariamente, sendo que já não ia há 5 dias. Perguntei ainda se tinha mais alguns sintomas como sensação de inchaço, flatulência ou hemorroidas e ela falou que se sentia inchada. Interroguei ainda sobre os hábitos alimentares e a ingestão de água e se já tinha tomado algum medicamento ou se fazia alguma medicação habitual. Respondeu que costuma fazer refeições leves e faz baixa ingestão de água. Sobre a medicação, faz um anti-hipertensor e um antidislipidémico.

Após recolher toda a informação, falei primeiramente das medidas não farmacológicas como aumentar a quantidade de fibras consumidas diariamente (ex: cereais, leguminosas e frutas e legumes frescos) e a ingestão de líquidos; ajustar os hábitos intestinais, tentando manter um horário regular de ida à casa de banho, preferencialmente após as refeições, destinando esse tempo para o efeito, sem pressas nem interrupções e nunca ignorando a urgência de defecar; não usar roupas apertadas e, dentro das limitações físicas, fazer algum exercício.

Enquanto estas alterações do estilo de vida não surtem efeito, a utente poderá necessitar da toma de um laxante de forma ocasional e transitória de modo a não mascarar nenhuma patologia nem criar hábitos prejudiciais. Assim sendo, e uma vez que ela já não defeca há muito tempo, comecei por aconselhar a administração de uma bisnaga de *Microlax* (Citrato de sódio + Laurilsulfoacetato de sódio), que devido a ser um laxante de contacto administrado na forma de clister vai atuar mais rapidamente. Posteriormente, e sendo que o laxante anterior apenas se deve usar em SOS, aconselhei um laxante osmótico como o *Duphalac* xarope, que é constituído pela lactulose, acabando por tornar as fezes mais moles. Este deve ser tomado sempre à mesma hora (de manhã ou à noite) numa dose diária de até 45mL, e após 2-3 dias, reduzir para, no máximo, 30mL por dia até se verificar a regularização da defecação. Este laxante vai ajudar a normalizar a função intestinal de uma forma mais suave e natural, sendo geralmente bem tolerado. Mencionei ainda que com a toma destes medicamentos poderiam surgir alguns efeitos adversos, tais como flatulência e dores abdominais, e mais uma vez salientei a importância de beber água. Caso a utente não melhorasse ou houvesse agravamento dos sintomas, deveria ser avaliada por um médico.

Caso Prático 2

Mulher, 28 anos, dirige-se à farmácia queixando-se de prurido, ardor e irritação vulvar.

Questionei a utente quanto à duração da sintomatologia, se tinha corrimento e quais as suas características (alteração da cor, odor ou textura) e se existiu alguma alteração no quotidiano que se pudesse relacionar com o surgimento dos sintomas. Ela refere que o corrimento é branco, espesso e grumoso, não sentindo alterações de odor. Para além disso tinha começado um novo relacionamento há 1 mês

Ao perceber que se podia tratar de uma candidíase vaginal, comecei por aconselhar um produto de higiene íntima para fazer a lavagem diária pois a zona genital da mulher é diferente da restante pele do corpo, pelo que na higiene íntima devem ser utilizados produtos adequados. No caso aconselhei o *Lactacyd Pharma suavizante* que ajuda a aliviar o desconforto vaginal, uma vez que quando questionada sobre os hábitos de higiene íntima referiu que apenas utilizava gel de banho corporal. Para além desta medida não farmacológica, referi ainda que é importante ir à casa de banho após ter relações sexuais para prevenir eventuais infeções urinárias, após lavagem da zona íntima é essencial que esta fique bem seca de modo a evitar humidade e utilizar roupa interior de algodão.

Como medida farmacológica apresentei as formulações que tínhamos disponíveis (creme, comprimido vaginal ou óvulo) solicitando que a utente indicasse a forma farmacêutica preferível: creme e comprimido vaginal - *Gino-Canesten* (Clotrimazol) e óvulo - *Gyno-Pevaryl* (Econazol). Acabando por optar pelo creme, expliquei que devia aplicar através da via vaginal, ao deitar, durante 7 dias consecutivos e durante esse período não ter relações sexuais. Caso o parceiro também tivesse algum desses sintomas deveria fazer também o tratamento, sendo, no seu caso, 14 dias.

Caso Prático 3

Homem, 36 anos, dirige-se à farmácia com os olhos vermelhos e refere ser um "terçolho".

Após observar o olho coloquei algumas questões ao utente acerca do contacto com algum agente irritante (poeiras, agentes químicos, entre outros), se passava muito tempo à frente de ecrãs (computador, telemóvel), se utilizava lentes de contacto ou se tinha frequentado a piscina recentemente. Adicionalmente, perguntei se sentia alguma comichão ou dor. O utente respondeu que não tinha dor nem comichão, no entanto, sentia os olhos mais "secos" e

cansados que o habitual, provavelmente devido ao seu trabalho de escritório. Ademais, o olho não apresentava inchaço comum nem indício de foliculite.

De acordo com as respostas do utente, presumi que se poderia tratar apenas do olho irritado. Desta forma, dispensei ao utente o *Relieve Hypo*, um colírio que tem como o objetivo equilibrar a osmolaridade do filme lacrimal contendo glicerina (ação humectante) e povidona (aumenta o tempo de retenção da água na superfície ocular) aliviando a sensação de irritação.

Caso Prático 4

Mulher, dirige-se à farmácia para solicitar uma nova embalagem de Nizoral para o filho de 14 anos, uma vez que a que tinha em casa estava a acabar. Indicou ainda que o seu filho estava a usar o champô há mais de três semanas sem ter os resultados pretendidos.

Dada a situação, resolvi colocar algumas questões à utente. Uma delas estava relacionada com o tipo de caspa (seca ou oleosa e aspeto) e como estava a fazer o respetivo tratamento. A estas questões a utente respondeu que a caspa era oleosa e tinha aspeto de placas espessas. Mencionou que o champô estava a ser utilizado duas vezes por semana e em alternância usava o champô habitual que comprava no supermercado. Referiu ainda que o filho andava muito stressado por causa da escola e que, por isso, podia estar pior devido a essa situação.

Como resultado da informação recolhida concluí que poderia tratar-se de uma dermatite seborreica agravada pelo fator *stress*. Desta forma aconselhei o champô *Tarmed* constituído pela solução de coaltar, que diminui a proliferação dos queratinócitos promovendo o retorno a uma queratinização normal. Além disso, o coaltar tem também atividade anti-inflamatória, antibacteriana, queratolítica, queratoplástica, vasoconstritora e antipruriginosa. Para complementar o tratamento sugeri o kit crosta láctea da *Babé* para ajudar na remoção das placas espessas. Além do champô levemente queratolítico, incluí um champô suave que o filho poderia utilizar como alternância ao tratamento que iria fazer.

Por fim, sugeri ainda um multivitamínico em xarope, o *Tonosol vitalidade*, para reforçar o organismo nesta fase de maior *stress*. Para além disso, mencionei ainda a importância de apoiar o filho nesta fase mais complicada, dando-lhe o devido apoio.

Caso Prático 5

Utente, de 23 anos, dirige-se à farmácia solicitando algo que a faça respirar melhor. Indica que andou constipada e que, embora a tosse já tenha passado, o nariz entupido ainda a perturba e lhe tem dificultado o sono. Refere que já anda assim há 2 dias.

A congestão nasal caracteriza-se por uma vasodilatação dos vasos sanguíneos das fossas nasais. Entre outras, as suas causas podem ser sinusite, rinite alérgica, constipação, gripe ou desvio do septo.

Como medidas não farmacológicas indiquei o aumento da ingestão de líquidos, evitar assoar com demasiada força e lavar as fossas nasais diariamente. Para isso sugeri o *Sinomarin adultos* (água do mar hipertónica pressurizada) numa primeira fase e depois caso quisesse lavar as fossas nasais regularmente, podia usar soro fisiológico isotónico em spray ou uma água do mar isotónica como o *Rhinomer força 2*.

A terapêutica farmacológica passou pela indicação de um descongestionante nasal. Estes apresentam-se em forma de gotas para idades inferiores a 6 anos e nebulizadores/sprays, preferencialmente para idades superiores a 6 anos. Os descongestionantes nasais são aminas simpaticomiméticas que estimulam os recetores adrenérgicos α dos vasos sanguíneos nasais e provocam a contração do músculo liso, aliviando a congestão nasal. Assim sendo, aconselhei o *Vibrocil actilong* em spray, constituído pela oximezolina. Mencionei o modo de utilização, sendo a sua aplicação de um puff em cada narina, no máximo 3 vezes por dia, inclinando ligeiramente a cabeça para a frente e utilizando o braço contrário à narina, utilizando no máximo durante 5 dias seguidos. Antes de aplicar o spray descongestionante, é aconselhado lavar o nariz com água do mar e assoar bem. Referi ainda que a sua utilização prolongada (superior a 7 dias) pode levar ao aparecimento do efeito *rebound*, provocando tolerância e irritação da mucosa nasal.

5. CONSIDERAÇÕES FINAIS

O estágio em farmácia comunitária constituiu uma etapa fundamental do meu percurso académico. Na verdade, este possibilitou, por um lado, visualizar e aplicar na prática diversos conhecimentos adquiridos ao longo dos 5 anos do MICF e, por outro lado, aprofundar e adquirir outros conhecimentos, bem como desenvolver novas competências que certamente terão grande utilidade a nível profissional.

Efetivamente, este estágio permite obter uma visão do mundo real de trabalho, sendo algo desafiante, mas simultaneamente muito gratificante. Assim sendo, sinto que esta experiência me enriqueceu bastante enquanto futura farmacêutica.

Por fim, realço o papel de toda a equipa, que desde o início me acolheu e que fomentou um ambiente de constante aprendizagem e melhoria. A eles, o meu agradecimento por toda a disponibilidade e pelos conhecimentos transmitidos.

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Parte II

Relatório de Estágio em Assuntos Regulamentares do Medicamento

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INFARMED, I.P.

Sob orientação da Dra. Dina Lopes

LISTA DE ABREVIATURAS

AS	Alterações substanciais
CAM	Comissão de Avaliação de Medicamentos
DAM	Direção de Avaliação de Medicamentos
EC	Ensaios clínicos
EM	Estados-Membros
MICF	Mestrado Integrado em Ciências Farmacêuticas
PAS	Pedido de alteração substancial
PE	Pedido de elementos
SWOT	*Strengths, Weaknesses, Opportunities and Threats*
UAC	Unidade de Avaliação Científica
UEC	Unidade de Ensaios Clínicos
UIM	Unidade de Introdução no Mercado
UMM	Unidade de Manutenção do Mercado

1. Introdução

O Mestrado Integrado em Ciências Farmacêuticas (MICF) forma profissionais de saúde com capacidade de atuar em áreas como análises clínicas, investigação, farmácia comunitária, farmácia hospitalar, indústria farmacêutica, assuntos regulamentares do medicamento e produtos de saúde, entre outros.

A pluralidade de saídas profissionais nesta área faz com que o estágio curricular seja fulcral e o momento da tomada de decisão preponderante. Assim, a Faculdade de Farmácia da Universidade de Coimbra realiza acordos com diversas entidades, garantindo aos alunos a oportunidade de experienciar as diversas vertentes da profissão farmacêutica. O INFARMED - Autoridade Nacional do Medicamento e Produtos de Saúde, I.P. (INFARMED, I.P.) é uma das entidades vinculadas neste acordo, possibilitando a realização de um estágio no âmbito de assuntos regulamentares do medicamento.

Os assuntos regulamentares do medicamento são uma área de fundamental no mundo farmacêutico, uma vez que estão envolvidos em todas as etapas do medicamento, desde o seu desenvolvimento até à sua comercialização, não esquecendo que enquanto o medicamento está no mercado é necessária uma contínua monitorização do mesmo e a informação deve ser disponibilizada prontamente aos profissionais de saúde. Os farmacêuticos que trabalham nesta área são confrontados com níveis de exigência de qualidade, segurança e eficácia impostos pelas autoridades competentes [2].

No meu caso em particular, o estágio realizou-se no departamento da Direção de Avaliação de Medicamentos (DAM), na Unidade de Ensaios Clínicos (UEC), sob orientação da Dra. Dina Lopes e orientação direta da Dra. Telma Fortunas, a quem agradeço por todo o apoio e ensinamentos.

O presente relatório incide sobre o estágio curricular realizado no Infarmed durante o período de 2 de maio a 28 de julho de 2023, e nele se descreve e analisa de forma crítica e pessoal o conhecimento e experiência adquiridos durante o estágio, este relatório assume a forma de uma análise SWOT (do inglês: *Strengths, Weaknesses, Opportunities and Threats*).

2. INFARMED

O Infarmed é a autoridade regulamentar nacional que contribui na formulação da política de saúde do país. As suas áreas de atuação englobam os medicamentos de uso humano, dispositivos médicos e produtos cosméticos. Isso envolve atividades como estabelecer regulamentos, realizar avaliações, conceder autorizações, supervisionar e manter vigilância sobre esses produtos. Tem como principal missão "regular e supervisionar os setores dos medicamentos de uso humano e dos produtos de saúde, segundo os mais elevados padrões de proteção da saúde pública, e garantir o acesso dos profissionais da saúde e dos cidadãos a medicamentos e produtos de saúde de qualidade, eficazes e seguros" [3].

Sendo uma estrutura dotada de autonomia administrativa e financeira, encontra-se organizado por Órgãos de Gestão e Unidades Orgânicas com Funções de Negócio e com Funções de Suporte. Na Figura abaixo encontra-se esquematizada a estrutura e organização interna do Infarmed.

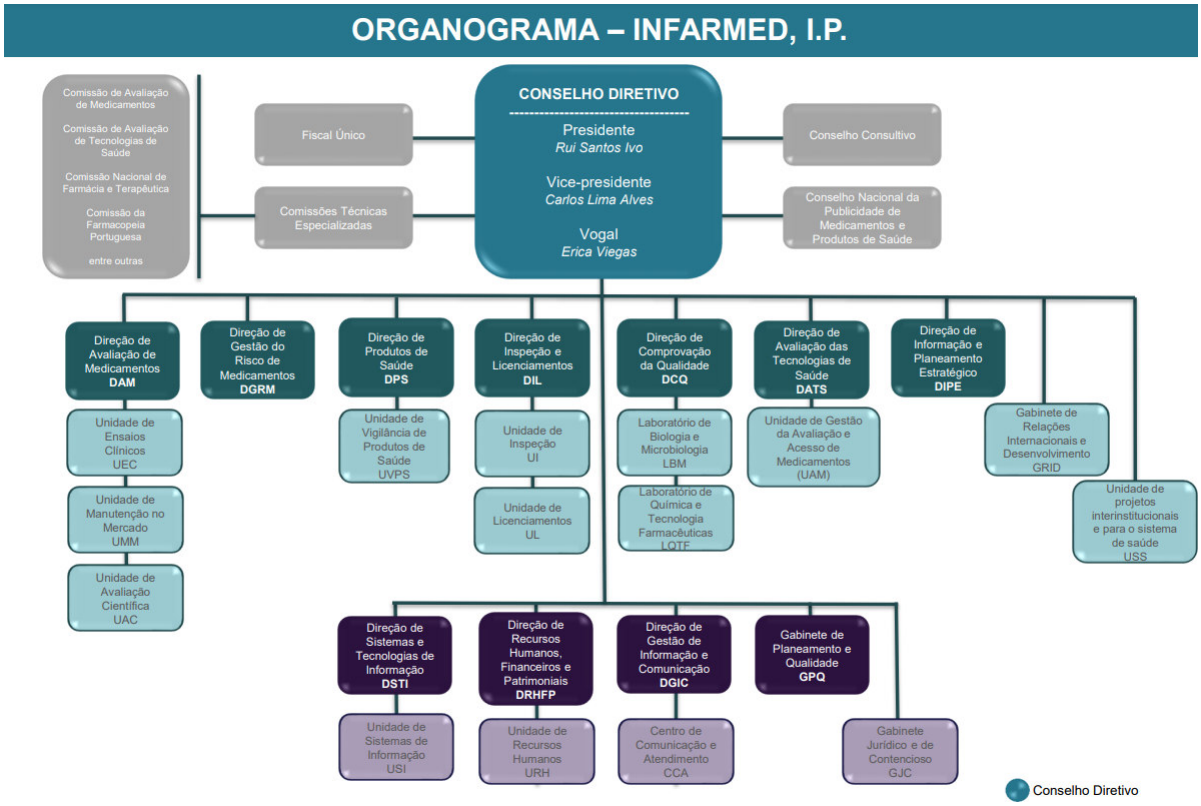

Figura 1- Organograma do INFARMED, I.P. [1]

2.1 DAM

A DAM, dirigida pela Dra. Marta Marcelino, tem como principais objetivos assegurar as atividades necessárias aos procedimentos de registo, avaliação e autorização de introdução no mercado de medicamentos de uso humano e, também, garantir as atividades relacionadas com a sua manutenção no mercado. Divide-se em 3 subunidades distintas, especializadas por área de intervenção: a Unidade de Ensaios Clínicos (UEC), a Unidade de Manutenção no Mercado (UMM) e a Unidade de Avaliação Científica (UAC) [4]. Como referido anteriormente, o meu estágio realizou-se na UEC que tem como função principal autorizar a realização de ensaios clínicos (EC), monitorizando a segurança da utilização dos medicamentos experimentais, acompanhando os mesmos de acordo com a legislação em vigor [5].

O trabalho que desenvolvi passou pela gestão de pedidos de alteração substancial (PAS), em que após a sua receção era verificada a documentação e validado o pedido, sendo posteriormente enviado para avaliação e consequentemente autorizado.

3. ANÁLISE SWOT

3.1 Pontos Fortes

3.1.1 Acolhimento

A receção no Infarmed foi bastante acolhedora, realizada pela Dra. Dina Lopes que, de um modo simplificado, nos mostrou os vários departamentos da DAM e explicou o seu funcionamento. A meu ver o acolhimento foi um ponto forte do estágio porque permitiu-me ter uma visão geral do seu funcionamento, saber onde se localizavam os serviços e, mais importante, dotou-me de ferramentas necessárias para o restante estágio.

3.1.2 Equipa

Dada a complexidade da área regulamentar é comum surgirem dúvidas nas quais a comunicação e o trabalho em equipa desempenham um papel essencial. Nesse contexto, a troca de conhecimentos e experiências é essencial para uma resolução rápida e simples.

Assim, ao longo dos três meses de estágio, pela envolvência que o trabalho requer, pude vivenciar a dinâmica de trabalhar em equipa num ambiente profissional. A postura da equipa, caracterizada pela prontidão e profissionalismo na explicação ou esclarecimento quanto aos procedimentos, desempenhou um papel crucial para o sucesso do meu estágio, uma vez que me permitiu ampliar os conhecimentos nesta área, contribuindo para me tornar mais segura e confiante nas tarefas que realizava.

3.1.3 Duração da frequência do estágio

A duração dos estágios em Indústria Farmacêutica ou estágios equiparados tem uma duração mínima pré-estabelecida de 420h. Na unidade em que fui integrada e no trabalho que desenvolvi, considero esta duração um ponto forte, uma vez que o processo de aprendizagem e o trabalho desenvolvido não teria sido possível de alcançar com uma duração inferior. Durante este período consegui desenvolver autonomia no trabalho e contactar de forma próxima com o papel desempenhado pelo Infarmed na gestão de PAS.

3.1.4 Competências adquiridas em processos de alterações substanciais (AS)

O processo de gestão de alterações substanciais de um EC é um processo complexo, que requer uma análise minuciosa. Os conhecimentos que adquiri relativamente à validação e avaliação das alterações permitiram-me uma maior compreensão acerca de todo o processo, das pessoas envolvidas e do papel que o farmacêutico poderá ter neste contexto.

Na minha opinião, o processo de gestão de PAS encontra-se muito bem delineado, com profissionais competentes que reconhecem a necessidade de, por vezes, pedir uma segunda opinião a avaliadores farmacêuticos que se encontrem mais integrados no assunto. No processo de validação dos pedidos de alteração, foi frequentemente necessário fazer um pedido de elementos (PE) ao promotor, pois os documentos fornecidos não estavam completos ou não eram os corretos. Já no processo de avaliação, inicialmente verificava se era possível fazer referência cruzada da informação com outro EC a decorrer em Portugal ou se essa documentação já tinha sido aprovada em, pelo menos, 2 Estados-Membros (EM), caso contrário, a documentação prosseguia para avaliação.

3.1.5 Existência de documentos internos

O Infarmed desenvolveu diversos documentos para servirem como modelos para uso próprio, escritos em português, de modo a harmonizar e agilizar o trabalho dentro da própria instituição. Os *templates* que utilizei foram para fazer o PE de validação, enviar o e-mail aos avaliadores, o PE de avaliação, o despacho da autorização da PAS e para a notificação da autorização.

3.1.6 Inglês técnico

Atualmente é imprescindível um bom domínio da língua inglesa, sendo, nesta área, bastante importante o conhecimento de termos técnicos no âmbito farmacêutico e regulamentar, dado que muitas vezes é difícil encontrar uma tradução adequada e que seja

reconhecida por todos de igual modo como sendo correspondente a determinado termo em inglês. Assim, sinto que o contacto diário com o inglês "técnico" foi um ponto forte do estágio.

3.1.7 Condições de trabalho

De um modo geral, as condições de trabalho são bastante apropriadas. Os edifícios estão bem conservados, contando com um sistema de ar condicionado eficaz. Todas as ferramentas e materiais necessários para o meu estágio foram prontamente disponibilizados. Ademais, o edifício da DAM possui ainda uma sala para os funcionários almoçarem que se encontra devidamente equipada com micro-ondas e frigoríficos.

Outro ponto forte é a flexibilidade de horários permitida, que oferece ao trabalhador a possibilidade de gerir os seus tempos de trabalho dentro de limites estabelecidos, escolhendo horas de início e fim do período normal de trabalho.

3.2 Pontos Fracos

3.2.1 Falta de oportunidade para aplicar os conhecimentos adquiridos no MICF

O trabalho que me foi atribuído incidiu na gestão de PAS, tal como mencionado anteriormente. Apesar do conhecimento adquirido relativamente à documentação necessária para realizar um EC e o contacto com estas ferramentas poderem ser úteis futuramente, não me permitiu aplicar os conhecimentos adquiridos ao longo do curso. Do mesmo modo, pelo facto de o meu trabalho ter incidido sempre no mesmo tipo de alterações, foram poucas as oportunidades de inter-relacionar conhecimentos.

3.2.2 Falta de planificação do estágio

Uma das dificuldades sentidas no início do estágio foi o facto de não haver um delineamento prévio das tarefas a executar durante a primeira semana. Após esta, as tarefas passaram a estar delineadas e concretizou-se a integração em contexto de trabalho. Acredito que esta questão da falta de planeamento inicial do estágio se deva ao facto da UEC não receber muitos estagiários. No entanto, mesmo reconhecendo pontos fracos neste aspeto, considero que esta situação não teve impacto negativo no desempenho final das funções que me foram requeridas.

3.2.3 Não rotatividade de unidades

Do meu ponto de vista, penso que seria um estágio mais dinâmico e interessante se fosse possível experienciar outra unidade dentro do mesmo departamento de modo a perceber o trabalho que compete a cada uma. No entanto, tenho consciência que apenas 3 meses não seria viável uma vez que com este intervalo de tempo não é possível fazer uma profunda integração sobre o trabalho desenvolvido e ganhar posteriormente autonomia para a realização de tarefas.

3.3 Oportunidades

3.3.1 Teletrabalho

À semelhança de toda a equipa da DAM, o estágio foi realizado, maioritariamente, em teletrabalho. Neste sentido, o teletrabalho, permitiu-me desenvolver competências tais como a gestão de tempo, organização de tarefas e ainda a aquisição de competências nas plataformas utilizadas para comunicar entre a equipa. Tendo em conta que, atualmente, grande parte das empresas do setor farmacêutico funciona em teletrabalho estas competências adquiridas durante o estágio poderão vir a ser valorizadas no futuro.

3.3.2 Reunião da Comissão de Avaliação de Medicamentos (CAM)

A CAM é um órgão consultivo do Infarmed a quem "compete genericamente, sempre que solicitada, emitir pareceres em matérias relacionadas com medicamentos, designadamente no domínio da avaliação da qualidade, eficácia e segurança, bem como sobre quaisquer outros assuntos de carácter técnico-científico, que lhe sejam submetidos pelo Conselho Diretivo do Infarmed" [6]. Mensalmente realiza 1 a 2 reuniões plenárias com o principal objetivo de avaliar medicamentos, nomeadamente autorizações de introdução no mercado e outros assuntos de carácter técnico relacionados com os mesmos. Assim, considero que a oportunidade dada de poder assistir a esta reunião constituiu uma oportunidade ao meu estágio.

3.4 Ameaças

3.4.1 Acumulação de trabalho

O elevado volume de trabalho relativamente aos recursos humanos disponíveis para o executar, leva a que existam muitos processos submetidos ao Infarmed que não são analisados em tempo razoável, sendo frequente a existência de longos tempos de espera até à sua finalização. Considero assim uma ameaça não só ao posicionamento externo do Infarmed, podendo transparecer falta de competência dos profissionais, como ao próprio trabalho

realizado pelo Infarmed e pela Indústria Farmacêutica/Hospitais, já que, muitas vezes precisam de uma resposta rápida para conseguirem avançar com os EC.

Outro fator que contribui para que não seja dada uma resposta atempada da autorização das PAS, são os pareceres dos avaliadores. Quando não é possível fazer referência cruzada com os EM ou com outro EC, é enviada a documentação para avaliação com uma data limite, sendo que vários avaliadores protelam a resposta pondo em causa o cumprimento dos prazos estabelecidos.

3.4.2 Recursos humanos

Nos últimos anos, Portugal tem sofrido sucessivas crises económicas e políticas, tendo estas impacto no Infarmed traduzindo-se numa escassez de recursos humanos. A quantidade de profissionais que trabalham nesta instituição é reduzida quando pensamos nas incumbências e necessidades da mesma. Para além disso, existem muitos casos em que os profissionais que trabalham no Infarmed são atraídos para a indústria farmacêutica que oferece melhores condições de trabalho e remuneratórias, constituindo outra ameaça à afetação/manutenção de recursos humanos.

3.4.3 Sistema informático

O Infarmed possui um sistema informático bastante complexo, sendo necessário recorrer a vários softwares para realizar o trabalho devidamente.

Ao longo do estágio deparei-me com alguns problemas a este nível. Na UEC utiliza-se muito a SECL, um programa constituído por *software* bastante antigo e que dava imensos problemas, sendo que, quando o sistema falhava, impossibilitava a realização das tarefas. Embora esta instituição disponha de uma equipa técnica para a resolução deste tipo de incidentes, a resposta era por vezes demorada. No meu ponto de vista, deviam ser tomadas medidas para melhorar estas plataformas e assim minimizar a ocorrência destas situações que revelam ser uma forte ameaça ao trabalho desenvolvido.

4. CONSIDERAÇÕES FINAIS

Atualmente, o farmacêutico está presente em diversas áreas de atuação do setor da saúde, tornando-se, por vezes, difícil adquirir um conhecimento aprofundado sobre todas estas áreas. Neste sentido, é de realçar a oportunidade que este estágio me deu para experienciar outra área do setor farmacêutico além da tradicional Farmácia Comunitária ou Farmácia Hospitalar.

Terminado este estágio posso afirmar que foram vários os fatores que contribuíram para a minha aprendizagem e crescimento, quer a nível pessoal quer a nível profissional. Considero que foi uma experiência extremamente enriquecedora que me permitiu adquirir competências que serão, certamente, uma mais-valia para o meu futuro profissional. A possibilidade de trabalhar com uma equipa empenhada, altruísta, e com brio profissional contribuiu também, de forma bastante positiva, para o percurso do meu estágio, uma vez que me fizeram sentir à vontade para colocar qualquer tipo de dúvida e expor as minhas dificuldades de forma a melhorar as minhas aptidões e capacidades profissionais.

Apesar de, como em qualquer experiência, existirem aspetos negativos, a minha experiência foi deveras gratificante. Além disso, fiquei a conhecer melhor o papel do farmacêutico na área dos assuntos regulamentares e a sua importante contribuição para a cadeia do medicamento.

Em suma, considero que esta experiência me permitiu adquirir conhecimentos e competências técnicas fundamentais, que serão muito úteis na minha vida profissional, tornando-me agora mais apta e capacitada a ingressar no mercado de trabalho.

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Parte III

Monografia

NATURAL-DERIVED PIGMENTS: FOCUS ON TOPICAL APPLICATIONS

Sob orientação da Mestre Laura Ferreira

LIST OF ABBREVIATIONS

ABTS.+	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AE-K	Hydrogel of algae extract
AE-L	Lipogel of algae extract
AST	Astaxanthin
Curc-GS-HS	Curcumin loaded gel-core hyalusome
DPPH	2,2-diphenyl-1-picrylhydrazyl
EE	Encapsulation efficiency
ES_{Hp}	Extractive solution
HK-2 cells	Human kidney 2 cells
IHC	Immunohistochemical
LED	Light emitting diode
MLC	Microstructured lipid carrier
NC- ES_{Hp}	PLGA nancapsules loaded with total carotenoids in *H. pluvialis* solution
NLC	Nanostructured lipid carrier
SC	Stratum corneum
SNEDDS	Self-nanoemulsifying drug delivery system
SP	Extracts from decorticated seeds
SPF	Sun protection factor
PDT	Photodynamic therapy
PEG	Polyethylene glycol
PLGA	Poly(lactic-co-glycolic acid)
PWCCS	Purple waxy corn cob extract
Rho-PE	1,2-Dioleolyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl)
ROS	Reactive oxygen species
TP	Extracts from peels
UV	Ultraviolet
η	Flow behavior index

FIGURES CAPTIONS

TABLES CAPTIONS

ABSTRACT

To make their products more appealing to consumers, the industry uses pigments and dyes. With industrialization, synthetic pigments have become more prevalent, but in recent years they have been associated with several harmful effects on both public health and the environment, which boosted the exploration of alternatives that are unprocessed, and with less anthropogenic environmental impact. Nowadays, and with the emergence of new social standards and patterns of behavior, consumers tend to select products of natural origin, with an ethical and sustainable design. Specifically, natural-derived pigments besides their coloring properties, they possess topical therapeutic applications, particularly antioxidant, antimicrobial and anti-inflammatory activities.

Skin is a complex multilayered structure and a remarkable route for the application and delivery of active molecules. However, owing to its protective barrier, imposes physical, chemical, and biological restrictions on molecules that may cross the stratum corneum. In this context, several nanodelivery systems have been used to transport pigments into deeper layers of the skin, to improve their release, bioavailability, and retention into the skin.

The present work focuses on the applicability of natural-derived pigments in topical applications, highlighting their outstanding bioactivities through several in vitro and in vivo studies, and disclosing the sustainable exploration thereof.

Keywords: natural-derived pigments, topical application, nanodelivery systems, antioxidant activity, sustainability.

RESUMO

De modo a tornar os produtos mais apelativos ao consumidor, a indústria recorre a pigmentos e corantes. Com a industrialização os pigmentos sintéticos ganharam predominância, no entanto, nos últimos anos, têm-lhes sido associados vários efeitos nocivos, tanto para a saúde pública como para o meio ambiente, o que impulsionou a necessidade de se procurarem alternativas não processadas e com menor impacto ambiental antropogénico. Atualmente, e com o desenvolvimento dos novos paradigmas sociais, os consumidores tendem a optar por produtos de origem natural, com um design ético e sustentável. Ademais os pigmentos de origem natural, para além das suas propriedades corantes, apresentam aplicações terapêuticas quando aplicados topicamente, destacando-se as suas atividades antioxidantes, antimicrobianas e anti-inflamatórias.

A pele é um órgão complexo composta por múltiplas camadas, dispondo de uma grande área de superfície para a absorção e veiculação de ingredientes ativos. No entanto, devido à sua barreira protetora, impõe limitações físicas, químicas e biológicas às moléculas que tentam atravessar o estrato córneo. Neste sentido, vários nanossistemas têm sido utilizados para transportar pigmentos para as camadas mais profundas da pele, a fim de melhorar a sua libertação, biodisponibilidade e retenção na pele.

O presente trabalho foca-se na aplicação tópica de pigmentos de origem natural, dando destaque às suas excelentes atividades biológicas através de vários estudos in vitro e in vivo, assim como à sua exploração sustentável.

Palavras-chave: pigmentos naturais, aplicação tópica, nanossistemas, atividade antioxidante, sustentabilidade.

1. INTRODUCTION

Pigments are solid substances and are classified as organic or inorganic. As they are insoluble, pigments are dispersed into a binder that keeps them on the substrate. Additionally, these particles have larger sizes, ranging from 1 to 2μm. Dyes are soluble organic substances that can be easily dissolved in the material on which they are applied, giving it color. In these, the particle size ranges from 0.025 to 1.0μm. Thus, pigments consist of larger molecules that remain suspended while dyes consist of smaller molecules that dissolve in the medium. In short, the main difference between pigment and dye lies in the particle size and solubility in the medium in which it is inserted [1-3].

Pigments have been used by humans since prehistoric times. Back then, man used the colors of nature, whether from minerals in the earth or from the plants and animals that surrounded his environment, to paint walls, trees, or rocks. Over time, there were advances in the technology of civilizations which led to the discovery and production of new pigments. Progressively, pigments that faded with light or certain atmospheric conditions were replaced by more stable products. The constant evolution of science during the 19th century led to the discovery of techniques that made it possible to produce synthetic pigments and led to a huge expansion in the pigments and colors available, facilitating their commercialization and increasing their use in various types of industry. At present, the search for pigments that fade color or intensity drives to continuous research into these field [3, 4].

In recent years, several pigments and dyes of synthetic origin used in the production of food, cosmetics, and medicines, have shown several adverse effects. As far as public health is concerned, one of their main consequences is that they can cause cellular damage due to oxidation, may lead to immunosuppression, or even involve carcinogenic activity [5]. In addition, their toxic properties create a problem for the environment, especially the marine environment, requiring effluent treatment and bioremediation [6]. Therefore, to solve this problem, pigments from natural sources have been increasingly explored. These pigments play an important role in the physiology and molecular processes of microorganisms as they act as a means of adaptation to various extreme environments, have a protective function against solar radiation, and are also involved in functional processes such as photosynthesis. In addition, they have health-promoting properties, such as antioxidant, anti-inflammatory, anticancer, antiviral, and radical scavenging activities [5, 7]. These health benefits make natural pigments valuable compounds, increasing the market value of products that incorporate them, thus increasing their applications in the industry.

The importance of skin goes far beyond its role as a physical protective barrier between the body and the environment, and it is essential to take care of it. Given the growing awareness of the population about the importance of skin health and appearance, there is an increasing demand for efficient solutions to prevent and treat skin damage and disorders [8]. Hence, the use of pigments of natural origin for topical application has grown, due to a clear tendency of the consumer's for products more environmentally friendly, which drives the search for innovative combinations with this ingredients [9]. Most topically applied pigments have mostly antioxidant and healing properties (see Figure 1). For example, astaxanthin provides protection of the ocular surface against UV (ultraviolet) radiation exposure; anthocyanins derived from purple waxy corn, due to its antioxidant activity, possesses anti-aging and anti-wrinkle effects; and carotenoids and phycocyanins extracted from *Spirulina platensis* have shown wound healing activity owing to its high proliferative effect of skin cells [10-12]. Currently, beauty products incorporating microalgae pigments are already available on the market [13]. However, it is still a challenge to discover and obtain new potent pigments for application in pharmaceutical products.

Therefore, throughout this manuscript, it will describe several pigments of natural origin, with a focus on *in vivo* and *in vitro* studies that assess and support the remarkable biological effects of these pigments for topical applications, and subsequently will be discussed the sustainability and valorization of the natural resources exploited for their production and/or extraction.

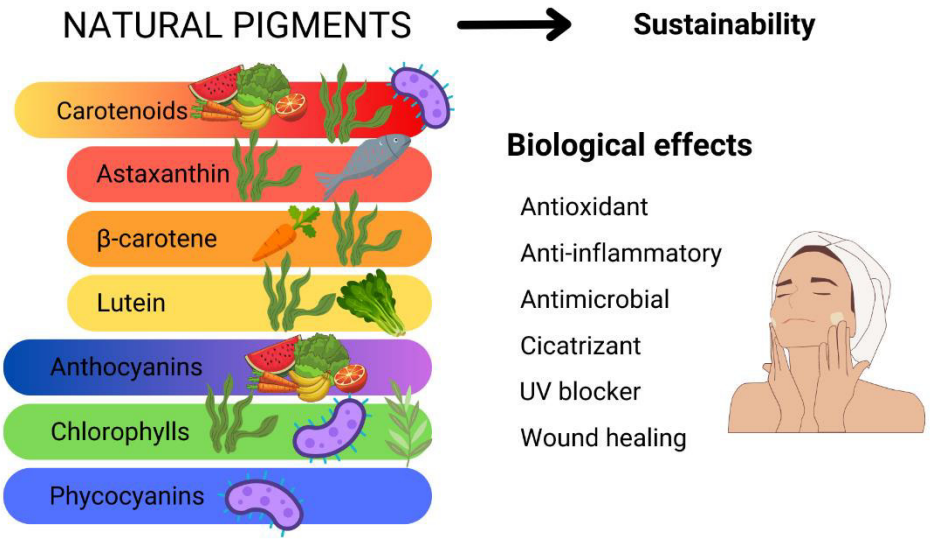

Figure 1 - Illustration of the pigments used for topical application and their respective activities.

2. NATURAL-DERIVED PIGMENTS AS METABOLITES OF INTEREST

Pigments are responsible for producing the colors we see every day, which are assessed through sensorial analysis to relate to product acceptability and quality. Therefore, industries tend to incorporate them in their formulations to make their products more and more attractive, being added to medications, supplements, and cosmetics, with a wide range of colors [14].

At present, synthetic pigments are broadly applied in products. Their advantages are the existence of a wide range of colors, which are more vivid and uniform, resistant to light, oxidation and reduction, heat, pH changes and microbial contamination. On the other hand, associated to these pigments is a great concern due to adverse effects on health such as allergy, asthma, and carcinogenic potential [15]. Thus, the replacement by natural-derived pigments has been progressively increasing, and their use is encouraged by consumers, industries, and authorities.

Natural-derived pigments are substances with various colors that selectively absorb light in a specific wavelength range. They occur naturally in plants (roots, seeds, fruits, leaves, flowers, bark, and wood), animals (crustaceans, insects, among others) and microorganisms (fungi, bacteria, and algae). Pigments of animal and plant origin are frequently light-sensitive, unavailable, and poorly water-soluble, contrary to microbial pigments, which are highly stable, and easily produced in a short period at a reduced cost [6, 16].

The worldwide trend for products considered healthy and environmentally friendly has led cosmetic and pharmaceutical industries to finance the research and development of new products containing compounds or extracts from natural sources, one of the most promising future developments being the sustainable exploitation of marine resources [17].

Currently, there are already products on the market with natural-derived pigments incorporated in their formulation, and some of their properties are already known. Among the pigments used for topical application, it can be highlighted the carotenoids, anthocyanins, chlorophylls, and phycocyanins (see Table 1).

3. CAROTENOIDS

Carotenoids are lipophilic pigments that are responsible for the yellow, orange, and red colors and are present in seeds, leaves, flowers, fruits, vegetables, crustaceans, fungi, bacteria, and algae. This group of pigments is subdivided into: xanthophylls (oxygenated molecules such as lutein and zeaxanthin) and carotenes (non-oxygenated molecules such as α-carotene and fucoxanthin) [18]. In addition to being natural-derived colorants, some carotenoids have provitamin A activity, i.e., their activity occurs only after their conversion from vitamin A to retinol [19].

3.1 Astaxanthin

Astaxanthin (AST) belongs to the xanthophyll group and is a red-orange pigment, responsible for the reddish-pink color. The green microalgae *Haematococcus pluvialis* is it's the richest natural source of AST, accumulating up to 5.0% of its dry weight, being consequently the main source of this compound for human consumption and industrial biological production [20]. Due to its structure (presence of terminal carbonyl groups conjugated to its polyene skeleton), AST is a more potent antioxidant than the carotenoids of carotene, such as β-carotene [21]. In addition to its antioxidant activity [22-24], it also has anti-inflammatory activity [24].

Photoaging is caused in part by UV radiation since in contact with the skin it will lead to ROS formation. This oxidative stress can be prevented using antioxidants. Eren, Tuncay Tanrıverdi [22], elaborated a study in which was evaluated the antioxidant properties of AST in topical formulations against skin aging. For this, oleoresin and algal extract containing AST in hydrogel and lipogel forms were used as antioxidants as anti-aging products. After characterizing the formulations (pH, rheological analysis, mechanical properties, and stability), *in vitro* release studies of the formulations, antioxidant activity and cytotoxicity test were carried out. The results obtained from the *in vitro* release studies show that the consistency and firmness of the gels affect the rate and amount of AST that is released over time. The algal extract lipogel formulation showed lower hardness and viscosity values, resulting in a higher amount of AST available (403.0 μg after 24 h); and the oleoresin formulations released less amount of AST due to the presence of the resin (99.0 μg for the lipogel and 88.3 μg for the hydrogel). In addition, the effects of the formulations on cell proliferation were also studied using HK-2 cells, and the formulations of only algal extract and oleoresin induced cell proliferation in a dose-dependent manner, which suggests that AST is have a more proliferative

effect in lipogel formulations when compared to hydrogel formulations, where AST possess a more controlled release. Moreover, the antioxidant activity was evaluated using two different radicals: DPPH and ABTS.+, with each formulation containing 0.5 mg/mL of AST. Regarding DPPH, the percentage of radicals scavenged was 29.0 ± 3.9% for oleoresin lipogel, 18.5 ± 2.3% for oleoresin hydrogel, 77.0 ± 4.3% for algae extract lipogel and 95.6 ± 4.2% for algae extract hydrogel. These results suggest that formulations containing AST possess a more pronounced antioxidant capacity towards the DPPH radical. Similarly, with respect to the ABTS.+ radical, it is found that all formulations scavenged the ABTS.+ radical in a dose-dependent manner of AST, and its addition resulted in higher antioxidant activity, highlighting the algae extract lipogel formulation. In summary, the algae extract lipogel formulation could be a promising strategy for topical application, that combine AST with a delivery system highly efficient due to its strong antioxidant activity, easy application and rapid release profile.

As aforementioned, AST possess and antioxidant activity, however, it has characteristics that restrict its application in pharmaceutical products, mainly their low water solubility and stability when exposed to oxygen, light, and high temperatures. Therefore, it is essential to develop a formulation that optimizes its stability and bioavailability in the skin. Vieira, Derner [25] carried out a study to develop a novel formulation through the encapsulation of carotenoid extracts, mainly AST, from the microalgae *Haematococcus pluvialis*. Extracts from the microalgae were prepared and then incorporated into PLGA (Poly lactic-co-glycolic acid) nanocapsules, with their correspondent characterization and determination of the amount of AST and their antioxidant activity. Free, and total AST was determined by HPLC, demonstrating an encapsulation efficiency superior to 98.0%. Moreover, the DPPH scavenging activity of the free microalgae extract against the extract incorporated in the PLGA nanocapsules were evaluated and compared the results with the positive control (ascorbic acid). Free extract showed a 3.0-fold superior activity regarding ascorbic acid, while extract into PLGA nanocapsules showed a 9.0-fold superior antioxidant activity (PLGA nanocapsules loaded with total carotenoids in *H. pluvialis* solution: IC_{50}=0.5; extractive solution: IC_{50}=2; and ascorbic acid: IC_{50}=6). Moreover, a thermosensitive hydrogel was also prepared when poloxamer 407 was added to the formulations with and without nanocapsules, to understand whether it would be possible to form a film of carotenoids on the skin with a sustained release profile. The hydrogel was characterized, showing a decrease in the zeta potential value from -40.0 mV to -27.5 mV, which indicates a reduction in the electrostatic stability of the suspension. Also, the rheology profile of the hydrogel was assessed, and at 4.0°C, the hydrogels showed Newtonian behavior, with a simple linear relationship between shear stress

and shear rate, and low viscosity; and at 35°C, they exhibited pseudoplastic behavior, with a significant increase in viscosity. The flow behavior index (η) at 4°C was 0.96 and at 35°C was 0.07, and with these values being less than 1, it can be confirmed the pseudoplastic nature of the hydrogel, i.e., its viscosity decreases as the shear rate increases, which is advantageous for skin application as it facilitates spreadability. Finally, the release profile of the carotenoids in the nanocapsules was evaluated because they the PLGA wall acts as a barrier, resulting in a lower amount released in the first 12h. After and once the carotenoids reach molecular dispersion in the hydrogel, they are released rapidly. Thus, it was concluded that the developed formulation can be applied topically to deliver carotenoids and produce a prolonged antioxidant effect, with the aim to prevent skin photoaging.

In another study, novel self-dispersing nanodelivery systems based in nanoemulsions containing AST were investigated for their physical characteristics, stability, skin permeation and distribution, and to assess if antioxidant activity of AST was enhanced (see Figure 2). Furthermore, the incorporation of terpenes as skin penetration enhancers was also evaluated. The initial formulation (SNEEDS-L1) is composed of an oil phase, a non-ionic surfactant, and a co-surfactant, being these compounds in the ideal proportion of 1:4:1. This ratio gave rise to a transparent formulation that was immediately dispersed in water to produce a nanoemulsion. The SNEDDS-L1 formulation was further adapted to include a terpene in the oil phase, forming SNEDDS-T1, consisting of D-limonene; T2, consisting of geraniol; and T3, consisting of farnesol. SNEEDS-L1 showed about 2.2-fold higher penetration of AST into the SC compared with positive controls: marketed AST-containing product (Facial serum: Astarism®) and ascorbic acid. Moreover, it was observed a higher deposition of AST in the skin in deeper layers, about 3.6-fold higher than the marketed AST product and the ascorbic acid. Considering its antioxidant activity, it was assessed through ABTS.+ radical scavenging capacity for the self-nanoemulsifying drug system formulations and their based nanoemulsion when compared with the marketed AST-containing product and ascorbic acid. All the formulations showed antioxidant activity at all concentrations tested. The results showed that the self-nanoemulsifying drug systems had an elimination percentage between 66.8% and 68.1% and the nanoemulsion 57.8%. To determine the stability of AST, the formulations were stored for 30 days at 22-25°C in dark and light conditions. The percentage of AST remaining after 30 days was in the range 86.7-93.3% for both light and dark conditions for the formulations that included the self-nanoemulsifying drug system; the control was relatively stable in the dark (80.7 ± 11.6%) but reduced significantly when exposed to light (58.8 ± 9.6%). In summary, the developed nanodelivery systems showed satisfactory results in the cutaneous penetration of

AST, in the stability of the formulation which prevents AST degradation and improved their antioxidant activity [26].

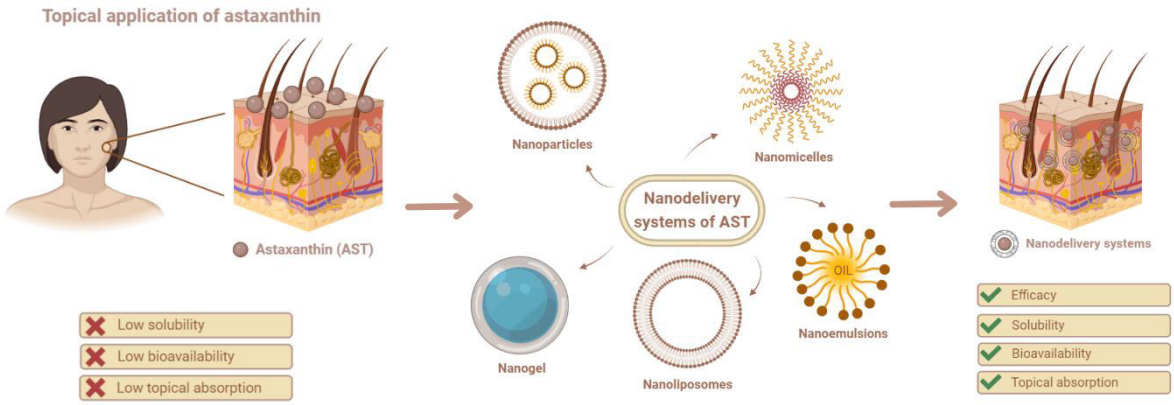

Figure 2 - Illustrative scheme of astaxanthin nanodelivery systems for topical applications.

Besides the effects on the skin, UV radiation can provoke inflammation in the eye, and particularly, UVB exposure causes photokeratitis, which is characterized as an inflammation of the cornea caused by excessive exposure to UVB radiation. Therefore, the effect of AST was studied to understand its effect on photokeratitis in mice. For this purpose, AST was administered diluted in polyethylene glycol in the right eye of mice, while in the left eye, only the vehicle was administered. To evaluate the effects of AST on the corneal surface, it was given different concentrations of AST in the right eye and subsequently subjected to UVB radiation. In the left eye, controls received an instillation of 1.0 mg/mL AST without irradiation. After irradiation of UVB for 24 h, the corneas of both eyes were analyzed by hematoxylin-eosin staining. Corneal epithelial thicknesses in treated eyes were 25.6 ± 2.9 μm for 1.0 mg/mL AST, 18.8 ± 3.5 μm for 0.1 mg/mL AST and 8.2 ± 3.6 μm for 0.01 mg/mL AST; and in untreated eyes, it was 8.9 ± 5.3 μm. Thus, it is found that for the 1.0 mg/mL AST concentration, the epithelium thickness remained close to normal values (29.6 ± 0.5 μm), and the photoprotective effect depends on the concentration of AST. Cytotoxicity was much lower (17.6% ± 2.0) for AST at 1.0 mg/mL, compared to the other concentrations (29.5% ± 2.2, for 0.1 mg/mL, 31.7% ± 2.8 for 0.01 mg/mL and 32.7% ± 1.7 for untreated eyes). In conclusion, topical AST showed noteworthy ocular photoprotection without severe adverse effects on the skin [24].

Furthermore, since topical application of AST can provide photoprotection due to its antioxidant activity, its effect on skin wound healing in mice has been studied. Wounds were treated 2 times a day until complete closure and were evaluated their wound area and

histopathology images. On day 1 post injury, the wound area in the AST treated group decreased by 28.2% of the original wound area, while the control group showed a reduction of 18.1%. On day 7, the AST treated group (90.0%) showed a more pronounced closure than the control group (82.4%). On day 9, the wounds treated with AST were already fully closed and those in the control group did not close completely until day 11. Additionally, histopathology images expose that AST promotes proper re-epithelialization, formation of well-organized granulation tissue, reduction of inflammatory cells and proper epidermal regeneration in mice. Hence, it is clear that topical AST treatment seems to accelerate the healing of skin wounds in mice, and may be a suitable strategy for wound care in humans [23].

3.2 β-carotene

β-carotene is a lipophilic carotenoid with a yellow-orange color. It is generally responsible for capturing light during photosynthesis but can also function as a photoprotective pigment under critical conditions. This pigment is extracted industrially from *Dunaliella salina* and possesses antioxidant activities [28].

Topical application of UV filters and antioxidants is recommended to minimize UV damage to the skin after sun exposure, when antioxidant levels are significantly depleted [29]. Since UV filters must remain in the SC and antioxidant compounds must be able to reach viable skin layers to provide adequate photoprotection, when developing a sunscreen, the critical part lies in skin penetration. Therefore, Freitas, Praça [28] conducted a study in which the aim was to evaluate the skin penetration of commercialized UV filters combined with trans-resveratrol and β-carotene in their composition. The experimental formulations were developed based on a self-emulsifying wax and a liquid polymer containing four combined sunscreens: octocrylene, octyl methoxycinnamate, avobenzone and bemotrizinole. In addition, these formulations were supplemented or not with trans-resveratrol, with β-carotene or with trans-resveratrol and β-carotene in combination. After assessing the solubility, *in vitro* skin penetration and recovery of trans-resveratrol and β-carotene in the SC and epidermis + dermis, the results showed that the extraction recovery of UV filters in the corneal extract and epidermis + dermis were between 84.2 and 114.8%. On the other hand, the recovery of antioxidants were 59.8% (β-carotene) and 88% (trans-resveratrol) in the SC, while values higher than 97.8% were recorded in the epidermis + dermis. With regard to penetration, after application of the formulations to the experimental porcine ear skin membrane, it was observed that more than 90.0% of the amount of UV filters that penetrated the skin was restricted to the SC. Among the UV filters tested, bemotrizinole exhibited the lowest

penetration rate. Antioxidants penetrated the skin layers, and higher amounts were found in the SC (>80.0%), showing them to be ideal for topical application as they were able to permeate through the SC. In addition, the amount of UV filters delivered to the SC for formulations containing β-carotene alone or combining it with trans-resveratrol were reduced on average by 63%. In conclusion, this study demonstrates the advantages of combining β-carotene and trans-resveratrol with UV filters when skin penetration is considered. The combination reduces UV filter retention on the skin, thus improving sunscreen safety.

Human skin contains carotenoids, mainly β-carotene and lycopene, from the diet. In the skin, carotenoids are mainly distributed in the adipose tissue and in the intercellular lipid bilayers of the SC due to their lipophilic nature. Their concentration depends on the layer of skin we are considering, the region of the skin and the lifestyle of each person. β-carotene is a natural antioxidant that when applied topically is susceptible to external factors such as light, oxygen and heat, which can lead to cytotoxic degradation products [30]. Additionally, its high lipophilicity results in retention in the lipid matrix between the cells of the SC for a limited period, as due to the natural process of skin desquamation, without significant penetration into the deeper layers, it leads to its depletion [31]. In order to overcome this disadvantage of instability and storage of β-carotene in the SC, Maretti, Leo [32] investigated Nanostructured Lipid Carriers (NLC) incorporated with β-carotene to evaluate their ability to facilitate permeation through the SC. Thus, in this study, two samples of NLC of different sizes applied *in vivo* on human skin were compared with Microstructured Lipid Carriers (MLC) and pure β-carotene. Regarding particle size, MLCs show an average size of about 32.1 ± 10.3 μm for the main dimensional class, and a second class with size up to 65.1 μm. On the other hand, the NLCs samples showed a significant difference between NLC_1 and NLC_2. The average size of the main size class of NLC_1 particles was about 812.0 ± 201.0 nm, with a smaller population ranging from 1.0 to 1.3 μm; whereas NLC_2 particles had an average size of about 222.8 ± 87.3 nm, with the smaller population ranging up to 475.7 nm. Thus, the loading levels of β-carotene within the particulate carriers and the encapsulation efficiency (EE%) were evaluated. The MLC showed a loading of β-carotene of 0.68% w/w ± 0.02, while the NLC showed values of 0.23% w/w ± 0.01 and 0.23% w/w ± 0.03. These results show that the ability to incorporate β-carotene into the lipid matrix was significantly lower for both samples of NLC compared to MLC, and this result can be explained by the finite solubility of β-carotene in the lipid matrix. The decrease in particle size, results in a larger surface area of the oil exposed to the aqueous phase of the emulsion containing the surfactant, which may increase the leaching of the compound from the oil nanografts during the production process.

However, even with the reduction in incorporation, the formulations may still be viable to deliver relevant dosages of β-carotene for certain applications, and controlled release can be achieved through the process of lipid matrix erosion on the skin. Regarding EE%, MLC showed an efficiency of 70.8% ± 2.1, while NLC showed values of 24.0% ± 1.0 and 24.0% ± 3.1. This difference can be attributed to the distinct properties of both systems under evaluation, including particle size and structure, the solubilization capacity of β-carotene in the lipid matrix and the leaching of the compound during the production process. To assess skin penetration *in vivo*, ten tapes with the ability to remove a thickness of the SC of about 40% were used. The tapes involving permeated β-carotene were combined into two groups, group 1 and group 2, covering stratum disjunctum and stratum compactum, respectively. 45.0% of pure β-carotene permeated the SC. Furthermore, it is evident that the concentration of β-carotene decreased according to the stratum depth, showing values of 32.4% ± 2.8 in group 1 and 13.2% ± 2.8 in group 2. These results indicate a preferential accumulation of β-carotene in the outermost part of the SC. When encapsulating β-carotene, modifications to the permeation level were observed according to the particle size. The permeation of β-carotene incorporated inside MLC was 81.5% ± 10.9 in the stratum. Regarding the depth along the stratum, it showed values of 6.2% ± 2.1 for group 1 and 2.2% ± 2.0 for group 2. These results suggest that these micrometre-sized systems have difficulty in penetrating deeply into the SC, except for microparticles up to 10 μm that are able to enter the follicle orifices, in addition to being very retained on the skin surface. Thus, this may be a strategy to improve the effectiveness of protection against UV radiation and other harmful effects of the sun by ensuring that β-carotene remains on the surface of the skin, where it is most effective at absorbing radiation. In turn, NLCs had a promoting effect on the penetration of β-carotene into the SC, due to their size. For NLC_1, the permeation of β-carotene was 57.6% ± 1.3, while that of NLC_2 was 48.9% ± 0.9. Regarding the groups, NLC_1 for group 1 showed values of 18.3% ± 0.9 and for group 2 7.8% ± 0.9; while NLC_2 for group 1 showed values of 12.6% ± 3.9 and for group 2 20.9% ± 9.6. These results are consistent with previous studies suggesting that skin penetration depends on the size of the nanoparticles. The smaller size of NLC_2 particles facilitated their distribution to the deeper layers of the SC, allowing a higher concentration of β-carotene to reach the underlying viable epidermis. In addition, this smaller size may also result in an increased occlusion effect on the skin surface, increasing the gaps between corneocytes and facilitating easier passage of the nanosystems into the deeper layers. These observations are relevant for the formulation of cosmetic and pharmaceutical products that aim at the efficient delivery of bioactive compounds into the skin. Selecting the appropriate size of nanoparticles

can influence the penetration, distribution, and retention of compounds, allowing them to be targeted to specific layers and improving treatment efficacy.

3.3 Lutein

Lutein is a yellow xanthophyll, found in most green vegetables, orange fruits, and some species of microalgae [33]. In addition to other characteristics, it possesses a potent antioxidant activity [34, 35].

To assess its effects on skin aging, a study was conducted in which crocin-rich (apocarotenoid, responsible for the color of saffron) extracts derived from *Nyctanthes arbor-tristis* and lutein extracts from *Tagetes patula* encapsulated in phytosomes (phyto-phospholipid complexes) to enhance the stability and efficacy of the carotenoids in a gel, and it was evaluated using a galactose-induced skin aging model. After standardizing the extracts, phytosomes were prepared and evaluated for their entrapment capacity showing values of 60.2% w/w for crocin and 50.8% w/w for lutein. Despite this, two gel formulations with different concentrations of crocin and lutein were developed and evaluated. To conduct this study, mice were divided into 4 groups: group 1, which was the normal control that received treatment with the saline solution; group 2, was the disease control, which received treatment with galactose; group 3 that received treatment with formulation 1 (phytosome with 2.0% w/w crocin and 1.0% w/w lutein); and group 4 that received treatment with formulation 2 (phytosome with 1.0% w/w crocin and 0.5% w/w lutein). During the study, galactose was administered for 42 days, except control group, which provoked skin aging. Thus, the control group received saline once daily for 42 days. The application of the gel increased the epidermal layer thickness from 32.0 µm to 48.2 µm in group 3, and to 55.1 µm in group 4. Also, dermal layer thickness increased from 581.2 µm to 739.5 µm in group 3, and to 627.1 µm in group 4. Finally, antioxidant parameters were evaluated through glutathione and malondialdehyde content. In the control group, a significant reduction in glutathione levels from 103.0 nmol/mL to 77.0 nmol/mL was observed; and in the gel-treated groups, there was a restoration in glutathione levels, presenting values of 83.9 nmol/mL and 84.0 nmol/mL in groups 3 and 4, respectively. Galactose administration led to an increase in malondialdehyde levels. In the control group, these increased from 0.06 µmol/mL to 0.10 µmol/mL; in the gel-treated groups, levels decreased to 0.08 µmol/mL. These results suggest that the gel formulations used in the study possess antioxidant activity and can restore glutathione levels and reduce malondialdehyde levels, indicating a potential protective effect against galactose-induced oxidative stress. The study also analyzed the expression of elastin and collagen genes in response to treatment with the gel. The results showed a 0.03-

fold and 0.02-fold increase in collagen expression in groups 3 and 4, respectively, compared with the control group. Furthermore, its topical application showed an up-regulation of the elastin gene in the skin. Therefore, these results indicate that crocin and lutein have the potential to prevent skin aging by up-regulating the expression of collagen and elastin genes and reducing oxidative stress [34].

Since visual impairments are a major problem for most people, affecting their quality of life, a study was conducted to deliver lutein directly to the eye to reduce the rate of selenite-induced cataract progression. As lutein possesses antioxidant activity, could rapidly counteract the oxidative stress directly involved in the pathogenesis of cataracts, and other serious age-related degenerative eye diseases. In this study, a formulation was developed using polymeric nanoparticles made of PLGA or zein incorporated into a thermosensitive gel to deliver lutein to the eye for enhanced antioxidant activity compared to the free form. These polymeric nanoparticles constitute an ideal vehicle for the topical administration of hydrophobic substances into the cornea due to their hydrophilic properties and PLGA and zein allow the administered nanoparticles to reach the lens, being able to penetrate the cornea, and diffuse through the aqueous humour. When assessing the release profile of lutein, there is an initial rapid release in the first 48 h and then the release becomes slower and more stable. In the groups treated with free lutein, there was an insignificant decrease in the advancement of cataracts, while in the groups treated with lutein-loaded PLGA nanoparticles at moderate (p=0.00145) and lutein-loaded zein nanoparticles at moderate (p=0.0458) and high (p=0.0476) doses, a noticeable decrease in cataract progression was noted. Moreover, it was showed a negative correlation between malondialdehyde levels (a marker of oxidative stress) and cataract severity, as far as the remaining markers (glutathione peroxidase, carbonyl proteins, catalase and antioxidant activity) were positively correlated with cataract severity. Based on the results obtained, PLGA and zein nanoparticles may be able to significantly reduce cataract progression with a consistent posology and application [35].

3.4 Other studies with carotenoids

The pulp of the Palmyrah fruit integrates carotenoids in its composition, which through its antioxidant activity protect the skin from damage caused by free radicals. Thus, a moisturizing face cream, a solid soap, and a liquid soap with pulp in its composition were developed. To assess the antioxidant activity of each of these formulations, these were compared with controls (formulation without carotenoids) and each with correspondent commercialized products, considering the percentage of DPPH inhibition. Of all the

formulations, the commercialized product was the one that presented the lowest activity, followed by the control and finally, the formulation prepared with the oil enriched with carotenoids. For the formulations enriched with carotenoids, the cream showed inhibition of 41.6% ± 2.4, the solid soap 66.4% ± 2.7, and the liquid soap 60.5% ± 0.1. Briefly, the formulations that included the pulp with carotenoids in their composition, showed significantly higher antioxidant activity compared to the others [36].

Hamdi, Feki [37], used blue crab chitosan enriched with carotenoids to develop a hydrogel with wound healing properties. For 13 days, 5 groups of 6 rats each with wounds of similar aspects were evaluated, with the first group being the control, the second group being the reference who received treatment with MEBO® (reference medication for wound healing tests), and the rest being the groups treated with synthesized hydrogels. On the fifth day, the wounds of hydrogel-treated rats showed a brown coloration regarding other group that showed perilesional erythema. By the seventh day, the inflammatory response was detected around the damaged skin in the untreated wounds. The brown crust observed in the treated groups (test and reference), showed thicker and more rigid in hydrogel-treated rats, persisting until the ninth day. From day eleven onwards, the crusts in rats treated with hydrogel-based patches composed of blue crab chitosan enriched with carotenoids, began to fall off, revealing a pinkish-colored, healthy tissue. Thus, it is concluded that the use of this hydrogel composed of blue crab chitosan enriched with carotenoids is effective in wound healing treatment as it accelerates wound closure with enhanced angiogenesis.

The microalgae *Neochloris oleoabundans* was studied to verify its application as an active ingredient for cosmetic products, since it has significant quantities of carotenoids in its composition. A gel formulation composed of aqueous extract of *Neochloris oleoabundans* was produced and then an *in vivo* experiment was conducted in healthy male and female volunteers, with skin phototypes II-IV, to evaluate its antioxidant and anti-inflammatory activity. To determine the antioxidant activity, DPPH method was used, where compared to the control, the aqueous extract showed higher antioxidant activity (54.8% ± 2.1). To evaluate the anti-inflammatory activity, a gel was applied on the forearm of the participants in two random zones, in one the control (without extract) was applied and in the other, the gel with 0.1% of the extract in its composition was applied. A less marked increase in blood flow was observed in the area pre-treated with the microalgae than in the untreated area, and the onset of action was longer in the treated areas. These results seem to confirm the potential of using microalgae derivatives in cosmetic products with anti-aging effects [38].

4. ANTHOCYANINS

Anthocyanins are a group of water-soluble plant pigments that are widely distributed throughout nature in seeds, leaves, fruits, and vegetables and are responsible for the blue, purple, red, and orange colors of many fruits and vegetables. They are flavonoids and occur mainly as glycosides or acylglycosides [39]. Several studies have shown that the chemical structures of anthocyanins have a positive impact on human health through their antioxidant activity [11, 40, 41].

Kanpipit, Nualkaew [11], conducted a study to develop 6 sericin-hydrogel formulations incorporating purple waxy corn (*Zea mays L.*), a natural compound rich in anthocyanins, at different concentrations to develop potential topical cosmetic products, such as anti-aging products, wound healing ointments, sunscreens, among others. The anti-melanogenic effects were studied through the percentage of tyrosinase inhibition (enzyme that limits the rate of melanin synthesis and in the melanogenesis pathway) and melanin content in treated B16F10 melanoma cells. The formulation that showed the highest tyrosinase inhibition was the one consisting of 0.2% alginate and 0.15% purple waxy corn cob extract (PWCCS), with 72.2% inhibition and this being significantly higher than the others. The α-melanocyte stimulating hormone can be used as a marker or inducer of melanin, thus B16F10 cells treated with this compound will produce a higher melanin content (135.0 ± 3.3%). In cells treated with 0.2% alginate and 0.15% PWCCS, the content was 101.3 ± 2.2% and in those treated with 0.2% alginate and 0.5% PWCCS, the content was 93.2 ± 4.7%. Thus, the formulation composed of 0.2% alginate and 0.15% PWCCS presents a higher potential to inhibit tyrosinase activity and melanin production. Regarding UV protection, the morphology of HaCaT cells pretreated with both formulations and some components was observed. As a result, the formulation composed of 0.2% alginate and 0.15% PWCCS showed a positive effect on cell protection after UVB irradiation, more than the formulation composed of 0.2% alginate and 0% PWCCS. To evaluate their effect on anti-aging, the formulations were studied for elastase and collagenase inhibition. The formulation consisting of 0.2% alginate and 0.5% PWCCS was the one that presented the highest inhibition of elastase (86.1 ± 2.3%), however, the formulations that contained purple corn extract in their composition, presented an inhibition higher than 75%. Regarding collagenase inhibition, all the samples presented a lower collagenase inhibition than the positive control. The formulations composed of 0.2% alginate and 0.15% PWCCS and 0.5% PWCCS showed higher collagenase inhibition (68.5 ± 3.4% and 65.5 ± 1.8%, respectively) than the formulations without alginate, so this compound may improve the release or permeation properties of anthocyanin in the skin. Collagen production in NHDF cells was also

studied, and the results indicated that sericin combined with purple waxy corn extract may increase collagen production. Overall, the formulation consisting of 0.2% alginate and 0.15% PWCCS is able to inhibit collagenase and elastase, making it a promising anti-wrinkle and anti-aging product. Whitening effects have also been found, including inhibition of tyrosinase and reduction of melanin content. Additionally, it has shown protection against UVB radiation, thus it can be used in sunscreen products.

Pistachio (*Pistacia vera* L.) it is abundant in phenolic compounds known for their strong antioxidant activity, and stands out for being the only variety of nut that has anthocyanins. Martorana, Arcoraci [40], conducted a study with to verify the potential use of Bronte pistachio peel and decorticated seeds extracts as ingredients in topical photoprotective formulations. The phenolic profile of the two extracts, showed that peel extract is about ten times richer in phenols than the extract obtained from the seeds, and only the peel extract contains anthocyanins. Tests were carried out to evaluate the antioxidant activity *in vitro*, through homogeneous and heterogeneous chemical systems. Homogeneous chemical systems (Folin-Ciocalteau method and Reducing Power test) were used to evaluate phenolic compounds in pistachio seed and peel extracts. Under alkaline conditions, the Folin-Ciocalteau method showed the presence of high levels of phenolic compounds in the extracts (seed extract: 19.5 ± 1.2 mg gallic acid equivalents/g; peel extract: 320.4 ± 25.2 mg gallic acid equivalents/g); it was also demonstrated the redox potential of the antioxidants present (seed extract: 1.1 ± 0.1 mmol of ascorbic acid equivalent/g of extract; peel extract: 2.7 ± 0.2 mmoles of ascorbic acid equivalent/g of extract). Also, the β-carotene bleaching test assessed the ability of antioxidants to break the lipid peroxidation chain, to study the efficacy in scavenging lipoperoxyl radicals. The IC_{50} values obtained were 0.3 ± 0.02 mg/mL for the peel extract and 2.1 ± 0.2 mg/mL for the seed extract. The UV-IP test allowed to evaluate the ability of antioxidants to protect against damage caused by ROS generated during the process. This presented IC_{50} values of 2.5 ± 0.2 mg/mL for the peel extract and 4.1 ± 0.4 mg/mL for the seed extract. According to these results, the peel extract showed greater antioxidant activity, however, both extracts provided positive results as topical photoprotective agents, being subsequently tested *in vivo* for their ability to improve skin erythema caused by exposure acute exposure to UV-B in human volunteers. In this test, three formulations containing pistachio peel and seed extracts or tocopheryl acetate (used as a reference compound) were applied to the skin immediately after exposure to UV-B radiation. All formulations were able to protect the skin against erythema, leading the formulation with peel extract (66.8%), then the formulation with seed extract (33.2%) and, finally, the formulation with tocopherol (22.6%).

Those findings suggests that peel extract can be considered as a valuable ingredient for cosmetic and pharmaceutical products with photoprotective features.

Blackberries (*Rubus* sp.) contain a large number of anthocyanins in its composition. Raspberry is also a source of natural antioxidants due to its phenolic content and the presence of flavonoids. Thus, Cefali, Franco [41] conducted a study with the aim of evaluating the *in vitro* antioxidant activity and the SPF of a topical formulation constituted with blackberry and raspberry extracts. After characterizing and standardizing the extracts, the *in vitro* SPF was calculated. The blackberry had an SPF value of 54.6 and the raspberry of 37.3, and the minimum value required for a sunscreen after being tested *in vivo* is 6.0. Regarding antioxidant activity, this was evaluated *in vitro* using the DPPH radical. At a concentration of 0.14% (v/v), raspberry extract exhibited an inhibitory effect of approximately 82.3%, while blackberry extract displayed an inhibition rate of around 74.0% at a concentration of 0.11% (v/v), which means that both can neutralize free radicals. Furthermore, quercetin, an efficient antioxidant flavonoid, was used as a reference standard at different concentrations. The results showed that concentrations between 1.0 and 1.75 μg/mL of quercetin showed an inhibition rate greater than 50.0%. Although the antioxidant activity of raspberry and blackberry extracts is lower compared to quercetin, this does not diminish the relevance of these extracts, as they still have significant antioxidant activity, which can be attributed to the presence of flavonoids. Based on these results it is possible to state that both blackberry and raspberry demonstrated antioxidant activity and sun protection factor during *in vitro* tests. Therefore, the extracts of these fruits can be considered as promising natural-derived product candidates for investigation as potential sunscreens agents and to prevent skin photoaging.

5. CHLOROPHYLLS

Chlorophylls are lipid-soluble natural pigments, pigments that are green in color and are found in algae, plants, and cyanobacteria. There are four types: chlorophyll a, which absorbs most of the energy of the wavelengths of blue-violet and red-orange light and is responsible for photosynthesis; chlorophyll b, which functions as a photosynthetic pigment that helps absorb light energy in photosynthesis; chlorophyll c, which is a substitute for b and is present in brown algae and diatoms; and chlorophyll d, present in some red algae. These compounds stand out for their antioxidant [42] and antimicrobial [42, 43] activities.

Song, Lee [42], conducted a study to evaluate the clinical efficacy and safety of chlorophyll a with photodynamic therapy (PDT) to treat acne, owing to better benefits regarding conventional treatments. Gold standards, e.g., antibiotics and retinoids can be

associated with microbial resistance, irritation, and systemic adverse effects. Thus, in this study, one side of the face received chlorophyll a PDT treatment, while the other side was subjected to Light Emitting Diode (LED) phototherapy as a control. After 1 week, a significant improvement in acne severity was already noticeable on the chlorophyll a PDT treated face, from 3.1 at baseline to 2.9, and at week 2, the LED phototherapy face also went from 3.1 at baseline to 2.6. At week 6, the average acne grade of the chlorophyll a PDT and LED treated sides was 1.8 and 2.2, respectively. Also, the comedon count decreased significantly on both sides after 6 weeks: LED phototherapy had a considerable improvement in the closed comedones count from 18.4 at baseline to 13.3, and chlorophyll a PDT produced a significantly greater improvement from 18.4 at baseline to 8.5. Also, sebum levels decreased significantly as early as week 2 on the chlorophyll a PDT treated side, while on the other side, we only observed this significant reduction from week 4. In the end, it is noticeable that the acne improved on both treated sides, however, on the side treated with chlorophyll a, have been observed significant reductions in acne lesions, degrees of severity, and sebum levels.

Cutaneous leishmaniasis is an infectious disease that affects the skin and mucous membranes. Conventional treatments are expensive and require hospitalization. In this context, research has been conducted in order to find more efficient, cheap, and tolerable alternatives. To this end, a study was carried out in which a thermal and photoresponsive emulgel was developed that incorporates microdroplets of copaiba oil extracted from *Copaifera reticulata* Ducke and chlorophylls, evaluating the way it acts as a leishmanicidal, healing and antibiotic agent, reinforcing the action of PDT. When carbomer 934P (0.25%, w/w), thermoresponsive copolymer F127 (18.0%, w/w) and different concentrations of copaiba oil-resin (from 8.0 to 12.0%, w/w) were tested, they showed promising results in the treatment of leishmaniasis [44]. Therefore, two emulgels with different concentrations of chlorophyll (0.5 g and 1.0 g) were formulated and tested. Bioadhesion, fundamental to improve wound healing since it prolongs the contact of the drug with the wound, was evaluated by the strength and work of bioadhesion. The addition of copaiba oil reduced the bioadhesion strength by 17.0% compared to gels with 0.5% chlorophyll, but there were no significant differences in gels with 1.0% chlorophyll. The incorporation of chlorophyll increased the overall bioadhesiveness of the gels, improving their performance. In permeation studies, emulgels showed the ability to cross the SC barrier and reach deep layers of the skin. In the analysis of the epidermis, permeation is superficial, with emphasis on copaiba oil, which, due to the interaction with the SC, presents a significant interaction. In the dermis, all formulations were able to permeate. With increasing exposure time, permeation also increased. The combination of copaiba oil

with chlorophylls showed the potential to favor healing and antimicrobial treatment, therefore, the antimicrobial activity of the emulgel composed of 0.5% chlorophyll was evaluated. The results showed that, in the absence of light, this formulation has bactericidal effects against *Staphylococcus aureus*, which was amplified when red light activated the photosensitizers, resulting in bacterial destruction. Hence, emulgels can penetrate deep into the skin and PDT provide enhanced bactericidal effect. These findings highlight the potential of the formulation designed for the treatment of wounds and cutaneous leishmaniasis [43].

6. PHYCOBILIPROTEINS

Phycobiliproteins are fluorescent pigments that occur in phycobilisomes, which are protein complexes that collect light energy in cyanobacteria, red algae, and glaucophytes. There are three different types of phycobiliproteins: blue phycocyanin, blue-green allophycocyanin, and red phycoerythrin [45].

6.1 Phycocyanins

Phycocyanins are water-soluble pigments characterized by their intense blue color. They come from cyanobacteria, rhodophytes, and cryptophytes, being one of the most abundant constituents of *Spirulina platensis*, which possess antioxidant [46, 47] and antimicrobial activities [12, 46].

A study conducted by Gunes, Tamburaci [12] aimed to develop and evaluate the wound healing capacity of a skin cream incorporating an extract of *Spirulina platensis* in its composition. Initially, the proliferative capacity of the crude extracts of this alga was evaluated, and the highest activity stood out in the concentrations of 0.1% and 0.05%, attributing these results to the high content of phycocyanins, which represent about 4.5% of the extract composition. To evaluate the wound healing effect, creams with different concentrations of *Spirulina platensis* extracts (0.5% and 1.13%) were applied in human fibroblasts and keratinocytes for 5 and 10 days through an *in vitro* scratch test. The 0.5% extract showed a wound closure of 56.6%, while the extract with 1.13% closed the wound by 74.9%, thus exhibiting a greater proliferative effect of skin cells, and this result was further supported by the results of the immunohistochemical assay (IHC). Based on these findings, and owing mainly to its proliferative activity, it can be affirmed that the incorporation of *Spirulina platensis* into skin creams could be a suitable and feasible strategy, particularly for wound healing.

Nihal, Gupta [46] conducted a study to develop an anti-acne ointment of C-phycocyanin extracted from *Spirulina platensis* and its antimicrobial and antioxidant activities were tested. Concerning antioxidant activity, the percentage of antioxidant activity is dependent on the concentration of extracts, i.e., for a concentration of 0.05 µg/mL of C-phycocyanin, the extract have 20.0% antioxidant activity; for 0.3 µg/mL of C-phycocyanin, the activity achieved is about 110.0%. Concerning the antimicrobial activity, the minimum inhibitory concentration of aqueous coriander extract had values of 1.7 mg/mL and 2.1 mg/mL, and the zone of inhibition of 21.5 ± 1.4 mm and 20.6 ± 1.1 mm against *Propionibacterium acne* and *Staphylococcus epidermidis*, respectively. In this study, the aqueous extract of *Spirulina platensis* has minimum inhibitory concentration values of 1.5 ± 0.1 mg/mL and 1.8 ± 0.2 mg/mL, and the zone of inhibition zone of 26.1 ± 1.2 mm and 24.6 ± 1.6 mm respectively. These results indicate that *Spirulina* aqueous extract possesses antimicrobial activity against the microorganisms that contribute to inflammation and lesions in acne vulgaris, therefore, this extract has anti-acne properties.

In another study regarding phycocyanins, Pagels, Almeida [47] investigated the potential of pigments (carotenoids and phycobiliproteins) present in *Cyanobium sp*. extracts through the development of a skin serum. Therefore, 2 types of *Cyanobium sp.* extracts were evaluated, ethanolic (carotenoid-targeted extract) and aqueous (phycobiliprotein-targeted extract). The ethanolic extract was able to inhibit hyaluronidase, with IC_{50} of 108.7 ± 5.7 µg/mL, while the aqueous extract was able to inhibit both hyaluronidase and collagenase, with IC_{50} of 67.3 ± 1.2 µg/mL and 582.8 ± 56.9 µg/mL, respectively. Likewise, no inhibition of tyrosinase and elastase was found in either of the two extracts. Thus, due to their bioactivity, both extracts could be potential ingredients for cosmetic use, namely in anti-aging products.

Due to their high molecular weight, phycocyanins have low bioavailability. Thus, a study was designed where phycocyanin was encapsulated in hyalurosomes, or alternatively in PEG hyalurosomes. Unencapsulated phycocyanin demonstrated an approximate EE of 52.0%. In contrast, in hyalurosomes and PEG-hyalurosomes, the measured efficiency rates were 65.0% and 61.0%, respectively. Subsequently, an *in vitro* study was conducted to examine how fluorescent vesicles are distributed in pig skin. These vesicles were prepared with a lipid compound called 1,2-Dioleolyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (Rho-PE) together with phycocyanin. The results showed that the fluorescence from Rho-PE was predominantly identified in the superficial layers of the skin after 2h, increasing, however, gradually at the 4 and 8 h markings, propagating towards the deeper layers, including the epidermis and dermis. Using hyalurosomes, after 2h, Rho-PE was accumulated in the

outermost layer of the skin (SC) and, after 4h, managed to penetrate the epidermis and dermis, remaining in these layers until 8h. Finally, the biocompatibility of encapsulated phycocyanin was explored, considering its ability to protect keratinocytes and endothelial cells against oxidative stress. Cell viability was greater than 95.0% when incubated with liposomes, but decreased when cells were exposed to the phycocyanin solution alone (about 75.0% for 20.0μg/mL and about 59.0% for 400.0μg/mL). Regarding the effectiveness of formulations containing liposomes and hyalurosomes loaded with phycocyanin (100.0μg/mL) to combat oxidative stress, it was found that cells treated only with hydrogen peroxide resulted in a significant decrease in cell viability after 3 and 6h, while that the simultaneous presence of hydrogen peroxide and phycocyanin-loaded vesicles increased cell viability in a time-dependent manner. Hyalurosomes exhibited the most notable protective effect, resulting in cell viability of about 108.0% and 118.0% after 3h and 6h of treatment, respectively. PEG-hyalurosomes also demonstrated efficacy in restoring stress conditions, with cell survival of approximately 100.0% at 3h and 105.0% at 6h. Liposomes recorded the lowest viability rate, but still provided higher cell viability than the control group treated with hydrogen peroxide alone, maintaining about 90.0% at 3h and 100.0% at 6h. In summary, phycocyanin-loaded hyalurosomes, especially PEG hyalurosomes, could be a feasible strategy to improve the bioavailability of phycocyanin, and consequently, exert its protective and restoring effects on the skin [48].

7. OTHERS NATURAL-DERIVED PIGMENTS

As far as hair loss is concerned, pharmacological treatments have several limitations, particularly, products of topical application, where the active substances that have a high onset of action on skin. It is therefore of great interest to investigate an approach, which may involve for example, a combination of 5α-reductase inhibitors with substances that promote hair growth [49]. Hence, compounds of natural origin, such as fatty acid, phytoestrogens, and proanthocyanidins have shown some efficacy on hair growth and hair loss [50, 51]. These compounds are found in various plants, notably Thai rice, with anthocyanins being responsible for the pigmentation of black rice and proanthocyanidins for red rice. Thus, Thitipramote, Imsonpang [52] carried out a study to evaluate the *in vitro* inhibition of 5α-reductase, hair growth, and hair cycle conversion activity *in vivo* in mice, of native Thai red (Sang-Yod and Mun-Poo) and black (glutinous black and Hom-Nil) pigmented rice extracts. Red pigmented Thai rice extracts (Sang-Yod: 18.5 ± 9.0 and Mun-Poo: 11.5 ± 3.1mg FEA/g extract) showed significantly higher 5α-reductase inhibitory activity than black rice (glutinous black: 9.3 ± 4.8 and Hom-Nil: 2.9 ± 0.6mg FEA/g extract), being a more attractive option to include in anti-

hair loss formulations. Also red rice extracts (Sang-Yod: 216.2 ± 0.7% and Mun-Poo: 159.2 ± 16.5%) promoted greater proliferation of human hair matrix cells than black rice at the same concentration. Therefore, the results demonstrate that red pigmented rice has a high potential as an *in vitro* 5α-reductase inhibitor and hair growth promoter, being a naturally sourced raw material available and adequate for hair care and treatment.

Curcumin is a polyphenol found in the rhizome of the turmeric plant *Curcuma longa L.*, being quite relevant due to its antioxidant, antibacterial and anti-inflammatory activities, among others [53]. Since wound healing can be influenced by prolonged inflammation and increased oxidative stress, curcumin, due to its therapeutic activities, may help to improve this condition. Therefore, a study was conducted on rat skin with the aim of evaluating the potential of nanogels, namely curcumin loaded gel-core hyaluosome, to increase the delivery of curcumin to wound sites, the healing rate and decrease scar formation. The efficacy of curcumin loaded self-assembled gel-core hyaluosomes in the treatment of dermal burns was compared with other formulations: conventional transfersomal gel, curcumin in hyaluronic acid gel, curcumin in pluronic gel, gel-core hyaluosome and hyaluronic acid gel. On day 2, all treated groups showed a noticeable difference in wound reduction compared to the control group, however on day 10, only the groups treated with curcumin loaded self-assembled gel-core hyaluosomes, with conventional transfersomal gel and gel-core hyaluosome without curcumin showed a statistically significant difference compared to the control group. Curcumin loaded self-assembled gel-core hyaluosomes is noteworthy as it was the system that stood out for a marked significant improvement on day 7 of treatment (with 99.7 ± 0.3% efficacy) and little or no scarring on day 10. This positive result can be attributed to the combination of curcumin's anti-inflammatory and antioxidant action, combined with the effect of hyaluronic acid, which has scarring reduction potential. In addition, this new formulation has the ability to direct the drug to the dermal region, ensuring that the therapeutic action occurs at the necessary site, improving the effectiveness of the treatment. In addition, a skin deposition study was conducted, demonstrating the potential of the new gel-core hyaluosomes compared to other formulations to improve the penetration of curcumin into the skin and protect it against metabolism, resulting in greater bioavailability and effectiveness in the treatment of wounds or other chronic skin diseases. In this study, 6h after the use of curcumin loaded self-assembled gel-core hyaluosomes, 0.8% ± 0.3 of the applied dose of curcumin was found deposited on the skin; and after 24h the deposition was significantly higher, from 4.6% ± 1.3, compared to the curcumin gel formulation conventional transfersomal gel (0.9% ± 0.4) and there was no detection of curcumin in the simple gel formulations. This indicates that the gel-core

hyaluosomes was able to efficiently protect curcumin against metabolism by increasing its stability and location in the skin. The results highlighted the benefits of topical application of curcumin loaded self-assembled gel-core hyaluosomes in rat burns, and represent a promising approach to improve outcomes in the treatment of burns and other skin conditions that require healing and scar reduction [54].

Lignin is one of the most abundant natural aromatic polymers on Earth, being the main component of lignocellulosic biomass. It is a compound of interest due to its antioxidant and UV-blocking properties [55]. The study by Antunes, Mota [56] aims to investigate the potential of sugarcane bagasse lignin as a multifunctional ingredient acting as a pigment, antioxidant, and UV blocker. For that, lignin was extracted from sugarcane bagasse and its safety and its biological potential were assessed. In order to evaluate the safety of topical application of lignin, an *in vivo* skin irritation test was performed, in which a patch with 5.0% lignin was used and kept on the skin for 48h. After this time the acute irritation index was calculated, presenting a value of 0.00, which confirms its safety for topical application. A Blemish Balm cream with 5.0% lignin from sugarcane bagasse was formulated, where its antioxidant activity was evaluated through ABTS.+ and DPPH methods. The lignin presented a similar capacity to sequester the ABTS.+ radical in comparison with the reference standard BHT, which is commonly used in cosmetic products. In the case of the DPPH method, the IC_{50} of the lignin was slightly higher than that presented by BHT. Furthermore, the SPF *in vitro* and *in vivo* was also evaluated, together with the UVA-PF *in vitro* and the critical wavelength, presenting an identical SPF *in vitro* and *in vivo* (9.5 ± 2.9 and 9.6 ± 0.8, respectively). With these results, the bioactivity of this compound is confirmed and can be considered a good ingredient to include in cosmetic formulations, as its topical application is safe.

Lastly, a study conducted by Shiva Krishna, Sudha [57] intends to evaluate the wound healing properties of the red pigment isolated from marine Bacterium *Vibrio sp.* on injuries in albino rats, simultaneously with its antimicrobial activity. In this study, there were 4 groups: group 1, control without treatment or application; group 2, in which only the cream base was applied; group 3 which was treated with a 2.0% framycetin ointment; and group 4 which was topically treated with a 10.0% red pigment ointment. Each group was treated for 14 days, with cream applied twice daily. None of the groups exhibited skin irritation. Considering wound healing, the red pigment ointment, and the ointment with framycetin showed significant wound healing activity when compared to the control and ointment base. At day 6, the groups treated with the ointments presented a wound healing of about 50.0% (49.4% for the red pigment ointment and 58.4% for the ointment with framycetin), while the control and the ointment

base had values of about 10.0%. Further, complete wound healing was observed after 14 days for framycetin and 16 days for red pigment ointment. Partial wound healing was observed for the remaining groups. Also, in the evaluation of antibacterial activity, red pigment showed significant antibacterial activity against gram-positive and particularly, gram-negative bacteria. Thus, these results support the wound healing potential of the red pigment, namely, a positive influence on the different stages of wound repair.

Table I - Compilation of studies addressing the therapeutic activities of topically applied natural-derived pigments.

Pigment	Biological effects	Relevant results	Ref.
Carotenoids			
Astaxanthin	Antioxidant	Scavenge capacity - DPPH radical: - 95.6 ± 4.2% AE-K - 77.0 ± 4.3% AE-L Scavenge capacity - ABTS.+ radical: ↑ AE-L [1]	[22]
	Antioxidant	Scavenge capacity - DPPH radical: - NC-ES_{Hp}: IC_{50}=0.5 - ES_{Hp}: IC_{50}=2 - Ascorbic acid: IC_{50}=6	[25]
	Antioxidant	SNEDDS: ↑ 2.2x AST penetration into the SC and 3.6x in skin in deeper layers[2] Scavenge capacity - ABTS.+ radical: 66.8% - 68.1%	[26]
	Antioxidant and anti-inflammatory	Mean epithelial thickness in eyes without radiation: 29.6 ± 0.5 μm. - Eyes treated with 1 mg/mL AST: 25.6 ± 2.9μm - Eyes treated with 0.1 mg/mL AST: 18.8 ± 3.5μm - Eyes treated with 0.01 mg/mL AST: 8.2 ± 3.6μm - Untreated eyes: 8.9 ± 5.3μm	[24]
	Antioxidant and wound healing activity	Day 1: ↓ 28.2% of the wound area Day 7: ↓ 90.0% Day 9: 100.0%	[23]
***β*-carotene**	Antioxidant	- More than 90.0% of the amount of UV filters that penetrated the skin was restricted to the SC. - ↓ 63.0% UV filters in the SC[3]	[28]
Lutein	Antioxidant	- After gel application, ↑ epidermal and dermal layer thickness[4] - Restored glutathione levels in the skin - Positive regulation of collagen and elastin expression	[34]
	Antioxidant	- Free lutein: ↓ small and statistically non-significant[5] - Lutein-loaded PLGA nanoparticles: ↓ clear and statistically significant[5]	[35]
Other carotenoids - *Palmyrah* fruit pulp	Antioxidant	Scavenge capacity - DPPH radical: - Cream: 41.6% ± 2.4 - Solid soap: 66.4% ± 2.7 - Liquid soap: 60.5% ± 0.1	[36]
Other carotenoids - Blue crab chitosan	Antioxidant and wound healing activity	Day 5: wounds showed a brown coloration Day 7: the crust showed thicker and more rigid, persisting until day 9 From day 11: the crusts began to fall off, revealing a pinkish-colored, healthy tissue	[37]
Other carotenoids - *Neochloris oleoabundans*	Antioxidant and anti-inflammatory	- Aqueous extract showed ↑ antioxidant activity (54.8% ± 2.1)	[38]

		- In the site pre-treated with the microalgae, a less marked increase in blood flow was observed and the start of action was longer.	
Phycocyanins and Carotenoids			
Spirulina platensis	Antioxidant and wound healing activity	- The extract with 0.5% showed a wound closure of 56.6% - The extract with 1.13% showed a wound closure of 74.9%	[12]
C-phycocyanin - *Spirulina platensis*	Antioxidant and antimicrobial	- ↑ antioxidant activity, ↑ concentration of C-phycocyanin - IC_{50}=1.5±0.1mg/mL against *P. acne*(6) - IC_{50}=1.8±0.2mg/mL against *S. epidermidis*(6)	[46]
Cyanobium sp	Antioxidant	- Ethanolic extract inhibited hyaluronidase, with IC_{50}=108.74 ± 5.74 µg/mL - Aqueous extract inhibited, hyaluronidase and collagenase, with IC_{50}=67.3 ± 1.2 and 582.8 ± 56.9 µg/mL, respectively.	[47]
Anthocyanin			
Waxy purple corn cob - *Zea mays* L.	Antioxidant	- Tyrosinase inhibition of 72.2%(7) - Ability to inhibit melanin(7) - Positive effect for cell protection after UVB irradiation(7) - Elastase inhibition of more than 75.0%(7) - Collagenase inhibition was 68.5 ± 3.4%(7)	[11]
Pistachio - *Pistacia vera* L.	Antioxidant	Folin-Ciocalteau assay - SP: 19.5 ± 1.2 mg *GAE/g*; TP: 320.4 ± 25.2 mg *GAE/g*(8) Reducing power test - SP: 1.1 ± 0.1 *mmoles AAE/g*; TP: 2.7 ± 0.2 *mmoles AAE/g*(8) β-carotene bleaching test - SP: IC_{50} = 2.1 ± 0.2 mg/mL; TP: IC_{50} = 0.3 ± 0.02 mg/mL(8) UV-IP test - SP: IC_{50}=4.1 ± 0.4 mg/mL; TP: IC_{50} = 2.5 ± 0.2 mg/mL(8) % inhibition of erythema - SP: 33.2%; TP: 66.8%	[40]
Raspberry and blackberry	Antioxidant	SPF - blackberry: 54.6; raspberry: 37.3 Scavenge capacity - DPPH radical: - Blackberry: 74.0% at 0.11% (v/v) - Raspberry: 82.3% at 0.14% (v/v)	[41]
Chlorophyll			
Chlorophyll-a	Antioxidant and antimicrobial	- At week 1, a significant improvement in acne severity was already noted, from 3.1 to 2.9. - Week 6, the average grade of acne was 1.8. In addition, the number of comedones decreased significantly from 18.4 at baseline to 8.5. - Sebum levels ↓ significantly as early as week 2.	[42]
Copaiba oil extracted from *Copaifera reticulata Ducke* and chlorophylls	Antimicrobial	- Chlorophyll ↑ the bioadhesiveness of the formulation - Copaiba oil combined with chlorophylls, ↑ exposure time of the drug on the wound, offering physical protection and antibiotic effect. - With PDT, the effect against *S. aureus* ↑ significantly (↓ of 3CFU/mL).	[43]

Others			
Proanthocyanidin from pigmented Thai rice	Hair Growth Promoter	- Red rice showed ↑ 5α-reductase inhibitory activity and promoted higher proliferation of capillary matrix cells.	[52]
Curcumin	Antioxidant	For Curc-GC-HS: Day 2: noticeable difference in wound contraction Day 7: marked improvement in healing Day 10: little or no scarring	[54]
Lignin from sugarcane bagasse	Antioxidant and UV blocker	Scavenge capacity - DPPH radical: IC_{50} of lignin was ↑[(9)] Scavenge capacity - ABTS.+ radical: similar capacity[(9)] - Identical *in vitro* and *in vivo* SPFs: 9.5 ± 2.9 and 9.6 ± 0.8, respectively	[56]
Red pigment from *Vibrios sp.*	Antimicrobial and wound healing activity	Day 6: healing of about 50.0%. Day 14: complete wound healing for framycetin Day 16: complete wound healing with red pigment ointment. - The red pigment showed significant antibacterial activity	[57]

Legend:

(1) Regarding AE-K;
(2) Regarding to Facial serum (Astarism®) and ascorbic acid;
(3) Regarding formulations containing β-carotene;
(4) Regarding groups 3 and 4;
(5) Regarding cataract progression;
(6) Regarding antimicrobial activity;
(7) Regarding formulation with 0.2% alginate and 0.15% PWCCS;
(8) Regarding antioxidant activity;
(9) Regarding BHT.

Abbreviations: AE-K - hydrogel of algae extract; **AE-L** - lipogel of algae extract; **Curc-GC-HS** - Curcumin loaded gel-core hyalusome; **ES_{Hp}** - extractive solution; **NC-ES_{Hp}** - PLGA nanocapsules loaded with total carotenoids in *H. pluvialis* solution; **SC** - stratum corneum; **SNEDDS** - self-nanoemulsifying drug delivery systems; **SP** - extracts from decorticated seeds; **TP** - extracts from peels.

8. SUSTAINABILITY AND VALORIZATION OF RESOURCES FOR THE PRODUCTION OF NATURAL-DERIVED PIGMENTS

Nowadays, the concept of sustainability has gained prominence in various industrial areas. Interest in this concept goes beyond mere environmental concerns, covering three broad areas: social, economic and environmental [58]. With industrialization, the use of synthetic pigments has become widespread due to their high stability, low production costs and ease of application. However, their excessive use has harmful effects on human health, as they are mostly carcinogenic, and on the environment, as they are toxic to ecosystems and are not biodegradable. [59].

Based on the assumption that the current thinking of most consumers is to prioritize the environment and the purchase of sustainable products, new strategies have been developed. Among these, the introduction of natural-derived pigments can be seen as a sustainable innovation capable of bringing numerous associated benefits. Researchers have therefore focused on researching and developing new, more ecological approaches that can be applied at the various stages of the product life cycle, from sourcing of raw materials, preformulation and design, to production, packaging, distribution and the use and post-consumption phase [60, 61]. The raw material selection phase is crucial to obtain a sustainable product and therefore deserves greater attention. The concept of sustainability goes beyond the use of natural, green, or organic-derived ingredients. The classification of a raw material as natural, organic and/or ecological is based on the type of agriculture and/or the absence of synthetic substances used for its production. On the other hand, an ingredient is considered sustainable if it also has environmentally preferable attributes that meet ethical, social and economic responsibility. Consequently, it is essential to assess each ingredient individually with a holistic approach [61].

As natural-derived pigments can be extracted from a variety of plants, animals, and microorganisms, they could provide an alternative to synthetic pigments, however, each natural source has its own limitations and cannot currently compete economically with synthetic counterparts. The advantages and disadvantages of each of these pigments are therefore listed in the following table:

Table 2 - Advantages and disadvantages between natural-derived and synthetic pigments.

	Natural-derived pigments	**Synthetic pigments**
Advantages	- The scientific literature is favorable, associating its use with health benefits - "Clean labelling" - Viewed positively by consumers - They are biodegradable	- High stability against heat, light and oxygen - High dying power - Accessibility of all color tones with saturation of bright and vibrant colors - Low cost of use - It does not lose quality - Application techniques are more reproducible
Disadvantages	- Low stability against heat, light and oxygen - Low color saturation and brightness - Not all color tones are accessible at the desired color depth - High cost of use - Interactions with other compounds - Need for complex analytical techniques for authenticity and quality control	- Not well considered by consumers - The scientific literature is unfavorable, associating certain pigments with potential adverse health effects - Since they remain quite stable to common oxidation and reduction processes according to their production, they are very difficult to remove from industrial effluents - Most are not biodegradable, accumulating in land and rivers causing ecological problems

Due to the increase in industrial food production to meet consumer needs, there has been a significant increase in the amount of food waste generated [62]. The processing of food of plant origin produces large quantities of by-products, which are potential sources of natural-derived pigments, including carotenoids, anthocyanins and chlorophylls, which are often discarded as waste [63]. Recovering natural-derived pigments from food waste is therefore important not only for economic and environmental reasons, but above all for their colouring properties and potential effect on human health (see Figure 3).

To extract natural-derived pigments, the first step is to obtain the raw pigment from plant resources. The conventional extraction techniques used to recover these compounds are often time-consuming, expensive and therefore unsustainable [63]. In addition, these techniques use high temperatures and long processing times, making the natural-derived pigments susceptible. To overcome these limitations, new green extraction methodologies have been developed in recent years, such as pulsed electric field, ultrasound assisted extraction, microwave assisted extraction, and high pressure assisted extraction methods, in response to increasing market demand for sustainable products. The advantages of using these new technologies include better insulation, greater selectivity, lower energy consumption and low environmental impact. However, they also continue to have some limitations, including the high cost of equipment and the low possibility to scale-up for industrial applications [64].

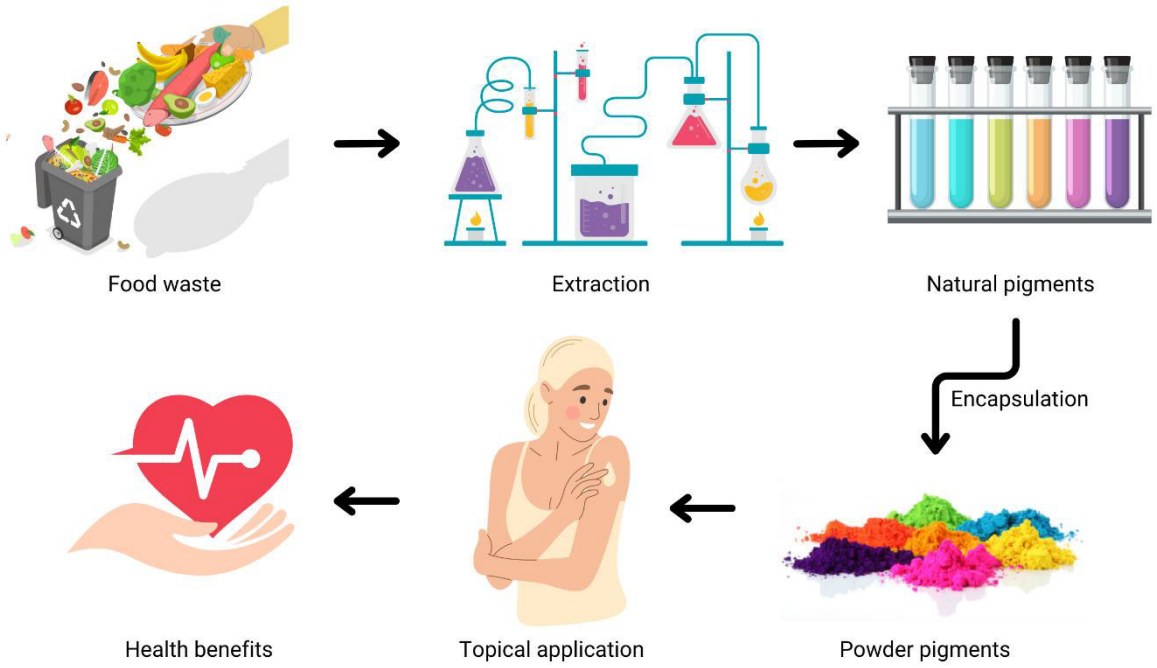

Figure 3 - Illustrative scheme of the process of extracting pigments from food waste.

Another sustainable alternative for obtaining natural-derived pigments is biotechnological which use microorganisms (fungi, yeasts, bacteria) which are known to produce a variety of pigments, which are preferable to those from plants due to their availability for cultivation, low cost, efficiency, high yield and easy processing [65, 66]. Among microorganisms, bacteria have a high potential for producing pigments, being one of the emerging fields of research in various industrial applications, due to their natural character, level of safety of use (non-toxic), medicinal and nutrient properties, production independent of the time of year and geographical conditions and a controllable and predictable yield [67].

In addition to bacteria, algae and marine microorganisms are also of particular interest as they provide a sustainable and abundant source of pigments that can persist under extreme conditions of pH (4>pH<9), temperature (-2°C to -15°C and 60°C to 110°C), and under limited substrate availability [68, 69]. Hence, the marine environment is considered an attractive source of natural-derived pigments.

9. CONCLUSION AND FUTURE PERSPECTIVES

The use of natural-derived pigments in products for topical application has gained prominence due to their multifunctional properties. These pigments not only provide an attractive appearance, but also offer significant benefits for skin health. Many natural-derived pigments contain compounds with antioxidant activities, which will help neutralize free radicals, protecting the skin against photodamage caused by oxidative stress, contributing to a youthful and healthy appearance; and/or antimicrobial activity that helps to control bacterial proliferation on the skin, contributing to the prevention of problems such as acne. It also has benefits for wound healing and protection against UV radiation.

The future prospects for natural-derived pigments for topical application are very promising, as the industry evolves towards more sustainable and personalized approaches. Possible directions that research in this field could take in the coming years:

- Innovation in nanodelivery methods, allowing better stability, safety and efficacy of natural-derived pigments in topical formulations [70].
- Ongoing research can identify new natural-derived pigments with unique properties for topical application, or even identify new activities. For example, there are pigments that, due to their ability to inhibit COX-2 expression, reduce PGE_2 levels and induce apoptosis in tumor cells, could be considered as potential cancer treatments [71].
- Regarding to existing extraction techniques, it is necessary to be optimized and new ones developed in order to reduce the need to purify the compounds [72].
- Large-scale microalgae culture techniques have not yet been fully developed, limiting their production to the experimental stage, and preventing industrialization. It is therefore essential to carry out further research into the characteristics of microalgae, pigment properties and biosynthetic metabolism [73].

Therefore, natural-derived pigments have an impressive future as prominent ingredients in the cosmetics and pharmaceutical industries. As consumer preferences evolve towards more natural and sustainable products, natural pigments can play a key role in creating effective formulations that benefit skin health and the environment.

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