Mariana Sofia Marques Ribeiro

Relatório de Estágio e Monografia intitulada "Skin applications of bakuchiol – a review" referentes à Unidade Curricular "Estágio", sob a orientação de Professora Doutora Filipa Alexandra Mascarenhas Melo e de Dr. Nelson Armando Pereira Gomes da Silva apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Setembro de 2022

Eu, Mariana Sofia Marques Ribeiro, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o nº 2017253774, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatório de Estágio e Monografia intitulada "Skin applications of bakuchiol – a review" apresentados à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade curricular de Estágio Curricular.

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Coimbra, 09 de setembro de 2022

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(Mariana Sofia Marques Ribeiro)

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A todos vós, a minha gratidão.

Índice

PARTE 2

Resumo

No âmbito da unidade curricular "Estágio Curricular" do Mestrado Integrado em Ciências Farmacêuticas da Faculdade de Farmácia da Universidade de Coimbra, o presente documento evidencia, sob a forma de análise SWOT, (*Strenghts, Weaknesses, Opportunities,* and *Threats*), o relatório de estágio em farmácia comunitária, desenvolvido na Farmácia Moderna, localizada na Marinha Grande, no período de 10 de janeiro a 18 de junho de 2022. Este documento integra ainda a monografia intitulada "Skin applications of bakuchiol – a review".

O bakuchiol é um meroterpeno paradigmático encontrado em diversas plantas. Esta revisão descreve as propriedades físico-químicas do bakuchiol para compreender melhor as suas atividades biológicas e a sua incorporação em sistemas de libertação na pele. O bakuchiol foi isolado pela primeira vez em 1973 por Mehta *et al.* de sementes de *Psoralea corylifolia* (Babchi), a sua principal fonte. Por se tratar de uma erva medicinal em vias de extinção, também serão abordados estudos que descrevem a regeneração da planta a partir de fragmentos de raízes. Além disso, será relatada uma técnica que pode ser usada como técnica-padrão para verificar a autenticidade da planta e evitar falsificações, bem como, será descrito em detalhe um método de extração "verde" para obter o bakuchiol. O bakuchiol tem sido sintetizado quimicamente desde 1967. Serão apresentadas cronologicamente as sínteses químicas conhecidas até o momento. Possui um amplo espectro de atividades biológicas, com especial relevância para a pele, sendo, portanto, considerada uma biomolécula líder. As principais atividades cutâneas incluem antifúngica, antibacteriana, antioxidante, anti-inflamatória, antienvelhecimento, despigmentante e anticancerígena, devidamente fundamentadas em estudos experimentais. Além disso, serão descritos e caracterizados sistemas de liberação cutânea de bakuchiol, começando com a microescala (microesponjas de etilcelulose), passando para a nanoescala (nanoesponjas à base de β-ciclodextrinas e nanoemulsões de surfactina e coco-betaína) para aplicação terapêutica e cosmética. Esta monografia também apresentará questões regulamentares, considerações metabólicas do bakuchiol e preocupações toxicológicas tanto para o usuário quanto para o meio ambiente.

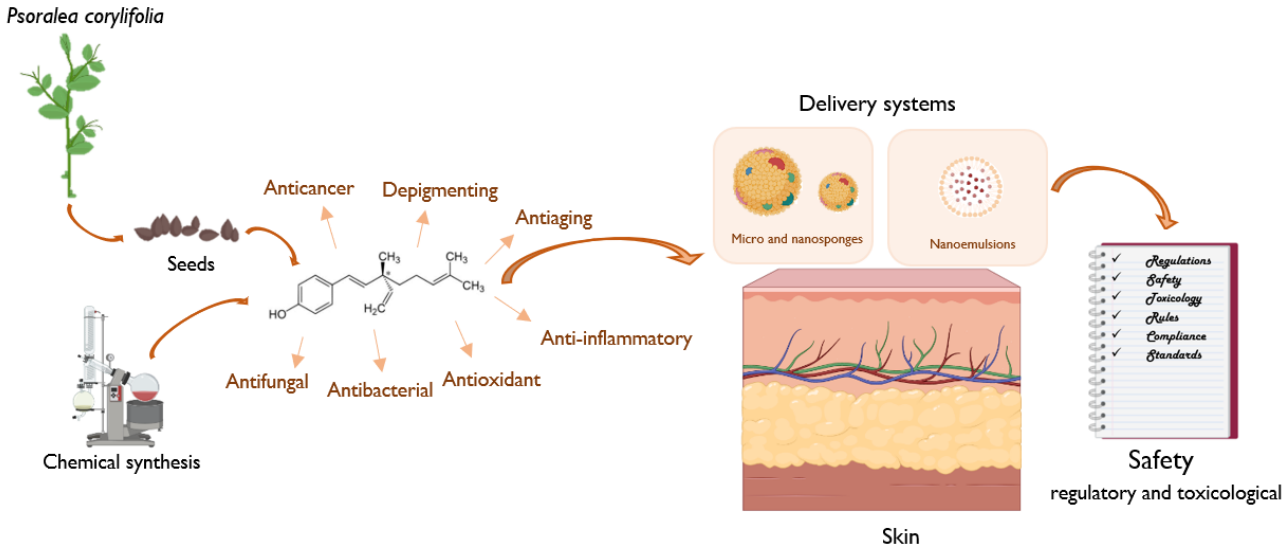
Palavras-chave

Bakuchiol, efeitos na pele, sistemas de entrega na pele, terapêutica, cosmética.

Abstract

Within the context of the curricular unit of "Curricular Internship" of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy of the University of Coimbra, the present document evidence, in the form of a SWOT analysis (*Strenghts, Weaknesses, Opportunities,* and *Threats*), the internship report in community pharmacy, developed at the Farmácia Moderna located in Marinha Grande, in the period of 10th January until 18th June 2022. This document integrates the monograph entitled "Skin applications of bakuchiol – a review".

Bakuchiol is a paradigmatic meroterpene found in several plants. This review describes the bakuchiol physicochemical properties to better understand its biological activities and incorporation into delivery systems. Bakuchiol's first isolation occurred in 1973 by Mehta *et al.* from *Psoralea corylifolia* (Babchi) seeds, its main source. As it is a medicinal herb that is threatened with extinction, studies that describe the regeneration of the plant from root fragments will also be addressed. In addition, a technique that can be used as a standard technique to verify the plant's authenticity and prevent counterfeiting will be reported, as well as a "green" extraction method to obtain bakuchiol will be described in detail. Bakuchiol has been chemically synthesized since 1967. The synthesis routes known to date will be presented chronologically. It has a broad spectrum of biological activities, with particular relevance to the skin, and is therefore considered a leading biomolecule. The main cutaneous activities include antifungal, antibacterial, antioxidant, anti-inflammatory, antiaging, depigmenting, and anticancer, duly supported by experimental studies. Besides, it will be described and characterize skin delivery systems for bakuchiol, starting with the microscale (ethyl cellulose microsponges), passing to the nanoscale (β-cyclodextrin-based nanosponges and surfactin and coco-betaine nanoemulsions) for therapeutic and cosmetic application. This monography will also present regulatory issues, metabolic considerations of bakuchiol, and toxicological concerns, both for the user and the environment.



Highlights:

-*Psoralea corylifolia* (*Cullen corylifolium*) is the major source of bakuchiol, and it is its main constituent.

-Bakuchiol has antibacterial, antifungal, antioxidant, anti-inflammatory, antiaging, anticancer, and depigmenting activity on the skin.

-Bakuchiol shows better antiaging results than retinol, with fewer side effects.

-Micro and nanosystems provide bakuchiol a stable delivery, reduce dermal toxicity and protect it from UVA-radiation degradation.

Keywords

Bakuchiol, skin effects, skin delivery systems, therapeutic, cosmetic

Parte I

Relatório de Estágio em Farmácia Comunitária

Farmácia Moderna

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"Usus magister est optimus",

A experiência é o melhor professor.

Abreviaturas

FC – Farmácia Comunitária

FM – Farmácia Moderna

LVMNSRM – Locais de Venda de Medicamentos Não Sujeitos a Receita Médica

MICF – Mestrado Integrado em Ciências Farmacêuticas

1. Nota introdutória

O Mestrado Integrado em Ciências Farmacêuticas (MICF) é um curso muito completo que, através das preleções teóricas e práticas visa dotar os estudantes com conhecimentos, de modo a que desenvolvam um espírito crítico, capazes de avaliar por si tudo o que respeita ao medicamento. Para tanto, possibilita a oportunidade de realizar um estágio no último ano, para que nós, os futuros profissionais de saúde, "especialistas do medicamento", possamos alcançar tão desejado desiderato. O estágio em farmácia comunitária (FC), é obrigatório, contudo, pode ser conciliado com outro, que pode ser realizado em áreas clássicas, tais como: indústria farmacêutica, farmácia hospitalar, análises clínicas, distribuição grossista. Eu optei por dedicar todo o meu período de estágio somente à FC, para poder tirar o máximo proveito deste primeiro contacto com o mundo profissional, pois é uma área com a qual me identifico e onde pretendo aprofundar os meus conhecimentos. Não se julgue que a farmácia é apenas um estabelecimento de preparação e venda de medicamentos, não, está muito para além dessa designação simplista. É um espaço projetado e direcionado para a prestação de cuidados de saúde e promoção de bem-estar. O farmacêutico é dos profissionais de saúde mais acessíveis à população e que estabelece mais contacto com a sociedade, dada a sua proximidade e relação interdisciplinar com outros profissionais de saúde. Assim, a minha jornada académica culminou com o meu estágio em FC, que teve lugar entre o dia 10 de janeiro até ao dia 18 de junho de 2022, perfazendo um total de 810 horas, com uma média de 8 horas de trabalho diário, na Farmácia Moderna (FM), sita na cidade da Marinha Grande, pertencente ao distrito de Leiria. O meu estágio foi orientado pelo Dr. Nelson Gomes Silva, diretor técnico da FM, organizada segundo as regras de Boas Práticas Farmacêuticas para a FC [1].

2. Análise SWOT

Com o propósito de apresentar as aprendizagens e atividades desenvolvidas no âmbito da unidade curricular "Estágio Curricular" do MICF, abordarei as mesmas crítica e sinteticamente, sob a forma de análise SWOT. Este acrónimo resume os pontos fortes (Strenghts), pontos fracos (Weaknesses), oportunidades (Opportunities) e ameaças (Threats), que se encontram discriminados na tabela I.

Tabela I - Análise SWOT relativas ao período de estágio na Farmácia Moderna

Pontos fortes	Pontos fracos	Oportunidades	Ameaças
• Organização do estágio • Diversidade de casos clínicos • Dermocosmética • Única estagiária • Estágio prévio • Equipa técnica da farmácia	• Lacunas em algumas áreas • Pouco feedback	• Contexto real de trabalho • Formações complementares • Programa informático	• LVMNSRM. • Contexto Pandémico

3. Pontos fortes

3.1 Organização do estágio

Desde o momento em que iniciei o estágio, foi notório que o meu percurso tinha sido pensado e estava estruturado de forma a permitir-me passar por todos os setores, seguindo o circuito do medicamento dentro da farmácia.

Na primeira semana estive no *backoffice*, onde adquiri uma visão geral e integrada do funcionamento da farmácia. Aprendi os procedimentos da receção e gestão das encomendas, como realizar a encomenda diária, bem como as encomendas diretas aos laboratórios. A contagem física de produtos e o controlo rigoroso de validades são dois pilares para a minimização de erros e para a boa gestão de *stocks*. É fundamental ter em conta os cuidados necessários no armazenamento de alguns produtos, nomeadamente aqueles que necessitam de ser conservados no frigorífico e os psicotrópicos (guardados num local específico). Apliquei conceitos como "first in, first out", abordados na faculdade, ao dispor os medicamentos e outros produtos nas gavetas ou lineares, usando o critério de "validade" para o fazer, ou seja, colocando os produtos de menor validade à frente, de forma a saírem primeiro. Ao fazê-lo, tive a possibilidade de me ir familiarizando com as diferentes embalagens, permitindo-me consolidar a associação dos nomes comerciais aos respetivos princípios ativos, paulatinamente. O mesmo com as diferentes apresentações e dosagens. Posteriormente, passei para a organização dos produtos *over-the-counter*, onde fui estabelecendo relação entre a composição e a indicação terapêutica. Além disso, como a disposição desses produtos na farmácia tinha como base a sua área terapêutica, foi mais fácil para mim correlacioná-los.

Na segunda semana, comecei a assistir a atendimentos. Foi o momento em que pude ver como a bordar certas questões, assim como que perguntas colocar e em que sentido é que nos orientava na seleção de uns produtos em detrimento de outros. Aos poucos iniciei o atendimento. No começo atendia apenas os clientes que iam comprar medicação habitual. Fui-me adaptando progressivamente às funções do programa informático usado. Inicialmente tive receio, pois mesmo nesses atendimentos eram-me colocadas questões relativas à indicação terapêutica ou o mecanismo de ação de certos medicamentos. Dessa forma, fui aplicando conhecimentos adquiridos ao longo do meu percurso académico e ao mesmo tempo, adaptando a linguagem à pessoa em causa. Foi um aspeto que eu gostei muito, pois senti haver ali um propósito maior e que podia de facto ajudar os utentes. Outro aspeto de que gostei foi o de descortinar os cenários com os quais era confrontada. Muitas vezes as pessoas já tinham uma ideia formulada, ora dos *media* ora porque eram indicados por conhecidos. Nesses casos foi necessário explicar o porquê de não serem adequados para a pessoa/situação concreta e,

completava apresentando alternativas. Exige uma "ginástica mental" e pode ser desafiante, o que torna este trabalho dinâmico, o que para mim, é outro aspeto positivo.

Um tempo depois comecei o atendimento ao balcão, com a supervisão do meu orientador. Ao final de algumas semanas já me sentia mais autónoma e confiante. Ao longo do estágio recorri aos vários elementos da equipa técnica, isso foi fantástico, uma vez que me auxiliaram em diversos aspetos, como por exemplo, a posologia e o modo de administração de medicamentos tais como insulinas ou a colocação de cartuchos em inaladores como o Spiriva®. Ao falar com a equipa, soube mais feedbacks de clientes, o que era uma mais valia, porque essas experiências permitem-nos aconselhar melhor, explicar sabores e texturas e fazer um acompanhamento mais próximo dos doentes que fazem este tipo de terapêutica. Como é o caso de certos inaladores, em que não se sente sabor, nem "vento", apesar de ter sido dispensada a dose. Fui recordando as situações em que devo acrescentar explicações como "bochechar com água após cada administração" ou "aplicar uma camada fina", no caso de inaladores e cremes ou pomadas, respetivamente que contenham corticosteroides. Determinei parâmetros como a pressão arterial, frequência cardíaca, perfil lipídico e glicémia. No caso de doentes medicados para patologias como hipertensão e diabetes, estas simples avaliações permitem prever a adesão à terapêutica, se a medicação está a ser bem tomada, a sua eficácia e, se necessário, reforçar medidas não farmacológicas. Nos doentes não medicados, permitem fazer o controlo dos valores ou alertar para um possível problema de saúde desconhecido. No final de cada mês é feito o fecho do receituário, que consiste, resumidamente, no envio das receitas manuais e eletrónicas, às entidades responsáveis, conforme previsto no Decreto-Lei n.º 242-B/2006, de 29 de dezembro [2], de forma a que a farmácia receba o pagamento da comparticipação do Estado ou das respetivas entidades, subsistemas de saúde e seguradoras. É necessário confirmar se as receitas são válidas, sob pena de não serem aceites, e atrasar ou ser negado o pagamento das comparticipações devidas. Além disso, é obrigatório proceder ao registo dos psicotrópicos e estupefacientes, seguindo os passos que constam na circular informativa Nº 166/CD/100.20.200, de 15 de setembro de 2015 [3]. Mensalmente até ao dia 8, e anualmente até ao dia 31 de janeiro, referente ao mês e ano transato, respetivamente.

3.2 Diversidade de casos clínicos

A FM sita na avenida Vítor Gallo, na Marinha Grande, uma das principais avenidas. Encontra-se a cerca de 1 km do Centro de Saúde e o hospital mais perto fica a 16 km. Era de esperar que este fator representasse uma desvantagem, no entanto, é uma grande vantagem, já que a maior parte das pessoas se dirige à farmácia como primeiro ponto de saúde. Deparei-

me com diversas situações. No início, traduziu-se num fator de insegurança para mim, pois nem sempre me senti suficientemente confiante para resolver os casos sozinha. A elevada afluência permitiu-me contactar com muitas pessoas, de diferentes idades, classes socioeconómicas, com necessidades variadas e com diversas culturas. Exigiu uma personalização do atendimento e da informação transmitida. Foi clara a evolução dessa capacidade de adequação da comunicação e linguagem no decorrer do meu estágio. A equipa técnica foi a minha bússola. Foi necessário um grande trabalho de equipa para harmonizar a elevada afluência de utentes com a minha orientação e aprendizagem. Nem sempre foi possível aprofundar questões durante o atendimento, no entanto, esses esclarecimentos eram prestados mais tarde, de forma a consolidar conhecimentos e a dar-me confiança para futuras ocorrências. A multiplicidade de situações reais com que fui confrontada favoreceu, em muito, a minha preparação. Tive oportunidade de aplicar conhecimentos adquiridos no meu percurso académico ou outras formações que tive ao longo do curso.

3.3 Dermocosmética

A dermocosmética foi uma área bastante procurada pelos clientes da FM. A farmácia possui uma ampla variedade de marcas cosméticas. Isso permite dar resposta a peles mais sensíveis, onde encontramos marcas como *La Roche Posay*®*, Uriage*® e *Avene*®. Para as peles mais maduras, marcas de referência no antienvelhecimento como a *Lierac*® e *Vichy*®. Estas marcas são subsegmentadas, providenciando peculiaridades que permitem responder a um vasto espetro de necessidades dos utentes. A disposição dos produtos por marca facilita o reconhecimento visual e transparece maior variedade e organização. Inicialmente tive receio, porque eram muitas apresentações para cada marca e dentro de cada marca várias particularidades. Posicionar os produtos tendo por base as respetivas necessidades e segmentos foi um desafio. Robusteci as minhas competências e fui-me sentindo mais confiante aquando destes aconselhamentos. A elevada procura destes produtos pelo público em geral, de diferentes faixas etárias e com requisitos sui generis facilitou o processo de aprendizagem. Dada a crescente procura associada ao desenvolvimento deste domínio em FC, considero que foi um tema bem abordado no meu estágio. Sem dúvida, uma mais valia para a minha carreira e uma excelente ferramenta para o meu futuro profissional.

3.4 Única estagiária

A observação e execução de tarefas, ao longo do tempo, permitiu-me adquirir autonomia e, dessa forma, ganhar confiança e consolidar melhor os conhecimentos. O facto de ter sido a única estagiária facilitou esse processo, já que, os elementos da equipa puderam passar mais tempo comigo. Considero, por isso, que foi um ponto forte no meu estágio.

3.5 Estágio prévio

No fim do terceiro ano estagiei numa farmácia na minha zona de residência. Uma farmácia com um contexto social, tipo de pedidos e público alvo completamente distinto do da FM. Esta última encontra-se no centro de uma cidade, enquanto que, a primeira se situa numa vila. Para mim foi uma grande vantagem, na medida em que me permitiu ter um primeiro contacto com os medicamentos, receitas, contexto real de trabalho, entre outros aspetos. Na faculdade fomos relacionando os nomes comerciais com os princípios ativos, escritos na Denominação Comum Internacional. Na FC somos confrontados com uma imensidão de produtos e apresentações e nem sempre temos tempo para correlacioná-los. Eu tive essa possibilidade e o facto de ter sido numa farmácia com menos movimento, deu-me abertura para me ir habituando, gradualmente, aos princípios ativos, nas suas diferentes formas farmacêuticas e dosagens, assim como, às suas diversas embalagens de acondicionamento. Nesse estágio de verão fui tendo um contacto mais próximo com o receituário, onde pude ver as diferenças entre as receitas médicas desmaterializadas (eletrónicas) e as materializadas (eletrónicas – não renováveis ou renováveis - e manuais – não renováveis). Foi importante para mim ter esta visão realista sobre o trabalho que é desenvolvido numa FC. Temos uma posição privilegiada, já que temos conhecimento em diversas áreas e podemos contribuir para o acompanhamento e gestão da terapêutica dos utentes. Podemos ainda, fazer a determinação de parâmetros, o que permite a identificação de pessoas de risco e a deteção precoce de doenças. Somos o ombro amigo de muitos, os conselheiros e confidentes de tantos outros. Somos o último elemento da cadeia de saúde (outras tantas, o primeiro e único) e temos, por esse motivo, uma responsabilidade acrescida. Este estágio prévio foi uma vantagem, no entanto, tomei-o como uma referência, ao invés de fazer uma transposição *ipsis verbis* para o novo estágio. A FM tem uma realidade diferente, equipa com métodos de trabalho e dinâmicas próprias. O meu interesse e humildade deram abertura à aquisição e desenvolvimento de competências e know-how. O meu conselho para os estudantes, que tenham, tal como eu tive, a possibilidade de estagiar no verão numa FC é que aproveitem essa oportunidade, retirem o maior proveito e adquiram o máximo conhecimento!

3.6 Equipa técnica da farmácia

A equipa técnica foi um pilar base para mim. Estou grata pela preocupação em me integrar e transmitir conhecimentos que foram adquirindo ao longo das suas carreiras. O Dr. Nelson Silva é quem assume a direção técnica da farmácia, sendo um elemento fulcral da equipa. Foi nítido o grande conhecimento em relação à realidade profissional, o domínio de todas as áreas, medicamentos e produtos e requisitos legais, mas também a entrega nas suas

tarefas, a paixão pelo ato farmacêutico. Foi muito positivo tê-lo como minha referência. O profissionalismo de todos fez com que eu própria me sentisse entusiasmada em cada atividade que desempenhei. Fui, desde o primeiro momento, bem acolhida e todos os elementos, sem exceção, mostraram-se disponíveis para me esclarecer todas as dúvidas. Senti que o meu trabalho era reconhecido e isso tornou a minha experiência mais enriquecedora. A FM conta com uma equipa muito dinâmica e o facto de atribuir diferentes atividades a cada um dos profissionais, permite tirar partido das particularidades de cada um, de forma a orquestrar as tarefas diárias de forma harmoniosa, contribuindo para a prestação de um serviço de excelência ao utente. Esta divisão é flexível o bastante de forma a que os profissionais partilhem ideias e opiniões. Durante os atendimentos, ajudavam-me na resolução da questão junto do cliente, e posteriormente explicavam-me mais detalhadamente. A equipa proporciona um acolhimento familiar aos utentes, estabelecendo relações de maior confiança farmacêutico-utente, facilitando, por um lado, a fidelização do utente e, por outro, um melhor acompanhamento farmacoterapêutico. Um bom ambiente de trabalho é meio caminho andado para o sucesso e isso acaba por transparecer para os clientes.

4. Pontos fracos

4.1 Lacunas em algumas áreas

Eu considero que o MICF se encontra bem estruturado e confere aos estudantes muitas bases úteis para aplicação profissional, nas mais diversas áreas onde o farmacêutico pode atuar e ser diferenciado enquanto profissional de saúde. No entanto, senti algumas lacunas no meu conhecimento em áreas bastante requisitadas no dia-a-dia, designadamente, dietética, homeopatia e puericultura. Embora tenha tido unidades curriculares como nutrição e de terem sido tratados conceitos importantes, foram abordados superficialmente e a sua aplicabilidade prática não ficou muito clara. Numa situação simples como a medição de colesterol total, em que este esteja elevado, há certas dicas de alimentação que podemos indicar antes de recorrer à medicação. Os medicamentos homeopáticos foram mencionados, mas pouco aprofundados. A homeopatia baseia-se no princípio da semelhança e são produzidos de acordo com os métodos descritos na Farmacopeia Europeia, ou na sua falta, de modo oficial num Estado-Membro. Apesar da controvérsia e ceticismo que a sua utilização gera, são vários os utentes que os procuram de forma regular. Considero uma área que mereça um olhar mais atento, já que, o Infarmed aprova a sua comercialização. A puericultura é uma área muito ampla no que respeita a produtos e informação. Muitas vezes tive de confirmar se a minha seleção estava correta, com receio de comprometer a terapêutica ou colocar em causa a segurança do bebé ou criança. Apesar de ser abordada em várias unidades

curriculares, considero que deva ser estudada mais detalhadamente, tendo em conta a frequência com que fui abordada. De qualquer das formas, considero que este ponto fraco seja facilmente convertido em ponto forte, uma vez que, depois de o identificar sei onde posso posteriormente investir as minhas formações e enriquecer a minha bagagem.

4.2 Pouco feedback

A farmácia é, inúmeras vezes, o primeiro local onde as pessoas recorrem à procura de ajuda e informação. Simultaneamente, é o último ponto de contacto entre o utente e um profissional de saúde. O farmacêutico assume uma posição determinante para o sucesso terapêutico e o nosso papel passa pelo acompanhamento farmacoterapêutico do utente. Este torna-se difícil nos casos em que o cliente está apenas de passagem, por exemplo. Para os clientes habituais é possível acompanhar a evolução terapêutica. Durante o meu estágio senti claramente o impacto positivo que esse seguimento tem nos doentes. No entanto, reconheço que o feedback de determinados produtos ou medicamentos, exigiam que eu permanecesse mais tempo para inferir sobre a eficácia do tratamento em cada caso concreto. Considero este fator um ponto fraco, já que, tendo o feedback desenvolveria novas competências. Claro está que é inevitável, mas ultrapassável com a prática profissional.

5. Oportunidades

5.1 Contexto real de trabalho

Este estágio foi o despertar para a realidade da FC, o aplicar de conhecimentos, tanto teóricos como práticos e o acumular de novas experiências. Foi o culminar de um longo percurso. Considero que o estágio no último ano é crucial e na altura chave! Não para aprendermos tudo, mas para termos a oportunidade, de ainda em fase de aprendizagem e com um tutor, gradualmente, sermos inseridos no mundo do trabalho. O estágio foi uma excelente oportunidade, pela qual estou muito grata.

5.2 Formações complementares

A FM recebe, frequentemente representantes das diversas marcas de produtos de venda livre. Além de estabelecerem uma relação de proximidade com a farmácia e facilitarem encomendas diretas, preparam várias vezes pequenas formações para os profissionais na farmácia. Durante o período de estágio tive a oportunidade de assistir e participar em formações de marcas como Telfast®, Canesten®, Bepanthene®, Dulcosoft®, Brainkin®, que contribuíram para a minha aprendizagem e confiança pessoal, aquando da seleção e aconselhamento dos produtos em questão. Nessas formações foram apresentados quer produtos novos, quer já existentes. Foram estabelecidas comparações entre produtos

semelhantes, de modo a salientar as vantagens e as desvantagens entre eles, em que situações optar por um em detrimento de outro, as populações de risco e as contraindicações. Além disso, pude tocar, experimentar e simular administração/aplicação de alguns produtos, algo que nunca tinha feito. Essa experiência facilitou muito o posicionamento desses produtos. Um outro aspeto profícuo, foi que, todas as formações eram muito concisas, interessantes, e seguiam um fio condutor. É com esse tipo de formações que vamos, aos poucos, estruturando o nosso raciocínio e "arrumando em gavetas" a informação. Foram muito proveitosas. Em suma, as formações periódicas são formas práticas de atualizarmos conhecimentos, contribuindo para uma melhor atuação e prestação de um serviço de qualidade. Não podemos estagnar no tempo, o progresso científico e tecnológico é inevitável e temos de o acompanhar.

5.3 Programa informático

O *software* que é usado é o Sifarma 2000, onde são executadas a maior parte das atividades como receção de encomendas, devoluções, gestão de *stocks*, controle de mínimos e máximos dos produtos, atendimentos ao público, entre outras. Na opção do atendimento existem as fichas dos utentes, que personalizam o atendimento e ajudam na parte de indicação farmacêutica, já que temos acesso ao perfil medicamentoso e sabemos desde logo, quais marcas o doente prefere e quais medicamentos a não ceder, de forma a evitar interações medicamentosas, contribuindo para a segurança do utente. Outro aspeto é a informação científica de que o programa dispõe: indicações terapêuticas, posologia, precauções, possíveis interações, efeitos adversos. Temos acesso a informações que constam no Resumo das Características do Medicamento, de forma mais simples. Otimiza e agiliza o atendimento. O Sifarma 2000 é uma ferramenta que me vai ser muito útil no futuro, visto que é o programa usado na maior parte das farmácias. Para mim foi uma excelente oportunidade, já que no estágio que eu realizei anteriormente o programa utilizado era diferente.

6. Ameaças

6.1 LVMNSRM

Locais de Venda de Medicamentos Não Sujeitos a Receita Médica (LVMNSRM), são locais fora das farmácias onde é possível a venda de medicamentos que não necessitam de receita. Onde se incluem as vulgarmente designadas parafarmácias. Esses locais têm de estar registados no Infarmed e cumprir os requisitos legais, de acordo como Decreto-Lei n.º 134/2005, de 16 de agosto [4]. Normalmente encontram-se em grandes superfícies comerciais, o que lhes permite negociar grandes volumes de compras, com melhores condições comerciais, podendo oferecer ao cliente preços mais em conta do que aqueles

praticados nas farmácias. Considero-os uma "ameaça", para a saúde dos doentes, se não forem corretamente aconselhados, podendo colocar em causa a sua terapêutica e/ou segurança, e para a saúde financeira da farmácia, já que, é nesses produtos que podem praticar os preços de acordo com a margem de lucro estabelecida. Lembrando que no caso dos medicamentos sujeitos a receita médica, é o Infarmed quem regula e aprova os preços.

6.2 Contexto pandémico

Dois anos depois da Organização Mundial de Saúde declarar a pandemia, ainda se fazem sentir algumas consequências. No enquadramento da FC foram sentidas várias alterações. Com o distanciamento obrigatório, inicialmente os clientes deixaram de poder tocar nos produtos, ler atentamente as letrinhas pequeninas nas caixas de cosmética, viram-se separados do seu farmacêutico, quantas vezes o seu ombro amigo. Este ponto apresenta uma ameaça principalmente nos doentes mais velhos, na medida em que, com as máscaras e o acrílico, é difícil perceber e fazermo-nos entender. Senti várias vezes que o doente não conseguiu ouvir parte das informações que lhe tinha dado e, por vergonha ou receio de ter de me fazer repetir não pedia para voltar a explicar. Isso pode comprometer a adesão à terapêutica, segurança na administração e até mesmo a eficácia do tratamento. O facto de termos de falar mais alto, influenciou negativamente utentes mais reservados por perda de privacidade, por de terem de se "expor" perante outros clientes. Dificultou a minha intervenção pela dificuldade em entender o cerne da questão.

7. Considerações finais

Como diz o provérbio popular, "mais vale experiência que ciência". De que serve ter os conhecimentos teóricos se não souber aplicar nem quando aplicar? Não descurando, de forma alguma, a importância do conhecimento teórico. O que pretendo dizer é que, por mais unidades curriculares que existam, só "no terreno" é que se ganha calo. É o momento de "ver e aprender". O estágio em FC na FM possibilitou a aquisição e desenvolvimento de novas competências, que me vão ser muito úteis para o bom desempenho da minha função no meu futuro profissional. Todas as experiências vividas foram extremamente valiosas e contribuíram, direta ou indiretamente para a minha formação como farmacêutica, a nível profissional e pessoal. Foram importantes momentos de transmissão de valores, pelo que agradeço, uma vez mais, à FM, à sua equipa técnica e direção, em particular ao meu orientador, Dr. Nelson Silva, pela sua dedicação e oportunidade dada.

Caso prático I

Uma senhora de cerca de 75 anos, dirigiu-se à farmácia à procura de um "champô para a comichão na cabeça". Fiz algumas perguntas para perceber a origem do prurido. Não tinha piolhos nem apresentava descamação. Há mais de um mês que estava a usar o Nizoral® [5]. O uso prolongado do Nizoral® poderia ser a causa do prurido. A substância ativa é o cetoconazol, um antifúngico e está indicado para o tratamento e profilaxia de infeções como pitiríase versicolor, dermatite seborreica e pitiríase capitis (caspa). Tem posologias diferentes consoante a situação concreta, sendo que de todos, o período máximo é de 4 semanas. Selecionei o D'aveia® Champô Neutro [6], um champô suave com pH fisiológico e sem tensioativos agressivos. Apropriado para o uso diário do couro cabeludo sensível e fragilizado. Dada a sua composição à base de proteínas de trigo e farinha integral de arroz associadas às propriedades da aveia, possui propriedades hidratantes e calmantes ao nível do couro cabeludo. Além disso, recomendei que quando secasse o cabelo reduzisse a temperatura do secador, pois o excesso de calor pode contribuir para a comichão.

Caso prático II

Uma senhora com cerca de 40 anos, dirigiu-se à FM queixando-se de comichão entre os dedos do pé e pele a descamar. Além disso, as unhas estavam amareladas e ligeiramente mais grossas. A senhora acrescentou que trabalhava numa fábrica e usa botas de biqueira de aço diariamente. Aconselhei medidas não farmacológicas: lavar e secar muito bem os pés, especialmente entre os dedos. Se for possível, trocar de meias quando estiverem húmidas. Preferir meias de algodão em detrimento das de tecido sintético. Na altura do banho, tendo em conta que a senhora toma banho no balneário da fábrica, usar chinelos. Em casa, preferir calçado aberto, como chinelos. Evitar usar consecutivamente o mesmo par de botas de trabalho. Há vários fatores que promovem o crescimento fúngico, dois deles são a humidade e temperatura. Portanto, se conseguirmos minimizá-los, mais eficazes vão ser as medidas farmacológicas. E medidas farmacológicas: primeiro, o tratamento fúngico na pele, necessita do uso de um creme com antifúngico, como o Canesten Unidia® [7] (princípio ativo: bifonazol). Aplicado 1 vez por dia, quando se fosse deitar. Durante 2 a 3 semanas. O mesmo antifúngico existe na formulação de creme, solução para pulverização e creme com aplicador (boa opção, uma vez que, permite aplicar em locais difíceis de alcançar, evita o contacto direto com a zona infetada e ainda apresenta uma superfície rugosa, que permite aliviar a comichão). Segundo, uma vez que, o creme não tem capacidade de penetrar na unha, é necessário um verniz. Sugeri o Locetar® EF [8] (princípio ativo: amorolfina). A embalagem contém limas descartáveis e "toalhetes" (compressas embebidas em álcool). Primeiramente, limar as unhas afetadas.

Depois limpar com um "toalhete". Aplicar o verniz com a espátula. Dicas importantes, não limpar a espátula no frasco, mas sim no toalhete usado. A espátula deve ser limpa entre cada unha, para evitar contaminações. O tratamento é para ser feito 1 vez por semana. Por fim, de forma e prevenir uma possível reinfeção indiquei o Canesten® pó cutâneo [9], que absorve a humidade e desinfeta, pois, contém um antifúngico: clotrimazol. Pode ser aplicado de manhã e à noite (até 3 vezes por dia), mas nesta situação indiquei apenas antes de ir trabalhar, tanto nas meias, como no calçado. Se tivesse de aplicar o pó mais vezes podia complicar o tratamento e comprometer a adesão à terapêutica. Tendo em conta a quantidade de informação que lhe tinha dado considerei que uma vez era mais simples e o suficiente.

Caso prático III

Uma senhora, perto dos 30 anos apresentava uma mancha vermelha grande no peito, tinha comichão, já tinha tomado o Lergonix® e tinha iniciado as cápsulas Heliocare® em maio. O lergonix® [10] (substância ativa é a bilastina) é um anti-histamínico H1 não sedativo. O heliocare® [11] é um suplemento alimentar à base de plantas e vitaminas, com antioxidantes de origem natural. Confere proteção UVA e UVB. No final, a senhora mostrou-me um spray que estava a usar à base de lavanda. Eu aconselhei-a a parar de aplicar o spray, pois poderia estar a sensibilizar a sua pele, já fragilizada pelo sol. Recomendei a aplicação do gel Benaderma® [12, 13] para aliviar os sintomas. Ele é indicado para alergias cutâneas, queimaduras solares, urticária. Neste caso optei especificamente pelo gel em detrimento do Benadermacalm® creme, porque o gel forma uma película protetora da pele. Posteriormente, fiz uma pesquisa sobre o spray [14]. Este, apresenta efeitos calmante devido à presença de hidrolatos aromáticos. Não contém óleos essenciais. Além da água da flor de lavanda, contém linalol, considerado, pela Agência Europeia do Medicamento, uma substância alergénica [15].

Caso prático IV

Um jovem, perto dos 25 anos relatou que tinha diarreia e vómito. Manifestou preferência por produtos naturais e acrescentou que tinha ansiedade e hiperatividade. Para atuar ao nível da ansiedade cedi o Valdispert Stress® [16]. É um medicamento tradicional à base de plantas – Valeriana e Lúpulo – indicado para o alívio dos sintomas de stress mental. Recomendei 1 comprimido 3 vezes por dia. O nosso "segundo cérebro" é muito afetado pelo stress e a causa da diarreia poderia ser mesmo a hiperatividade associada à ansiedade que o mesmo referiu. Nesse sentido, cedi o Debridat® [17]. É um medicamento com ação antiespasmódica do trato gastrointestinal. A sua substância ativa é a trimebutina, tem atividade moduladora da motricidade digestiva, com indicação terapêuticas para o trato digestivo superior (náuseas, digestões lentas) e inferior (dor e distensão abdominal, alterações do

trânsito intestinal como diarreia e/ou obstipação). Recomendei que tomasse 1 comprimido 3 vezes por dia, por 3 dias. Eu optei pelo Debridat® em detrimento do Imodium®rapid [18] (substância ativa, loperamida). Este último, liga-se aos recetores opiáceos que estão na parede intestinal, inibindo a libertação de acetilcolina e das prostaglandinas, diminui o peristaltismo propulsivo e aumenta o tempo de trânsito intestinal. Atua para parar a diarreia diretamente, ou seja, é um antidiarreico. Faz sentido usar numa situação em que a causa é exógena, como vírus ou intoxicações alimentares, onde a diarreia é um sistema de defesa com o intuito de eliminar o agente patogénico. O stress pode causar-nos diarreia porque há alteração da motilidade intestinal, cólicas e distensão do intestino, são causas endógenas. O Debridat® atua a esse nível, é um antiespasmódico, o que justifica a minha escolha. Não diretamente como antidiarreico, mas no sentido de tentar minimizar os fatores que estavam a provocar a diarreia. Não recomendei probióticos nesta situação, no entanto, podiam ser usados como complemento, por "regularem o intestino", ao reporem a microbiota intestinal.

Caso prático V

Um cliente habitual com os seus 70 anos, dirigiu-se à FM à procura de "pastilhas para a garganta inflamada". O senhor "sentia a garganta arranhada e doía a engolir". Questionei se já estava a tomar alguma coisa, se tinha asma ou bronquite, diabetes, úlcera, alergia à aspirina, insuficiência cardíaca, renal ou hepática, as respostas foram todas negativas. A tensão arterial estava controlada. Orientei o meu pensamento para pastilhas com anti-inflamatório, como Strepfen® [19] (substância ativa: flurbiprofeno). Por "descargo de consciência" fui ao histórico e verifiquei que tomava o Eliquis® [20] (substância ativa: apixabano). Pertence ao grupo farmacoterapêutico anticoagulantes e antitrombóticos, inibidores diretos do fator Xa. Não atua diretamente na agregação plaquetária, mas ao inibir o fator Xa, previne a formação da trombina e acaba por interferir indiretamente na agregação plaquetária. Os doentes que tomam esta medicação têm, *per si,* uma maior predisposição para hemorragias. A associação entre o Eliquis® e um anti-inflamatório, representa uma interação major e aumenta o risco de hemorragia [21]. Apresentei outras alternativas, sem anti-inflamatórios: TantuNatura® [22]. Tem na sua constituição própolis (bom antibacteriano natural), mel (acalma e nutre a garganta irritada), vitamina C e zinco (reforçam o sistema imunitário). Recomendei 3 a 5 pastilhas por dia. Disse para beber mais líquidos mornos (ajudam a lubrificar a garganta e diminuir a irritação). Eu escolhi este caso, não por ser complicado, mas porque, para mim foi um "abre olhos" e, desde esse atendimento, incluí nas minhas questões iniciais se a pessoa toma algum medicamento "para o sangue", pela gravidade e risco que pode resultar da interação desses medicamentos com um "simples" Strepfen®, por exemplo.

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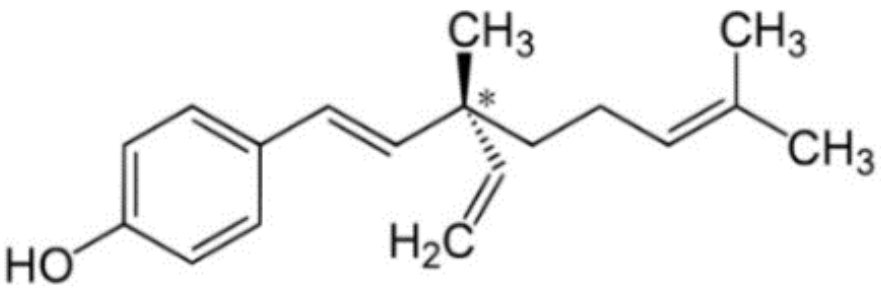
Parte 2

Monografia



Skin applications of bakuchiol – a review

1 2 9 0

UNIVERSIDADE D COIMBRA

"Se eu vi mais longe, foi por estar sobre ombros de gigantes"

Isaac Newton

Abbreviations

AA - American Academy

AP - Antioxidative Power

AQP3 - Aquaporin 3

AU- Antioxidative Unit

BAK - Bakuchiol

β-CD – β-Cyclodextrin

β-CDNS - β-Cyclodextrin-Based Nanosponge

BGM - Bakuchiol, Ginkgo Biloba extract and Mannitol

BO – Babchi Essential Oil

BOMS - Babchi Essential Oil in Microsponge

BONS - Babchi Essential Oil in Nanosponge

BPO - Benzoyl peroxide

CB - Coco-Betaine

CDR - Cumulative Drug Release

CLL – Collagen

CR - Claisen Rearrangement

DCM - Dichloro Methane

DEJ - Dermal-Epidermal Junction

DPC - Diphenyl Carbonate

DPPH - 2,2-diphenyl-1-picrylhydrazyl

EC - Ethyl Cellulose

ECM - Extracellular Matrix

EL – Elastina

EMA - European Medicines Agency

FCL – Fluconazole

FDA - Food and Drug Administration

FTIR - Fourier Transform Infrared Spectroscopy

GAG – Glycosaminoglycan

GC-MS - Gas Chromatography-Mass Spectrometry

GR - Grignard Reagent

HaCaT - Human Keratinocyte

HDF - Human Dermal Fibroblast

HPLC - High-Performance Liquid Chromatography

ICL - Itraconazole

IGA - Investigator Global Assessment

IL-6 - Interleukin-6

IL-8 - Interleukin-8

INF-γ - Interferon-γ

iNOS – Inducible Nitric Oxide Synthase

KCL - Ketoconazole

LDA - Lithium Diisopropylamide

LDH - Lactate Dehydrogenase

LPS – Lipopolysaccharide

MIC - Minimum Inhibitory Concentration

MIF - Macrophage Migration Inhibitory Factor

MMP - Matrix Metalloproteinase

MRSA - Methicillin-Resistant *Staphylococcus aureus*

MS - Microsponge

NHEM - Normal Human Epidermal Melanocytes

NF-Kβ - Nuclear Transcription Factor-Kβ

NO - Nitric Oxide

NOS - Nitric Oxide Synthase

NRR - Nuclear Retinoid Receptors

NS – Nanosponge

OD - Optical Density

PDI - Polydispersity Index

PGE_2- Prostaglandin E_2

PIH - Postinflammatory Hyperpigmentation

POFR - Phenolic Oxygen Free Radical

PTU - Phenylthiourea

PVA - Polyvinyl Alcohol

RAR - Retinoic Acid Receptor

RET - Retinol

ROS - Reactive Oxygen Specie

RXR - Retinoid X Receptor

SC - Stratum Corneum

SCFE - Supercritical Fluid Extraction

SUR - Surfactin

TCA - Trichloroacetic Acid

TDNA - Percentage of Fragmented DNA

TEWL - Transepidermal Water Loss

TMOM - Tail Moment

TNF-α- Tumor Necrosis Factor α

TRP - Tyrosinase-Related Rrotein

TS – Tyrosinase

UP – UP256 cream

UV – Ultraviolet

VTE - Vanilla Tahitensis Extract

1. Introduction

The field of herbal medicine has stood out for being a cheap and easily accessible source. Plant-derived products have been widely used for many years. *Psoralea corylifolia* is a well-known plant, especially for Chinese and Indian people, due to its natural geographical distribution. Given its years of use by the people and its diverse chemical constitution, several activities have already been assigned to *P. corylifolia*, namely estrogenic, antidepressant, hepatoprotective, immunomodulatory, osteoblastic, neuroprotective, and pesticidal [1-4]. It is the source of more than a hundred compounds [2-5], such as the famous psoralen, used since the decade of the '70s in psoriasis treatment [6]. Bakuchiol (BAK) is often considered one of its main compounds [7-14]. Chemically it has an aromatic ring and a long hydrocarbon chain which negatively influences its water solubility, a relevant aspect for its practical application in carrier systems [15]. More physicochemical properties are inherent to the BAK molecule, allowing it to identify and predict major biologic activities.

Several extraction techniques have been described over time, of which one "environmentally-friendly" was reported by a research group, which used a supercritical extraction to obtain BAK. A pretty promising strategy with good isolation yields and is eco-friendly [16]. It is not always easy to isolate plant-derived products due to their high number of active compounds. Plant extraction and isolation methods often result in lower yields. In these instances, chemical synthesis can be used. Although some steps entail a certain difficulty, several scientists reported varied BAK chemical synthetic routes [17].

Organisms that are part of skin microbiota – commensal organisms – are accountable for maintaining homeostasis and contribute to strengthening skin immune competence [18, 19]. Changes in the commensal balance allow fungal and bacterial species to grow. Several *in vivo* and *in vitro* studies confirmed BAK's effectiveness in treating these pathologies [9, 18, 20-23]. For example, *Staphylococcus aureus* is the opportunistic pathogen responsible for the most acute and chronic bacterial infections on the skin. It is estimated that around 20 to 30% of healthy and adult people are colonized, asymptomatically, by *S. aureus*. Moreover, about 76% of skin infections have *S. aureus* as an etiological agent [18]. The fact that BAK has antibacterial properties may be further applied due to growing antibiotic resistance [24].

Antioxidant and anti-inflammatory activity can be helpful to retard the natural aging process [25]. Free radicals are produced naturally and result from biological processes [26]. Solar radiation acute overexposure causes sunburn. Chronic exposure can promote skin alterations such as wrinkle formation, plaque-like thickening, deep furrowing, and loss of skin tone. This "photoaged skin", also designated "solar scar", has a particularly negative effect: the generation of reactive oxygen species (ROSs) associated with cellular oxidative damage [27],

in DNA, lipids, and proteins, culminating in cell viability loss [22, 28, 29]. Human exposure to factors like allergens, microbes, and pollutants contributes to the amplification of the generation of ROSs. Oxidation–antioxidation processes need balance [30]. It is necessary to have systems to monitor the formation of these radicals and promote their scavenging [26].

On the other hand, there is the anti-inflammatory component where BAK may act. RAW 264.7, a murine monocyte/macrophage cell line, has been widely used for over 40 years. In the human body, macrophages are part of the innate immune response and have immunomodulatory properties. They are crucial for inflammatory response and immune surveillance. Lipopolysaccharides (LPSs) can activate them, resulting in the activation of transcription factors, such as mitogen-activated protein kinase, nuclear transcription factor-kB (NF-kB), and secretion of cytokines and proinflammatory mediators, including nitric oxide (NO), prostaglandin E_2 (PGE_2), tumor necrosis factor α (TNF-α), interleukin-1β, and interleukin-6 (IL-6) [31-33]. Blood circulating monocytes are the first and major source of dermis macrophages, which undergoes tissue maturation and differentiation. They are not present in the epidermis [34, 35]. Macrophages may act like inflammatory mediators in some inflammatory skin diseases, such as atopic dermatitis [36, 37] and psoriasis [38]. They become attractive therapeutic targets by suppressing inappropriate or extensive activation [39].

Skin is divided into three main layers: epidermis, dermis, and hypodermis. In the dermis is an extracellular matrix (ECM), a non-cellular macromolecular network constituted by elastin (EL), collagens (CLLs), fibronectin, laminins, proteoglycans/glycosaminoglycans, and other glycoproteins. These components establish links between them and the cell surface receptors. This complex network regulates cellular functions, for instance, growth, survival, migration, and differentiation. ECM is a dynamic structure undergoing continuous remodeling orchestrated by multiple matrix-degrading enzymes [40]. Skin is exposed to ultraviolet (UV) radiation lifelong, which contributes to skin aging recognized by density decline and slacking. ECM degradation is related to skin aging. These could be genetically determined as a natural process of aging – intrinsic factors – or could be controllable - extrinsic factors, including include UV sunlight, pollution, diet, and smoking. This damage causes dermal connective tissue degeneration and is marked by the degradation of CLL fibers, elastic fibers, and hyaluronic acid. Probably the result of the increased expression of matrix metalloproteinase (MMP), elastase, and hyaluronidase. MMPs are accountable for ECM molecule degradation and tissue remodeling. MMP family includes the majority of collagenases, which break peptide bonds of CLL fibers, the major insoluble protein in the connective tissue and the ECM. About 80 to 90% of human body CLL is types I, II, and III, which ensure skin firmness [27]. By providing

EMC integrity, BAK is delaying the appearance of aging signals, making it a candidate molecule for the antiaging market, a billion-dollar industry [41].

The global market for skin-lightening is expected to reach US $12.3 billion by 2027 [42]. Skin hyperpigmentation can be summarized as increased secretion and deposition, in the skin, of melanin. [43]. This dark macromolecular pigment confers skin protection against UV radiation and photocarcinogenesis and is even responsible for hair color. [27, 44] Hyperpigmentation can have many causes: age, imbalance of hormones, ROSs, UV radiation, among others. Skin injuries such as burns, cuts, and wounds may lead to hyper or hypopigmentation. Melanin synthesis occurs within melanosome granules by the melanocytes - specific dendritic cells [43]. This natural mammalian process is called melanogenesis. Two types of melanin exist eumelanin (brownish black) and pheomelanin (reddish yellow) [27]. The key steps are melanocyte proliferation, followed by synthesis and activation of tyrosine, and finally, transference of the produced pigments to keratinocytes through the melanocyte´s dendrites, which allow them to contact with some neighboring keratinocytes [43]. This process is regulated by enzymes such as tyrosinase (TS), TRP-1, and TRP-2, which are tyrosinase-related proteins 1 and 2, respectively [43]. TS plays a critical role in mammalians once it oxidizes L-tyrosine to melanin [44]. TS superactivity leads to melanin overproduction, culminating in skin hyperpigmentation [27]. Skin-lightening products are available for cosmetic and therapeutic purposes. They can act on multiple targets: hyperplastic melanocytes or inhibiting TS, preventing melanin synthesis [43].

Within skin cancer, melanoma is among the most deadly and invasive ones. In the metastatic stage, it becomes difficult to control. The lack of response to treatment is associated with high mortality values. Sometimes the difficulty of obtaining anticancer therapy is related to the fact that it is not sufficiently targeted and ends up having a cytotoxic effect in normal cells. The earlier the diagnosis, the greater the chances of successful treatment [14]. The constant need to research new molecules gave BAK a chance to deepen its knowledge regarding anticancer properties.

As a larger organ, the skin is excellent for drug delivery. However, molecules with low penetration capacity must suffer changes to make this delivery possible [16]. Particle size reduction is one of the strategies. Micro and nanosponges and nanoemulsions formulations allow overcoming limiting characteristics such as volatile nature, hydrophobicity, and viscosity. They also enhance physical stability and extend drug release time, possibly reducing the dose and decreasing side effects. [16, 45, 46].

Regulatory issues are a critical factor in ensuring the safe use of nanosystems. Regulatory organizations must clarify specific regulations referring to manufacture,

determination of pharmacodynamic and pharmacokinetic profiles, and evaluation of toxicological profiles, to ensure efficiency and safety of these nanoformulations to enable sustained approval for placing on the market [47].

2. Physicochemical properties

BAK has a styryl moiety associated with a monoterpene. The set is known as meroterpene [11]. In 1986, Cornforth used for the first time the term "meroterpenoid" to designate products resulting from mixed biosynthesis, which derives partially from terpenoids. It results from adding the prefix "mero" to "terpenoids". "Mero" derives from the Greek: "merus", which signifies a "fragment, part or partial". [48] Succinctly, these natural products consist of terpenoid and nonterpenoid portions. This class of hybrid compounds is constituted by secondary metabolites originating from several organisms, [49] namely, higher plants, fungi, and marine organisms [50].

BAK's chemical composition, structure, and molecular arrangement are fascinating and provide diverse biological activities [48]. Looking closely at the chemical structure, it is possible to identify phenolic and terpene parts, which allows the characterization of BAK as meroterpene phenol. The chemical skeleton consists of an aromatic ring with a hydroxyl group and, at para-position, has an unsaturated long hydrocarbon chain with three alkenes and one all-carbon (tetra-alkylated) quaternary stereocenter [15]. Figure 1 shows an asymmetric stereocenter, and its absolute configuration has been evidenced to possess (S) – chirality.

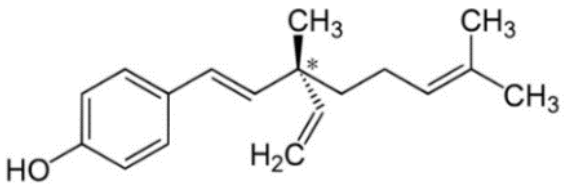


Figure 1 – BAK structure.

Table 1 summarizes BAK physicochemical properties. Because of its long hydrophobic chain, BAK has low aqueous solubility and poor availability. Besides that, it has a high first-pass metabolism, given the ease of establishing covalent bonds between the phenolic hydroxyl group and endogenous molecules such as glycine and glucuronic acid [15, 51].

Table 1 – BAK physicochemical properties.

Property	Result	Reference
Molecular formula	$C_{18}H_{24}O$	[15, 23, 52, 53]
Chemical name	4-[(1E,3S)-3-ethenyl-3,7-dimethylocta-1,6-dien-1-yl] phenol	[53-55]
CAS	10309-37-2	[53]
Molecular weight	256,38 g mol^{-1}	[23, 52, 53, 57-59]
Molecular volume	238,38	[59]
Organoleptic characteristics [A]	Pale yellow oil	[15, 23, 52, 55, 56, 58, 59]
	Viscous liquid. Brownish yellow, odor characteristic of aromatic compounds	[60]
Boiling point [B]	>260°C	[61]
Flash point [C]	184°C	[62]
Partition coefficient [D]	5.09 (octanol-water)	[63]
Explosiveness	No exothermic decomposition peak observed up to 430°C, the test item is considered to be nonexplosive.	[64]

Property	Result	Reference
Solubility in 20% PEG400	0,02 mM	[59]
Water solubility [E]	Slightly soluble (0.1-100 mg/L)	[65]
Density [F]	0.969 g/cm3	[66]
Rotatable bond	6	[15, 53, 57]
Bioavailability score	0,55	[57]
Maximum absorption UV	262 nm	[23, 58]
Optical rotation [G]	+37.2°	[23]
iLog P [H]	3.54	[15, 57]
Alog P [I]	5,35	[59]
Log K [J]	0,73	[59]
Molar Refractivity	84.10	[15]
Polar Surface Area	20.23 $Å^2$	[53, 59]
Surface tension [K]	43.92 mN/m	[67]
Hydrogen bond accept number	1	[15, 53, 57, 59]
Hydrogen bond donor number	1	[15, 53, 57, 59]

[A] Organoleptic characteristics, determined at 20° C. [B] Boiling point, determined at 300 mm Hg atmospheric pressure. [C] Flash point, determined at 760 mm Hg atmospheric pressure. [D] Partition coefficient (log Pow), determined at 20° C, 6.31 pH, by HPLC. [E] Water solubility, determined at 20° C, 5 pH. [F] Density, determined at 20° C. [G] Optical rotation, $[\alpha]^{30}_{D}$ = + 37.2°, exhibited by (+)-BAK. [H] iLog P is the n-octanol/water partition coefficient, accepted as a measure of lipophilicity of a substance. [I] Alog P is the partition coefficient, determined by molecular modeling. [J] Log K is the logarithm of t_r-t_0, where tr is the retention time of the compost peak and t_0 is the retention time of the solvent peak. [K] Surface tension, determined at 20° C (1g/L).

3. Bakuchiol Sources

3.1 Natural Sources

In nature, BAK presents its chemical and structural diversity. It is obtained within the plant due to a mixed biosynthetic route. BAK is part of a particular rare terpenoid group whose aromatic ring is derived from the phenylpropane pathway. This phenolic compound has a carbon side chain (monoterpene chain) derived from the mevalonate pathway [8, 15,]. Figure 2 represents schematically one of the described biosynthesis pathways. This mixed pathway starts, on the one hand, from a compound that is phenylalanine to origin p-coumaric acid. On the other hand, the reaction between acetyl CoA and acetoacetyl CoA results in the general pyrophosphate. In the end, these two compounds react and give rise to BAK.

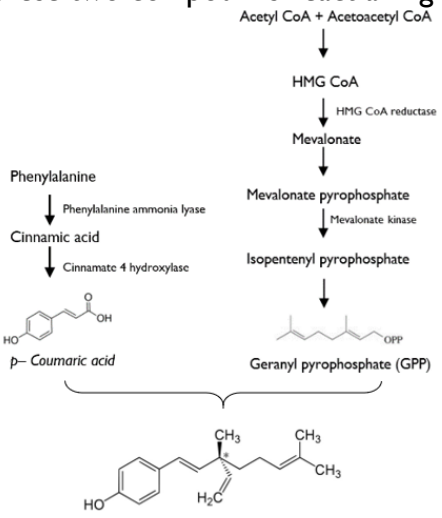


Figure 2- BAK biosynthesis.

It is a natural plant-derivative product, and despite being typically found in *P. corylifolia*, BAK has also been extracted from other species, for instance: *P. drupacea* [17], *Psoralidium tenuiflorum* [23], *P. glandulosa* [68, 69], *Piper longum* [70], *Aerva sangulnolenta* [71], *Otholobium pubescens* [72], *Ulmus davidiana* [73, 74].

BAK's first isolation occurred in 1973 by Mehta *et al.* from *P. corylifolia* seeds [75]. *P. corylifolia* is, in fact, the major source of BAK [71]. It is also known as a synonym of *Cullen corylifolium (*L.*) Medik* [76-80] Taxonomically, it forms part of Kingdom: Plantae, Division: Angiospermae, Class: Dicotyledoneae, Order: Rosales, Family: Leguminosae, Subfamily: Papilionaceae, Genus: *Psoralea*, Species: *corylifolia* Linn [5]. The name "psoraleos" has a Greek origin and means "affected by itching or leprosys" [3]. It has already been assigned several regional names due to its distribution and properties, Babchi is the most common [2, 3]. It is a plant with wide geographical distribution. It is native to Asia, but it is also cultivated in America, Africa, and Australia. This plant grows in plains in tropical and subtropical regions. It is well known and has been used, for many years, by the Chinese and Indian folk [3, 4]. It was a pretty used plant in Ayurvedic, a millennial Indian health care system with concepts from 2.500 and 500 B.C. The name derives from the Sanskrit words "ayus (r)" and "veda", which mean life and knowledge, respectively. Thereby, this Indian system of longevity stands for "science of life". Plant-derived products are divided depending on their pharmacologic activity in this system. In this sense, P. corylifolia has been used due to psoralen and BAK as active components, which justifies its antileucoderma and antibacterial action [81].

Herbal medicine has gained enhancement, and it becomes effortless to understand the reasons, which relate mainly to the fact that they are economical sources, naturally derived, which provides them safety margins greater than conventional drugs. [82] However, *P. corylifolia* is endangered [3, 82]. This medicinal herb is rough to propagate in one part due to low seed germination, and in the other, due to elevated seedling mortality [3]. Despite being valuable sources to obtain constituents like BAK, it is fundamental to learn how we could produce and conserve this species before being excessively explored, avoiding its extinction [3, 82]. To face these challenges, various studies have emerged, one of them aiming to obtain a system that allows plant regeneration *in vitro* from root fragments. In that study, several mediums with different supplement concentrations were tested. The maximal response, translated into a higher percentage of response (65.57%), higher shoot bud/explant, and elongated shoot number, was observed when the medium was supplemented with 2,22µM BAP (6-Benzylaminopurine) and 6,98µM Kin (Kinetin), both growth regulators which exert a

positive synergistic effect. As a footnote, the difficulty of seed propagation is so critical that it requires the use of cytokinin to promote bud formation starting from explants [82].

In addition, factors including geographical location, climatic differences, and environmental conditions of the area cause variations in the chemical composition and may, therefore, result in different pharmacological effects. Therapeutic activities and active ingredient concentration are directly related to plant quality [83]. It is paramount to ensure high-quality products do not compromise customer safety and to guarantee the intended therapeutic effect [84, 85]. It becomes necessary to use a standard technique to verify the authenticity and prevent counterfeiting, which might occur with species such as *Abutilon theophrasti* Medic. and *Crotalaria pallida*. Although their morphological similarities, from the point of view of chemical composition, they have distinct characteristics. Accordingly, Wu *et al.* have recently suggested an analytical method that might serve as a fingerprint: high-speed countercurrent chromatography. Chromatograms present six peaks that amount to certain indicator compounds that allow inferring plant quality [83].

An extensive range of extraction techniques has been described, with different methods, solvents, and experimental conditions. Lewinska *et al.* designed "environmentally-friendly" nanoemulsions with a "green" extraction method. BAK was extracted from *P. corylifolia* seeds through a supercritical fluid extraction (SCFE) with pure carbon dioxide – SC-CO_2. Seemingly, this could be a great sustainable alternative to conventional solvent-based extraction methods. This new "green" method has some advantages, including low extraction temperature and short operating times, thereby decreasing thermal degradation and oxygen decomposition of bioactive compounds. Besides including pure CO_2, a solvent with interesting characteristics such as odorless, inert, non-toxic, and non-inflammable is inexpensive and allows a good solubility of hydrophobic compounds. SCFE was performed at 280 bar and 40°C with a CO_2 flow rate equivalent to 3,6g/min. These experimental conditions allow obtaining an extract with a high content in BAK, with a low percentage of psoralens and isopsoralen (require higher pressure and temperature values). It used the static-dynamic method, which consists of alternate cycles of static extraction times of 10 to 15 minutes and dynamic extraction times of 15 to 50 minutes. The highest yield, about 8.58%, was obtained with 10/20 intervals at 280 bars for 330 minutes. BAK extraction occurs at the beginning. Consequently, a high process yield is not required to get an extract rich in BAK. Thus, the key step to obtaining high BAK content is to optimize the extraction process conditions. It was used in the oil active phase. The extract is oily dark red (liquid), and contains nearly 80% BAK [16].

To achieve a final product of excellence, it is necessary to establish quality standards, such as qualitative and quantitative composition, that must be respected and between an acceptable range of values. There are already documented several separative techniques, and depending on the technique, there is extraction and isolation of different compounds. The most common is high-performance liquid chromatography (HPLC), which can be coupled to varying detectors. Chen *et al.* have focused more closely on this matter. They concluded that HPLC coupled with electrochemical detection seems to be the most appropriate for BAK's separation, identification, and quantification due to its high sensitivity [12].

3.2 Chemical Synthesis

BAK might be isolated from diverse plant species. Nonetheless, this molecule can be obtained by resorting to chemical synthesis. Since 1966, the year of its discovery, have been carried out several approaches to BAK chemical synthesis. Nevertheless, they are complex routes and frequently originate racemic BAK (±) instead of (+)-isomer, optically active, found in nature [17]. There are some crucial steps in total synthesis: achievement of stereochemistry and all-carbon quaternary (tetra-alkylated) stereocenter. Moreover, obtaining alkenyl groups between carbons 17 and 18, sterically hindered position, entails a certain difficulty [15, 86-89].

In 1967, Damodaran and Dev announced the first chemical synthetic process to obtain methyl ether BAK derivate with only three steps. Initially, geraniol interacts with ethyl vinyl ether (in mercuric acetate presence) and originates geranyl vinyl ether, which undergoes a thermal reaction (Claisen rearrangement (CR)), resulting in an aldehyde with a quaternary carbon center. Then, it reacts with p-methoxyphenyl magnesium bromide and forms an alcohol later dehydrated with alumina, which culminates in *rac*-BAK methyl ether [15, 75]. Carnduff and Miller, also in 1967, announced BAK's first total synthesis. In this process, geraniol reacts with p-methoxyacetophenone diethyl acetal (in mercuric acetate presence), originating in methoxyacetophenone enol ether. *In situ* CR turns enol ether into the corresponding ketone, with a quaternary carbon center. Then, reduction with sodium borohydride results in alcohol. After, it is dehydrated in refluxing pyridine with phosphorus oxychloride, giving *rac*-BAK methyl ether (76% yield), which is demethylated under reflux with methylmagnesium iodide (Grignard reagent (GR)), culminating in *rac*-BAK [15, 90]. Both Damodaran and Dev and Carnduff and Miller's works have the key step to obtaining the BAK skeleton focused on CR of geraniol enol ethers [91].

Until 1990 there was no progress in the synthesis methods because of the challenges behind this synthesis. But, at this time, Takano *et al.* reported the first enantioselective synthesis of (+)-BAK. (S)–O-benzylglycidol reacts with methyl, originating methyl (R)-5-

hydroxyhex-2-ynoate. That cyclizes and forms α-β-unsaturated-∂-lactone, which reacts with vinylmagnesium bromide in copper(I) iodide presence to form a quaternary center, enantio-enriched BAK, which gives rise to (S)-(+)-BAK [15, 92]. In 1991, Araki and Bustugan used another strategy to obtain *rac*-BAK, a three-step synthesis. To obtain C-C bonds, allylic organometallic reagents must couple with carbonyl compounds. They proposed using various metals like Mg, Zn, Al, Li, and Cd. Geranylindium sesquibromide reacts with 2-(4-methoxyphenyl) acetaldehyde to form 1-(4-Methoxyphenyl)-3,7-dimethyl-3-vinyvloct-6-en-2-ol, which reacts with mesyl chloride-pyridine. The resulting product is treated with Potassium tert-butoxide and gives methyl ether BAK. Then it is demethylated to form BAK [15, 91]. In 1999, Asaoka *et al.* developed a new synthesis of (+)-BAK, with only a 5% yield. Sequential alkylation of cyclohexanone with Lithium diisopropylamide (LDA)-allyl bromide and LDA-methyl iodide originate corresponding cyclohexanone with chiral center. Undergo a chemoselective reduction that originates (+)-BAK [15, 93].

In 2008, Fukuyama *et al.* reported another methodology to obtain a chiral all-carbon quaternary center. This total synthesis had a yield of 20%. Chiral Michael acceptor is prepared in three steps. It reacts with copper lithium reagent to create the quaternary carbon center in a diastereoselective manner. It is necessary for phenyl oxazolidinone auxiliary to set the stereochemistry: R enantiomer in a ratio of 95:5 with S enantiomer (less favored). The result is (+)-BAK [15, 94]. In the same year, Chen and Li reported a synthetic pathway for *rac*-BAK, with an overall yield of 50%. They prepared an intermediate with vinylmagnesium bromide, using CU(I) as a catalyst. The GR reacts with the product obtained previously. After, the hydroxyl group and methyl protecting group are eliminated. This process culminates in the BAK racemate [15, 95]. Li *et al.* also, in 2008, synthesized (S)-BAK through a ten-steps strategy with a yield of 51%. They also synthesized (R)-BAK with a yield of 40%. Geraniol undergoes Sharpless epoxidation and originates oxygen silyl ether. The TBS group protects the hydroxyl group. The product obtained previously passes through silyl ether rearrangement to obtain a chiral quaternary carbon center. That, suffer Wittig reaction removing TBS, oxidation, and GR addition and elimination culminate in (S)-BAK. They also get (R)-enantiomer synthesis [15, 96]. Then, Novikov *et al.* (2009) found a conversion method that could be used in the total synthesis of BAK with a yield of 45%. To obtain a quaternary center is installed an intramolecular insertion of diazosulfonate C-H. Initially, there is a conversion of (-)-citronellol into citronellol-based chiral ∂-sulfone, used in BAK total synthesis [15, 97]. In 2010, Hoveyda *et al.* used an enantioselective allylic substitution reaction to synthesize the BAK enantiomer, with a yield of 72%. Firstly, there is geraniol phosphonation, and β-selective Ni catalyst is attached to the end portion of alkyne, followed by a hydroalumination to obtain an alkenyl

aluminum reagent. Allyl phosphate asymmetric substitution reaction occurs through azacarbene silver complex addition, which controls chiral center stereo configuration. Unfortunately, the chiral silver azacarbene complex is challenging to prepare [15, 98].

Tadano *et al.* (2012) did the asymmetric synthesis of (S)-(+)-BAK. In this case, they used Oppolzer's chiral auxiliaries (sulfur-based) for asymmetric synthesis and CR. Geraniol reacts with N-propioloyl Camphorsultam (Michael addition reaction). The product of this reaction is heated with butylated hydroxytoluene, resulting in a mixture of diastereomers. The chiral auxiliary is removed by basic conditions hydrolysis with decarboxylation simultaneous. The resulting aldehyde reacts with GR originating alcohol, lately dehydrated with pyridine and $POCl_3$, followed by demethylation. The result of this method is (+)-BAK [15, 52]. After the success in total synthesis, in 2013, Fukuyama *et al.* developed another strategy to obtain a chiral quaternary center with a yield of 64%. Geranic acid reacts with triethylamine and pivaloyl chloride. Then, it reacts with (2'R)-2'-phenyloxazolidinone (chiral auxiliary). The resulting product suffers an asymmetric 1,4-addition. After that, it reacts with sodium hexamethyl disilazane, and by p-methoxy benzaldehyde addition, results in BAK methyl ether, which is demethylated, culminating in (S)-BAK [15, 99]. In same year, Lei *et al.* reported another asymmetric synthesis of (S)-BAK. Designed an asymmetric synthesis pathway to obtain (S)-BAK by introducing an intermediate that contains a chiral all-carbon quaternary carbon center, involving stereoselectively unconjugated alkylation of an α,β-unsaturated imide. This route has key steps: Evans' auxiliary, Takai–Utimoto, Negishi, and Heck reactions [15, 56].

In 2016, Battilocchio *et al.* prepared a synthesis of BAK precursor in a single operation using an interactive coupling method. Geranial diazo is added to 4-methoxy phenyl boronic acid solution, a pretty selective addition, resulting in a boronic acid intermediate. Subsequent aldehyde removal allows generating the desired precursor. They demonstrated this method's applicability by forming the C-C bond through a controlled sequence [15, 100]. Also, in 2016, Xiong and Zhang demonstrated a way of obtaining (S)-BAK, starting from an allylation product. They established an asymmetric allylation to form two contiguous stereocenters with one quaternary center. Chiral oxazoline sulfonamide ligand and a chromium salt provide allylation products with good characteristics: high enantio and diastereo selectivities and functional group with good tolerance. This synthetic method allows transforming allylation products into (S)-BAK. 2-(4-methoxyphenyl) acetaldehyde reacts with geranyl bromide and originates in a salicylic product. Via two-step sequence gave dehydroxylated. Later, it demethylated, culminating in (S)-BAK [15, 101]. Then, in 2017, Chakrabarty and Takacs showed high enantioselective hydroboration and its application in (S)-BAK methyl ether synthesis. Their rhodium-catalyzed hydroboration of pinacolborane with allylic phosphonates allows obtaining

chiral tertiary boronic esters. β-borylated phosphonates are converted into chiral β- and γ-amino phosphonates/hydroxy phosphonates and phosphonates with a quaternary carbon stereocenter. The asymmetric catalytic hydroboration of trisubstituted alkene provides a chiral boronic ester. It suffers oxidation to form corresponding tertiary alcohol. Later, cross-coupling with vinyl magnesium bromide originates chiral tertiary boronic esters (vinylation). Chiral vinyl phosphonate undergoes a silyl ether deprotection and then, Parikh-Doering oxidation. Witting olefination originate chiral diene phosphonate, treated with Lawesson's reagent, to form thionophosphonate. Further, it is deprotonated by n-BuLi and, subsequently, is added 4-methoxy benzaldehyde and culminates in (S)-BAK methyl ether [15, 102]. Recently, in 2020, Khan *et al.* described a formal synthesis of *rac*-BAK using tertiary allylic electrophiles, molybdenum-catalyzed, and allylic substitution (regioselective). They are used as hetero-nucleophile reagents, sodium sulfonates, with tertiary allylic electrophiles to obtain C-S bonds. Tertiary allylic carbonate reacts with sulfinate salt to give branched allylic, the key intermediate. This tertiary allylic sulfone goes through a Suzuki–Miyaura cross-coupling with styryl boronic acid to form BAK methyl ether derivate, later phenol deprotection. Then, it culminates in *rac*-BAK [15, 103]. Table 2 (in the annex) summarizes chronologically syntheses pathways previously described.

4. Skin activities

4.1 Antifungal activity

Madrid *et al*. studied BAK's antifungal activity. It was isolated from *P. glandulosa* and was tested *in vitro* against various strains of *Candida* spp. Minimum Inhibitory Concentration (MIC_{80}) was determined as the lowest concentration inhibiting yeast growth greater than 80%, calculated by the microdilution method for yeasts. Fluconazole (FCL), ketoconazole (KCL), and itraconazole (ICL) were tested under the same conditions [55]. They are all well-known antifungals used in treating candidiasis [104]. BAK had a MIC_{80} of 0.125µg/mL, at 24h of incubation, against *Candida guilliermondii* (2204), greater than for tested antifungals, given that BAK showed the lowest MIC_{80} value. FCL and KCL had a MIC_{80} of 0.5µg/mL and ICL had 4.0µg/mL [55]. *C. guilliermondii* is an uncommon pathogen rarely associated with invasive infection but often related to onychomycosis and superficial cutaneous infections, and here, BAK with the topical application may act due to the antifungal properties demonstrated [20].

Lau *et al*. assessed the antifungal activity of BAK against *Trichophyton mentagrophytes* ATCC 9129 [21]. One of the causes of athlete's foot is also known as tinea pedis. It affects almost 15% of the population and involves interdigital spaces of the foot's toes and/ or lateral sides, soles, and heels. It could be a recurring or chronic condition [105, 106]. *In vitro* studies

were conducted using the broth dilution method to determine MIC, the lowest concentration that substantially inhibits organism growth. FCL was employed as a positive control [21]. It is an antifungal drug recognized for dermatophytosis treatment caused by this specie under study [107]. The results showed that BAK at 3.83μM (MIC) inhibited 100% *T. mentagrophytes* growth, which is optically clear, while FCL needed 52.20μM. These results support BAK antifungal efficacy against those dermatophytes [21].

Years later, Lau *et al.* continued their studies about BAK antifungal effectiveness against *T. mentagrophytes*-induced tinea pedis ATCC 9129. The MIC endpoint assumed the lowest concentration that completely inhibited microorganism growth and was determined by the broth dilution method after 24h of incubation. *In vitro* studies on *T. mentagrophytes* culture found that BAK MIC was 3,91μg/mL. The terbinafine and nystatin were used as positive controls. They explored *in vitro* underlying mechanisms of action and concluded that BAK does not induce DNA fragmentation in *T. mentagrophytes*. It increases fungal membrane permeability dose-dependently and generates ROSs [22]. Enhancing ROSs levels can result in oxidative stress, which can cause damage to DNA, RNA, lipids, and proteins, may culminate in cell viability loss. Besides, ROSs can change mitochondrial membrane potential resulting in apoptotic cell death [22, 28, 29]. The intensity of fluorescence emitted by SYTOX® Green was measured, reflecting its uptake, consequently reflecting the increase of membrane permeability. BAK, terbinafine, and nystatin were tested at 0.25-, 0.5-, and 1-fold MIC. BAK was the compound with the higher permeability increase. BAK, at 3,91μg/mL, increased ROSs levels in fungal cells by about 11, 114, and 187% after 0.5, 1, and 3h of incubation, respectively. In the same experimental conditions, both terbinafine and nystatin increased ROSs levels. However, nystatin produced a higher ROSs percentage increase, while terbinafine produced less than BAK. *In vivo* study consisted of applying an aqueous cream containing BAK at 1, 5, and 10% (w/w), once daily for 2 weeks, in guinea pigs´ feet previously inoculated with conidial suspension [22]. Lamisil® cream, which contains 1% terbinafine [108], was a positive control. It proved to be able to eradicate fungal hyphae and eliminate the fungal burden. Although BAK was not effective as Lamisil®, it showed a significant reduction in fungal burden. The discrepancy between BAK's activity *in vitro* and *in vivo*¸ was attributed to the absence of transdermal enhancers or issues related to the formulation that did not enable an appropriate drug delivery. BAK dosages used *in vivo* were thousands folds its MIC, previously determined on dermatophytes culture, *in vitro*. Therefore, it would not be due to an insufficient dose. These findings corroborate Lau *et al.* previous achievements [22].

4.2 Antibacterial activity

Cui *et al.* studied the antibacterial activity of the *P. corylifolia* constituents against two *Methicillin-Resistant S. aureus* (MRSA) strains, OM481 and OM584 [9]. Clinical manifestation of infection caused by MRSA may range from nasal mucosa asymptomatic colonization to mild soft tissue and skin infections to invasive fulminant disease [109]. BAK was the major extracted compound in this study, accounting for 16.49% (w/w) of ethyl acetate extract. Using the liquid dilution method, the researchers determined MIC *in vitro*. Several compounds, such as flavones, isoflavones, meroterpenes, and coumarins, were isolated from *P. corylifolia*. Among them, BAK was the constituent with the lowest MIC value (8µg/mL), proving its activity against both MRSA strains. They completed the study by comparing the activity of the compounds and establishing a relationship between structure and activity. This relationship concluded that when the prenyl group is oxidized, there is a reduction in antibacterial capacity, as well as when the methoxyl group substitutes the hydroxyl group at carbon 7. The study found that the antibacterial effect against MRSA depends on the presence of phenolic hydroxyl groups and lipophilicity provided by the benzene ring [9]. Although this study was not conducted on the skin, it was only performed *in vitro*. It can be a valuable tool in further studies focused on BAK antibacterial activity to treat skin infections, the MRSA etiological agent.

Hsu *et al.* reported BAK activity against Gram-positive bacteria, *Staphylococcus epidermidis*, one of the main bacterial skin colonizers in healthy humans. Depending on strain and context, they can be beneficial or harmful for homeostasis and maintenance of the protective barrier [110]. *S. epidermidis* was documented as an etiological agent of skin infections, most of them opportunistic infections, often related to permanently implanted medical devices by biofilm formation, which may disseminate and evolve into sepsis [111, 112]. Hsu *et al*. proceeded to an ethyl acetate extraction from *Psoralidium tenuiflorum* to obtain BAK. *In vitro* study was performed by well diffusion assay with media agar inoculated with *S. epidermidis*. BAK displayed cytotoxicity against *S. epidermidis* and IC_{12}= 123±11µg/mL [23].

Yin *et al.,* in their *in vitro* study performed to evaluate the antibacterial effect of prenylflavone derivates against *S. epidermidis* ATCC 12228 and *S. aureus* ATCC 25923, used BAK as the positive control, as "well-known natural antimicrobial agent", assuming BAK MIC of 0.018mM and 0.037mM for *S. epidermidis* and *S. aureus*, respectively [113], which already demonstrates the recognition of BAK in this context.

Trompezinski *et al.* carried out a study to assess the properties of a biologic complex, composed of BAK, ginkgo biloba extract, and mannitol (BGM), and evaluate, among other, *in vitro* antibacterial activity against *Cutibacterium acnes* (previously *Propionibacterium acnes*) of its isolated compounds. It was determined MIC, corresponding to the lower concentration with

no observable visible culture (considered irrelevant up to 3 colonies). Obtained MICs were compared with benzoyl peroxide (BPO) and zinc gluconate [114]. BPO is one of the topical agents used as first-line in acne treatment, included in Standard guidelines of both the American Academy (AA) of Dermatology and AA of Pediatrics. It acts in the pilosebaceous unit (where is bacteria *C. acnes*) and produces free radicals. Then, they damage pathogenic bacteria cell walls [115]. Zinc is also used in acne management and possesses several activities, including the bacteriostatic effect against *C. acnes* [116]. The results of the Trompezinski study showed that BAK, BPO, and zinc MICs were 0.0005, 0.008, and 0.12%, respectively. The BAK inhibited *C. acnes* growth at a concentration of 0.0005% and was the compound with the best activity once it needed a lower concentration [114].

4.3 Antioxidant activity

Adhikari *et al.* demonstrated BAK's antioxidant activity and established a relationship between structure and activity. It possesses the potential to protect biological components, namely proteins and lipids, from oxidative damage. It was mainly attributed to the presence of the hydrogen atom on the terpenoid chain, effortlessly abstractable, adjacent to the trisubstituted alkene group, as well as the presence of the phenolic group, given that phenolic bond dissociation enthalpy has been related to the antioxidant effect [117]. Additionally, Jiangning *et al.* considered bakuchiol an unhindered phenol, given that it has no substituted groups in any of the ortho-positions of the hydroxyl group. An essential double bond connects phenol of phenolic hydroxyl group in para-position. That allows extending the conjugation system of BAK phenolic oxygen free radical (POFR) after donating to active free radicals a hydrogen atom. This form resonance structure stabilizes POFR and fortifies antioxidant action. Briefly, more than phenolic hydroxyl groups number, antioxidant properties depend on the environmental conditions of this functional group. Essential characteristics: first, the presence of hydroxyl groups within the aromatic ring, second, electron-donating groups fortify, especially at ortho- and para-positions, while electron-withdrawing weakens these antioxidant properties, and third, stereo-hindering groups of hydroxyl groups, particularly at ortho-position, increase antioxidant action [58].

In the Trompezinski *et al.* study they evaluated the efficacy of BGM complex in acne vulgaris. The compounds were evaluated individually and together, and their efficacy against inflammation, oxidation, and *C. acnes* proliferation was studied. *In vitro* study evaluated BAK antioxidant activity, determined through squalene, previously oxidized by hydrogen peroxide at 15%. BAK was compared to vitamin E at the same concentrations (3.9 and 19mM). Gas chromatography-mass spectrometry (GC-MS) subsequently quantified squalene. Test results

concluded that BAK prevented squalene from oxidation by 30.0%, whereas vitamin E, in an equal concentration, only prevented 15.2%, both at 3.9mM. At 19mM, BAK prevents squalene oxidation by 36.9%, while vitamin E prevents 40.3%. BAK's squalene protection index was twice higher than vitamin E [114]. Within the follicular canal, multiple porphyrins are produced by *Propionibacteria* spp., the very efficient pigment, photocatalytic in oxygen presence. Absorbs UVA and visible radiation and photo-oxidize squalene (sebaceous lipid), prone to oxidation due to six double bonds in its chemical structure. Squalene peroxides are involved in various skin conditions, such as acne, by activating comedones, and lipoxygenase, which is involved in lipoperoxide formation that participates in inflammatory skin diseases associated with keratinocyte hyperproliferation (atopic dermatitis and psoriasis, for example) [118]. In addition, it can increase the production of the pro-inflammatory cytokine IL-6 [119, 120]. To prevent squalene oxidation, skin secrets itself vitamin E, lipophilic, present in normal human sebum. Its production is directly related to the amount of squalene. In subjects with acne, vitamin E levels are low, increasing oxidized squalene levels [114, 121]. Thus, *in vivo* studies with BAK on the skin should be conducted to confirm this benefit and promising effect.

Bluemke *et al.* studied BAK multidirectional and holistic approaches against cellular aging. They assessed BAK (at 100µM) antioxidative capacity *in vitro*, measuring 2,2-diphenyl-1-picrylhydrazyl (DPPH) reduction through its absorption decay. The absorbance was read at 524nm. BAK demonstrated an augmented antioxidative capacity, recorded in a significant absorbance reduction in all time points analyzed. They also assessed BAK antioxidative power (AP), *in vitro*, by resorting to electron spin resonance spectroscopy. The comparison established was that 1 ppm vitamin C corresponds to an antioxidative unit (AU). They also tested retinol (RET). Here, the reduced quantity of free radicals (DPPH) was evaluated and characterized by the number of free electrons (spins). The calculation of AP follows an equation $AP = \frac{RA \times Nspins\ (DPPH)}{Tr \times Wc}$. Where RA is the reduction amplitude constant ($1/e^2$), N_{spins} are spins (free electrons) of DPPH, t_r is the reduction time, and w_c is the antioxidant product characteristic weight. The reduction time and characteristic weight are inversely proportional. BAK had a reaction time of 0.99min, while RET had 2.59min, meaning that BAK had better reactivity with free radicals than RET. In addition, BAK and RET w_c were 0.028 and 0.151mg, respectively. Confirming BAK has an increased capacity to reduce free radicals compared to RET. BAK resulting antioxidant power was 12125 AU and 848 AU for RET [25]. ROSs lead to a consecutive inflammation culminating in reduced cell viability of both dermal and epidermal cells. It results in ECM damage, as mentioned before, one of the cornerstones of skin aging. These data support BAK's antiaging effect through its antioxidant activity [25].

4.4 Anti-inflammatory activity

Chen *et al.* evaluated, *in vitro*, the anti-inflammatory activity of *P. corylifolia* fruit compounds. To do an initial screen of possible natural anti-inflammatory agents, they used RAW 264.7 cells, previously exposed to LPS, to produce NO, a common inflammatory mediator responsible for host defense. The anti-inflammatory response is related to suppressing the ability of NO generation by macrophage murine cell line. They determined the IC_{50} to compare components. Quercetin was used as a positive control [39]. It is a flavonoid component with anti-inflammatory properties [122]. BAK was the most effective among all tested compounds, once it had the lowest IC_{50} value (21.57μM), while quercetin had an IC_{50} value of 33.08μM. Moreover, researchers used the MTT assay to assess cytotoxic effects. Given the great cell viability (>93%), they concluded that BAK inhibitory activity against NO generation LPS-induced was not a consequence of its cytotoxicity [39].

Pae *et al.* studied BAK influence, *in vitro*, on inhibition of inducible NO synthase gene expression [35]. Both endothelial and neuronal isoforms of NO synthase (NOS) are usually constitutively expressed. Inducible NOS (iNOS) has a key role in activated macrophage cytotoxicity. NO synthesized is a critical homeostasis regulator [123]. iNOS expression may be stimulated by proinflammatory cytokines, including interferon-γ (INF-γ) and LPS. Once synthesized, it produces long-term and high yields of NO. Although its cell is beneficial, sustained production has been involved in inflammatory disease pathogenesis. iNOS gene has a promoter with consensus sequences and an initial repeated sequence of the DNA bases thymine adenine (TATA box) that allow transcription factors such as NF-kB linking to induce its expression. INF-γ/LPS-stimulated RAW cells assessed NO synthesis by nitrite accumulation in the culture medium. The results presented a lag phase of around 5 to 6h followed by a gradual increase in accumulation, culminating in 44.0μM nitrite. Later, pyrrolidine dithiocarbamate (at 10μM), which inhibits NF-kB activation, was incubated with activated macrophage cells resulting in potent inhibition of nitrite accumulation. BAK was also incubated at 10μM, in the same experimental conditions. BAK showed similar, slightly higher, iNOS protein suppression in a dose-dependent manner. This synthesis inhibition effect was not related to BAK cytotoxicity, given that there was no cell viability impairment. To verify whether the decrease was due to transcriptional regulation, the researchers studied mRNA iNOS expression. INF-γ/LPS incubation has considerably increased mRNA expression. Then, BAK decreased its expression, indicating that BAK acts at the transcriptional level to regulate iNOS gene expression. NF-kB activation proved to play a functional role in iNOS induction.

Once BAK inhibited dose-dependently NF-kB binding, this finding suggests that the BAK effect on the iNOS gene is due to NF-kB inhibition [35].

Bluemke *et al.* focused their studies on BAK's anti-inflammatory capacity. To determine BAK's anti-inflammatory effects, *in vitro*, full-thickness human skin was incubated to separate the epidermis from the dermis to obtain human dermal fibroblasts (HDFs), cells mimic the cutaneous route of BAK after topical application. This cell culture was used to assess both PGE_2 and Macrophage migration inhibitory factor (MIF) both proinflammatory cytokines. HDF was incubated with LPSs from *Salmonella Minnesota*. PGE_2 levels suffer a significant elevation, a synonym of successful induction of stress. BAK and RET were added at 1.25, 2.5, 5, and 10μM. Diclofenac was used, at 25ng/mL as positive control. BAK considerably reduced the PGE_2 level for all tested concentrations, while RET decreased only at concentrations higher or equal to 2.5μM. BAK at 10μM had a similar reduction as a positive control. In addition, HDF was stressed by Dulbecco's phosphate-buffered saline, raising MIF protein levels, a synonym of successful induction of stress. BAK and RET were used at 1 and 10μM. Both have considerably reduced MIF levels. Interestingly, at 1μM, BAK showed a slightly higher reduction than at 10μM [25]. BAK proved to affect the expression of two proinflammatory cytokines: PGE_2 and MIF. Both cytokines levels are elevated in skin aging due to UVA and UVB chronic irradiation. However, they have distinct signaling pathways. PGE_2, the major prostaglandin produced in human skin, can reduce CLL synthesis and increase the expression of MMP-1 in fibroblasts [25]. MIF is expressed in the skin, particularly in dermal fibroblasts, keratinocytes, and multiple other organs. It is a potent macrophage activator and upregulates MMP-1 UVA-induced in dermal fibroblasts [124]. BAK decreases these cytokines proving its anti-inflammatory activity and also showing its antiaging effect [25].

4.5 Antiaging

Bakuchiol's antioxidant and anti-inflammatory activities showed very interesting results and demonstrates impact on aging. In future research, these properties may be directed to treating other pathologies such as psoriasis and acne, the latter in particular, given the holistic approach required [125-129].

4.5.1 Non retinol-like

Bacqueville *et al.* studied the *in vitro* benefits of BAK in preventing human skin photoaging, performed on the HDF. Controls were non-irradiated/non-treated and UVA-irradiated/non-treated. They used as skin aging markers: actin network to assess morphology, interleukin-8 (IL-8) for inflammation, and P16 for senescence. Actin staining showed that HDFs

lost their star-shaped pattern after UVA irradiation and acquired a fusiform pattern. BAK at 0.5µg/mL was demonstrated to prevent the fibroblast morphological changes. The results were comparable to the non-irradiated/non-treated control. After UVA irradiation, IL-8 expression increased, reflecting inflammation. BAK at 0.5µg/mL reduced about 88.3% IL-8 expression. UVA irradiation increased P16 protein expression, which is related to cellular senescence. BAK at 0.5µg/mL protected about 44.4%. All the results were statistically significant [130].

Bacqueville *et al.* continued their study of BAK's benefits and vanilla tahitensis extract (VTE) properties. Compounds were tested alone and combined to prevent human skin photoaging. *In vitro* studies with BAK at 0.5µg/mL and VTE at 0.05% combined showed an important synergistic reduction, about 95.1% of IL-8 expression (individual reductions: BAK 88.3% and VTE 83.8%), and reduction in P16 levels, about 95.2% (individual reductions: BAK 44.4% and VTE 29.2%) [130]. To better understand this combination, they formulated a dermo-cosmetic serum (1.5% BAK + 1% VTE) and conducted an *ex vivo* study in a human skin photodamaged model (explants with 1.12 cm^2) induced by chronic UVA irradiation. They used glycosaminoglycan (GAG) as an ECM marker to evaluate dermal density. GAG is common, organized in an intense blue network located near dermo-epidermal junctions (DEJ) and underlying papillary dermis. They used photon microscopy to compare GAGs staining intensity. Controls were non-irradiated/non-treated and UVA-irradiated/non-treated. Chronic UVA exposure causes photoaging stress associated with a decline in ECM production, namely GAGs content, and disrupts the network. The staining was less intense and more diffuse. GAGs loss was correlated with altering CLL/elastic fibers in the papillary dermis. This serum (5mg/cm^2) recovers GAGs content and network organization, improving dermal density. The results were comparable to the non-irradiated/non-treated control. Serum proved re-densifying effect and protected skin from GAGs alterations UVA-induced [130-132]. They completed their study with clinical evaluation in 43 healthy Caucasian women (aged 45 to 65 years with naturally aged skin phototypes II / III) who applied the serum twice daily for 56 days. This clinical trial evaluated skin remodeling, evaluated using FaceScan®. Firmness was measured by DynaSKIN®, while clinical score and radiance were assessed on standardized photographs of subjects full-face. Baseline parameters were used as a comparison. After 56 days, 63% of subjects noted a meaningful remodeling effect, with the face contour line more defined, particularly on the jowl part. Skin firmness assessment showed a decrease from baseline of about 17 and 16% of depth and volume, respectively, of skin deformation. 73% of subjects improved depth of skin deformation, and 78% showed a reduction of skin volume deformation, assessed with DynaSKIN®. Dermatologist evaluation revealed that 95% of

subjects had improved skin firmness. Skin radiance was felt significantly in 80% of subjects. After 56 days, improvement was about 20%. Results about global tolerance/safety judged the serum as classified as "very good" [130]. The main results of this study found schematically illustrated in Figure 3 (in the annex).

Bluemke *et al.* conducted an *in vivo* study on 34 individuals with healthy mixed skin types (dry, oily, normal, and combination), Fitzpatrick type I to III. Individuals applied the 0.5% BAK cream and the vehicle twice daily for 12 weeks. Individuals visually evaluated it regarding radiance, freshness, and signs of skin aging. They assign a score from 1 (very fatigued) to 10 (very fresh). The results were calculated as the difference between the last and 1st day (t_1-t_0). The mean value was 2.57±2.14 for BAK cream and 2.06±1.89 for the vehicle, significantly considered. BAK showed a significant improvement in the perceived appearance of the skin compared to the facial skin treated with the vehicle. Besides, BAK was well tolerated [25].

4.5.2 Retinol-like

For many years, vitamin A has played a vital role in the skin. Their deficiency or excess leads to a disturbance of the skin's natural balance and disruption of homeostasis, impairing the barrier function of the skin [133]. Apart from vitamin A (retinol), there are other molecules involved: retinoids. Within this group of natural compounds, there are two important elements: vitamin A aldehyde – retinal – and vitamin A acid - retinoic acid [8]. RET derives from *all*-trans retinoic acid or just retinoic acid [134]. They are active derivates from this vitamin and play a key role in different parts of the normal cell life cycle - differentiation, proliferation, and apoptosis [135]. Retinoic acid in the dermis may have two origins. Firstly, it may result from *in situ* metabolisms of fibroblasts and, secondly, from the epidermis delivered by keratinocytes. In the dermis, fibroblasts obtained retinoic acid from RET and retinal. Retinal and RET are oxidized in the dermis and the epidermis, leading to retinoic acid [134].

As noted above, RET is a driving force in controlling and regulating homeostasis and natural cell processes. Thus, the research for a molecule that exerts the same effect with minimal adverse effects is a premise. BAK does not have structural similarities to retinoids. Nevertheless, it can perform similar functions, so it is considered a functional analog [8].

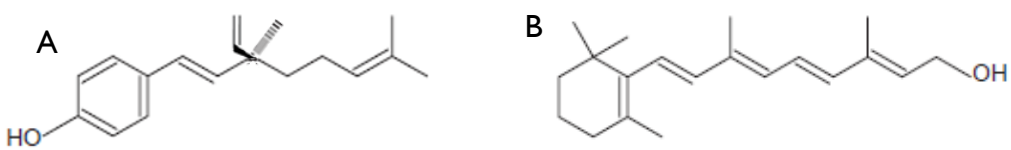


Figure 4– (A) Structure of BAK; (B) Structure of RET.

Chaudhuri *et al.* studied the resemblance between BAK and retinoids. Structurally they are not similar, as shown in Figure 4. The reasoning line was to compare gene expression profiles with those of RET (known) to identify possible compounds RET-like. They analyzed the whole genome and evidenced it through a volcano plot for BAK and RET. A volcano plot is a scatter graph that presents statistical significance versus magnitude of change (*p*-value VS fold-change, y and x axes, respectively). It analyzes meaningful changes in DNA microarray data. Upregulated genes are on the right of the graph the same way downregulated genes are on the left. The most statistically significant, low *P*-values are on top. Points statistically significant are above 1.3 on the y-axis. Overall shapes similarity of the volcano plots of the two compounds was proof of the functional analogy of these molecules, later confirmed by similar modulation of genes such as retinoid-binding and metabolizing [8].

The nuclear retinoid receptors (NRR) are responsible for translating retinoid signals, which result in gene transcription. As the name suggests, NRRs are placed in the core/nucleus when the ligand (retinoid) is absent. This superfamily of ligand-dependent receptors includes retinoic acid receptors and retinoid X receptors, RARs, and RXRs, respectively [135]. In normal epidermis (human skin) are expressed some RARs and RXR isotypes (α, β, and γ). Amongst RARs proteins, the majority are RAR-γ [133]. The RAR and RXR receptors interact directly with epidermal genes or interfere with transcriptional factors [8]. Briefly, they are generally involved in the mediation of retinoid actions. Chaudhuri *et al.* found that RAR-β and RAR-γ were both upregulated by RET. BAK does not affect their modulation. It can be advantageous in terms of adverse effects. Moreover, BAK had significant upregulation of cellular RET-binding protein II and IV, whereas RET does not. The same happened with the cellular all-trans-retinoic acid binding protein I gene, where RET had downregulation, whereas BAK had upregulation [8]. These proteins encoded by these genes are essential for retinoid function and homeostasis, responsible for facilitating and shepherding them during uptake and metabolism [136]. Lecithin-retinol acyltransferase is responsible for the esterification of RET with a long chain of fatty acids, crucial for RET absorption and storage. RET showed a 12.3-fold upregulation increase. However, BAK augmentation was even more significant, with a dramatic 82.2-fold upregulation. These results support the idea that BAK is a RET functional analog and that, in addition to this, indicate that BAK can increase endogenous RET bioavailability. We highlight the tazarotene-inducible gene 1, a retinoid acid receptor-responsive gene downregulated in acne, psoriasis, rosacea, and multiple human cancers. Here, BAK and RET have a similar upregulation effect, making BAK an attractive molecule for the treatment of these health conditions [8].

Similar gene modulations occurred in genes that encode ECM components as DEJ. BAK showed an elevated upregulation in CLL genes (1A2, 4A6, 9A2, and 9A3). BAK also upregulates EL microfibril genes. Both CLL and EL microfibrils are famous for their close relationship with skin scaffolding structure tensile strength and elasticity, respectively [137]. Its degradation is related to the appearance of wrinkles and fine lines. Fibronectin provides matrix stability and is responsible for maintaining cell shape. BAK upregulates the fibronectin-like gene. Hyaluronan synthase 3 is responsible for maintaining an ECM highly hydrated in tissues and was also upregulated by BAK. Also, aquaporin 3 (AQP3) is a channel protein involved in water/glycerol transport, present in the epidermis, and maintains skin hydration, barrier recovery, and elasticity. BAK regulation was superior to that of RET [8].

DEJ confers cohesion between the epidermis and dermis. BAK had a higher upregulation on CLL α-6 (IV), of the main constituents of the lamina densa, also involved in cell migration, differentiation, and adhesion, and α-2 (XVII), which fortifies epidermis to the dermis fixation [8]. Plectin 1 and integrins (α-6, and β-4, 6, 8) are components of hemidesmosomes and are associated with cellular functions such as the organization of the cytoskeleton. They were all upregulated by BAK. Laminin (subunit α-3 and γ-2 precursors), non-collagenous, major lamina densa's proteins are associated with cell survival and phenotypes. They were all upregulated by BAK. Finally, upregulation of the E-cadherin gene is responsible for maintaining the solid tissue and cell differentiated state [8].

ECM and DEJ weaken with age, resulting in morphological flattening and skin thinning. RET is renowned for its ability to inhibit those processes. BAK represents a potential candidate for antiaging effects, given that it showed upregulation in most cases, higher than RET [8]. Dermal fibroblasts secrete CLL, main skin ECM (type I and III), and basement membrane (type IV) constituents. In photodamaged and aged skin, CLL synthesis diminishes, consequence of quantitative and qualitative reduction of fibroblasts. DNA microarrays confirmed that BAK at 10μg/mL displays CLL stimulating effect in DNA microarray (CLL type I and IV) and a model of mature fibroblast (CLL type III), expressed as 147, 150, and 119% stimulation for CLL type I, II, and IV, respectively. Comparatively, RET showed 119, 148, and 100% stimulating effects in the same conditions [8]. A 3D model of a skin tissue substitute (EpiDerm FT) was used to assess if the stimulation of CLL type IV corresponded to robustness in CLL expression. The tissue was incubated with RET and BAK at 10μg/mL. The result revealed a strong signal near the DEJ. It was visible by using an anti-type IV CLL antibody. These findings support previous DNA microarray data. These effects are attributed to CLL synthesis and selective metabolic activation in fibroblasts, once BAK does not promote cell proliferation [8]. Epidermal water homeostasis is crucial for normal skin function and *stratum corneum* (SC) hydration. Essential

for mechanical properties, metabolism, barrier function, and healthy appearance. SC dehydration is typical of photoaged skin and diseases such as psoriasis, eczema, and atopic dermatitis. As previously seen, the AQP3 gene, a water channel, was upregulated by RET 3.5-fold, while BAK was 4.3-fold. EpiDermFT was incubated with BAK to verify if DNA microarrays corresponded to an increase in protein amount. The results confirmed that BAK increased AQP3 expression [8].

When RET suffers skin penetration, it is oxidized and origins retinoic acid, which acts like it but is remarkably less irritating. Although the usual concentration is lower or equal to 0.1% of RET, even so, it can cause some irritation. BAK has better acceptability and tolerability in the skin. Chaudhuri *et al.* concluded their research with a clinical study on 16 subjects to whom formulation with 0.5% BAK was applied twice daily for 12 weeks. To compare the parameters such as skin elasticity, tone, brightening, dryness, wrinkles, radiance, and eye area appearance, the researchers have established a semi-quantitative scale, where 0 corresponds to none and 4 to severe. The reference was the baseline, where the parameters were scored on day zero (with no treatment). The scores were assigned and registered by experts (appraisers) and the individuals submitted to the study. Comparing expert and individual evaluations, it is clear that parameters such as radiance, roughness, and dryness improvement percentage were higher for experts. On the other hand, eye area appearance, fine lines, and wrinkles parameters had higher scores when classified by individuals. Most of the parameters were markedly enhanced more in the 8th week compared with the 4th week [8]. That means that there is cumulative BAK beneficial effects over time. In addition, profilometry (wrinkle depth and skin roughness) was analyzed through a silicone replica. Wrinkle depth reduction was 7, 13, and 20% after 4, 8, and 12 weeks, respectively, compared with the baseline. On equal terms, 2, 10, and 21% skin roughness decrease. All these findings of significant improvements in photodamage signs and overall reduction observed 12 weeks after BAK treatment validates *in vitro* results, supporting BAK retinoid-type functionality [8]. Figure 5 (in the annex) describes the main results of this study.

Topical retinoids have been used for many years. The most common adverse effects reported include irritation, burning sensation of application sites, erythema, pruritus, and peeling [138]. In sensitive skin, these effects may be even more severe. Accordingly, Draelos *et al.* [139] clinically evaluated BAK as a nature-based antiaging product in sensitive skin. It was assessed in 60 women (ages 40 to 65) with Fitzpatrick skin types I to V, with mild or moderate photodamaged skin. This study has three dermatologic conditions: atopic dermatitis/ eczema (barrier disruption), rosacea (vascular hyperreactivity), and cosmetic intolerance syndrome, defined as subjects with a previous episode of noxious sensorial stimuli associated with topical

application. BAK has retinoid-like effects due to the modulation of genes, in common with RET, that regulates the formation of DEJ and ECM compounds. The product cleanser and moisturizer BAK-based (1% w/w) were applied twice daily for 4 weeks. The evaluation was based on two pillars, both determined by the investigator and by the subject: efficacy (clarity, radiance, overall global photoaging, overall skin appearance, tactile softness, and visual smoothness) and tolerability (redness, peeling, burning, itching, dryness, tightness, erythema, flaking, irritation, roughness, stinging). That was assessed according to an ordinal scale (0=none to 4=severe). Moreover, transepidermal water loss (TEWL) was measured by a noninvasive instrument. BAK has enhanced all the parameters evaluated, effectively improving photoaging [139]. Shortly after the application, 6 eczema subjects felt a little stinging, which persisted through study time. This effect is related, perhaps, to weak skin barrier function. Few individuals experienced slightly dry skin, which may be due to winter weather, which coincided with the study time. Overall, the tolerability profile was rated as excellent. TEWL did not change at the end of the 4 weeks, meaning that the products caused no damage to the skin barrier. That is essential to skin health in people with sensitive skin. In addition, this product increased about 16% moisture content in the skin (corneometry) [139]. Briefly, individuals with atopic dermatitis and eczema using these formulations had no worsening of their skin barrier function. Individuals with rosacea had no increased inflammatory papules or flushing. Individuals with cosmetics intolerance syndrome could use these products without feeling facial sensory discomfort. These results were promising, associated with the fact that BAK is photostable, allowing greater ease of formulation. Remember that it is necessary the use sunscreen, given that BAK did not show phototoxicity [139].

4.6 Depigmenting

Kang *et al.* conducted a study involving UP256 (UP), a cream comprised of 77.02% BAK, which contributes to reducing hyperpigmentation. The effect of UP on inhibition of melanin synthesis was analyzed *in vitro* in normal human epidermal melanocytes (NHEM). Phenylthiourea (PTU), a melanogenesis inhibitor, was a positive control. UP showed no significant cytotoxicity up to 5μM. UP inhibited melanin production at this concentration by about 15%, similar to PTU inhibition at 10μM. Besides, the L-3,4-dihydroxyphenylalanine stain was used to detect TS activity *in situ*. A reduction of the stained area was visible with the increase of UP concentration. The effect of UP on the expression of melanogenic enzymes was analyzed, *in vitro*, by western blot. At 5μM, UP inhibited about 43% TS expression, 32 and 47% TRP-1 and 2, respectively. UP also inhibited microphthalmia-associated transcription factor in approximately 19%. UP was more effective than PTU in inhibition of TRP-1 and 2.

The effect of UP on signaling proteins was analyzed, *in vitro*, by pull-down assay. It showed a marked inhibition of Rac1 and Cdc42 activation and reduction in α-PAK expression. These GTP-binding proteins are involved in melanocyte dendrite formation. The effect of UP on the inhibition of cilia formation was analyzed *in vitro*. Melanin content and cilia formation were measured on melanocytes treated with UP. Melanocytes non-treated were the control. The researchers read the results after 24, 48, and 72h. All the parameters increased in the control group. UP decreased cilia formation by about 20%. The effect of UP on inhibition of melanogenesis was determined *in vivo* (zebrafish embryo model) and *ex vivo* (3D human skin model). PTU was used as the positive control. The Zebrafish model was incubated for 72h with UP and PTU at 30μM. Skin tissue was incubated for 14 days with 0.05% UP (w/v) and 0.1% PTU (w/v). The results showed a clear inhibition of melanogenesis by the UP *in vivo* and *ex vivo* models. However, UP presented a better depigmenting effect on the skin than the zebrafish model. Once, in this last model, the amount of melanin synthesized was slightly lower, but not much compared to the positive control, while in the skin model, the inhibition of melanogenesis was higher with UP [43].

Lyons *et al.* conducted an *in vivo* study to demonstrate BAK anti-hyperpigmentation action. It was a prospective and non-randomized study, including 20 individuals, 6 males and 14 females, all aged 18 or over, with skin phototypes ranging from IV-VI (Fitzpatrick classification) and acne-induced postinflammatory hyperpigmentation (PIH) history. PIH is an hypermelanosis, which arises after the resolution of skin conditions such as acne, eczema, and psoriasis, after infections, contact dermatitis, allergic reactions, medications use, inflammatory diseases, and burns. It occurs mainly in the skin of color. Three facial lesions (acne consequence) were selected in every individual. Besides, three selected gluteal skin areas have suffered PIH lesion induction using 35% trichloroacetic acid (TCA). Twenty-eight days later, TCA-induced PIH lesions were already formed, spectroscopically, histologically, and clinically indistinguishable from acne lesions. Individuals were instructed to use the vehicle or bakuchiol cream (blinded to which cream they were using) twice daily for 28 days. Exception for third lesion, both of the face and buttock, where no product was applied, used as control. Investigator Global Assessment (IGA) score was used to classify hyperpigmentation from 0 (clear) to 5 (very severe, very dark brown, and black in quality). The change in the IGA score reflects an improvement degree. A negative score indicates a PIH decrease and, therefore, improvements. The results showed significant improvement in acne lesions after bakuchiol treatment (IGA score -1.06±0.23), comparatively with vehicle (IGA score -0.69±0.18). However, the results did not reach statistical significance. TCA-induced lesions showed statistically significant results [140].

4.7 Anticancer

A study conducted by Madrid *et al.* investigates BAK anticancer potential. Resinous exudate from *P. glandulosa* was tested *in vitro* against human melanoma cancer cell lines (A2058) and fibroblast cells. The exudate was incubated at increasing concentrations and measured by MTT assay. After 48h, the preliminary results revealed significant inhibition of A2058. The same did not happen with fibroblast cells, where cellular viability was not affected, remaining constant even with the increase of resinous exudate concentration. BAK, extracted from aerial parts of *P. glandulosa*, was studied against A2058. It showed cytotoxicity, observed by the measurement of lactate dehydrogenase (LDH) in the culture medium, given that it is an enzyme found in the cytosol. That was an indicator of impairment of cell membrane integrity, hence cytotoxicity. Its IC_{50}, defined as the concentration that inhibits 50% cell viability, was 29.3±0.4 µM. After 48h of incubation with pure BAK at 40µM, the percentage of LDH released in A2058 cells was 5.9±0.6%, not statistically significant. The negative control showed 6.5±0.9% LDH released. These dismiss the idea of membrane breakdown. As there was no cell damage, it was thought that the exudate could be related to apoptosis [14]. Hanahan and Weinberg described, in their work, six hallmarks of human cancer cells, one of them is resisting cell death. Programmed cell death – apoptosis – is a natural defense to tumor development. Cancer cells act through many mechanisms in several stages of the apoptosis cascade to limit and circumvent it. [141] This is the basis for the progression and development of a tumor. Besides allowing tumor growth, making them resilient to human defense mechanisms and therapy [14]. A detailed analysis of DNA fragmentation patterns through single-cell gel electrophoresis makes it possible to distinguish between necrotic and apoptotic cells. Percentage of fragmented DNA (TDNA) and in the tail moment (TMOM) are parameters used to determine DNA damage. An increase in these parameters suggests cell death by apoptosis. Hydrogen peroxide, an apoptotic inductor to cancer cell lines, was used as the positive control. The results revealed an increase of TDNA and TMOM related to the increasing BAK concentration after 48h incubation. That means that there was a high DNA fragmentation. These findings suggest that BAK triggers apoptotic cell death [14]. Caspase-3 is the major caspase. When it is activated, it participates in cell growth inhibition. Active caspases are involved in protein cleavage important for apoptosis. In this experiment, hydrogen peroxide at 1µM was a positive control. Caspase-3 activity was measured by the amount of p-nitroaniline released *in vitro* and was reported as optical density (OD) spectrophotometrically read at 405 nm that is, OD_{405}nm/mg protein. P-nitroaniline is released upon the cleavage of a caspase substrate. The results show that after 48h of incubation of A2058 cells with bakuchiol at 5µM, it resulted in 0.45 OD_{405}nm/mg protein, at 10 and 20µM

originate 0.63 and 0.89 OD_{405}nm/mg protein, respectively. This increase in caspase-3 activity related to increasing BAK concentration corroborates with the prior results of apoptotic cell death [14]. Western blot analysis evaluated, *in vitro*, the expression of specific proteins. Bcl-2, an anti-apoptotic protein family, plays a crucial role in apoptosis and cell survival. They regulate the activation or not of particular caspases cell death proteases. *In vitro* studies showed that BAK down-regulates Bcl-2 expression in A2058 cells. Besides that, there is Bax protein, pro-apoptotic. Bax/Bcl-2 ratio allows us to infer whether or not it is favorable for apoptosis. High values of Bax protein combined with low values of Bcl-2 shifted in the apoptosis direction. *In vitro*, BAK at 20μM showed a higher ratio than the control, A2058 cells not incubated with BAK. The p53 family acts in the cell like a stress sensor and promotes the activation of pro-apoptotic genes. BAK showed a p53 upregulation. ROSs are associated with the induction of cell death. A fluorescent probe was employed to analyze intracellular ROSs levels, which can diffuse through the cell membrane. After being oxidized by ROSs, the probe emits fluorescence proportional to ROSs levels. The results showed an apparent increase in fluorescence in a concentration-dependent manner after 48h incubation with BAK in A2058 cells. Related to all previously described findings, we conclude that the high DNA fragmentation in the A2058 melanoma cell was not associated with membrane lysis (not significant LDH release) [14]. The increase of activity of the caspase-3 enzyme reinforced the idea of apoptosis induction. BAK increased the generation of ROSs, which triggers the cascade of apoptosis, in a concentration-dependent manner. BAK proved to be effective *in vitro* in reducing A2058 cells viability. Taken together, these findings support the biocompatibility of bakuchiol and its selective toxicity against cancer cells [14]. Table 3 (in the annex) summarizes BAK's activities on the skin.

5 Skin delivery systems

The skin is known as the larger organ in the human body. Topical delivery is an attractive strategy compared to oral, parenteral, and intravenous via due to its low painfulness and invasiveness. Regardless of the therapeutic or cosmetic application, active molecules face some challenges, particularly low penetration capacity through the SC, compromising their permeation [16]. Several strategies emerged to overcome this issue.

BAK has the potential to be used as a therapeutic substance for skin application. However, to this day, no drugs are available, at least on the Portuguese market containing BAK as active substance, following the Infarmed database. Nonetheless, BAK was included as a cosmetic ingredient in some marketed formulations, namely, Lierac Paris Cica-filler serum [142] and cream [143], where BAK is used as a RET-like effect by its action on CLL I synthesis

stimulation; Lierac Paris Sébologie regulating gel [144], where BAK acts on shine by reduction on sebum secretion; Bioderma Sébium global [24, 114, 145] has BAK as part of SeboRestore technology. That helps restore natural sebum quality; ISDIN Melatonik serum [146-150] has BAK for its effect on restoring skin elasticity and firmness (stimulation on CLLs and EL fibers synthesis), reducing fine lines appearance and wrinkles. Unfortunately, no delivery system is publicly known. In this section, three nanosystems for dermal delivery will be described and well characterizes so that they may serve as the basis for further delivery studies.

5.1 Therapeutic applications

5.1.1 Microsponges

Wadakwa *et al.* have invested in research of a new way to deliver *P. corylifolia* (Babchi) essential oil (BO) through the skin, encapsulating it in microsponges (MS). The essential oil of Babchi was widely used in traditional medicines. Despite its phytotherapeutic properties, it has volatile nature, poor solubility, and stability, hindering its pharmaceutical application [45]. To produce Babchi oil microsponges (BOMS), resorted to the quasi-emulsion solvent evaporation method, given BO hydrophobic properties, as Pawar *et al.,* [151] a technique highly reproducible. Ethyl cellulose (EC) was the polymer, a hydrophobic cellulose derivative, non-swellable, and suitable to offer structural integrity. As emulsifier and stabilizer, polyvinyl alcohol (PVA) and dichloro methane (DCM) as solvent. BO and EC were dissolved in DCM and later progressively added to an aqueous solution of PVA under continuous magnetic agitation. The final mixture suffered filtration to isolate MS formed and was dried under a vacuum dryer. GC-MS confirmed that BAK was the principal constituent of BO, reflecting 65.37%. Essential oils composition may have slight variations depending on the growing environment, pluck and collection time, and extraction technique, among other factors [45].

The entrapment efficiency was determined as follows: first, MS were weighed and then triturated. After solubilized in ethanol and filtering, the resulting particles were measured by a UV-visible spectrophotometer at 262 nm. That corresponds to released drug concentration (C_R). Applying the formula of Wadakwa *al.*, encapsulation efficiency is calculated. %Encapsulation efficiency=$(C_r x V_r/M_{mp})/(M_d/M_d+M_p)x100$ Where V_R corresponds to released volume, M_{mp} is microparticles mass, M_D and M_P are encapsulated BO and polymer mass used initially. The maximum value was 87.70% [45].

Keeping fixed PVA concentration and varying EC concentration, entrapment efficiency presents a pattern similar to bell-shaped, higher in mean values zone. Increased EC concentration is accompanied by enhanced internal phase viscosity and intramolecular forces. Larger globules are formed during emulsification, yielding bigger MS and, therefore, greater

particles able to entrap larger BO quantities. High values of polymer ratio lead to a reduction of oil diffusion from EC solution to the external phase, giving more time for the droplet formation, reflecting higher entrapment efficiency. Higher EC concentration, *in vitro*, led to the thickening of the polymeric matrix wall, which hinders the posterior oil entry. MS porosity appears to positively affect entrapment efficiency due to the increase in surface area. Further, the homogeneous distribution of the pores is associated with the fact that, when DCM quantity is constant, they are connected to each other favoring BO loading in MS [45].

BO Fourier transforms infrared spectroscopy (FTIR) spectrum presents several typical peaks with main compounds, one of which is BAK, that confirms BO purity. Comparing BO, EC, and PVA spectrum, no peaks appeared or disappeared, meaning there was no chemical interaction between BO and EC, translating compatibility between BO and polymers and other selected excipients. Accordingly, these findings corroborate with BO encapsulation and stability in porous microstructures [45]. Differential scanning calorimetry is helpful for the recognition of guest molecules that are entrapped into MS porous cavities. Some points in thermal curves can shift or disappear due to melting, boiling, and sublimation. BO thermogram has an exothermic peak of around 400°C, which corresponds to the temperature at which the oil evaporation process occurs. In the BOMS thermogram, this peak is reduced with sharpness loss, which confirms success in imprisonment and demonstrates that BO thermal properties did not change during MS formulation. Field-emission scanning electron microscopy revealed that MSs are predominately spherical and highly porous (spongy structure). DCM diffusion from the MS surface results in pore formation. MS internal structure has multiple annulled spaces where BO will stay [45].

MS particle size should be in the range of 5 to 300µm. In this study, MS ranged from 20.44 to 41.75µm. Logically, the more EC and PVA are used, the bigger the MS size, and the reverse is also true. When the viscous organic phase has a greater concentration of EC, it results in larger emulsion droplets, therefore, larger-sized microporous particles. PVA variation has a similar influence on particle size. Higher PVA concentrations make emulsion droplets challenging to split into smaller droplets, culminating in larger MS [45].

The researchers studied, *in vitro*, the BOMS drug release profile. The highest cumulative drug release (CDR) value was 86.21% after 8h. In general, lower EC concentrations results in higher CDR percentage. The same for the reverse. High EC values make the pores larger with low %CDR. Increasing EC amount leads to a decrease in drug quantity on the particle surface of microporous, and simultaneously, an increase in drug quantity in the polymer matrix. In practice, it is observed that retardation in drug release rate from MS. Low EC amount forms small MS with a high surface area. Suppose some fluid contact with MS needs to cross a shot

route (small size MS), improving the release rate. Keeping EC concentration constant, an increase in PVA leads to a decrease in drug release rate. Drug release extent decreases with the rise of both polymer and emulsifier concentration. EC high amount origins a thick layer that allows controlled drug release [45].

Although having excellent properties for topical application, some essential oils have shown skin toxicity and irritation. Wadakwa *et al.* studied BOMS compatibility with skin cells to better understand and find more data. It was performed, varying free BO and BOMS concentrations in immortalized human keratinocytes (HaCaT) cell lines using MTT assay. The results showed a cellular viability reduction in a dose-dependent manner. BOMS, at 320µg/mL, did not have a significant cellular cytotoxic effect (26.34% inhibition). Free BO at 320µg/mL caused 51.05% inhibition. These findings support that developed microformulation is safer for dermal cell application than free BO, showing skin cells compatibility [45].

Previous studies demonstrated BAK's antibacterial effect against a variety of bacteria. Namely, *P. corylifolia* ethanolic seed extracts inhibitory effect against *S. aureus* and *S. epidermidis* [1], MRSA [152], *Pseudomonas aeruginosa* [153], *Escherichia coli* [51]. *In vitro* study against skin bacteria, showed that BOMS had good growth inhibitory activity. The growth of dermal bacterias *P. aeruginosa, E. coli*, and *S. aureus* was analyzed using free BO, BOMS, and streptomycin, positive control. The results for free BO were 11.67, 11.33, and 12.67mm zone inhibition for P. aeruginosa, E. coli, and S. aureus, respectively. The results show that inhibition zones for streptomycin were 14.67, 16.00, and 10.00mm, respectively, and for BOMS were 16.77, 15.33, and 16.67mm, respectively. *S. aureus* was the most susceptible. BOMS had good antimicrobial activity *in vitro*, comparable action to streptomycin (standard drug), and notably better when compared with free BO [45].

It is known that BO exhibits a peak in the UV region at 262nm, and its intensity is diminished after UVA irradiation due to the BO constituent's photolysis. The photodegradation studies showed that BOMS were more photostable than free BO. That was attributed to BO encapsulation within the MS system forming a physical barrier, thus protecting BO from UV-induced degradation and enhancing its photostability. These findings represent added value for pharmaceutical applications, given that MS can protect bioactive substances from UVA-radiation degradation. In addition, stability studies were carried out for 3 months. The results showed no color change of MS, meaning no significant difference in its content. FTIR spectrum revealed microformulation stability without oil degradation. MS represents more of an advantage than free BO [45].

This microformulation appears to be a good, stable, and effective system for dermal delivery of BO, which proved antibacterial properties. Given the minimal trend of developing

antibiotic resistance, it may be employed for dermatological disorder treatment. This new formulation allows overcoming limiting characteristics such as volatile nature, hydrophobicity, high viscosity, and degradation susceptibility during storage due to its low stability to air, light, and high temperature, which hinder BO's practical application. In addition to improving stability, it reduces dermal toxicity, a relevant point for adherence to therapy. It proves no cytotoxicity, corroborating with skin cell compatibility. BOMSs dermatological potential can be enhanced by loading them in creams, gels, lotions, or other suitable dermal carriers, strengthening BO skin advantages and surpassing skin toxicity issues resulting from BO's direct contact with the skin. This carrier system allows for extended drug release time, thus, reducing the dose and side effects. On the other hand, it improves cost-effectiveness and payload [45].

5.1.2 Nanosponges

After Wadkwa *et al.* studies on the encapsulation of *P. coryfolia* essential oil into MS, two collaborators, notably Kumar and Rao, continued the research and developed a delivery system of this essential oil, passing from microscale to nanoscale [45, 46]. Kumar *et al.* devote themselves to studying the encapsulation of Babchi oil (BO) in β-cyclodextrin-based (β-CD) nanosponges (NS) (β-CDNSs) [46]. Within cyclodextrin exist three types: α, β, and γ, with six, seven, and eight glucopyranose units, respectively [154]. This solid mesh-like network structure has nano-cavities that allow the imprisonment of complex chemical substances, which are later controlled and sustainably released by adjustment of cross-linker to polymer ratios. NS generally are very efficient and greatly enhance stability. Cross-linking peptides and polyesters (biodegradable poly-glycolic acid, for instance) interaction make this lipophilic structure act like a transporting fluid, able to disperse in water. They can mask less pleasant flavors and change the encapsulated physical state from liquid to solid, among other advantages, as presented by Pawar *et al.* [154].

Quantitative analysis of BO by GC-MS proved that the main component was BAK (65.37% percentage area [46]). NS were synthesized by melt method β-cyclodextrin that suffers cross-linking with diphenyl carbonate (DPC). It was noted that the increase in a molar ratio (β-CD:DPC) increases practical yield, which is thought to be due to a rise in reactive functional groups number [46]. Later, NSs were loaded with essential oil by freeze-drying method [46]. BO loading capacity was calculated as the ratio between BO weight in NS and NS weight. Encapsulation efficiency was calculated as the ratio between BO weight in NS and BO weight initially fed. Their experiments showed that complexation efficiency is affected by cross-linking degrees. For example, the 1:2 molar ratio represents a low cross-linking degree, which results in few nanochannels, and may be insufficient for a good guest complexation.

Conversely, a higher degree leads to hyper cross-linking, which hinders the interaction between BO and β-CD cavities. The most appropriate degree was a 1:4 ratio with 21.47% w/w loading efficiency. Encapsulation efficiencies ranged from 61 to 93%, whereas the 1:4 ratio NS recorded the highest value (93.05%). This molar ration appears to involve inclusion and external interactions simultaneously, and this optimum cross-linking provides a significant quantity of BO encapsulated both in the NS matrix and CD cavity [46].

BO-βCD and (Babchi Essential Oil in Nanosponge) BONS solubilization capacity was established using the free BO solubility in water. The researchers tested several molar ratios. All NS had higher solubility values than free BO (223.2μg/mL). BONS4 (1105μg/mL) achieved the maximum value of solubilization efficiency in BONS4 (1105μg/mL). BO-βCD exhibited 851.1μg/mL, lower than β-CDNSs. BO enhanced solubilization may have resulted from including complex formation associated with BO encapsulation in the NS matrix. The low cross-linking degree may result in insufficient nanochannels, reflected through low solubilization. On the other hand, the high cross-linking degree may result in complex and tortuous nanochannels, leading to BO entrapping into NS structures. Optimized β-CDNS solubilization was 4.95 times higher than free BO [46].

NS by themselves already indicates particle size less than 1000nm, that is, <1μm. Physically, have the appearance of a free-flowing and fine powder. In NS, the size range varied between 234 and 484nm. Zeta potential (surface charge measurement) ranged from -15.5 to -22.0mV. High values mean more repulsive forces, resulting in greater stability with less aggregation trend. The polydispersity index (PDI) ranged from 0.188 to 0.509. Low values mean homogeneous and stable nanocolloidal suspensions. Nanoformulation with 1:4 molar ratio (BONS4) presents 360.9 particle size, -16.0 zeta potential and 0.311 PDI. Despite not having the best values for these three characteristics, encapsulation and solubilization efficiencies were chosen as a model in subsequent characterization [46].

Blank NS, BO, and BONS4 were analyzed by FTIR. The Blank NS spectrum showed typical absorption bands confirming NS constitution CD-based. When the β-CD (NS synthesis starting material) FTIR spectrum is examined, the absence of a specific peak in the NS spectrum signifies interaction with DPC (cross-linker) with carbonate bond formation in NS. BO spectrum presented characteristic peaks. Comparing all analyzed FTIR spectrums, it is evident that the shifted or broadened of some BO peaks in nanoformulations reflect interactions between essential oil and NS [46]. Later, thermo gravimetry was used to verify possible physical and chemical material properties alterations. The results showed an inclusion compound presence and a β-CD and DPC physical mixture. The degradation of cross-linked

structures was required 240 to 300°C, synonymous with considerable thermal stability [46]. X-ray powder diffraction showed that the product obtained lost crystallinity after freeze drying, resulting in a fluffy powder characterized by a very porous structure. Blank NS has characteristic peaks whose intensity has been reduced after BO loading. That depicts a decrease of β-CDNSs crystallinity [46]. The surface topography of NS was studied using field emission scanning electron microscopy. The images depict CDNSs crystalline morphology. Then, a single NS crystal was observed by transmission electron microscopy. Its morphology shows porous nature, high crystallinity degree, and homogeneous size distribution [46].

These CDNSs were studied as to their cytotoxicity, *in vitro*, against human skin cell HaCaT line. Posteriorly, they were submitted, at different concentrations, to the MTT assay. This assessment is critical since essential oils can potentially cause toxicity and skin irritation. Free BO and BONS4 were compared, and the results showed that BONS has slightly less cytotoxicity than free BO. Cellular viability reduction observed was associated with increased dose. Furthermore, the IC_{50} value when cells were treated with BONS and BO at 320µg/mL was 191.4µg/mL and 172.3µg/mL, respectively. Nevertheless, this difference was insignificant, despite the greater cellular toxicity associated with NS. Hence, these findings support that BONS formulation is safer than BO for human skin cells [46].

It is known that BO exhibits a peak in the UV region at 265nm, and its intensity is diminished after UVA irradiation due to the BO constituent's photolysis. The photodegradation kinetics were determined according to the irradiation time for free BO and BONS. Comparing photodegradation rate constants obtained for free BO ($6.909x10^{-3}min^{-1}$) and BONS ($2.303x10^{-3}min^{-1}$), it is clear that NS retard photo-oxidative process once they form a physical barrier, thus protecting BO from UV-induced oxidation. In other words, NS complexation prevents UV photodegradation [46]. BO proved to be effective, *in vitro*, against several bacteria, including *E. coli, P. aeruginosa*, and *S. aureus*. This inhibitory effect was markedly improved when tested with BONS, verifiable by an increase in the growth inhibition zone. This difference may be due to the volatile nature and water insolubility of free BO. As previously seen, BO entrapment by CDNSs significantly increases its water solubility, which may be the cause of the increased antibacterial effect [46].

In this formulation, the advantages are similar to MS. It is a skin delivery system that aims to overcome essential oil inherent and limiting characteristics. Therapeutic response enhancement was expected, given that, as previously seen, drug release time extension permits reduced dose and drug consumption, consequently minimizing side effects due to the localized and directed drug delivery, specifically for the skin. It also has dermatological potential, being able to be loaded in suitable dermal carriers, strengthening BO skin advantages

without skin irritation/toxicity. The scale reduction can be an asset to improve parameters such as solubility and permeability, depending on the required purpose, given the significant increase of particle surface area [46].

5.2 Cosmetic applications

A recent study by Lewinska *et al.* involved an in-depth survey of "environmentally-friendly" nanoemulsions to explore the possibility of improving BAK transdermal delivery. They designed and described a new colloidal stable system: oil-in-water nanoemulsion. The "green" nanosystem (o/w) used, surfactin (SUR) and coco-betaine (CB) (1:4), are two hybrid-surface actives (stabilizers) [16]. They are ionic surfactants crucial to obtaining stable formulations, especially when electrostatic effects are involved. Anionic SUR is a bioinspired rapeseed origin. It is a highly surface-active cyclic lipoheptapeptide and biotechnologically obtained by *Bacillus subtilis* natto KB1 strains. While biosurfactant is considered more biodegradable, environmentally compatible, less toxic, and stands stable in a longer interval of pH and temperatures. Beyond that, they have high surface activity even when at low critical micellar concentration. CB, alkyl dimethyl betaine, is an amphoteric semi-synthetic surfactant from coconut oil. Its emulsifying properties may be increased in surfactin presence [16]. Physical stability involves the assessment of certain phenomena such as phase separation or inversion, creaming, sedimentation, flocculation, or coalescence. That is vital to ensure active payload maximum and long-lasting effectiveness. The idea is that none of the processes occur during storage since it may jeopardize formulation kinetic stability [16]. Extraction obtained about 80% BAK content. The researchers tested several systems with different concentrations of compounds (surfactant, oil, and water). One corresponds to a 5% surfactant mixture, 1% oil, and 94% water. It was characterized by small nanodroplet size and almost monodisperse size distribution (hydrodynamic diameter/ droplets size: 221±4nm; polydispersity index: 0,182±0,01) and high surface charge (zeta potential: -73±5mV). This combination of features allows them to be prospective candidates for transdermal transport and also were the conditions that exhibited minor modifications over time [16]. Some parameters, such as size distribution, zeta potential, backscattering profile, and UV-Vis absorbance (reflects the quantity of BAK loaded), were evaluated at the end of 30 days. Graphically, there was no evident separation of obtained curves, reflecting that did not occur particle growth or migration within nanoparticle dispersion. Thus, demonstrating great kinetic stability, later confirmed by resorting to the Turbiscan Stability Index, where low values are related to more stable formulations, 1.2 in this case. Droplets' morphology and shape were imaged by transmission electron microscopy. The result was spherical nanostructures, well distributed and nearly

uniform sizes. Confocal microscopy revealed a round shape with no aggregated nanodroplets, then, without the occurrence of flocculation or coalescence processes. The researchers used UV-Vis spectroscopy to prove that the extract present in the nanoemulsion was BAK-rich. After 30 days, the representative peak, at 480nm, remained almost unchanged, even at the end of the storage period. That was possible thanks to the efficient solubilization of hydrophobic molecules and, in part, also to the preservative applied [16]. The formulation containing BAK proved stable and was submitted to different experiments: *ex vivo* permeation, *in vitro* cytotoxicity, and *in vivo* contact study [16].

An *ex vivo* study was performed on pig skin (full-thickness) in a Franz cell. The formulation was applied to the donor chamber. After 1 and 7h, HPLC analysis of acceptor chamber fluid confirmed that surfactants (SUR and CB) had not entered the bloodstream due to the absence of compounds. This way, the formulation remained on some skin layers. Confocal microscopy fluorescent imaging revealed that BAK penetrated the epidermal barrier and reached the subcutaneous layer. 1h hour after BAK nanoformulation application was recorded fluorescence between 80 to 120µm skin penetration. After 7h, it was about 140µm in depth. The carrier was kept intact and provided stable transport [16]. *In vitro* study was performed in HaCaT and HDF. BAK concentrations were between 0.02 and 0.5mg/mL and were tested in cell cultures after 24 and 48h. MTT assay showed that encapsulated BAK formulation showed low cytotoxicity in both cell cultures, even at 0.5mg/mL, the higher concentration. Cell viability reduced about 60% after 24h and 55%, 48h after treatment with the BAK formulation. Within a concentration range of 0.02 to 0.2mg/mL, curiously, a beneficial effect has occurred in the cell, accompanied by excellent cell survival. BAK proved to be biocompatible in both cell lines [16]. *In vivo* study involved human volunteers, both men, and women, aged 30 to 50 years old, to assess nanoemulsion effectiveness on capillaries, skin discoloration, and wrinkles. BAK formulation at 0.05mg/mL was applied twice daily for 28 days. Reminding that younger people have slighter skin deterioration signs, smoother changes in the age groups are expected. For people over 40, skin changes are usually perceptible to the naked eye, which is where were notice the most difference. Over 50 years, some changes are permanent. Consequently, seen effects were even less observable. The results showed that BAK formulation enhanced skin conditions. BAK reduced wrinkles depth and skin blood vessels in subjects of all ages. In discoloration, for 30 and 50 years old, BAK showed a considerable decrease, particularly in the 50s [16].

This nanoemulsion has a great potential to enhance the solubility and effectiveness of hydrophobic compounds, given its rise in surface-to-volume ratio, decrease in particle size, and increased mobility. In addition, it has greater physical stability, and biosurfactants may

preserve the system against degradation either in production or storage. Nanoemulsions can penetrate the epidermis barrier. Once it arrives intact, it can adequately exert its effects. That could be useful for delivering active ingredients such as BAK in deep skin layers [16].

6 Safety: regulatory and toxicological concerns

Regulatory organizations, including European Medicines Agency (EMA) and Food and Drug Administration (FDA), are responsible for developing guidelines that orient to toxicity assessment. In the European Union, EMA supervises the use of medicines and monitors their risk-benefit balance and safety. In addition, before being used, all nanocarriers require a complete risk assessment evaluation and a previous authorization. Scientific Committee on Consumer Safety must be notified if there is a suspected lack of safety regarding the nanosystem. There are two databases with gathered information about toxicological information (eNanoMapper) and safety (NanoData) [47]. In Europe, EU Directive 2001/83/EC regulates medical products, and EU Directive 93/42/EEC regulates medical devices. It is necessary to decide if nanotechnology-based formulations are medical products or devices [155]. Toxicity assessment provides efficacy and safety results that determine acceptance or denial of regulatory approval. International Organization for Standardization, associated with Organization for Economic Cooperation and Development, has developed industry standards for toxicity assessment of nanoformulations. However, these regulations were only for application in industry [155-158]. FDA guidance draft related to the industrial nanomaterials does not clarify the toxicity assessment. Despite having updated the guidance document in April 2022 [159] (previously dated December 2017 [160]), remains unexplained the toxicity assessment. It only referred to the importance of establishing the safety profile [155].

The European Chemicals Agency has compiled the information relating to the BAK. Regarding physical hazards classification, the results were conclusive but insufficient to classify almost all parameters, such as explosive or self-reactive substances, except for desensitized explosives, whose reason for non-classification was the lack of data. Health hazards have conclusive results but are insufficient for classifying skin sensitization and irritation/corrosion. Dermal acute toxicity was not classified by lack of data [161]. Skin irritation/corrosion was assessed in 111 individuals by a patch test, and the results showed no irritation. The same was observed in skin sensitization.

Although it is said that BAK could offer an advantage over retinoids since it could prevent some side effects such as redness, peeling, itching, erythema, irritation, roughness, and stinging, some cases of adverse reactions were reported. A 33-year-old female with no atopic antecedents presented itchy and erythematous plaques, mainly located on the neck, perioral

area, and eyelids. Patch tests were performed on the products she used and Noreva Exfoliac Global 6 cream result was positive. All ingredients were submitted to patch tests, read on the 3rd and 7thdays. BAK was evaluated at 0,1%, corresponding to its concentration in the cream. The result was positive (+++) on the 3rdday. The patient was counseled to avoid products containing BAK [162]. In another case, a 23-year-old woman with antecedents of seasonal rhinoconjunctivitis presented a frequent situation of facial eczema. The woman mentions recurrent flares of edematous and erythematous itchy lesions. That coincided with the beginning of applying DermAbsolu Soin, antiage eye cream. Patch tests were performed and were read on the 2nd and 4thdays. The results were negative for all patch tests. The eye cream was investigated through the repeated open application test, shown positive since day one, presenting a follicular inflammatory pattern. BAK was evaluated at 1%, corresponding to its concentration in the cream. In the end, only the BAK test was positive (++). The patient was counseled to prevent products containing this compound [163].

Environmental hazards classified BAK as "very toxic to aquatic life" short-term (category acute 1) and "with long-lasting effects," long-term (category chronic 1). BAK labeling has an environment hazard pictogram (GHS09) [164, 165]. Short-term toxicity [166] was assessed in aquatic invertebrates (Daphnia magna), and the results were read after 48h. The effect concentration (EC_{50}) was ca. 0.2mg/L. Algae and cyanobacteria (Raphidocelis subcapitata) toxicity results were read after 72h, and EC_{50}/NOEC was > 2.108mg/L [167]. BAK's environmental fate is not completely known. There is still no data on photodegradation or bioaccumulation, for instance [168]. It was determined its biodegradation in water. A sample of water taken from the Daman Ganga River was made into an inoculum. To estimate the degradation percentage, BAK was first incubated, then measured based on the consumption of the dissolved oxygen (initial concentration was 7.98mg/L). The results were read on days 7, 14, 21, and 28, and the values obtained were 34.72, 66.89, 77.62, and 87.66%. BAK was considered not readily biodegradable. Potassium hydrogen phthalate was the reference substance (toxicity control) and showed similar degradation values. They concluded that BAK did not present adverse effects on the inoculum [168].

Toxicity is closely related to structural and physicochemical properties, among them size, shape, the tendency to agglomerate, and surface charge [169]. The nanometric dimension entails potential risks, on the one hand, increases the possibility of achieving systemic circulation, on the other, increases contact surface area. Therefore, it is expected that more interactions with biological systems, turning these systems more reactive and with higher toxicity potential, particularly harmful *in vivo* [156]. Cytotoxicity depends on exposure time and concentration of the nanosystem. It is important to note that contamination is possible

from the manufacturing process itself [169]. It is vital to assess the toxicological potential of BAK itself. However, greater attention should be taken to toxic features of the surface material once all the surrounding environment is influenced in some way [47]. The presence of surfactants, depending on their ratio, may induce some adverse effects, including irritation, erythema, or toxicity [155]. Some strategies, including nanosystems coating, are intended to overcome these toxic effects [170]. Further studies are required to conclude about BAK skin delivery safety and carrier systems' toxicological profiles, both short and long-term [156].

Although nanoformulations offer many advantages and possess great potential for therapeutic and cosmetic application, their practical utility entirely relays on their favorable safety profile. Therefore, a regulatory framework is required to elucidate nanotechnology-based formulations with specific manufacturing regulations, determine pharmacodynamic and pharmacokinetic profiles, and evaluate toxicological profiles. Only in this way is it possible to ensure the efficiency and safety of these nanoformulations to enable sustained approval for placement on the market [169].

Although it is for topical application, it is essential to know about BAK metabolic pathways. It occurs in the human liver microsomes, which include several isoenzymes such as CYP2C9, CYP2C19, and CYP3A4, which are responsible for BAK metabolism. This theme is relevant once these interactions may result in adverse reactions or a lack of therapeutic efficacy. That is even more important when there is the possibility of co-administration of some molecules able to activate or inhibit any of those isoforms, as is the case of glycyrrhetinic acid, licorice active metabolite. Inhibiting cytochrome P450 isoenzymes ultimately delays metabolic detoxification and extends the drug residence time in the organism. That could be dangerous because we are increasing the possibility of bioaccumulation and, in addition, cytotoxicity [157]. According to Kai Li *et al*., salt processing reduce the toxicity of *P. corylifolia* extract in the renal and cardiovascular systems. It is attributed to a decrease of volatile compounds, either of which is BAK, resulting from the heating process [158].

Hsu *et al.,* in their study concerning the antibacterial effect of BAK, found a curious aspect about its long-term storage. After 8 months at room temperature, BAK was degraded to 4-hydroxybenzaldehyde, which showed no antibacterial effect against *S. epidermidis* [23]. This formed compound is inactive. However, more studies will be needed to explore its toxicological potential and possible unexpected biological effects.

7 Conclusions and Future Prospects

Overall, this review summarized Bakuchiol's physicochemical properties, natural sources, synthesis pathways, biological effects, carriers for skin delivery, and toxicity. *P.*

corylifolia (*C. corylifolium*) is its major natural source. There is already a promise of a sustainable initial "green" path for its isolation, with a considerably good yield. In chemical methods, there is still no one that stands out for its performance, so the existing ones can be improved from a sustainable perspective. Furthermore, were thoroughly described and discussed its main skin activities found described in the literature, whose results were promising, especially as an antiaging agent and as bioretinol. BAK can replace the RET without its associated adverse effects, even in individuals with sensitive skin. Moreover, it presented potential antioxidant, anti-inflammatory, and depigmenting effects. Its anticancer potential is still little explored but appears to be prosperous. Bakuchiol also showed antibacterial and antifungal activity against *C. guilliermondii*, MRSA, *S. epidermidis*, and *C. acnes*, among others. It can be a solution given the emergence of antibacterial resistance. However, their mechanisms of action require further clarification. About skin delivery technology of the BAK, more studies should be performed because the delivery systems described show that there may be strategies that allow overcoming limiting characteristics such as volatility, hydrophobicity, viscosity and susceptibility to degradation. A future challenge will also be to see if there can be a interconnection between the type of nanosystem and its therapeutic or cosmetic use to further personalize the therapy. Besides, they reduce dermal toxicity and extend drug release time, thus, reducing the dose and side effects and could deliver active ingredients in deep skin layers. There are few studies, so it should be a future investigation area since BAK's properties are already known. Therefore, it is necessary to take "better benefit" from it by using delivery systems that increase its bioavailability and enhance its dermatological potential by loading them in creams, gels, lotions, or other suitable dermal carriers. Nanosystems risk assessment and its control are core requirements. Until now, there are not enough appropriate studies to evaluate the possible risks associated with nanocarriers. To accurately measure the toxicity is needed to wait for further clinical trials in humans. Most *in vitro* toxicological studies are focused only on a cell line. Nevertheless, it is necessary to create concrete guidelines to confirm the findings to develop good models to predict the nanosystems' effect on mammalians. Future studies, both *in vitro* and *in vivo*, must be performed in damaged skin instead of healthy skin. Once the barrier capacity may be compromised, it is reflected in an increase in permeability, consequently is to be expected a variation of pharmacodynamic and pharmacokinetic profiles. More intensive research is necessary to assess these systems' real skin penetration capacity and determine the time they remain and the possible problems they may cause. Finally, more research is required to determine ecotoxicity better and more appropriately.

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Annex I – Table 2

Table 2 – BAK synthesis pathways chronologically.

Year	*Authors*	*Final product*	*Notes*	*Reference*
1967	Damodaran and Dev	*Rac*-bakuchiol methyl ether	Three-step synthesis.	[15, 75]
1967	Carnduff and Miller	*Rac*-bakuchiol	First total synthesis of *rac-bakuchiol.* Prepared *in situ* Claisen rearrangement: key step to obtain the bakuchiol skeleton	[15, 90, 91]
1990	Takano *et al.*	(+)-bakuchiol	First enantioselective synthesis of (+)-bakuchiol. Twelve-steps synthesis.	[15, 92]
1991	Araki and Bustugan	*Rac*-bakuchiol	Synthesis *via* a geranylindium reagent. Three-step synthesis.	[15, 91]
1999	Asaoka *et al.*	(+)-bakuchiol	Synthesis *via* stereoselective alkylation using silyl group to obtain the chiral quaternary carbon center. Sixteen steps with an overall yield of 5%	[15, 93]
2008	Fukuyama *et al.*	(+)-bakuchiol	Synthesis *via* stereoselective alkylation using silyl group. 1,4 addition using vinylcopper (I) reagents. Ten-step synthesis.	[15, 94]
2008	Chen and Li	*Rac*-bakuchiol	Synthesis *via* 1,4 addition of citral, with vinylmagnesium bromide under Cu(I) catalyst. Four-step synthesis.	[15, 95]
2008	Li *et al.*	(S)-bakuchiol (R)-bakuchiol	Method of obtaining chiral center. Synthesis of (S)-enantiomer in ten steps. Synthesis of (R)-enantiomer in nine steps.	[15, 96]
2009	Novikov *et al.*	(+)-bakuchiol	Synthesis *via* intramolecular diazosulfonate obtaining C–H bond.	[15, 97]
2010	Hoveyda *et al.*	(+)-bakuchiol	Synthesis *via* Ni Catalyzed NCH–Cu enantioselective allylic substitution reaction.	[15, 98]
2012	Tadano *et al.*	(+)-bakuchiol	Synthesis *via* sulfur-based chiral auxiliaries-mediated Claisen rearrangement.	[15, 52]
2013	Fukuyama *et al.*	(S)-bakuchiol	Synthesis *via* asymmetric 1,4-addition.	[15, 99]
2013	Lei *et al.*	(S)-bakuchiol	Asymmetric synthesis routes. Approach using Evans' auxiliary.	[15, 56]
2016	Battilocchio *et al.*	(S)-bakuchiol	Synthesis of bakuchiol precursor in one single operation, using an interactive coupling method.	[15, 100]
2016	Xiong and Zhang	(S)-bakuchiol	Asymmetric allylation to obtaining quaternary center. Method through chromium-catalyst.	[15, 101]
2017	Chakrabarty and Takacs	(S)-bakuchiol methyl ether	Strategy *via* enantioselective rhodium-catalyzed hydroboration.	[15, 102]
2020	Khan *et al.*	*Rac*-bakuchiol	Used a regioselective molybdenum-catalyzed allylic substitution.	[15, 103]

Annex 2 - Figure 3

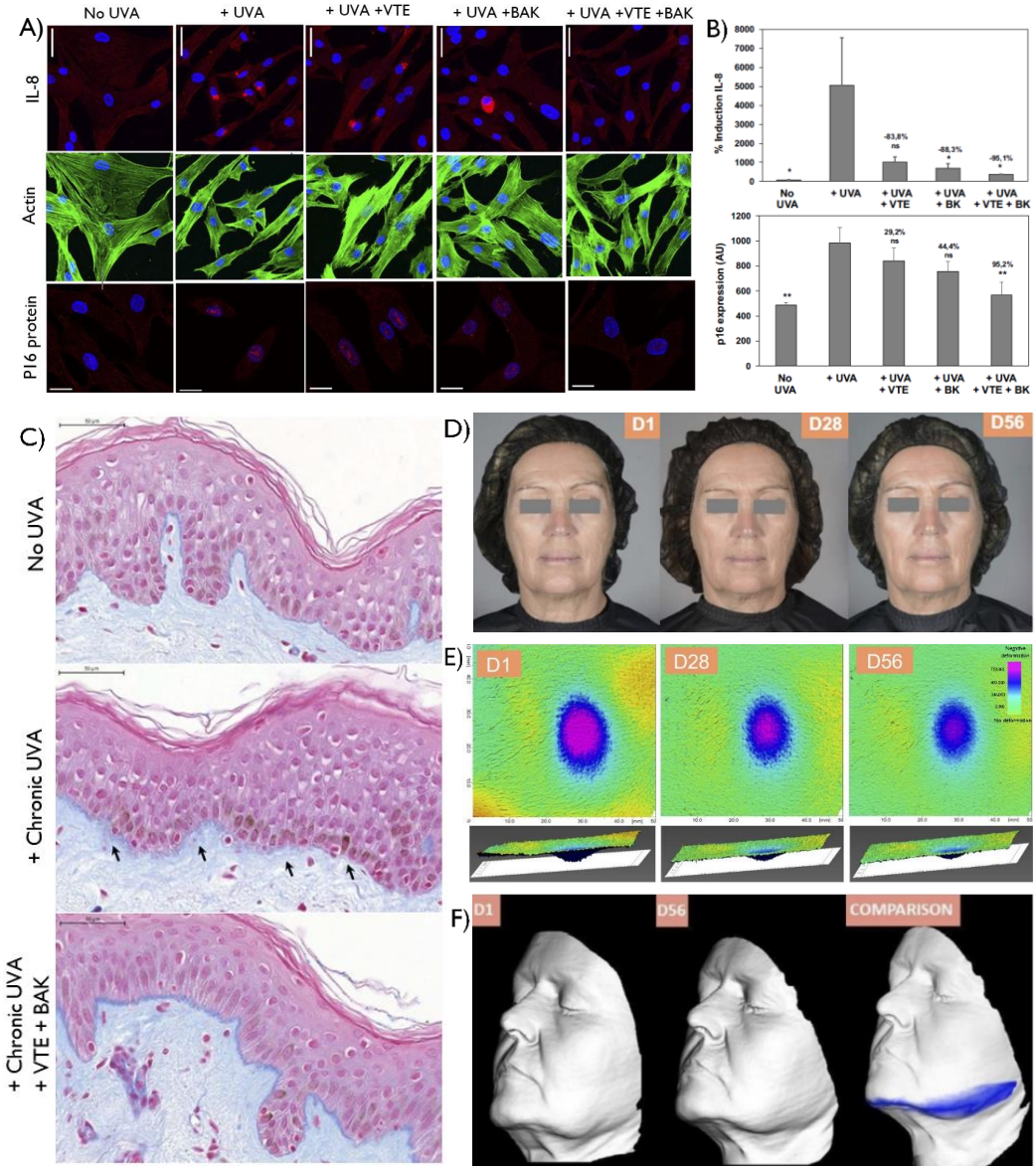
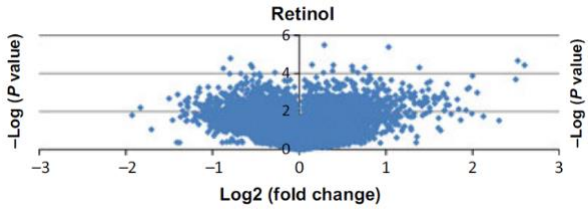
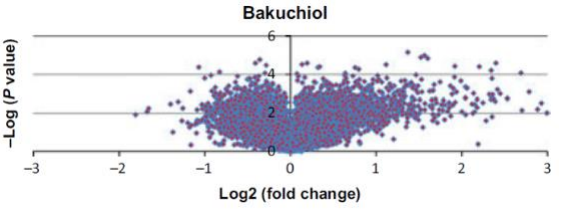


Figure 3 - BAK's antiaging effect. A) Immunofluorescence staining of actin network, interleukin-8, and p16 protein as markers of morphology, inflammation, and senescence, respectively. Performed in human dermal fibroblasts. Analyzed by laser scanning confocal microscopy; B) Quantitative analyses of IL-8 and p16 immunolabelling; C) Evaluation of dermal density in an ex vivo human skin aging assay. Analyzed by photon microscopy. Arrows indicate GAG (marker of the extracellular matrix) loss at the dermo-epidermal junction; D) Full-face macrophotographs show improvement of radiance after 28 days (+26%) and 56 days (+44%); E) Skin firmness improvement, analyzed by Dynaskin®. Cross-section showing the depth of skin deformation reduced after 28 and 56 days. In the image below, from day 1 to day 56, skin deformation depth 56 days (30.4%), and skin deformation volume 56 days (36.7%); F) Facescan®, ptosis volume decreased 56 days (22.8%). Defined face contour, showing a remodeling effect of the product. Adapted from [130]

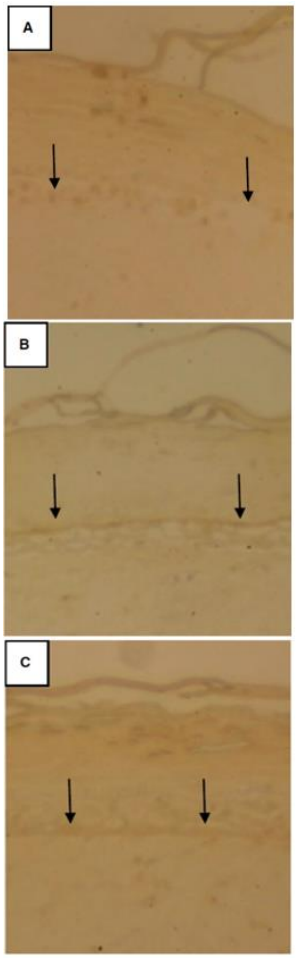
Annex 3 - Figure 5

A)

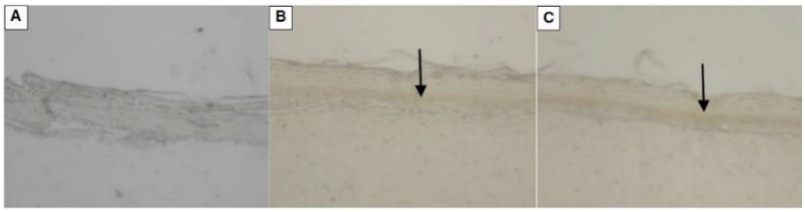




B)



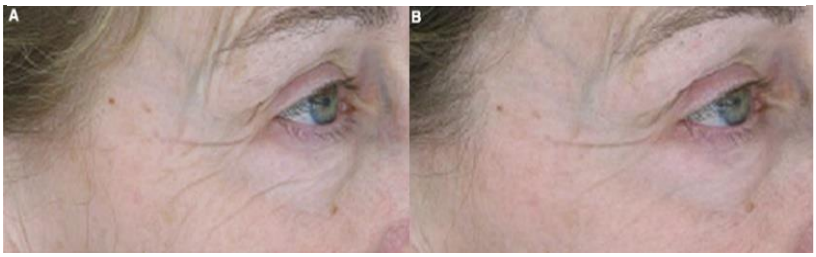
C)



D)

Parameters	4 Weeks E	4 Weeks I	8 Weeks E	8 Weeks I	12 Weeks E	12 Weeks I
Roughness & dryness	65	35	81	40	90	48
Fine lines/wrinkles	5	19	16	25	29	23
Skin tone	14	12	29	19	39	29
Skin elasticity/firmness	13	14	20	23	39	25
Radiance	24	11	40	17	52	30
Skin brightening	13	10	35	14	46	29
Eye area appearance	8	22	24	24	46	35

E)



F)

Parameters	4 Weeks	8 Weeks	12 Weeks
Wrinkle depth	−7%	−13%	−20%
Skin roughness	−2%	−10%	−21%

Figure 5 - A) Volcanic plot of DNA microarray data for RET and BAK; B) RET(b) and BAK (c) effect on collagen IV expression in human EpidermFT (full thickness), (a) is the control. Arrows indicate DEJ, where collagen IV is localized. Darker bands in (b) and (c) confirm collagen expression; C) RET(b) and BAK (c) effect on AQP-3 expression in human EpidermFT (full thickness), (a) is the control. Arrows indicate AQP-3 staining in the basal layer, where is mainly localized; D) Subjective evaluation by experts (E) and individuals (I) (% improvement vs. baseline); E) Right view day zero VS 12-week treatment; F) Results of silicone replica analysis using profilometry: % reduction vs. baseline. Adapted from [8].

Annex 4 - Table 3

Table 3 - BAK's activities on the skin

Skin activity	Study	Results	References
Antifungal	*In vitro*	↓ MIC80 against C. guilliermondii	55
	In vitro	↓ MIC against T. mentagrophytes	21
	In vitro	↑ fungal membrane permeability and ↑ROSs.	22
	In vivo	↓ fungal burden in guinea pigs´ feet	22
Antibacterial	*In vitro*	↓ MIC against MRSA	9
	In vitro	Cytotoxicity against S. epidermidis	23
	In vitro	↓ MIC against C. acnes	114
Antioxidant	*In vitro*	Prevent squalene oxidation 2x↑ vitamin E	114
	In vitro	↑ antioxidant capacity and power than RET	25
Anti-inflammatory	*In vitro*	↓ NO generation LPS-induced, without cytotoxicity	39
	In vitro	↓ iNOS gene via NF-kB binding inhibition	35
	In vitro	↓ PGE2 and MIF in HDF.	25
Antiaging	*In vitro*	↓ IL-8, p p16 protein expression. Prevent fibroblast morphological changes	130
	Ex vivo	Re-densifying effect and protect skin from GAG alterations UVA-induced in human skin photodamaged model	130
	In vivo	Remodeling effect. ↓ Depth and volume. Improved skin firmness and radiance.	130
	In vivo	Improved regarding radiance, freshness, and signs of skin aging	25
	In vitro	Up and downregulation of genes (ECM and DEJ). Similar RET gene modulation profile	8
	Ex vivo	↑ Collagen synthesis and metabolic activation in mature fibroblasts. ↑ AQP3 expression	8
	In vivo	Improve photodamage signs (skin elasticity, tone, brightening, dryness, wrinkles)	8
	In vivo	↑ Efficacy and tolerability in sensitive skin	139
Depigmenting	*In vitro*	↓ tyrosinase, TRP 1 and 2, MITF expression. ↓ Rac1 and Cdc42 activation and α-PAK expression. ↓ cilia formation	43
	In vivo *Ex vivo*	↓ melanogenesis	43
	In vivo	Improve TCA-induced lesions (hyperpigmented)	140
Anticancer	*In vitro*	Induced apoptotic cell death in A2058 cells. ↑ DNA fragmentation and ↑ caspase-3 activity. ↓Bcl-2 ↑Bax, p53, and ROSs	14