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Rodrigo Boscariol

**AVALIAÇÃO DO POTENCIAL DA CURCUMINA NO TRATAMENTO
DA PSORÍASE: ESTUDOS DE PERMEAÇÃO TRANSDÉRMICA *IN*
VITRO E *IN VIVO* FACILITADA POR GERANATO DE COLINA**

**Sorocaba/SP
2022**

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Orientadora: Profa. Dra. Marta Maria Duarte Carvalho Vila

Coorientador: Prof. Dr. Victor Manuel Cardoso Figueiredo Balcão

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“Mas em todas estas coisas somos mais
que vencedores, pela virtude daquele que
nos amou.”

Romanos 8:37-38

RESUMO

A psoríase é uma doença inflamatória crônica da pele com predisposição genética. Para o tratamento da psoríase, terapias alternativas têm sido avaliadas, como o uso da curcumina, extraída da cúrcuma (*Curcuma longa* Linn). Visando aumento da permeação dérmica, substâncias como líquidos iônicos têm sido utilizadas. Neste trabalho, objetivou-se desenvolver um gel de curcumina e geranato de colina, avaliar suas características físico-químicas e biológicas e verificar *in vivo* sua eficácia no tratamento da psoríase. Avaliou-se a atividade do geranato de colina como permeador cutâneo em estudos em célula de Franz e análises histológicas. A qualidade da curcumina foi determinada por ponto de fusão; atividade antioxidante por DPPH (2,2-difenil-1-picrilhidrazila), atividade antimicrobiana por difusão em ágar, infravermelho por transformada de Fourier (FTIR), contaminação metálica por fluorescência de raios-X por energia de dispersiva (EDXRF) e espectrofotometria (UV-VIS). Para estabelecer a concentração ideal de geranato de colina foram realizados ensaios de permeação cutânea em célula de Franz. Para analisar a estabilidade do gel foram feitos ensaio de estabilidade preliminar, análise por calorimetria diferencial de varredura (DSC) e teste de estabilidade acelerada (pH, espalhabilidade e análise sensorial). A capacidade de solubilização do geranato de colina foi determinada por espalhamento de luz dinâmico (DLS) e por Potencial Zeta. Para averiguar a eficácia do gel foram feitos ensaios *in vivo* em camundongos. Os resultados foram apresentados na forma de artigos, sendo: i) *“Performance of choline geranate deep eutectic solvent as transdermal permeation enhancer: An in vitro skin histological study”* comprovando-se que o geranato de colina promoveu aberturas transitórias na pele aumentando a permeação cutânea; ii) *“Physicochemical characterization of commercially available turmeric for use in pharmaceutical products and food supplements”* indicando necessidade de maior controle desta matéria-prima; iii) *“Transdermal permeation of curcumin promoted by choline geranate ionic liquid: potential for the treatment of psoriasis”*, apontando 2,0% (m/m) de geranato de colina como a concentração mais adequada para permeação de curcumina; iv) *“Gel de alfarroba e curcumina com geranato de colina: ensaios de estabilidade”* verificando-se estabilidade de gel; v) *“Transdermal permeation assays of curcumin aided by CAGE-IL: in vivo control of psoriasis”* que confirmou a eficácia do gel no tratamento da psoríase. Pelos resultados apresentados pode-se concluir que o gel desenvolvido se mostrou eficaz no tratamento da psoríase, comprovando a melhora da permeação transdérmica da curcumina pelo geranato de colina.

Palavras-chave: Psoríase. Curcumina. Permeação transdérmica. Líquido iônico. Geranato de colina

ABSTRACT

Psoriasis is a chronic inflammatory skin disease with a genetic predisposition. For the treatment of psoriasis, alternative therapies have been evaluated, such as the use of curcumin, extracted from turmeric (*Curcuma longa* Linn). In order to increase dermal permeation, substances such as ionic liquids have been used. In this work, the objective was to develop a curcumin and choline geranate gel, evaluate its physicochemical and biological characteristics and verify in vivo its effectiveness in the treatment of psoriasis. The activity of choline geranate as a skin permeator was evaluated in Franz cell studies and histological analyses. The quality of curcumin was determined by melting point; antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl), antimicrobial activity by agar diffusion, Fourier transform infrared (FTIR), metal contamination by energy dispersive X-ray fluorescence (EDXRF) and spectrophotometry (UV-VIS). To establish the ideal concentration of choline geranate, skin permeation tests were performed in Franz cells. To analyze the stability of the gel, preliminary stability assay, analysis by differential scanning calorimetry (DSC) and accelerated stability test (pH, spreadability and sensory analysis) were performed. The solubilization capacity of choline geranate was determined by dynamic light scattering (DLS) and by Zeta potential. To verify the effectiveness of the gel, in vivo tests were carried out in mice. The results were presented in the form of articles, as follows: i) *“Performance of choline geranate deep eutectic solvent as transdermal permeation enhancer: An in vitro skin histological study”* proving that choline geranate promoted transient openings in the skin increasing skin permeation; ii) *“Physicochemical characterization of commercially available turmeric for use in pharmaceutical products and food supplements”* indicating the need for greater control of this raw material; iii) *“Transdermal permeation of curcumin promoted by choline geranate ionic liquid: potential for the treatment of psoriasis”*, indicating 2.0% (m/m) of choline geranate as the most suitable concentration for curcumin permeation; iv) *“Locust bean gum and curcumin gel with choline geranate: stability tests”* verifying gel stability; v) *“Transdermal permeation assays of curcumin aided by CAGE-IL: in vivo control of psoriasis”* which confirmed the effectiveness of the gel in the treatment of psoriasis. From the results presented, it can be concluded that the developed gel was effective in the treatment of psoriasis, proving the improvement of the transdermal permeation of curcumin by choline geranate.

Keywords: Psoriasis. Curcumin. Transdermal permeation. Ionic liquid. Choline geranate

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LISTA DE ABREVIATURAS E SIGLAS

ADH - Álcool desidrogenase

AMPs - Peptídeos antimicrobianos

BB – UVB - Ultravioleta B de banda larga (BB-UVB, do inglês *broadband*)

BCG - Vacina Bacilo Calmette-Guerin

BSA - Área de Superfície Corporal (do inglês *Body Surface Area*)

CCL20 - Ligante quimiocina 20

COX-2 - cicloxigenase-2

CXCL1- Quimiocina 1

CXCL8 - Quimiocina 8

DES - Solvente eutético profundo (do inglês *Deep eutectic solvent*)

DHC - Dihidrodrocurcumina

DLQI - Índice de Qualidade de Vida em Dermatologia (do inglês *Dermatology Life Quality Index*)

DNA - Ácido desoxirribonucleico

DSC - Calorimetria diferencial de varredura

FDA - *Food and Drug Administration*

FLG - Filagrina

FTIR - Espectrofotometria de infravermelho

GRAS – Geralmente reconhecida como segura (do inglês *Generally Recognized as Safe*)

GSK3 β - enzima glicogênio sintase quinase 3 β

HDL - Lipoproteínas de alta densidade (do inglês *High density lipoproteins*)

HHC - Hexahidrocurcumina

IFA - Ingrediente farmacêutico ativo

IL - Líquido iônico

IL – 17 - Interleucina 17

IL – 17A - Interleucina 17A

IL – 17F - Interleucina 17F

IL – 17 21 - Interleucina 21

IL – 22 - Interleucina 22

IL – 23 - Interleucina 23

INV - Involucrina

LDL - Lipoproteínas de baixa densidade (do inglês *Low density lipoproteins*)

Labnus - Laboratório de Biomateriais e Nanotecnologia da Uniso

Labiton - Laboratório de Avaliação de Bioatividade e Toxicologia de Nanomateriais da Uniso

Lafinau - Laboratório de Física Nuclear Aplicada da Uniso

Lapetox - Laboratório de Pesquisas Toxicológicas da Uniso

Lapisus- Laboratório de Processamento de Imagens e Sinais da Uniso

MEV -Microscopia eletrônica de varredura

NB-UVB - Ultravioleta B de banda estreita (do inglês *narrowband*)

NF- κ B - Fator nuclear kappa B

NGF - Fator neurotrófico

PA - Para análise

Phagelab - Laboratório de Biofilmes e Bacteriófagos da Uniso

PASI - Índice de Gravidade da Área de Psoríase (do inglês *Psoriasis Area and Severity Index*)

RNA - Ácido ribonucléico

RNase - Ribonuclease

ROS - Espécies de oxigênio reativo

TCD4+ - Célula linfócito TCD4+ (Th 17)

TCD8+ - Célula linfócito TCD8+ (Th 22)

THC - Tetrahidrocurcumina

TNF- α - Fator de necrose tumoral- α

ton/ha – Toneladas por hectare

Uniso - Universidade de Sorocaba

UV - Radiação Ultravioleta

UVA - Radiação Ultravioleta A

UVB - Radiação Ultravioleta B

XRF- Espectrofotometria de fluorescência de raios-X

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APRESENTAÇÃO

O trabalho de pesquisa aqui desenvolvido e intitulado “Avaliação do potencial da curcumina no tratamento da psoríase: estudos de permeação transdérmica *in vitro* e *in vivo* facilitada por geranato de colina” fez parte de uma série de pesquisas iniciadas com o projeto de pesquisa “TransApplL- Permeação cutânea de entidades proteicas estabilizadas estruturalmente e funcionalmente, carregadas por líquidos iônicos” (Projeto Fapesp 2016/12234-4). O trabalho encaixa-se ainda dentro da linha de pesquisa “Avaliação de Substâncias Bioativas e Sistemas de Liberação de Fármacos” do Programa de Pós-Graduação em Ciências Farmacêuticas da Universidade de Sorocaba (Uniso).

Os ensaios, de maneira global, foram realizados no Laboratório de Biofilmes e Bacteriófagos da Uniso (Phagelab), no entanto, contou com a colaboração de outros laboratórios de pesquisa da Uniso. As análises de espectrofotometria de fluorescência de raios-X (XRF) foram feitas no Laboratório de Física Nuclear Aplicada da Uniso (Lafinau). Imagens da estrutura do gel foram obtidas por microscopia eletrônica de varredura (MEV) no Laboratório de Processamento de Imagens e Sinais da Uniso (Lepisus). No Laboratório de Avaliação de Bioatividade e Toxicologia de Nanomateriais da Uniso (Labiton) foram desenvolvidos ensaios de citotoxicidade. Já no Laboratório de Biomateriais e Nanotecnologia da Uniso (LaBNUS) foram feitas análises de espectrofotometria de infravermelho (FTIR) e de calorimetria diferencial de varredura (DSC). E, finalmente, no Laboratório de Pesquisas Toxicológicas da Uniso (Lapetox) foram feitos os ensaios *in vivo* e histológicos.

Esta tese de doutorado foi elaborada no formato de artigos científicos de acordo com as orientações para apresentação de dissertações/teses do Programa de Pós-graduação em Ciências Farmacêuticas da Uniso. Na “Introdução” foi feita apresentação do tema. Na “Revisão de Literatura” foi elaborada uma revisão bibliográfica abordando pele, psoríase, tratamentos da psoríase, curcumina e permeação transdérmica. Após o item “Objetivos”, o tópico “Resultados” foi produzido na forma de artigos. Por fim, em “Considerações Finais” discorreu-se sobre os manuscritos elaborados e feita uma avaliação geral do trabalho de pesquisa realizado.

O primeiro artigo científico **“Performance of choline geranate deep eutectic solvent as transdermal permeation enhancer: An *in vitro* skin histological study**” teve como foco buscar esclarecer como ocorre a facilitação da permeação dérmica da curcumina pelo líquido iônico geranato de colina, sendo publicado na revista *Pharmaceutics*, no ano de 2021. Em outros artigos e, mesmo em trabalhos realizados no nosso laboratório, já houve a comprovação da eficácia do líquido iônico como promotor de permeação dérmica. No entanto, havia ainda, uma lacuna a esclarecer sobre o “modus operandi” do geranato de colina na pele.

Outra preocupação foi avaliar a qualidade da curcumina comercialmente acessível para uso nos géis. Isto era um questionamento importante, pois não teria sentido desenvolver um produto para uso tópico, com curcumina de grau analítico (PA - para análise), em função do custo elevado do produto final. Assim, foram realizadas uma série de análises para a comprovação da qualidade de curcuminas obtidas como insumos, em farmácias de manipulação e em estabelecimentos que vendem produtos naturais. Estes ensaios estão apresentados no segundo artigo intitulado **“Characterization of commercially available turmeric for use in pharmaceutical products and food supplements”**, publicado em 2022, na revista *Journal Brazilian Chemical Society*.

O terceiro artigo **“Transdermal permeation of curcumin promoted by choline geranate ionic liquid: potential for the treatment of psoriasis**, foi publicado na Revista *Saudi Pharmaceutical Journal* em 2022. Este trabalho teve como propósito avaliar o efeito da concentração do geranato de colina na permeação da curcumina, visando definir a melhor concentração de líquido iônico no gel.

O quarto capítulo, na forma de artigo intitulado **“Gel de alfarroba e curcumina com geranato de colina: ensaios de estabilidade”** apresentou ensaios de estabilidade do gel visando garantir a qualidade, eficácia e a segurança do produto.

Por fim, o quinto artigo **“Transdermal permeation assays of curcumin aided by CAGE-IL: *in vivo* control of psoriasis** apresentou os resultados da eficácia do gel de curcumina no tratamento da psoríase através de ensaios *in vivo* utilizando camundongos como modelo experimental. Este artigo foi publicado na revista *Pharmaceutics* em edição especial *Curcumin in Biomedical Applications*.

Os resultados também foram divulgados em 4 congressos científicos, sendo: i) I Congresso Brasileiro Interdisciplinar em Ciência e Tecnologia - CoBICET (Evento on-line, Brasil, 31 agosto a 04 setembro de 2020, **ANEXO A**); ii) V Congresso da Associação Brasileira de Ciências Farmacêuticas - ABCF (Evento on-line, 1-3 outubro de 2020, **ANEXO B**); iii) V International Workshop on Rational Use of Medicines / III Workknowledge of Evidence-Informed Policy / III Symposium ISPE BrazInt RIG/ I Symposium of ISPE Brazilian Student Chapters (Evento on-line, 1-5 março de 2021 2021, **ANEXO C**); iv) 13th International Congress of Pharmaceutical Sciences - CIFARP (Evento on-line, 3-6 novembro de 2021, **ANEXO D**) (2 resumos).

Em conjunto, os testes realizados buscaram contribuir para a comprovação da eficácia da curcumina carregada pelo geranato de colina no tratamento tópico da psoríase leve a moderada em ensaios *in vivo* e *in vitro*.

1 INTRODUÇÃO

A pele é o maior órgão do corpo humano com uma área de superfície de aproximadamente 2m^2 , sendo a primeira linha de defesa contra inúmeros patógenos externos, traumas mecânicos e químicos e danos causados pela luz ultravioleta. Além disto é responsável por manter a homeostase, a temperatura corporal e exercer funções endócrinas e exócrinas (CHAMBERS; VUKMANOVIC-STEJIC, 2020; VOLLONO *et al.*, 2019).

A pele é constituída por três camadas distintas: epiderme, derme e hipoderme. A epiderme é formada por camadas de células e, está ligada a uma derme subjacente com uma membrana basal, que coordena a troca de substâncias químicas entre os vasos sanguíneos e a epiderme. A estrutura da membrana basal é determinada pela interação de células epidérmicas, queratinócitos e fibroblastos da derme papilar, que produzem uma vasta série de proteínas, incluindo o colágeno tipo IV. A hipoderme é considerada como uma camada subcutânea formada por células de gordura, os adipócitos (SHIRSHIN *et al.*, 2017; SINGER; FINK; ANGELOVA, 2019).

As doenças de pele são as desordens mais comuns afetando pessoas em todo o mundo. Embora as taxas de mortalidade sejam mais baixas do que as de muitas outras patologias, os problemas de pele têm um impacto significativo na qualidade de vida das pessoas acometidas (PEÑA-JUÁREZ; GUADARRAMA-ESCOBAR; ESCOBAR-CHÁVEZ, 2021). A incidência dessas doenças, que incluem alergias, dermatite atópica, psoríase, entre outras, está aumentando nos últimos anos, especialmente em países desenvolvidos (OWCZARCZYK-SACZONEK; CZERWIŃSKA; PLACEK, 2018).

A psoríase é uma doença sistêmica crônica, autoimune, recorrente, na qual os processos inflamatórios são caracterizados pelo aparecimento de erupções cutâneas vermelhas com coceira, lesões e escamas (KULAWIK-PIÓRO; MIASTKOWSKA, 2021). Do ponto de vista patológico, consiste em dermatite crônica, com rápida e descontrolada proliferação de células epiteliais, hiperemia e densa infiltração linfocítica. Essencialmente, pode começar em qualquer fase da vida e persistir por um longo período com erupções permanentes ou periódicas (CALARA

et al., 2017; CAMPANI et al., 2020). Quando a psoríase afeta as articulações (artrite psoriásica), manifesta-se em artrite periférica, dactilite, entesite e espondilite (SINGH et al., 2019).

Pode ser incapacitante tanto pelas lesões cutâneas (fator que dificulta a inserção social), quanto pela presença da forma articular que configura a artrite psoriásica (ARNONE et al., 2019). A psoríase é uma doença associada a várias comorbidades. Uma das comorbidades mais frequentes é a depressão (VARMA et al., 2017). Os afetados pela psoríase têm uma maior propensão a desenvolver depressão, principalmente pelos sintomas típicos como o prurido, sangramento, descamação, envolvimento ungueal e fatores como o próprio tratamento.

Além destes fatores, é uma doença visível e desfigurativa, contribuindo para uma baixa autoestima, níveis aumentados de ansiedade, disfunção sexual e, até mesmo, tendência suicida (PUIG et al., 2018). Tais situações podem resultar de uma interação entre fatores genéticos e fatores externos, incluindo infecções microbianas, lesões na pele, distúrbios imunológicos, influências ambientais, clima e estresse. Contudo, os mecanismos moleculares subjacentes à patogênese desta doença são ainda não totalmente compreendidos (GENDRISCH et al., 2021).

Embora exista uma variedade de terapias medicamentosas tópicas e sistêmicas para o tratamento da psoríase, não apresenta cura e os esquemas terapêuticos devem ser otimizados de maneira a alcançar conformidade e benefícios ideais (DUTTA; CHAWLA; KUMAR, 2018a). Apesar da disponibilidade de diferentes agentes tópicos e opções terapêuticas sistêmicas, nenhum dos tratamentos fornece excelentes resultados clínicos sem o risco de significativos efeitos colaterais.

Nesta perspectiva, terapias alternativas têm sido avaliadas, com o uso de espécies vegetais com potencial ação antipsoriática como: *Capsicum annum*/*Capsicum frutescens* (pimentas), *Aloe vera* (babosa), *Matricaria recutita* (camomila), *Melaleuca alternifolia* (árvore de chá), *Mahonia aquifolium* (uva do Óregon), *Nigella sativa* (cominho preto), *Wrightia tinctoria* (oleandro de tintureiro), *Ipinia galangal* (gengibre) e *Curcuma longa* (açafrão) (MALEŠ et al., 2019; PRADHAN et al., 2018).

A *Curcuma longa* L. cujos sinônimos científicos são *C. domestica* Valetton e *Amomum curcuma* Jacq é uma monocotiledônea perene pertencente à família

Zingiberaceae, sendo originária do sudeste da Ásia (FUGITA *et al.*, 2012; GUIMARÃES *et al.*, 2020). Popularmente, no Brasil, é conhecida como açafrão, açafroeira, açafrão-da-terra, açafrão-da-Índia, batatinha amarela, gengibre dourado e mangarataia (BORGES *et al.*, 2019). Do rizoma desta planta obtêm-se um composto denominado curcumina que apresenta um amplo espectro de potencialidades biológicas (DUTTA; CHAWLA; KUMAR, 2018a; NARDO *et al.*, 2018). Mais recentemente, estudos apontam para a curcumina como um ativo em potencial para o tratamento da psoríase e em processos anti-inflamatórios em geral (NARDO *et al.*, 2018; SKYVALIDAS *et al.*, 2020).

Contudo a curcumina é praticamente insolúvel em água e apresenta baixa disponibilidade (GUIMARÃES *et al.*, 2020). Assim, para o uso em preparações tópicas é necessário utilizar substâncias que favorecem sua permeação. A permeação transdérmica visa o transporte de ingredientes farmacêuticos ativos (IFAs) através do tecido cutâneo, até às camadas mais profundas, com vantagens terapêuticas quando comparada com a administração de IFAs por via oral ou parentérica (SUN *et al.*, 2017a).

A pele é uma barreira natural de proteção, com baixa taxa de permeação. Assim, o desenvolvimento de sistemas promotores de permeação que promovam uma alteração transiente da estrutura do estrato córneo, possibilitando permeação mais eficaz de moléculas bioativas é um desafio a ser vencido. Nesta perspectiva, tem se buscado substâncias facilitadoras da permeação cutânea, denominadas promotores químicos. Os promotores químicos são substâncias capazes de melhorar o transporte de fármacos através da pele, pela redução temporária e reversível de suas barreiras e, conseqüente aumento da permeabilidade (OTTERBACH; LAMPRECHT, 2021).

Os líquidos iônicos surgiram como proposta inovadora para aumento de permeação cutânea de entidades proteicas de interesse. Alguns líquidos iônicos, como o geranato de colina, têm a capacidade de provocar alterações momentâneas na organização lipídica e proteica inter e intracelular do estrato córneo, permitindo a passagem de moléculas bioativas por eles carregadas (ZAKREWSKY *et al.*, 2016). Além disto, os líquidos iônicos podem ser incorporados com sucesso em sistemas

de liberação de ativos como emulsões, nano e micropartículas, géis, hidrogéis, entre outros.

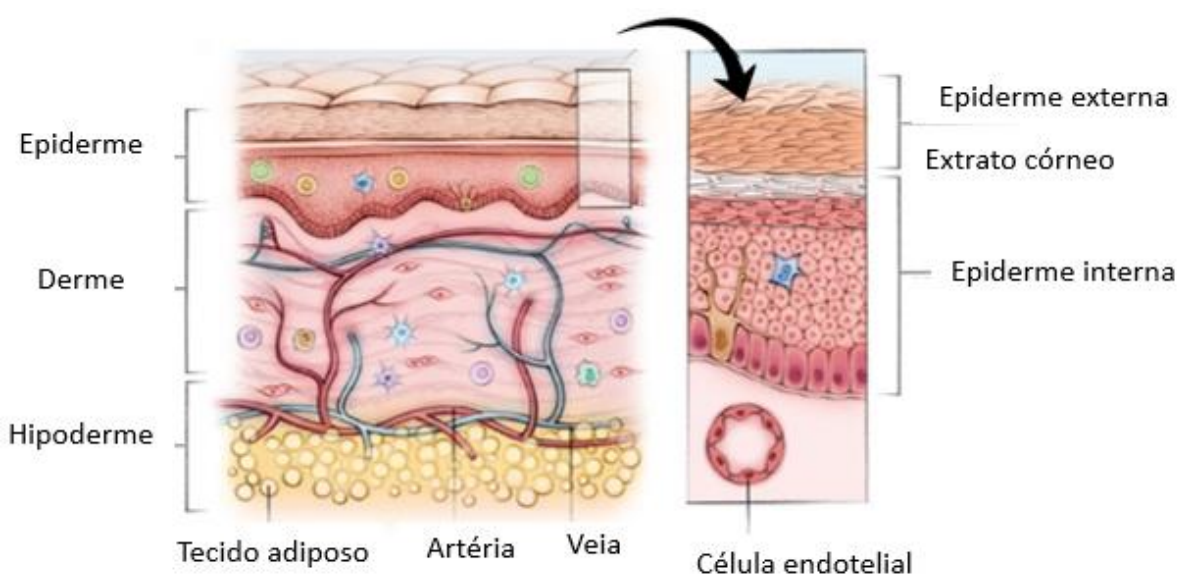
Com estes fatos em mente, objetivou-se o desenvolvimento de um gel com curcumina e o líquido iônico geranato de colina visando o tratamento de psoríase. A curcumina foi eleita em função de seu potencial para o tratamento da psoríase e o geranato de colina foi escolhido por suas características de biocompatibilidade e melhora na entrega transdérmica.

2 REVISÃO DA LITERATURA

2.1 Pele

A pele é composta por três camadas, sendo a epiderme, derme e hipoderme. A camada superior é a epiderme, uma fina camada de aproximadamente 0,1mm de espessura de epitélio escamoso estratificado, composta por quatro estratos de queratinócitos em estágios progressivos de diferenciação (DYRING-ANDERSEN *et al.*, 2020). A Figura 1 apresenta a morfologia da pele com suas camadas.

Figura 1. Ilustração das camadas da pele.



Fonte: Adaptado de DYRING-ANDERSEN *et al.*, 2020.

As células epiteliais são naturalmente programadas para desenvolver um crescimento que promove uma renovação da pele em fluxo contínuo e lento, com duração de aproximadamente um mês, desde o nascimento até à morte celular. A espessura de cada camada da pele varia de acordo com a região do corpo sendo classificada com base na espessura das camadas epidérmica e dérmica (BENHADOU; MINTOFF; DEL MARMOL, 2019; BOSCARIOL *et al.*, 2021).

O epitélio estratificado fornece uma barreira à prova de água do ambiente externo e evita a perda excessiva de água do corpo.

2.1.1 Epiderme

A epiderme é composta principalmente de queratinócitos. Também estão presentes os melanócitos, que formam uma barreira contra a radiação ultravioleta (UV) através da sintetização da melanina. A epiderme não possui suprimento sanguíneo próprio, mas, em contrapartida, é alimentada pelos vasos sanguíneos localizados na derme (BURAPAN *et al.*, 2020; NI; LAI, 2020).

Em sua constituição possui ainda uma variedade de grupos celulares e mediadores que, sinergicamente, promovem a proteção contra os agentes externos. Embora a ativação do sistema imune seja um mecanismo de proteção que objetiva remover os antígenos prejudiciais, a estimulação constante desta ativação através dos mecanismos celulares pode resultar num dano inflamatório crônico e iniciar estados patológicos onde se inclui, por exemplo, a psoríase (POLESE *et al.*, 2020; VON MEYENN; BERTSCHI; SCHLAPBACH, 2019).

As camadas da epiderme incluem o estrato basal, estrato espinhoso, estrato granuloso, estrato lúcido e estrato córneo (BENHADOU; MINTOFF; DEL MARMOL, 2019; DYRING-ANDERSEN *et al.*, 2020; SINGER; FINK; ANGELOVA, 2019; YOUSEF; ALHAJJ; SHARMA, 2021), sendo:

Estrato basal: conhecido como estrato germinativo, compreende a camada mais profunda, separada da derme pela lâmina basal, fixando-se à membrana basal por hemidesmossomos. Células células-tronco mitoticamente ativas, cuboidais a colunares estão presentes nessa camada e, produzem queratinócitos colunares em proliferação com uma extensa rede de queratinas K5 e K14. Melanócitos também estão presentes.

Estrato espinhoso: constituído de células espinhosas (8-10 camadas de células), possui células poliédricas irregulares com processos citoplasmáticos, (“espinhos”), que se estendem para fora e entram em contato com as células vizinhas por desmossomos. Os queratinócitos são diferenciados pela síntese das queratinas K1 e K10, involucrina e enzima transglutaminase. Células dendríticas também podem ser encontradas nesta camada.

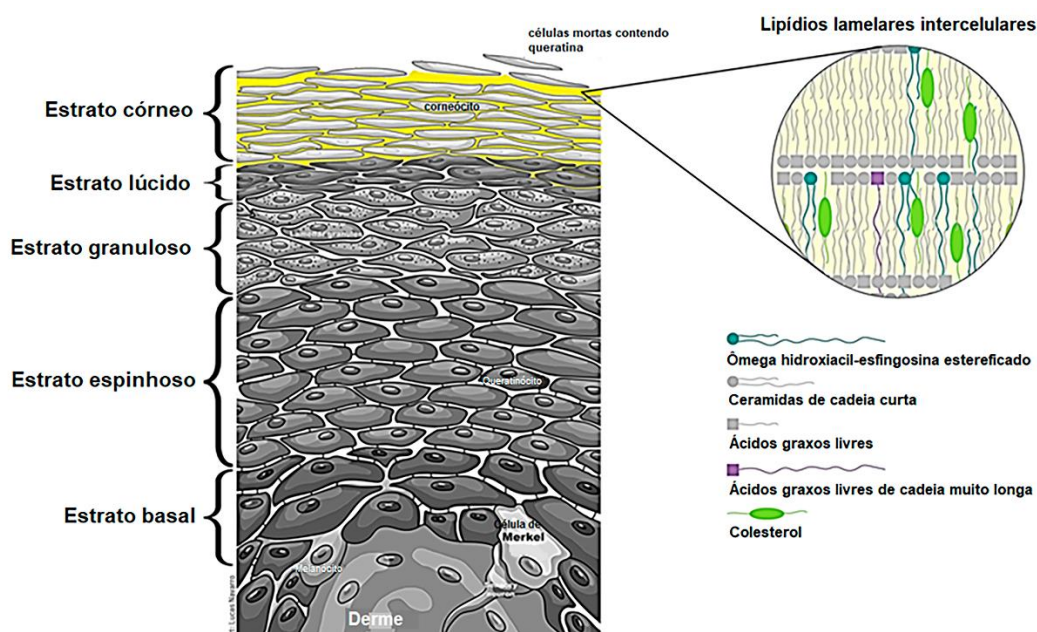
Estrato granuloso: células em forma de diamante com grânulos de querato-hialina e grânulos lamelares (3-5 camadas de células). Os queratinócitos são ricos em

grânulos de querato-hialina elétron-densos como o profilagrin e loricrina, que podem agregar-se, se reticulam e formam feixes. Os grânulos lamelares contêm glicolipídios que secretados para a superfície das células configurando uma cola, mantendo as células aderidas.

Estrato lúcido: presente nas palmas das mãos e plantas dos pés, é uma camada fina e transparente (2-3 camadas de células), que consiste em eleidina, um produto de transformação da querato-hialina.

Estrato córneo: representa a camada superior (20-30 camadas de células), composta de queratina e escamas córneas de queratinócitos mortos, conhecidas como células escamosas anucleadas. Esta camada difere em espessura, especialmente na pele calosa. Dentro dessa camada, há a secreção de defensinas pelos queratinócitos, representando a primeira defesa imunológica. A Figura 2 apresenta uma ilustração esquemática da epiderme.

Figura 2. Representação esquemática da epiderme



Fonte: Adaptado de BOSCARIOL *et al.*, 2021.

Os queratinócitos produzidos na epiderme tem como objetivo a diferenciação para corneócitos anucleados que compõem o estrato córneo. Como componentes do estrato córneo, a matriz lipídica extracelular e os corneócitos, possuem funções

diferentes. Os corneócitos, que são os queratinócitos diferenciados, fornecem reforço mecânico por protegerem células mitoticamente ativas a injúrias pelos raios UV, regulam a iniciação da inflamação mediada por citocinas e mantêm a hidratação (BENHADOU; MINTOFF; DEL MARMOL, 2019).

A matriz lipídica extracelular cria a organização do cemento hidrolípídico do estrato córneo, regula a permeabilidade, inicia a descamação do corneócito, possui atividade antimicrobiana, elimina toxinas e permite a absorção seletiva de substâncias químicas (POLESE *et al.*, 2020).

A manutenção da espessura da epiderme é mantida pelo equilíbrio fino entre a proliferação das células basais e a descamação dos corneócitos. O processo de descamação inicia-se com a destruição dos corneodesmossomos (desmossomos modificados) e é controlado por uma série de proteases e seus inibidores (YAMAMOTO, 2020).

2.1.2 Derme

A segunda camada da pele é a derme, uma camada mais espessa (até 3-4 mm dependendo do local do corpo), mas com um volume celular relativamente baixo em comparação com a epiderme. A derme é categorizada em subcamadas, sendo denominadas papilar e reticular (SHIRSHIN *et al.*, 2017).

A camada papilar produz extensões que alcançam a epiderme e contêm capilares para o transporte de nutrientes. A camada reticular possui apêndices cutâneos, como folículos pilosos, glândulas sebáceas e glândulas sudoríparas. A camada reticular apresenta-se mais espessa do que a derme papilar devido à densa concentração de fibras colágenas e reticulares que se entrelaçam. Ambas as camadas dérmicas abrigam fibroblastos, miofibroblastos, macrófagos, linfócitos e mastócitos. Os fibroblastos são responsáveis por sintetizar a matriz extracelular com colágeno, proteoglicanos e fibras elásticas conferindo integridade estrutural da derme (SHIRSHIN *et al.*, 2017; WOODLEY, 2017).

2.1.3 Hipoderme

A camada mais profunda da pele é a camada subcutânea, denominada hipoderme ou panículo adiposo. Esta camada consiste em tecido conjuntivo e adiposo, produtora de mediadores químicos, como fatores de crescimento, adipocinas e citocinas. Esta camada possui fibrócitos e adipócitos e é rica em proteoglicanos e glicosaminoglicanos. O tecido adiposo da pele armazena energia na forma de ácidos graxos e atua como órgão endócrino regulador da homeostase do metabolismo de lipídeos e carboidratos (RODRIGUES; KOSARIC; BONHAM, 2021).

Funcionalmente, a hipoderme, além de atuar como depósito nutritivo de reserva, participa no isolamento térmico e na proteção mecânica do organismo às pressões e aos traumatismos externos e facilita a motilidade da pele em relação às estruturas subjacentes (RIVITTI, 2018).

2.2 Permeação transdérmica

A administração transdérmica de moléculas bioativas oferece uma opção de administração indolor e ao mesmo tempo que evita o efeito de primeira passagem do metabolismo, em comparação com a via oral. No entanto, superar a barreira da pele é um considerável desafio (TANNER *et al.*, 2019a).

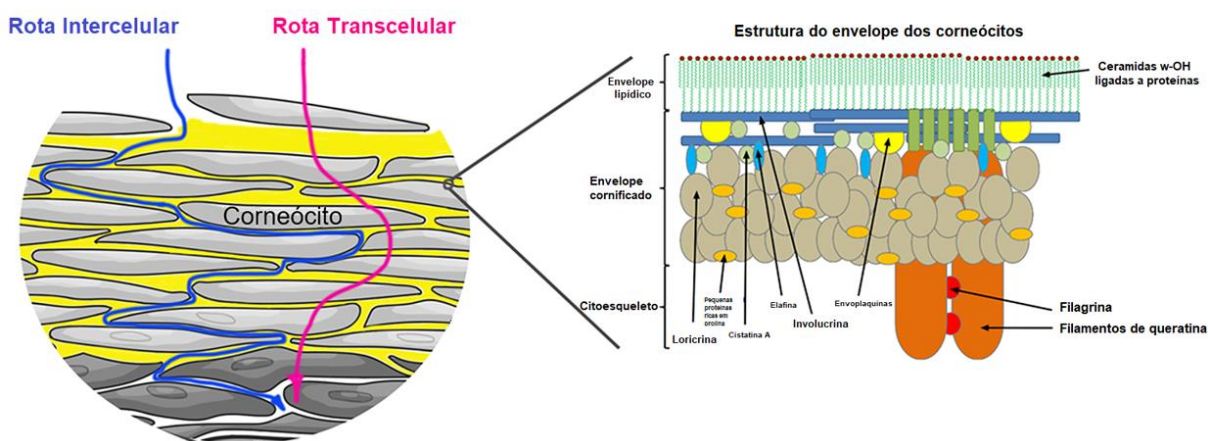
A ação de barreira da pele é exercida, sobretudo, pelo estrato córneo. Esta propriedade se baseia no conteúdo e na composição dos lipídios, em particular, no arranjo estrutural excepcional da matriz lipídica intercelular e no envelope lipídico ao redor das células (ISLAM *et al.*, 2020).

Os lipídios formam bicamadas ao redor da corneócito, que se assemelha ao modelo de "tijolo e argamassa" com os corneócitos como os tijolos e os lipídios intercelulares como a argamassa. Os lipídios intercelulares são, principalmente, as ceramidas, colesterol, ésteres de colesterol, ácidos graxos e uma pequena fração de sulfato de colesterol (BOSCARIOL *et al.*, 2021; JANTARAT *et al.*, 2018).

A permeação de substâncias químicas através da pele inclui a difusão através da epiderme intacta através de dois mecanismos propostos: a rota lipídica

intercelular entre os corneócitos e a rota trans celular atravessando os corneócitos e os lipídios intervenientes. A substância a ser permeada deve difundir em algum momento através da matriz lipídica intercelular (Figura 3) (ARCE *et al.*, 2020; JÚLIO *et al.*, 2017; MOSER *et al.*, 2001). Os anexos da pele, ou seja, folículos capilares e glândulas sudoríparas, podem também representar rotas para a permeação de substâncias químicas, no entanto, representam apenas 0,1% da superfície total da pele humana e a contribuição desse caminho geralmente é considerada pequena (AHAD *et al.*, 2021; ZSIKÓ *et al.*, 2020).

Figura 3. Mecanismos de permeação através do estrato córneo: através dos corneócitos e da matriz lipídica intercelular (rota ou via transcelular) e entre os corneócitos (rota ou via intercelular).



Fonte: Adaptado de BOSCARIOL *et al.*, 2021.

A difícil penetração de fármacos e medicamentos na pele, muitas vezes, reduz a eficácia das formulações tópicas. Entretanto, a penetração da pele pode ser aprimorada buscando-se aumentar a difusão dos fármacos na pele; aumentar a solubilidade do ativo na pele; e/ou aumentar a quantidade de saturação dessas substâncias na formulação (JÚLIO *et al.*, 2017).

2.3 Doenças da pele

As doenças de pele são um dos males mais comuns, afetando milhões de pessoas em todo o mundo. Existem mais de 4.000 doenças de pele e anexos cutâneos, sendo as mesmas responsáveis por 15% a 30% dos atendimentos ambulatoriais em sistemas de saúde (PIIPPONEN; LI; LANDÉN, 2020). Embora apresentem taxas de mortalidade mais baixas do que as de muitas outras doenças, os problemas de pele têm um impacto significativo na qualidade de vida das pessoas (PEÑA-JUÁREZ; GUADARRAMA-ESCOBAR; ESCOBAR-CHÁVEZ, 2021). A incidência dessas desordens, que incluem dermatites atópicas, alergias, infecções, doenças autoimunes, entre outras está aumentando nos últimos anos, especialmente em países desenvolvidos (NGUYEN; SOULIKA, 2019).

A homeostase cutânea é mantida pela coordenação e comunicação permanentes entre as células epiteliais e as células do sistema imunológico da pele. Qualquer ruptura da microbiota cutânea e/ou do mecanismo de reparo cutâneo leva a processos inflamatórios, desequilíbrios entre fatores pró-inflamatórios e anti-inflamatórios, ocorrendo as doenças dermatológicas e alérgicas (POLESE *et al.*, 2020; RODRIGUES; KOSARIC; BONHAM, 2021).

A doença de pele autoimune afeta o sistema imunológico e resulta na produção de autoanticorpos, ou seja, anticorpos contra autoantígenos. O conceito de sistema imunológico da pele pode ser visto como uma relação complexa entre células imunológicas, células epiteliais e o ambiente externo (VON MEYENN; BERTSCHI; SCHLAPBACH, 2019). As doenças autoimunes cutâneas ainda são um problema mundial e, muitas carecem de tratamentos eficazes (BENHADOU; MINTOFF; DEL MARMOL, 2019).

2.4 Psoríase

Esta doença pode ser definida como o desequilíbrio entre a proliferação e diferenciação celular na camada epidérmica da pele, que podem se multiplicar até 10 vezes mais rápido do que as células normais. Embora as principais causas da psoríase não sejam claramente identificadas, acredita-se que seja um distúrbio nos queratinócitos (DRVAR *et al.*, 2019; RAUT; WAIRKAR, 2018).

A psoríase era vista anteriormente como um distúrbio epidérmico que poderia ser tratado por agentes terapêuticos como metotrexato e arsênico, que podem limitar a hiper proliferação epidérmica. No entanto, a compreensão atual levou à conclusão de que a psoríase é uma doença inflamatória autoimune amplamente mediada pela ativação de células T auto reativas na pele (DAND *et al.*, 2020; GUIMARÃES *et al.*, 2020).

A psoríase é marcada por rápida recorrência, aparência peculiar, recidiva e com prejuízo social para o paciente, causando morbidade significativa. A psoríase não possui nenhum modelo animal específico para seu estudo, ao contrário de outras doenças autoimunes. Desta forma, a maioria das informações disponíveis é através de estudos clínicos, devido a essa dificuldade metodológica de estudo (TIMIS; ORASAN, 2018).

Este distúrbio dérmico acomete cerca de 2% da população mundial. No Brasil dados sobre a prevalência da psoríase apontam entre 1,10 a 1,31% da população, com destaque para as regiões Sul e Sudeste (DIBONAVENTURA *et al.*, 2018; ROMITI *et al.*, 2017; TODKE; SHAH, 2018). O registro de casos de psoríase não é obrigatório, o que significa que é difícil encontrar dados confiáveis (POLO *et al.*, 2020). Em 20% dos pacientes manifesta-se na forma de moderada a severa, embora não seja contagiosa nem mortal, diminuí o desenvolvimento social e profissional de seus portadores. O seu impacto negativo é significativo pois, estudos demonstram que 30% dos portadores da patologia, principalmente na forma de moderada a severa, já tentaram suicídio (BUDU-AGGREY *et al.*, 2019).

A etiologia exata da doença permanece desconhecida, entretanto, a origem é atribuída a vários fatores genéticos, imunológicos e ambientais que atuam como gatilhos, como trauma, estresse emocional e físico, alguns medicamentos e infecções. Apesar de estar associada a uma predisposição genética, a transmissão pais-filhos não segue um padrão Mendeliano, caracterizando-se como herança multifatorial, e não sendo explicada unicamente pela associação de antígenos a complexos de histocompatibilidade (NI; LAI, 2020; TABESHPOUR; HASHEMZAEI; SAHEBKAR, 2018). A origem genética da psoríase é evidente pela prevalência familiar da doença, sendo mais comum em parentes de primeiro e segundo graus de

pacientes com psoríase, em comparação com a população em geral. Aproximadamente 70% dos indivíduos com psoríase infantil relataram um histórico positivo na família (LIANG *et al.*, 2017; NEDOSZYTKO *et al.*, 2020).

Esta patologia correlaciona-se com a depressão, doenças crônicas cardiovasculares e diabetes. Devido a sua cronicidade, os tratamentos objetivam diminuir o impacto social e físico dos portadores, prolongando o tempo entre recidivas e potencializando a qualidade de vida (DRVAR *et al.*, 2019; RAHARJA; MAHIL; BARKER, 2021). Pesquisas atuais estabelecem que a psoríase é o produto de interações entre fatores ambientais e um fundo genético complexo (CAPON, 2017; DYRING-ANDERSEN *et al.*, 2020; JABEEN *et al.*, 2020).

2.4.1 Fisiopatologia

A patogênese da psoríase é controversa, acredita-se que ocorra como um distúrbio primário dos queratinócitos, ou como consequência de alterações na resposta imunológica, no combate contra um ou mais antígenos não identificados. A maioria dos autores faz referência a uma patologia imunomediada, devido a interação entre suscetibilidade genética individual e fatores ambientais frente um fator imunológico, através da inter-relação dos queratinócitos hiperplásicos epidérmicos com diversos tipos de células imunológicas envolvidas (CAMPANI *et al.*, 2020; ZHOU *et al.*, 2019).

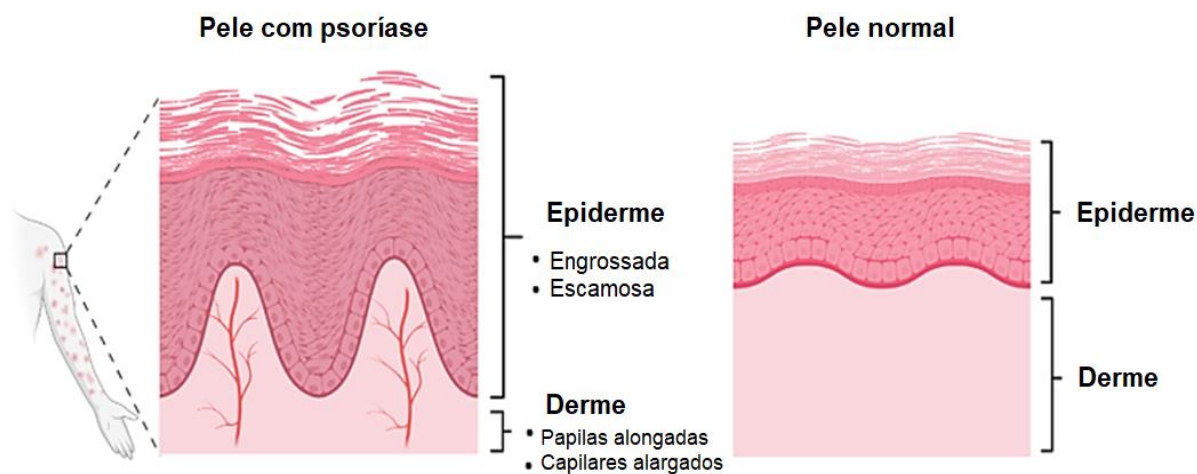
As características marcantes da psoríase incluem modificação profunda da epiderme, como hiper proliferação e diferenciação alterada de queratinócitos, concomitante à presença de um infiltrado inflamatório proeminente e neoangiogênese. Entende-se que as células T, juntamente com as células imunes inatas, produzam uma citocina efetora chave, IL-17A, que desencadeia a modificação epidérmica (BREMBILLA; SENRA; BOEHNCKE, 2018).

A fisiopatologia da psoríase é altamente complexa e considerada uma doença multifatorial com diminuição do tempo de renovação da epiderme, função de barreira prejudicada da camada epidérmica da pele e, diferenciação de queratinócitos, resultando no espessamento da camada epidérmica. Formas graves da doença afetam cerca de 10% dos pacientes; a artrite psoriática pode estar presente em 20%

a 30% dos casos. Cerca de 70% a 80% dos pacientes têm psoríase leve, controlada apenas por terapias tópicas (LUO *et al.*, 2021).

Dentre estudos a respeito dos processos fisiopatológicos da psoríase, duas hipóteses são consideradas, a primeira propõe o crescimento e reprodução excessiva dos queratinócitos devido a epiderme defeituosa resultando em psoríase (DUTTA; CHAWLA; KUMAR, 2018). O ciclo natural de crescimento celular varia em 28-30 dias, as células amadurecem e começam a se desprender da superfície da pele, na psoríase, as células começam a amadurecer em 3-6 dias e se transferem para a camada córnea da epiderme o que resulta em uma quantidade excessiva de células imaturas na superfície ilustrado na Figura 4 (MARANDUCA *et al.*, 2020).

Figura 4. Ilustração das diferenças na epiderme e derme em pele normal e com psoríase.



Fonte: Elaboração própria.

As características histológicas da placa psoriática fornecem uma visão da complexidade imunológica da doença. Há formação de acantose (hiperplasia epidérmica) devido a um aumento de queratinócitos, que recobrem infiltrados inflamatórios compostos de células dendríticas dérmicas, macrófagos, células T e neutrófilos, a retenção de núcleos de queratinócitos no estrato córneo (paraqueratose) destaca ainda mais a importância dessas células no desenvolvimento da psoríase (RENDON; SCHÄKEL, 2019).

A proliferação de queratinócitos induzida pelas citocinas contribui para o espessamento da pele, aparência superficial escamosa, hiperplasia epidérmica, hiperqueratose e paraqueratose. O desequilíbrio entre proliferação e diferenciação torna-se um ciclo de auto amplificação, onde as citocinas e a homeostase alterada atuam sobre as células imunes para perpetuar a resposta inflamatória (MIRON *et al.*, 2020).

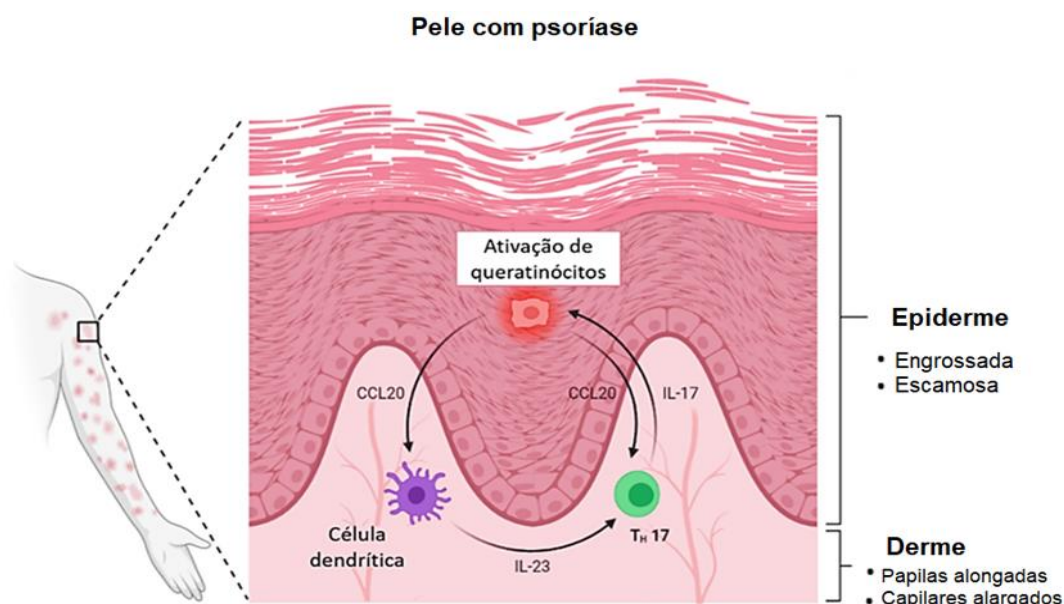
Outra hipótese atribui como uma doença imunomediada, que envolve interações dinâmicas entre vários tipos de células e numerosas citocinas em resposta aos gatilhos, culminando na interrupção na homeostase do sistema imunológico em indivíduos com predisposição genética (ZHANG; WU, 2018).

Na fisiopatologia da psoríase várias células estão envolvidas, incluindo as células de Langerhans, queratinócitos, células endoteliais e monócitos. As células linfócitos TCD4⁺ e TCD8⁺ (referidas como células Th17 e Th22, respectivamente), levam à produção de quantidades consideráveis de interleucinas IL-17 e IL-22 em placas psoriáticas, sendo consideradas a principal causa desta doença, onde a inflamação é o resultado do efeito combinado das células (VON MEYENN; BERTSCHI; SCHLAPBACH, 2019).

De acordo com sua função, as células Th17 podem ser categorizadas em células protetoras do hospedeiro ou células patogênicas inflamatórias. Sua função depende, primeiramente, das citocinas que promovem a diferenciação celular e, em segundo, do equilíbrio das moléculas efetoras produzidas (SRIVASTAVA *et al.*, 2017). As células Th17 ativadas por interleucina IL-23 desencadeiam autoimunidade e inflamação crônica, em resposta à inflamação do tecido mediada por IL-23 expressam e liberam citocinas efetoras, como interleucinas IL-17, IL-21, IL-22 (LIU *et al.*, 2020b).

As células Th17 geram interleucinas incluindo IL-17A, IL-17F e IL-22, representando fonte primária dessas moléculas. Induzem a proliferação de queratinócitos, e fator de necrose tumoral- α (TNF- α), quimiocina-1 (CXCL1) e quimiocina- 8 (CXCL8). O TNF- α acelera a infiltração de células pró-inflamatórias, linfócitos, monócitos e neutrófilos nas camadas da pele de acordo com a Figura 5 (POLESE *et al.*, 2020).

Figura 5. Mecanismo de ação da IL-23, que estimula células Th17 e induz a liberação de citocinas como IL-17 que ativam os queratinócitos.



Legenda: CCL20 – ligante quimiocina 20; IL-17 - interleucina 17; IL 23- interleucina 23; Th 17 – linfócito.

Fonte: Elaboração própria.

A identificação de células Th17 e Th22 com suas interleucinas IL-17 e IL-23, respectivamente, se revelaram como alvos para a descoberta de novos fármacos. A descoberta do mecanismo de ação da interleucina-17 (IL-17) e da IL-23 no desenvolvimento da psoríase acarretou a uma melhor compreensão dos eventos imunes patogênicos e levou a uma mudança de conduta no tratamento (YANG; OAK; ELEWSKI, 2021).

A interleucina IL-17 é uma citocina cuja família consiste em seis membros, de IL-17A a IL-17F, frequentemente o termo IL-17 se refere a IL-17A, que inicialmente era considerada uma citocina “única”, produzida exclusivamente por células T na psoríase. Outros membros da família IL-17 (IL-17C, E e F) também podem estar envolvidos na patogênese da psoríase. Recentemente, observou-se que a IL-17 não é produzida apenas por células adaptativas, mas também por basófilos, mastócitos e neutrófilos na pele (GALLAIS SÉRÉZAL *et al.*, 2018; SRIVASTAVA *et al.*, 2017).

Na pele, a IL-17A acarreta o aumento da proliferação e diferenciação dos queratinócitos e contribui para a ruptura da barreira cutânea ao diminuir a expressão de moléculas envolvidas na diferenciação dos queratinócitos. Além disto, participa na criação e amplificação da rede inflamatória, promovendo a liberação de peptídeos antimicrobianos, citocinas e quimiocinas pró-inflamatórias (EYERICH; DIMARTINO; CAVANI, 2017).

Verifica-se em pacientes com psoríase, que as concentrações de IL-17 aumentam nas lesões cutâneas e no sangue periférico, mas também na pele íntegra. Há evidências que indicam que as principais fontes produtoras de IL-17A em pacientes com psoríase são os neutrófilos, células T17, mastócitos, células T22 e células linfóides inatas nas lesões cutâneas (LIU *et al.*, 2020b). A IL-23 pode desempenhar seu papel independente da IL-17, promovendo a hiperplasia epidérmica e proliferação de queratinócitos através do aumento da expressão da queratina, em associação com IL-17, aumentando a acantose dérmica, recrutamento de neutrófilos e infiltração de células produtoras de IL-22 e IL-17 na pele lesionada (YANG; OAK; ELEWSKI, 2021).

Os neutrófilos são proeminentes na pele com lesões psoriásicas e, podem se acumular no estrato espinhoso e no estrato córneo. Dyring-Andersen e colaboradores (2017a; 2017b) sugeriram que os neutrófilos, contribuem para a inflamação da pele ao produzir IL-17 e IL-22. Além disto, os neutrófilos constituindo em mais de 50% dos leucócitos no sangue, podem participar de comorbidades cardiometabólicas associadas à psoríase grave. O aumento da frequência de neutrófilos circulantes foi observado em pacientes com psoríase, concomitante com um estado ativado dessas células, eles podem fornecer uma ligação entre a psoríase e a doença cardiovascular (DYRING-ANDERSEN *et al.*, 2017).

A repetição das placas psoriáticas em locais anteriormente acometidos propõem o papel da memória imunológica específica do tecido nos episódios da doença. Isso implica células de memória residente em tecido, frequentes em distúrbios inflamatórios crônicos, devido às suas capacidades de sobrevivência a longo prazo e à falta de migração de seu determinado local. Especula-se que essas células podem ser reativadas rapidamente para fornecer uma fonte precoce de IL-17

e contribuir para a recorrência de lesões da psoríase (DYRING-ANDERSEN *et al.*, 2017a; CLARK, 2015).

Estudos sobre a patogênese molecular da psoríase revelaram que os queratinócitos produzem proteínas e peptídeos antimicrobianos (AMPs), defensinas e catelicidinas, que estimulam a atividade das células do sistema imunológico por meio de múltiplos mecanismos (MA; SHAO; WANG, 2020).

A psoríase é uma condição na qual, tanto a imunidade inata como a adaptativa participam no processo patogênico. Os AMPs participam das principais funções da imunidade inata (ação direta sobre patógenos, ativação de células inflamatórias e mediadores teciduais e ação na imunidade adaptativa) sendo assim, estão envolvidos na iniciação e nos estágios iniciais da psoríase (LIU *et al.*, 2020c; SAULITE; PILMANE; KISIS, 2017).

Os AMPs integram primeira linha da defesa de um hospedeiro contra patógenos. São pequenos peptídeos compostos de 12-50 resíduos de aminoácidos e possuem cargas positivas e peptídeos anfipáticos com estruturas α e β -helicoidais ligadas por pontes dissulfeto. Mais de 1800 tipos de AMPs foram identificados e mais de 20 são encontrados na pele humana (MANGONI; MCDERMOTT; ZASLOFF, 2017). Essas características permitem que este compostos interajam quimicamente com grupos de fosfolipídios carregados negativamente e com as cadeias de ácidos graxos hidrofóbicos das membranas microbianas, destruindo os microrganismos ao romper a membrana celular e liberar componentes do citoplasma (ALECU *et al.*, 2020; BECHINGER; GORR, 2017). A carga catiônica está relacionada à atividade antibacteriana e à hidrofobicidade à atividade hemolítica. Alguns AMPs têm a capacidade de atravessar a camada lipídica, penetrando no interior da célula, bloqueando ou inibindo suas funções, promovendo destruição de organelas ou fragmentação do ácido desoxirribonucléico (DNA) (LEI *et al.*, 2019). Levando-se em conta as propriedades antibacterianas conhecidas dessas pequenas proteínas, explica-se a presença relativamente baixa de infecções bacterianas nas lesões psoriáticas (PRASAD *et al.*, 2020). As lesões psoriáticas expressam grandes concentrações de AMPs, como catelicidina, β -defensinas, proteínas S100, ribonuclease (RNase) 7, lisozima, elafina e lipocalina associada a neutrófilos

gelatinase (RAHARJA; MAHIL; BARKER, 2021). Os queratinócitos, sob a ação de diversos fatores traumáticos, secretam AMP (β -defensinas) em elevadas quantidades, além da ativação antibiológica, também têm forte ação pró-inflamatória (quimiocinas e promoção da secreção de citocinas) (ZHANG; GALLO, 2016).

A influência da microbiota da pele e do intestino na saúde humana, têm atraído a atenção dos pesquisadores nos últimos anos (COSTA, 2021). Os filos *Actinobacteria*, *Bacteroidetes*, *Firmicutes* e *Proteobacteria* e os gêneros bacterianos *Cutibacterium*, *Corynebacterium* e *Staphylococcus* residem na pele humana saudável. O microbioma intestinal é composto de bactérias anaeróbicas Gram-negativas e Gram-positivas, incluindo *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Fusobacterium*, *Ruminococcus*, com destaque para os mais dominantes na microbiota intestinal, *Bacteroidetes* e *Firmicutes*. A interação entre os microrganismos comensais e o hospedeiro ocorre através do reconhecimento dos padrões moleculares por receptores de reconhecimento específicos. O resultado dessa interação é a modulação do sistema imunológico humana realizada pela microbiota (LUGER; LOSER, 2018; OLEJNICZAK-STARUCH *et al.*, 2021).

O termo microbioma foi sugerido pela primeira vez pelo ganhador do Prêmio Nobel de Medicina (1958), Dr. Joshua Lederberg para descrever o genoma coletivo da microbiota do corpo humano, incluindo bactérias comensais, simbióticas, patológicas, arqueias e eucariotos que colonizam o corpo humano. A composição da microbiota pode variar significativamente dependendo do hospedeiro, tais como, idade, sexo, sistema imunitário, presença de comorbidades, nível de higiene, medicamentos utilizados e condições externas (por exemplo, clima e localização geográfica) (CARDOSO, 2015; KOMINE, 2020; TETT *et al.*, 2017).

Vários estudos mostraram que uma disbiose da pele e do intestino pode levar ao desenvolvimento de doenças, como a psoríase (HSU; FUNG; CHEN, 2020; OKADA *et al.*, 2020). Infecções por bactérias e fungos têm sido associadas à promoção da psoríase, sendo o mecanismo sugerido o da produção de enterotoxinas. Disbiose com exacerbação do crescimento de *Streptococcus pyogenes* e *Staphylococcus aureus* são observadas em 60% dos pacientes com psoríase. Fungos como a *Candida albicans*, que apesar de fazer parte da microbiota

humana, frequentemente são detectados na pele ou nas mucosas de pacientes com psoríase (LEE *et al.*, 2018; PIETRZAK *et al.*, 2018; YAN *et al.*, 2017).

Há uma interação constante entre receptores *Toll-like*, proteínas de reconhecimento de peptidoglicanos, peptídeos antimicrobianos, citocinas e a microbiota da pele humana. As catelicidinas, peptídeos antimicrobianos, são produzidos pelos queratinócitos como resultado do contato com os microrganismos comensais. As células dendríticas ativadas pela ligação com a β -glucana microbiana, expressa pela *Candida albicans*, produzem a interleucina 36 alfa, que leva ao desenvolvimento do fenótipo psoriasiforme, demonstrando o papel dessa interação na patogênese da psoríase (HASHIGUCHI *et al.*, 2018). A proteína M do *Streptococcus pyogenes*, colonizador da pele com psoríase, exibe mimetismo molecular com a queratina tipo I de 50 kDa (DEI-CAS *et al.*, 2020). Esse mimetismo ativa células T auto reativas estimulando a inflamação e ao desenvolvimento da psoríase. A beta defensina, é uma proteína antimicrobiana presente no sangue e na pele dos pacientes com psoríase sendo relacionada com a concentração de IL-17, demonstrado a participação do microbioma da pele com a doença (PRAST-NIELSEN *et al.*, 2019).

O ato de coçar causado pela coceira de lesões de pele psoriásicas, pode danificar a pele, portanto, algumas bactérias podem ser encontradas na derme ou mesmo no sangue periférico, onde facilmente encontram células imunes e causam inflamação congênita e adaptativa. Isso causa disbiose da microbiota da pele observada na redução significativa da população de *Corynebacterium spp.*, *Lactobacillus spp.*, *Burkholderis spp.* e *Cutibacterium acnes* na pele psoriática lesionada em comparação com a pele saudável (LANGAN *et al.*, 2018).

Na psoríase a microbiota intestinal exhibe aumento de *Actinobacteria* e *Firmicutes*, configurando um padrão de barreira epitelial intestinal prejudicada, resultando na estimulação de células T reguladoras, transporte de carboidratos e quimiotaxia bacteriana (LUGER; LOSER, 2018). Também, a excessiva ocupação do intestino por *Candida albicans*, *Malassezia* e *Staphylococcus aureus* pode resultar em piora do fenótipo da psoríase. As alterações na microbiota associadas à psoríase podem induzir uma

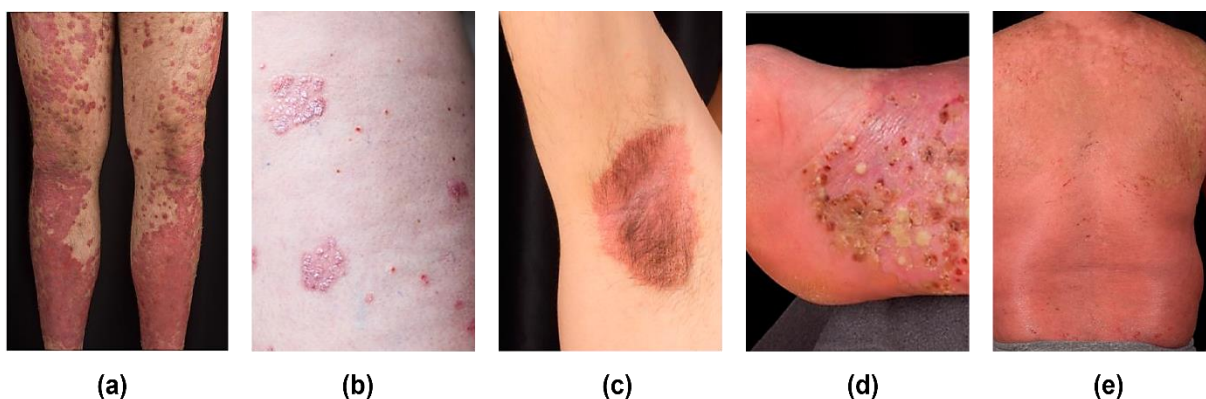
resposta inflamatória ativando as citocinas IL-23, IL-17, IL-22, interferon gama (IFN- γ), inibindo a produção das células T reguladoras, o que leva ao crescimento não desejado de queratinócitos (OLEJNICZAK-STARUCH *et al.*, 2021).

Infecções por vírus também estão associados ao início da psoríase. O vírus da Imunodeficiência Humana (HIV) é um fator de risco conhecido associado à psoríase (ARELLANO *et al.*, 2017). Outros vírus, como do papiloma, retrovírus também foram relacionados, mas os mecanismos precisos dessas correlações ainda precisam ser mais bem compreendidos (TAKESHITA *et al.*, 2017b).

2.4.2 Apresentações clínicas

A psoríase se manifesta de várias formas clínicas com base nas estruturas das lesões cutâneas, localização, idade de início e curso da doença (RAHARJA; MAHIL; BARKER, 2021). De acordo com as apresentações clínicas a psoríase é classificada em cinco tipos (LLAMAS-VELASCO *et al.*, 2017; RENDON; SCHÄKEL, 2019). A Figura 6 apresenta imagens com manifestações clínicas da psoríase.

Figura 6. Manifestações clínicas da psoríase. **(a)** psoríase vulgar, **(b)** psoríase eruptiva, **(c)** psoríase inversa, **(d)** psoríase pustulosa palmo plantar, **(e)** psoríase eritrodérmica.



Fonte: Adaptado de Rendon e Schäkel (2019).

a) Psoríase vulgar: conhecida por psoríase em placas, sendo a forma mais prevalente, afetando aproximadamente 80% dos indivíduos com psoríase.

Apresenta-se em placas rosa salmão bem demarcadas com escamas branco-prateadas, tipicamente em distribuição simétrica e afetando as superfícies extensoras (especialmente cotovelos e joelhos), tronco e couro cabeludo (CHANDRA *et al.*, 2015; FERRELI *et al.*, 2018; SCHÖN; ERPENBECK, 2018).

b) Psoríase eruptiva: é a segunda forma mais comum. Causa uma erupção simétrica aguda de pápulas/placas em gotas envolvendo principalmente o tronco e os membros, que podem evoluir posteriormente para psoríase em placas. Tipo mais comum observado em crianças e pessoas com menos de 30 anos, menos escamosa e com placas de tamanho menor (BOCHÉNSKA *et al.*, 2017; BUDU-AGGREY *et al.*, 2019).

c) Psoríase inversa: conhecida como psoríase intertriginosa ou flexural. Apresenta sem muitas descamações e pode afetar as axilas, áreas submamárias e genitais. Mais prevalente em indivíduos obesos e com sobrepeso e com dobras cutâneas profundas (NGUYEN *et al.*, 2018; TAKESHITA *et al.*, 2017a).

d) Psoríase pustulosa: assume a forma de pustulosa palmo-plantar. Os pacientes podem manifestar febre e os exames de sangue geralmente revelam neutrofilia e marcadores inflamatórios elevados (LIANG *et al.*, 2017; RAHARJA; MAHIL; BARKER, 2021; TVEIT, 2019).

e) Psoríase eritrodérmica: uma complicação rara, mas muito séria que pode se manifestar como resultado da progressão da psoríase crônica em placas, com risco de vida devido a complicações, incluindo hipotermia, infecção, lesão renal aguda e insuficiência cardíaca (RENDON; SHÄKEL, 2019).

Vários gatilhos estão associados aos episódios de psoríase, que podem variar de pessoa para pessoa e incluem, obesidade, infecções, lesões na pele, estresse, uso de anti-inflamatórios não esteroidais, β -bloqueadores, lítio e antimaláricos. Alergias, dieta, clima, tabagismo, e uso de álcool também podem ser desencadeadores da psoríase (LIU *et al.*, 2020c; REICH *et al.*, 2017). Tabagismo e consumo de álcool estimulam a produção de citocinas pró-inflamatórias, exacerbando gravidade da psoríase. Esses indivíduos apresentam alto risco de desenvolver psoríase, especialmente formas graves. Em contrapartida, dieta sem glúten, rica em vitaminas D, B12, selênio e ômega-3 tem benefícios em pacientes

com psoríase. Além disso, o estresse mental e a depressão facilitam o início e sua exacerbação (MIRON *et al.*, 2020; TAKAHASHI; YAMASAKI, 2020).

Diversos estudos relataram a coexistência da psoríase e doenças crônicas graves, destacando as doenças cardiovasculares, síndrome metabólica, hipertensão, dislipidemia, diabetes mellitus, esteatose hepática não alcoólica, esclerose múltipla, doença de Crohn, ansiedade, depressão e linfoma (LI *et al.*, 2021; TOKUYAMA; MABUCHI, 2020).

2.4.3 Comorbidades

Além de seus efeitos lesivos na pele, a psoríase está associada a várias comorbidades, incluindo doença cardiovascular, síndromes metabólicas (combinação de obesidade, dislipidemias, hipertensão e hiperglicemia), artrite psoriática, depressão, doença de Crohn, colite ulcerosa, nefrotoxicidade induzida por fármacos, doença renal crônica e doença hepática gordurosa não alcoólica (JOSHI *et al.*, 2018; NEKTALOVA; GOLDENBERG, 2019). Sendo assim, os fatores de risco para psoríase podem ser classificados em dois grupos, fatores de risco extrínsecos e intrínsecos (KAMIYA *et al.*, 2019).

As comorbidades psoriáticas têm sido associadas à natureza inflamatória crônica da psoríase, onde pacientes, geralmente, apresentam aumento de mediadores inflamatórios no sangue, como proteína C reativa, desregulação dos genes inflamatórios e do metabolismo lipídico relacionados a doenças cardiovasculares. Portanto, os pacientes com psoríase costumam apresentar taxas de mortalidade e hospitalização mais altas do que a população em geral (TAKESHITA *et al.*, 2017b).

Em pacientes com psoríase, as lesões cutâneas podem surgir em áreas não envolvidas, após algum dano na pele, por agentes mecânicos, químicos ou biológicos, sendo isso conhecido como fenômeno de Koebner. Exposição a radioterapia, radiação ultravioleta (UV) e, até uma leve irritação da pele foram relatados como desencadeadores de novas lesões (NEKTALOVA; GOLDENBERG, 2019). Após um choque cutâneo em uma lesão em desenvolvimento para psoríase, a proliferação de queratinócitos e a expressão de NGF (fator neurotrófico) em

queratinócitos basais são eventos iniciais e, que precedem a migração a epiderme de linfócitos T (ZHANG, 2019).

Vários poluentes do ar, como hidrocarbonetos aromáticos policíclicos, compostos orgânicos voláteis, óxidos, partículas micronizadas, ozônio, metais pesados e radiação UV agredem a pele induzindo estresse oxidativo que neutraliza as defesas antioxidantes da pele (FERNÁNDEZ-ARMENTEROS *et al.*, 2019). O cádmio é um dos poluentes atmosféricos que afetam a patogênese da psoríase e, estudos apontam que portadores de psoríase grave possuem mais cádmio no sangue em comparação com a população em geral (LIAW *et al.*, 2017).

A ação de alguns medicamentos e drogas também pode ser relacionada a psoríase. A psoríase ligada a medicamentos se manifestaria como psoríase em placas, psoríase palmoplantar, psoríase ungueal, psoríase do couro cabeludo, psoríase pustulosa e psoríase eritrodérmica. Na maioria dos casos, os estudos histopatológicos são indistinguíveis dos da psoríase convencional (AMIN *et al.*, 2020). No entanto, correlacionar causas da psoríase com o uso de medicamentos não constitui uma prática de fácil realização porque o período de latência entre o início da medicação e o aparecimento das lesões cutâneas psoriáticas pode variar, consideravelmente, entre os medicamentos (HAJDARBEGOVIC; BALAK, 2017). Há situações em que a exacerbação da psoríase pode persistir mesmo após a suspensão do medicamento suspeito (HAJDARBEGOVIC; BALAK, 2017; KAMIYA *et al.*, 2019).

Dentre os medicamentos associados com a manifestação de psoríase, destacam-se os β -bloqueadores, lítio, antimaláricos, interferons, imiquimode, inibidores da enzima de conversão da angiotensina, terbinafina, tetraciclina, anti-inflamatórios não esteroidais e fibratos. Os mecanismos da psoríase relacionada a medicamentos ainda precisam ser mais estudados e os mecanismos moleculares são complexos (HAJDARBEGOVIC; BALAK, 2017; LIU *et al.*, 2020a).

O uso de vacinação pode frequentemente desencadear e exacerbar a psoríase, como as vacinas para influenza, Bacilo Calmette-Guerin (BCG), tétano-difteria e adenovírus. Especula-se que esses imunizantes produzam respostas nos linfócitos T-Helper Th-1 e Th-17, células importantes para a fisiopatologia.

Entretanto, os mecanismos induzidos pela vacinação ainda não foram totalmente compreendidos e, a promoção de psoríase induzida por vacinação é muito baixa (LOPEZ *et al.*, 2017; YONEYAMA *et al.*, 2019).

A prática do tabagismo e o consumo de álcool têm sido associados à psoríase. O tabagismo está fortemente associado às lesões pustulosas da psoríase, com tendência no aumento do risco de psoríase com o aumento do consumo em anos ou maços de cigarros (NGUYEN *et al.*, 2019). Em relação ao consumo de álcool não há um consenso de evidências para estabelecer se o consumo de álcool é um fator de risco, entretanto, observa-se que o consumo de álcool é maior em pacientes com psoríase do que na população em geral. Os efeitos podem ocorrer por vários mecanismos, como aumento da suscetibilidade a infecções, estimulação da proliferação de linfócitos e queratinócitos e produção de citocinas pró-inflamatórias (SVANSTRÖM; LONNE-RAHM; NORDLIND, 2019; ZINK *et al.*, 2016).

Os distúrbios metabólicos são comuns em pacientes com psoríase e, a obesidade está fortemente associada ao início e exacerbação da psoríase (BARREA *et al.*, 2016; JENSEN; SKOV, 2017). Os níveis plasmáticos e teciduais de leptina, um regulador metabólico, são mais elevados em pacientes com psoríase, a leptina aumenta as funções imunológicas, produção de citocinas inflamatórias em macrófagos, quimiotaxia de granulócitos e aumento da proliferação de Th17, efeitos que contribuem para a exacerbação da psoríase (FRANCISCO *et al.*, 2018; NAYLOR; PETRI, 2016). Pacientes com psoríase têm uma prevalência maior de dislipidemia, que, provavelmente, aumenta com a gravidade da doença (MAHIL *et al.*, 2019). Os distúrbios lipídicos têm se mostrado em estudos como parte importante na patogênese da psoríase. Os resultados apontam que o aumento no plasma do colesterol total, colesterol LDL (Low density lipoproteins ou Lipoproteínas de baixa densidade) e/ou triglicerídeos e diminuição do colesterol HDL (High density lipoproteins ou Lipoproteínas de alta densidade) são características metabólicas de pacientes psoriáticos (WANG *et al.*, 2021).

A hipertensão e o diabetes mellitus tipo I e II são doenças crônicas que estão relacionadas fisiologicamente com a psoríase. Pacientes com psoríase possuem risco consideravelmente maior de diabetes mellitus tipo II, no entanto, a prevalência

não se correlaciona com a idade do paciente ou gravidade da psoríase (AMIN *et al.*, 2020; HAJDARBEGOVIC; BALAK, 2017).

Na patogênese do diabetes mellitus, citocinas pró-inflamatórias e TNF- α estão envolvidas. As células Th1 e Th17 e podem contribuir para o início do diabetes mellitus tipo I. Entretanto, não há comprovações que o diabetes mellitus tipo I contribua diretamente para a patogênese da psoríase. O conjunto TNF- α /IL-23/IL-17 desempenham função na patogênese da psoríase e do diabetes mellitus tipo I (EVANS *et al.*, 2020; KAMIYA *et al.*, 2019; ZHANG, 2019).

Pacientes com psoríase mostram maior prevalência e incidência de hipertensão, desenvolvendo o estágio mais grave da doença. Embora psoríase e hipertensão possuam fatores de risco compartilhados, como obesidade e tabagismo, os mecanismos dessa associação permanecem desconhecidos. Possíveis ligações etiológicas incluem desregulação do sistema renina-angiotensina, elevação dos níveis de endotelina1 e aumento do estresse oxidativo (KIM *et al.*, 2018). Estudos ainda correlacionam a deficiência de vitamina D em pacientes com psoríase. Entretanto a concentração sérica varia com alguns fatores, incluindo raça, nutrição e exposição à luz ultravioleta, e desta forma, os resultados dos estudos sobre a vitamina D e psoríase necessitam de interpretações mais cuidadosas (MALEKI *et al.*, 2016; MATTOZZI *et al.*, 2016).

2.5 Abordagem clínica

Pesquisas no campo da dermatologia obtiveram grandes avanços nos últimos 100 anos, desde o mapeamento do genoma humano (CAPON, 2017). Os estudos sobre a psoríase beneficiaram-se destas conquistas e influenciaram o desenho de várias terapias biológicas e inibidores moleculares (TIMIS; ORASAN, 2018; DAND *et al.*, 2020; OGAWA; OKADA, 2020).

Em pacientes com psoríase a escolha terapêutica baseia-se na avaliação da gravidade da doença, no impacto na qualidade de vida e na presença de comorbidades (artrite psoriática, distúrbios metabólicos e risco cardiovascular) (GORDON *et al.*, 2018).

A fim de se analisar e quantificar a gravidade da doença, utiliza-se o Índice de Gravidade da Área de Psoríase (PASI do inglês *Psoriasis Area and Severity Index*), um sistema de classificação desenvolvido em 1978, por Fredricksson e Pettersson (FREDRIKSSON; PETTERSSON, 1978). Atualmente, o PASI, a Área de Superfície Corporal (BSA do inglês *Body Surface Area*) e o Índice de Qualidade de Vida em Dermatologia (DLQI do inglês *Dermatology Life Quality Index*), índices desenvolvidos para medir a percepção do paciente sobre o efeito das doenças de pele, em seu dia a dia, ainda são considerados pelas diretrizes clínicas, como os instrumentos mais adequados para avaliar a gravidade da doença cutânea e seu impacto na qualidade de vida. Tais parâmetros de classificação são particularmente importantes para determinar estratégias para tratamentos terapêuticos (ARMSTRONG *et al.*, 2019; REICH *et al.*, 2017).

Parâmetros como o eritema, a espessura e a descamação são atribuídas valores de 0 a 4 - de acordo com a intensidade (0-ausente, 1-leve, 2-moderado, 3-intenso, 4-muito intenso) e a superfície da área corporal acometida (BSA) é expressa em porcentagem (de 1 a 100%), para o qual é dado um valor de 1 a 6. Dessa forma, 1 significa menos de 10% da área acometida; 2 de 10-29%; 3 de 30-49%; 4 de 50-69%; 5 de 70-89%; e 6 de 90-100% (ARMSTRONG *et al.*, 2019; HAWKES *et al.*, 2018).

As diretrizes estratificam a gravidade da psoríase em 2 grandes grupos de acordo com a intensidade dos sintomas como psoríase leve e psoríase moderada a grave (LLAMAS-VELASCO *et al.*, 2017). O PASI avalia as quatro regiões do corpo (cabeça, tronco, membros superiores e membros inferiores) em relação ao eritema, a espessura, a descamação das placas e a superfície da área corporal acometida (BSA) (ARNONE *et al.*, 2019; MATZA *et al.*, 2019).

Os valores do PASI, que classificam um quadro de psoríase como grave, tem variado. Para alguns autores, a gravidade do PASI foi definida como ≥ 18 , enquanto outros autores sugerem ≥ 12 (HÄGG *et al.*, 2017; HESSELVIG *et al.*, 2018; SAWYER *et al.*, 2019). Esta classificação, no entanto, enfrenta limitações importantes, pois não há um consenso sobre os limiares para PASI e DLQI, para fornecer uma diferenciação clinicamente coerente entre a psoríase leve e moderada

a grave. Uma definição mais precisa permitiria melhor correspondência entre a carga do tratamento e a gravidade da doença e as consequências extra cutâneas (HESSELVIG *et al.*, 2018; PUIG *et al.*, 2018).

2.6 Tratamentos

A psoríase é considerada uma doença inflamatória sistêmica e não somente uma doença de pele, sendo assim, requer uma terapia de longo prazo. A sua gravidade, comorbidades e acesso aos cuidados de saúde determinam a escolha do tratamento (DRVVAR *et al.*, 2019). O tratamento tem como objetivos alcançar uma remissão prolongada dentro de 12–24 semanas de terapia, controlar a atividade da doença com diminuição do PASI e BSA, melhorar a qualidade de vida do paciente, promover e manter sua capacidade de trabalhar, prevenir doenças concomitantes e reduzir a taxa de mortalidade (KULAWIK-PIÓRO; MIASTKOWSKA, 2021).

A interrupção da proliferação de queratinócitos, controle da diferenciação e apoptose são considerados alvos terapêuticos do bloqueio da psoríase (BARREA *et al.*, 2017). Neste sentido, são utilizados diferentes fármacos como corticóides, inibidores da calcineurina, anti-histamínicos, para aplicação tópica e, imunossupressores e moléculas biológicas para tratamentos sistêmicos (HÄGG *et al.*, 2017). Além disto, na psoríase, o mecanismo do estresse oxidativo já foi comprovado em estudos pré-clínicos e clínicos (TANAKA *et al.*, 2018). Assim, o uso de antioxidantes tem demonstrado ser uma estratégia importante para reduzir e controlar os danos causados pela psoríase e dermatite atópica (FURUE *et al.*, 2017).

A seleção de qual substância química medicinal utilizar, depende da localização das lesões, das preferências do paciente, do custo do tratamento, da probabilidade de remissão e dos possíveis efeitos colaterais. Em muitas situações, um tratamento com associações de medicamentos pode ser necessário (SHAH *et al.*, 2021). A localização em diferentes regiões do corpo, como couro cabeludo, mãos, pés e mucosas, afeta o tipo de substância química administrada, sua concentração e as características físico-químicas da formulação (DATTOLA *et al.*, 2020).

A psoríase leve, pode ser tratada com agentes tópicos. Já a moderada a grave geralmente requer tratamento sistêmico. A presença de comorbidades, como artrite psoriática, também é altamente relevante na seleção do tratamento (KULAWIK-PIÓRO; MIASTKOWSKA, 2021). Contudo, muitos pacientes, especialmente aqueles com psoríase generalizada, não são tratados adequadamente e, a longo prazo, as terapias apresentam efeitos colaterais significativos (HÄGG *et al.*, 2017).

2.6.1 Terapias tópicas

Os agentes tópicos representam a primeira linha de tratamento e, geralmente, são eficazes contra a psoríase em placas e, em formas mais leves que atingem até 25% da superfície corporal (SUN *et al.*, 2017b). Sua aplicação visa proporcionar a melhora dos sintomas e são recomendados em dosagens leves a moderadas, com uma combinação de glicocorticóides, análogos da vitamina D e fototerapia (PARTYKA *et al.*, 2018).

Os produtos de ação tópica Classificam-se em emolientes, inibidores da calcineurina (tacrolimus), corticosteroides, análogos da vitamina D3 (calcipotriol, clacitriol), retinóides e queratolíticos (ácido salicílico, ácido láctico e uréia) (BONESI *et al.*, 2017).

Dentre os tratamentos tópicos usuais, os emolientes atuam hidratando a camada externa da epiderme permitindo o desprendimento de escamas, minimizando a coceira, a sensibilidade ou o desenvolvimento subsequente de lesões. No entanto demonstram eficácia limitada e podem causar sensibilização (YAMAMOTO, 2020).

A aplicação de agentes queratolíticos antes de outros tratamentos aumenta a absorção de fármacos. Assim, são empregados em diversas formulações com concentrações variadas, como por exemplo, pomada de ácido salicílico (5-30%), pomada de ureia (10-20%), óleo com ácido salicílico (5-7%; para o couro cabeludo), entre outros. Entretanto, em soluções para o couro cabeludo e em xampus podem causar irritação (HERRERA-ACOSTA *et al.*, 2016; MARANDUCA *et al.*, 2020).

Os corticosteroides tópicos de potência média a alta, reduzem a inflamação rapidamente e, induzem regressão das lesões, principalmente quando aplicados sob oclusão. Todavia, o mecanismo ainda não foi totalmente elucidado, podendo estar relacionado com restauração da camada granular e a manutenção do gradiente de cálcio na epiderme para a correta diferenciação das células, além de permitir hidratação da epiderme e redução da atividade mitótica da epiderme (ZAGÓRSKA-DZIOK; SOBCZAK, 2020). Estes ativos apresentam boa eficácia na psoríase leve, permitindo o controle dos sintomas e o gerenciamento da doença. As associações de corticosteróides com derivados da vitamina D são consideradas padrão ouro no tratamento. Infelizmente, o uso a longo prazo pode levar à atrofia da pele, telangiectasia ou estrias (DATTOLA *et al.*, 2020).

Inibidores da calcineurina apresentam efeitos anti-inflamatórios e imunossupressores, enquanto os análogos da vitamina D3 e retinóides possuem como mecanismo de ação a redução da hiperproliferação dos queratinócitos. Porém, todos esses medicamentos tópicos causam efeitos colaterais locais, como vermelhidão, coceira, sensação de queimação, sensibilidade. Além disto, a aplicação tópica de corticosteroides, em longo prazo, pode causar atrofia da pele (YAMAMOTO, 2020). Embora as terapias tópicas sejam consideradas tratamento de primeira escolha, os tratamentos prolongados podem resultar em vários efeitos colaterais locais como prurido, sensação de queimação e eritema, entre outros (CHAMBERS; VUKMANOVIC-STEJIC, 2020).

2.6.2 Terapias sistêmicas

Terapias sistêmicas da psoríase incluem o metotrexato, ciclosporina, ésteres de ácido fumárico, e agentes biológicos. Os agentes biológicos inibem a atividade das células T (alefacepte e efalizumabe) e as vias mediadas pelo fator de necrose tumoral α (TNF- α) (etanercepte, adalimumabe, certolizumabe, golimumabe e infliximabe), IL-17 (brodalumabe, ixekizumabe e secucinumabe), IL-23 (guselkumabe), IL-12 e IL-23 (ustekinumabe e briakinumabe) causando imunossupressão significativa (DUTTA; CHAWLA; KUMAR, 2018; MAHIL; CAPON; BARKER, 2016; RAPALLI *et al.*, 2020).

A evidência do papel da IL-17 na patogênese da psoríase foi apoiada pela eficácia comprovada do tratamento com anti-IL-17 em pacientes com psoríase. Os antagonistas seletivos de IL-17 e IL-23 provaram ser altamente eficazes e resultaram em melhorias substanciais em aproximadamente 80–90% dos pacientes (HAWKES *et al.*, 2018; OWCZARCZYK-SACZONEK; CZERWIŃSKA; PLACEK, 2018).

Tratamentos por via oral e injetáveis podem servir como alternativas às terapias tópicas, mas apresentam como desvantagem a exposição sistêmica (HWANG; NIJSTEN; ELDER, 2017). Todas as terapias convencionais promovem efeitos colaterais, como atrofia, toxicidade de órgãos, imunossupressão, infecção e carcinogênese, que limitam o uso por longos períodos (MICHALEK; LORING; JOHN, 2017). Além disso, as terapias sistêmicas não têm o poder de cura da psoríase, apenas atenuam os sintomas da doença. Portanto, tem havido um interesse crescente por terapias baseadas em produtos naturais com maior eficácia, segurança e tolerabilidade para tratamento da psoríase (DANIYAL *et al.*, 2019; SALEEM *et al.*, 2020).

2.6.3 Fototerapia à luz ultravioleta (UV)

A fototerapia tem sido, frequentemente, empregada no tratamento da psoríase, com exposições repetidas das lesões psoriáticas à irradiação UVB ou UVA em combinação com os tratamentos farmacológicos tópicos ou orais. A fototerapia tem se mostrado eficaz em neutralizar a hiper proliferação dos queratinócitos. Entretanto, a fototerapia também está associada a vários efeitos colaterais, como queimação, coceira e maior risco de desenvolver câncer de pele (RENDON; SCHÄKEL, 2019).

Diversos tipos de fototerapia foram desenvolvidos e usados para o tratamento da psoríase ao longo dos anos. O tratamento pioneiro foi a fototerapia de luz ultravioleta B de banda larga (BB-UVB, do inglês *broadband*) que compreende a faixa entre 290-320nm. Posteriormente substituída pela ultravioleta B de banda estreita (NB-UVB, 311nm, do inglês *narrowband*), por se mostrar mais eficaz do que a anterior (ZHANG *et al.*, 2016). A psoríase responde favoravelmente à radiação ultravioleta UVB, por mecanismos de indução a apoptose de linfócitos T patogênicos

e queratinócitos, promoção de imunossupressão local e sistêmica. As ações dos linfócitos Th17 e T reguladoras (Treg) na patogênese da psoríase são suprimidos pela NB-UVB (DOPPALAPUDI *et al.*, 2017).

A fototerapia é um tratamento eficaz, seguro e acessível sem desenvolver quaisquer efeitos colaterais sistêmicos, quando comparados com os agentes biológicos ou outras substâncias químicas, especialmente para a psoríase em placas estável. Além disso, a fototerapia pode ser combinada com agentes biológicos para o tratamento da psoríase grave (DOPPALAPUDI *et al.*, 2017).

2.6.4 Fitoterapia

A fitomedicina, que é usada para pacientes com psoríase, oferece algumas vantagens, como a obtenção de ativos de fontes naturais cultiváveis, menores riscos de efeitos adversos, sendo uma alternativa aos tratamentos convencionais. Neste sentido, produtos naturais, como os agentes antiproliferativos, têm atraído cada vez mais atenção em investigações básicas e clínicas (MARTINEZ *et al.*, 2016).

Constituintes vegetais de várias classes químicas: alcalóides, ciclopeptídeos, flavonóides, furanocumarinas, derivados de ácidos orgânicos, polifenóis, esfingolipídeos e terpenóides foram considerados compostos líderes para o desenvolvimento de novos medicamentos antipsoriáticos (FAROOQUI; FAROOQUI, 2019; VOLLONO *et al.*, 2019).

Os polifenóis presentes nas plantas são eficazes no tratamento da psoríase devido à atividade antioxidante elevada. Reduzem o nível de ácido nítrico e hidroxila ou radical livre presentes no sangue de pacientes com psoríase. Outras propriedades dos polifenóis são antiproliferativas e anti-inflamatórias, além da capacidade de inibir os genes das calgranulinas A e B (proteínas de ligação ao cálcio) que estão envolvidos no processo da psoríase (AGUILAR-TOALÁ *et al.*, 2019; DANIYAL *et al.*, 2019).

Os medicamentos fitoterápicos são interessantes porque a diversidade estrutural dos produtos fitoterápicos e os múltiplos mecanismos de ação produzem sinergismo que atenua a psoríase (FORNI *et al.*, 2019; PALARETI *et al.*, 2018).

Contudo, os componentes químicos em extratos são geralmente complexos havendo, em muitos casos, dificuldade no controle de qualidade (SVENDSEN *et al.*, 2017).

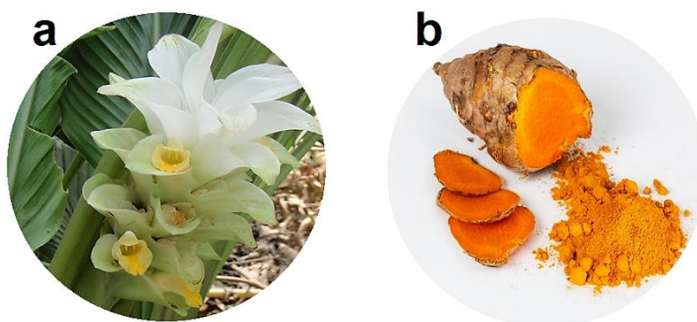
Ensaio clínico apresenta resultados positivos para o tratamento da psoríase com o uso de *Aloe vera*, *Indigo naturalis*, *Mahonia aquifolium*, *Curcuma longa* e capsaicina (composto químico encontrado nas pimentas) (FARAHNIK *et al.*, 2017; MAY *et al.*, 2015). Esses produtos podem melhorar as lesões psoriáticas através de mecanismos moleculares relacionados à apoptose, inibição da angiogênese e supressão da inflamação (ANDREICUT *et al.*, 2018). Dentre as diferentes moléculas antioxidantes naturais, a curcumina tem despertado atenção, devido ao grande número de importantes e benéficas atividades biológicas (ABRAHAMS *et al.*, 2019; PHULL *et al.*, 2018; RAUT; WAIRKAR, 2018).

2.7 Cúrcuma e curcumina

Dentre os vários fitoquímicos estudados no tratamento da psoríase, destaca-se a curcumina. A curcumina é um composto ativo derivado da cúrcuma que apresenta grande variedade de atividades biológicas, como antioxidante, anti-inflamatória, antitumoral, antimicrobiana, antiparasitária, entre outras (SHROTRIYA *et al.*, 2018; NARDO *et al.*, 2018; DUTTA *et al.*, 2018).

A cúrcuma é uma espécie vegetal de nome científico *Curcuma longa* L., da família Zingiberaceae, subordem Zingiberoidae, originária do sudeste asiático. Configura-se como uma das especiarias mais populares, dentre as que contêm antioxidantes naturais. Pode receber a denominação popular de açafrão, açafrão-da-índia, açafrão-da-terra, açafroa, açafroeira, açafroeiro-da-índia, batata-amarela, gengibre-amarelo, gengibre-dourada, mangarataia, entre outros (TSUDA, 2018; MÉLO *et al.*, 2021). Trata-se de uma planta perene de haste curta, atingindo em média, 120 a 150 centímetros de altura em condições favoráveis de clima e solo. Possui flores brancas e púrpuras, rizomas cilíndricos (Figura 7), sendo que os rizomas representam o interesse econômico e farmacológico da espécie (AGRAWAL; GOEL, 2016; KOTHA; LUTHRIA, 2019).

Figura 7. Imagens de *Curcuma longa* Linn: **(a)** planta com flor, **(b)** rizoma.



Fonte: **(a)** e **(b)** adaptado de SUETH-SANTIAGO *et al.* (2015)

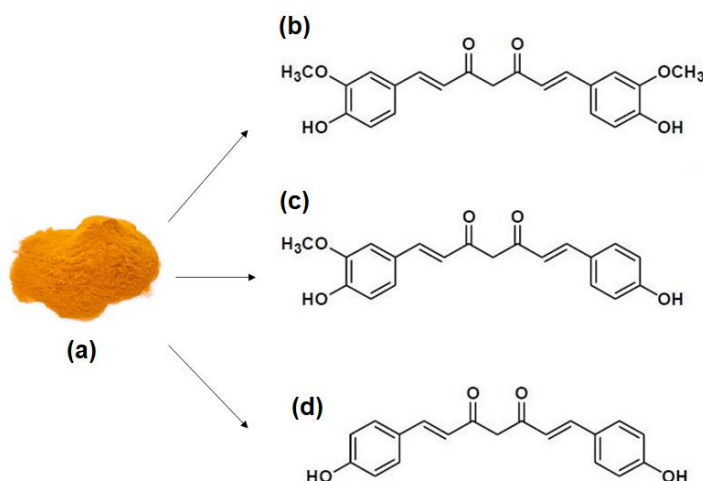
A Índia detém 80% da produção mundial de cúrcuma (3,22 hectares de cultivo, com uma produção total de 14 ton/ha), sendo o maior exportador mundial (HUSSAIN *et al.*, 2017; NARDO *et al.*, 2018). No Brasil, seu cultivo foi introduzido na década de 80, sendo cultivada principalmente nos estados de Goiás, Mato Grosso e São Paulo. A produtividade nacional é de 12 ton/ha, com destaque para o município de Mara Rosa em Goiás, considerado a capital do açafrão por responder por cerca de 90% da produção goiana (BORGES *et al.*, 2019).

O primeiro artigo científico referente à *Curcuma spp* foi publicado em 1748 e, 67 anos depois, a primeira revisão farmacológica foi apresentada (PULIDO-MORAN *et al.*, 2016; STOHS *et al.*, 2020). Em 1815, os cientistas Vogel e Pelletier realizaram experimentos para a purificação da curcumina e, próximo de um século depois, em 1910, foi estabelecida sua estrutura como um diferuloilmetano. Sua estrutura química foi confirmada em 1973 por Roughley e Whiting (ROUGHLEY; WHITING, 1973).

A *Curcuma longa* L. apresenta cerca de 235 compostos fenólicos, principalmente compostos terpenoides, incluindo curcuminóides, diarilpentanoides, monoterpênos, sesquiterpênos, diterpênos, triterpênos, alcalóides e esteróis (PRIYADARSINI, 2014; VIGATO *et al.*, 2019). A cúrcuma pode conter 3 a 15 % de curcuminóides; 1,5 a 5 % de óleos essenciais como tumerona, atlantona, zingiberona (GUIMARÃES *et al.*, 2020); açúcares; proteínas e resinas (BURAPAN *et al.*, 2020; STOHS *et al.*, 2020).

Os curcuminóides na cúrcuma são acumulados principalmente nos rizomas. A curcumina é obtida por extração com solvente alcoólico a partir de rizomas de cúrcuma e a purificação do extrato é feita por cristalização. A curcumina disponível comercialmente é composta pela mistura de curcuminóides, sendo 75-80% (m/m) de curcumina; 15-20% (m/m) de desmetoxicurcumina e 3-5% (m/m) de bisdesmetoxicurcumina. Estes curcuminóides são análogos estruturalmente, diferenciando-se apenas pela quantidade de grupos metoxila (OCH_3) em sua estrutura química. A curcumina possui dois grupos metoxila (OCH_3); a desmetoxicurcumina apenas um; e a bisdesmetoxicurcumina nenhum (Figura 8) (GUIMARÃES *et al.*, 2020; LOUREIRO; LIM, 2020; NELSON *et al.*, 2017; TABESHPOUR; HASHEMZAEI; SAHEBKAR, 2018).

Figura 8. Fórmulas estruturais dos curcuminóides. **(a)** curcumina em pó, **(b)** fórmula estrutural da curcumina, **(c)** fórmula estrutural da desmetoxicurcumina, **(d)** fórmula estrutural da bisdesmetoxicurcumina.



Fonte: Adaptado de NELSON *et al.* (2017).

Estudos apontaram efeito sinérgico entre estes curcuminóides. Além disso, a curcumina comercial tem um custo inferior a curcumina isolada (BAHRANI *et al.*, 2018; SHARMAN *et al.*, 2019; TSUDA, 2018; XU *et al.*, 2018). A curcumina isolada ou “pura”, tem o valor médio de R\$ 404,00 por 5 g (ALDRICH, 2021). Já a curcumina comercial, ou seja, a mistura dos curcuminóides, o valor é de cerca de R\$ 40,00 a cada 5 g em farmácias de manipulação, ou seja, aproximadamente 10% do valor da

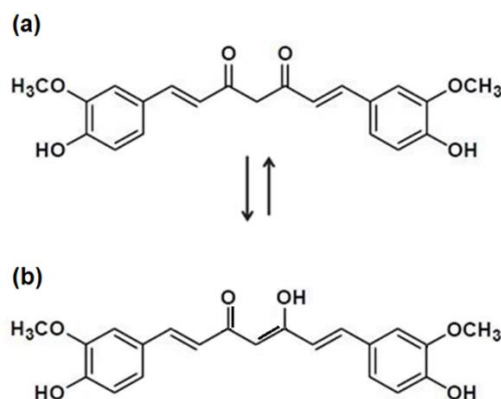
curcumina “pura”. Sendo assim, o emprego da curcumina comercial em produtos farmacêuticos poderá gerar produtos com um custo final mais acessível, além de manter ou potencializar suas propriedades medicinais.

A curcumina é uma molécula simétrica, possui nomenclatura oficial de 1,7-bis-(4-hidroxi-3-metoxifenil)-1,6-heptadieno-3,5-diona, com fórmula molecular $C_{21}H_{20}O_6$ e massa molecular de 368,38 g.mol⁻¹ e ponto de fusão de 183°C (ANINDYA *et al.*, 2015; KOTHA; LUTHRIA, 2019; PERAM *et al.*, 2019). Possui três entidades químicas em sua estrutura: dois sistemas de anéis aromáticos contendo grupos o-metoxifenólicos, conectados por um ligante de sete carbonos que consiste em uma porção α , β -insaturada β -dicetona (KANG *et al.*, 2016; KOCAADAM; ŞANLIER, 2017; SHROTRIYA *et al.*, 2018).

A ocorrência de transferência intramolecular de átomos de hidrogênio na cadeia de β -dicetona da curcumina leva à existência de conformações ceto e enol tautoméricas em equilíbrio. A forma enol é a mais estável tanto em fase sólida e líquida. As concentrações dos tautômeros de ceto-enol podem variar dependendo da temperatura e da polaridade do solvente. Em pH básico, a forma enólica está presente em maior quantidade, atuando principalmente como um doador de elétrons, o que caracteriza sua atividade antioxidativa (Figura 9) (LI *et al.*, 2020; MA *et al.*, 2019; SHAKERI *et al.*, 2019; VIGATO *et al.*, 2019).

Curcuminóides não fenólicos sintéticos não exibem atividade antioxidante, implicando que a curcumina é um antioxidante clássico de quebra de cadeia fenólica, que doa íons de H⁺ dos grupos fenólicos (ABRAHAMS *et al.*, 2019; AGRAWAL; GOEL, 2016; NELSON *et al.*, 2017; SILVA; SILVA; SIQUEIRA, 2020).

Figura 9. Equilíbrio dinâmico entre os tautômeros ceto e enol. **(a)** Forma di-ceto em condições neutras e ácidas. **(b)** Forma enol predominante em condições alcalinas.



Fonte: Adaptado de LI *et al.* (2020).

A natureza multimolecular da curcumina está bem descrita e apresenta status GRAS (do inglês Generally Recognized as Safe), ou seja, é considerada uma substância reconhecida como segura pela Food and Drug Administration (FDA) para uso como um aditivo alimentar em níveis de até 20 mg por porção (NELSON *et al.*, 2017). Alguns relatórios clínicos demonstram que a curcumina, mesmo em altas doses ($1000\text{-}2000\text{mg dia}^{-1}$), não produz nenhum efeito nocivo ao corpo humano. Entretanto, alguns efeitos adversos foram reportados pelo uso de curcumina, incluindo efeitos gastrointestinais e hematológicos (DHAKAL *et al.*, 2019).

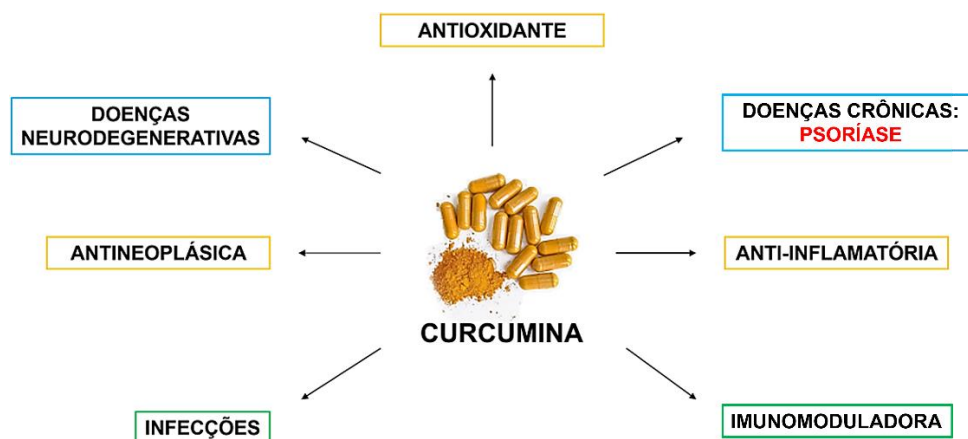
2.7.1 Atividade farmacológica

Por séculos, a curcumina foi utilizada na medicina tradicional indiana, conhecida como *Ayurveda*, para tratar diversas enfermidades, como anorexia, reumatismo, doenças de pele, doenças osteomusculares, parasitas intestinais, doenças hepáticas, dispepsia, doenças respiratórias, entre outras (BRUSCHI; DA SILVA; ROSSETO, 2019; KHALID *et al.*, 2020; TODKE; SHAH, 2018).

Devido sua estrutura química polifenólica, modula várias vias de sinalização e exerce um amplo espectro de atividades farmacológicas, como anti-inflamatória, antioxidante, imunomoduladora e anticarcinogênica (CERVEIRA *et al.*, 2021;

FILIPPONE *et al.*, 2020; GHASEMI *et al.*, 2019). A Figura 10 apresenta um esquema simplificado das possíveis aplicações farmacológicas para a curcumina.

Figura 10. Esquema das possíveis aplicações farmacológicas para a curcumina.



Fonte: Elaboração própria.

Estudos apontam que a curcumina apresenta atividade farmacológica contra várias doenças crônicas, diabetes tipo II, artrite reumatoide, fibrose cística, esclerose múltipla e aterosclerose (CAMPANI *et al.*, 2020; GUIMARÃES *et al.*, 2020). Também inibe a agregação plaquetária, regressão de trombose, proteção contra formação da catarata e promotor da cicatrização de feridas (VOLLONO *et al.*, 2019; WANG *et al.*, 2021).

Os efeitos biológicos se devem a habilidade de modulação de reações redox intracelular, bem como pela capacidade de ligar-se diretamente em diversas proteínas como a lipoxigenase, cicloxigenase-2 (COX-2), enzima glicogênio sintase quinase 3 beta (GSK3b) e várias outras enzimas regulatórias. Já a ação anti-inflamatória da curcumina deve-se, principalmente, a capacidade de inibição do fator de transcrição NF-κB (fator nuclear kappa B), que tem papel central nas vias de sinalização celular inflamatória e também na atividade antitumoral desse polifenol (TODKE; SHAH, 2018).

A potente atividade antioxidante da curcumina é decorrente de sua atividade regulatória de citocinas pró-inflamatórias e fatores de transcrição e, através disso, é capaz de eliminar espécies de oxigênio reativo (ROS), como os radicais $O_2^{\cdot-}$, $OH^{\cdot}$,

$\text{NO}\cdot$, $\text{ONOO}\cdot^-$ (AGUILAR-TOALÁ *et al.*, 2019). A atividade antioxidante da curcumina auxilia em sua ação anti-inflamatória, pois as espécies ROS estão envolvidas na fisiopatologia de inflamações crônicas e desordens degenerativas, como a psoríase. Já em nível molecular, a curcumina não só inibe a proliferação celular e a metástase, mas também induz a apoptose por modulação de vários fatores pró-inflamatórios, fatores de crescimento, receptores, fatores de transcrição e proteínas quinases (KOCAADAM; ŞANLIER, 2017).

A curcumina pode atravessar livremente a membrana celular devido sua lipofilicidade, contudo, essa substância tem uma solubilidade muito baixa em meio aquoso, sendo susceptível a degradação em meio alcalino e quando exposta à luz (KOCAADAM; ŞANLIER, 2017). A metabolização ocorre por enzimas em reações de fase I e II. Em reações de fase I o processo redutivo é catalisado por enzimas, incluindo a álcool desidrogenase (ADH), no citosol dos hepatócitos ou na mucosa intestinal. Os metabolitos desta fase, incluem dihidrodrocurcumina (DHC), tetrahydrocurcumina (THC) e hexahydrocurcumina (HHC), que existem na forma livre ou conjugada, principalmente em glucuronídeos (ALVES *et al.*, 2018; BORGES *et al.*, 2019). A formação de conjugados se processa através do metabolismo de reações fase II. A enzima UDP-glucuronosiltransferases presentes nos enterócitos ou hepatócitos catalisa a formação de glucuronídeo, enquanto sulfotransferases catalisam a formação de sulfatos (KOCAADAM; ŞANLIER, 2017).

2.7.2 Curcumina no tratamento da psoríase

No tratamento da psoríase a curcumina exerce efeitos inibitórios positivos através de vários mecanismos, como supressão de NF- κ B, regulação negativa de IL-1 β , IL-6 e TNF- α em macrófagos ativados, através da ligação direta em receptores levando à supressão de inflamação psoriática e hiperproliferação (VARMA *et al.*, 2017; VOLLONO *et al.*, 2019). O bloqueio da fosforilase quinase apresenta menor expressão na melhora dos sintomas da psoríase, enquanto a inibição dos canais de K^+ nos linfócitos T, contribui significativamente para a diminuição da inflamação (NARDO *et al.*, 2018; TSUDA, 2018).

A curcumina apresenta efeito antiproliferativo nos queratinócitos através da supressão de cascatas pró-inflamatórias e, ao reverter a função anti-apoptótica do TNF- α nas células da pele (XU *et al.*, 2018; YOUSEF *et al.*, 2019). Os bloqueadores do TNF- α vem sendo vastamente utilizados no tratamento da psoríase e, visto que a curcumina apresenta essas características, se justifica o interesse em seu potencial para tratamento da psoríase (HUANG *et al.*, 2019; PULIDO-MORAN *et al.*, 2016).

A curcumina também bloqueia a ação das interleucinas IL-17 e IL-23, ação importante, pois essas citocinas desempenham um papel crucial durante a patogênese da psoríase (OWCZARCZYK-SACZONEK; CZERWIŃSKA; PLACEK, 2018). A célula dendrítica ativada na pele libera IL-12 e IL-23, promovendo a diferenciação de linfócitos T auxiliares Th-1 e Th-17, que liberam citocinas Th-1 e Th-17, incluindo IL-17 e IL-23. Durante a evolução da doença, as citocinas IL-23, IL-17, IL-22, IL-1 β , IL-6 e o TNF- α interagem como uma rede integrada, seguida de ativação e indução da proliferação de queratinócitos (MA *et al.*, 2019).

Estudos com animais, que avaliaram a aplicação diária de gel de curcumina a 1% e observaram a redução da inflamação cutânea semelhante à psoríase, através do modelo de indução de psoríase por aplicação de imiquimode (quimioterápico tópico). Os autores propuseram que a ação ocorreu por meio da inibição dos canais de potássio expressos em linfócitos T e da redução de IL-17A, IL-17F, IL-22 por análises em pele de orelhas retiradas de camundongos (BAHRAINI *et al.*, 2018; KANG *et al.*, 2016).

VARMA e colaboradores (2017) demonstraram que o uso de curcumina nas concentrações de 25 e 50 μ M no tratamento de células do tipo psoriático (células HaCaT), *in vitro*, foi capaz de inibir a proliferação de células do tipo psoriático, pela regulação negativa de citocinas pró-inflamatórias, como interleucina-17 (IL-17), Fator de necrose tumoral- α (TNF- α), interferon- γ (IFN- γ), e interleucina-6 (IL-6). Além disso, a curcumina aumentou significativamente a função barreira da pele pela regulação positiva de involucrina (INV) e filagrina (FLG) (FAROOQUI; FAROOQUI, 2019).

Os pesquisadores Kang e colaboradores (2016) e Dutta, Chawla e Kumar (2018) em estudos com camundongos, demonstraram outro efeito importante da curcumina, que consistiu na inibição dos canais de potássio (subtipos Kv1.3)

expressos nas células T. Estas células parecem estar envolvidas no aparecimento da psoríase. As propriedades anti-inflamatórias da curcumina, foram confirmadas pela descoberta de que os camundongos apresentaram uma diminuição de mais de 50% do nível de fatores inflamatórios, incluindo TNF- α , IFN- γ , IL - 2, IL - 12, IL - 22 e IL-23.

A curcumina, embora promissora para o tratamento da psoríase, tem limitações como baixa biodisponibilidade oral, reduzida permeação dérmica e seu marcante pigmento amarelo (TSUDA, 2018). No entanto, há consideráveis progressos na superação dessas limitações, incluindo formulações com biodisponibilidade oral aumentada, preparações tópicas com promotores de permeação dérmica, etc. (MAHMOOD *et al.*, 2015). Esses sistemas configuram como potenciais tratamentos futuros para a psoríase (GAMRET *et al.*, 2018).

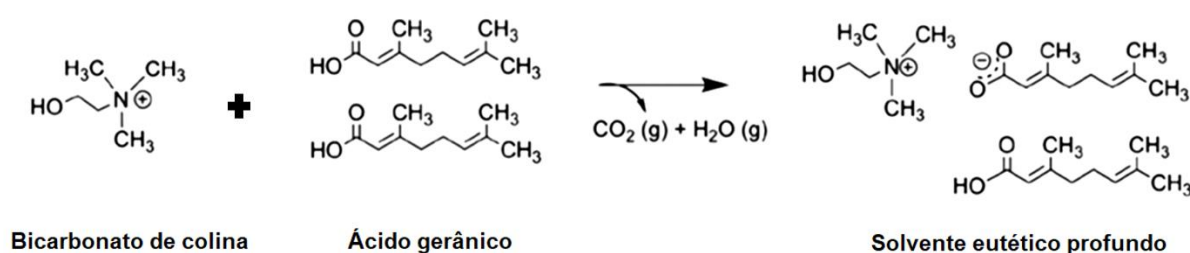
Dentre as estratégias para melhorar a permeação de ativos, como a curcumina, tem se aplicado a otimização da formulações, com a adição de substâncias facilitadoras da permeação cutânea, como os líquidos iônicos (JORGE *et al.*, 2020; TODKE; SHAH, 2018).

2.8 Líquidos iônicos

Líquido iônico (LI) são, essencialmente, sais em estado líquido e que apresentam ponto de fusão menor que 100°C. Os líquidos iônicos são preparados a partir de materiais pouco onerosos e com *status* GRAS (do inglês *Generally Recognized As Safe*), sendo constituídos por cátions orgânicos e ânions inorgânicos e/ou orgânicos (QI; MITRAGOTRI, 2019). Possuem propriedades ideais, tais como baixa pressão de vapor à temperatura ambiente, perfil de solubilidade extenso e variável, alta estabilidade térmica, não inflamável, polaridade adaptável, perfis químicos inertes, pontos de fusão abaixo de 100°C e viscosidades variáveis (ISLAM *et al.*, 2020). Devido às suas propriedades físico-químicas únicas, tornaram-se importantes em diversas áreas científicas e tecnológicas (JORGE *et al.*, 2020). Além dos líquidos iônicos também existem os solventes eutéticos profundos (DES do inglês *Deep eutectic solvent*), com características muito similares aos LI. Estabeleceu que a diferença entre um LI e um DES pela indicação de que líquidos

iônicos são classes de sais orgânicos com baixo ponto de fusão e os solventes eutéticos profundos são misturas de componentes que tem ponto de fusão mais baixo que o ponto de fusão de cada componente individual (DIAS, 2016). No entanto, alguns autores consideram os DES uma subclasse de LI (PŁOTKA-WASYLKA *et al.*, 2020). O produto da reação química apresentada na Figura 11 compreende as espécies iônicas (colina e geranato) e uma molécula de ácido gerânico neutro, portanto, exibe características de um líquido iônico devido a interações iônicas bem como um solvente eutético profundo devido à ligações de hidrogênio (TANNER *et al.*, 2019b), o que dificulta sua classificação como Li ou DES.

Figura 11. Síntese do solvente eutético profundo. Reação química na razão molar de 1:2 para produção do solvente eutético profundo produzido pela reação entre bicarbonato de colina e ácido gerânico.



Fonte: Adaptado de IBSEN *et al.* (2018).

No campo farmacêutico, os líquidos iônicos são utilizados no preparo de nano e microemulsões, como promotores de permeação cutânea, como antimicrobianos e, também em sistemas de administração oral para fármacos pouco solúveis (BANERJEE *et al.*, 2017; WILLIAN *et al.*, 2014; ZAKREWSKY *et al.*, 2014; ZHOU *et al.*, 2014).

Estudos realizados por Zakrewsky *et al.* (2016) mostraram que os líquidos iônicos aumentaram a permeabilidade de ativos pela barreira cutânea. O estudo revelou também que os líquidos iônicos podem romper os arranjos regulares e compactos dos corneócitos, alterar as propriedades superficiais do estrato córneo e tornar a estrutura da pele mais permeável. As moléculas de líquido iônico são capazes de deslizar através dos ácidos graxos que constituem as células da pele,

criando pequenas lacunas transitórias através das quais moléculas bioativas transportadas pelo líquido iônico podem permear (KO *et al.*, 2021).

Os resultados biológicos dos líquidos iônicos, incluindo a entrega transdérmica, atividade antibacteriana e citotoxicidade, estão totalmente relacionados com as propriedades químicas e seus cátions e ânions constituintes (CAPARICA *et al.*, 2018; ISLAM *et al.*, 2020). Quando comparados com solventes orgânicos tradicionais com cadeias alquila pequenas, tais como o etanol, são menos tóxicos para as células, minimizando assim problemas associados à irritação da pele induzida por solventes (YOUSEF *et al.*, 2019).

Líquidos iônicos e solventes eutéticos profundos têm se mostrado uma grande promessa em aplicações de entrega de moléculas bioativas, como proteínas, ácidos ribonucleicos (RNA) e polissacarídeos (DHARAMDASANI *et al.*, 2020; KO *et al.*, 2021; QI; MITRAGOTRI, 2019). Os LI a base de bicarbonato de colina e ácido gerânico têm sido utilizados para melhorar a entrega transdérmica de várias moléculas com relativo sucesso (BOSCARIOL *et al.*, 2021; IBSEN *et al.*, 2018; JORGE *et al.*, 2020; TANNER *et al.*, 2019b).

3 OBJETIVOS

3.1 Objetivo geral

Desenvolver um gel com curcumina e líquido iônico geranato de colina para o tratamento da psoríase.

3.2 Objetivos específicos

- ❖ Avaliar o processo de permeação dérmica da curcumina facilitada pelo geranato de colina através de ensaios de permeação *in vitro* e estudos histológicos;
- ❖ Aferir a melhor concentração de geranato de colina no gel de alfarroba e curcumina para a permeação dérmica da curcumina através de ensaios de permeação *in vitro*;
- ❖ Analisar parâmetros físico-química e biológicos de amostras de curcumina obtidas de diferentes procedências;
- ❖ Determinar a estabilidade do gel desenvolvido em ensaio de estabilidade acelerada (180 dias);
- ❖ Avaliar as propriedades físico-químicas e citotóxicas do gel;
- ❖ Determinar a efetividade da curcumina no tratamento da psoríase, permeada pelo líquido iônico geranato de colina em ensaios *in vivo*, tendo como modelo experimental o uso de camundongos.

4 RESULTADOS

Os resultados alcançados nesse trabalho de pesquisa foram estruturados em formato de artigo científicos e inseridos na íntegra, sendo:

- 4.1. Performance of choline geranate deep eutectic solvent as transdermal permeation enhancer: An *in vitro* skin histological study.
- 4.2. Physicochemical characterization of commercially available turmeric for use in pharmaceutical products and food supplements.
- 4.3. Transdermal permeation of curcumin promoted by choline geranate ionic liquid: potential for the treatment of psoriasis.
- 4.4. Gel de alfarroba e curcumina com geranato de colina: ensaios de estabilidade.
- 4.5. Transdermal permeation assays of curcumin aided by CAGE-IL: *in vivo* control of psoriasis

4.1 Performance of choline geranate deep eutectic solvent as transdermal permeation enhancer: An *in vitro* skin histological study

A trabalho *Performance of choline geranate deep eutectic solvent as transdermal permeation enhancer: An in vitro skin histological study* foi publicado na revista *Pharmaceutics* estando disponível em <https://www.mdpi.com/1999-4923/13/4/540>.

O objetivo do estudo, que culminou com a publicação, foi confirmar a atividade do geranato de colina como uma substância facilitadora da permeação cutânea de curcumina e, também procurar entender o mecanismo de facilitação da permeação pelo geranato de colina. Vários estudos mostram que os líquidos iônicos, como o geranto de colina, são compostos ideais para entrega transdérmica de biomoléculas, mas até o momento, não existiam estudos histológicos relacionando a ação de líquidos iônicos às mudanças na estrutura da barreira externa da pele.

Para isto foram feitos estudos de permeação *in vitro* utilizando células de Franz e realizadas análises histológicas em amostras de pele de orelha de porco, as quais ficaram em contato com as amostras. Os resultados obtidos mostraram que o líquido iônico atuou como um disruptor transitório da estrutura da pele, facilitando a passagem da curcumina na pele.



Article

Performance of Choline Geranate Deep Eutectic Solvent as Transdermal Permeation Enhancer: An In Vitro Skin Histological Study

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Abstract: In the present research work, we addressed the changes in skin by which deep eutectic solvents (DES) enhanced transdermal permeation of bioactive compounds and propose a rationale for this mechanism. Several studies showed that these unusual liquids were ideal solvents for transdermal delivery of biomolecules, but to date, no histological studies relating the action of DES to changes in the structure of the outer skin barrier have been reported. In the research effort described herein, we presented an in-depth analysis of the changes induced in the skin by choline geranate DES, a compound with proven capabilities of enhancing transdermal permeation without deleterious impacts on the cells. The results obtained showed that a low percentage of DES acted as a transient disruptor of the skin structure, facilitating the passage of bioactive compounds dissolved in it.

Keywords: choline geranate deep eutectic solvent; transdermal permeation; histological changes

1. Introduction

The transdermal permeation route received increased attention in recent years due to numerous advantages over the oral and injectable routes of drug administration [1]. Hence, transdermal permeation became an appealing route for the delivery of bioactive macromolecules [2,3]. The main advantages of the transdermal route encompassed the possibility of controlled release over time, elimination of drug degradation in the gastrointestinal tract (GIT) with the enhancement of therapeutic efficacy, and strikingly painless administration [4,5].

The skin is the largest organ in the human body, being a heterogeneous multilayer tissue that covers a surface area of ca. 2 m² and receiving approximately one-third of the blood circulation in the body [5], whose primary function is to protect the body from the external environment by functioning as an effective protective barrier to absorption of exogenous molecules. Such barrier characteristics of the skin are largely due to its composition, making it virtually impenetrable to exogenous large and hydrophilic molecules [1,6,7].

The outermost layer of the skin, termed *stratum corneum*, is responsible for such impermeability being constituted by an association between keratinocytes and lipids [4,6]. A unique class of ceramide lipids is essential for the natural barrier property of the skin, containing linoleic acid (C18:2) esterified to the w-hydroxyl group of a very-long-chain fatty acid linked itself at the N-acyl moiety to sphingosine (C18:1). These lipids, specific to the epidermis, are termed EOS to describe their chemical composition [7,8]: esterified omega-hydroxyacyl-sphingosine. The EOS lipid family integrates a group of structural analogs possessing different carbon chain lengths (viz. C30–C36) and the number of insaturations

in the amide-linked very-long-chain fatty acids. The EOS lipids are distributed within the *stratum corneum* alongside cholesterol and saturated fatty acids [8,9]: being organized into trilaminar units that surround corneocytes, the flattened dead cells containing keratin in the outermost layer of the skin. The extremely long fatty acid chain of EOS is essential for the barrier function of the skin. Neutron diffraction studies on *stratum corneum* lipid model systems revealed that one EOS molecule stretches from one lipid membrane layer and penetrates an adjacent layer [7–12], hence helping to tighten neighboring bilayers densely together and concomitantly decrease water diffusion. The exact lipid organization of the *stratum corneum* still remains, however, poorly understood due to the difficulty in studying an intact internal structure in native skin tissues in vivo. The skin is, therefore, a formidable barrier that protects nearly the entire human body.

Hence, the topical administration of many drugs requires appropriate technology for attaining successful transdermal drug delivery [13]. In order to overcome such formidable obstacles and to allow the non-invasive delivery of bioactive macromolecules via transdermal permeation, various approaches to weaken the *stratum corneum* have been tested over time, including absorption promoters, iontophoresis, sonophoresis, and micro-needles [4,6].

Absorption promoters increased the cutaneous permeability by increasing either the partition coefficient of the bioactive macromolecule or the thermodynamic action thereof, by modifying the composition of the *stratum corneum* via breaking the lipid structure [6,14] or by creating hydrophilic pores and/or establishing a drug reservoir in the *stratum corneum* itself [15]. In addition, permeation enhancers should exhibit the following characteristics: biocompatibility; nontoxicity; compatibility with the drug being administered; absence of any adverse pharmacological activity inside the body; not be expensive; and be devoid of color, odor, and taste [16]. Recently, ionic liquids (ILs) and deep eutectic solvents (DES) were used for this purpose with success [15,17–21].

ILs and DES are an interesting group of chemical compounds composed exclusively of ions that have melting points below 100 °C, low to negligible vapor pressure at room temperature, extensive and varying solubility profiles, high thermal stability, non-flammability, adaptable polarity, inert chemical profiles, variable viscosities, and many others properties [22,23]. Ionic liquids and their deep eutectic solvents are highly viscous materials made up exclusively of ions, exhibiting melting temperatures below the boiling point of water at atmospheric pressure, that are able to solubilize amphiphilic molecules [22,24]. By definition, the behavior of ILs is dominated by ionic interactions, whereas DES exhibit a strong contribution to hydrogen bonding [22,24]. Choline-based ILs with amino acids are amongst the mainly used ILs in pharmaceutical application studies [20,21]. Recently, choline geranate DES was reported to exhibit virtually nil toxicity to both epithelial cells and skin while greatly enhancing the transdermal delivery of bioactive moieties [17–19,25,26]. The transdermal delivery potentiation and the virtual absence of cytotoxicity of this DES are directly related to their chemical properties and constituent cations and anions [25,26].

ILs and DES are able to solubilize amphiphilic molecules and are, therefore, ideal solvents for the transdermal delivery of macromolecules. The molecules of these highly viscous fluids are hypothesized to slip around and through the fatty compounds that make up the skin cells, creating small transient openings through which bioactive molecules can permeate [22]. However, to date, no definitive proof of this putative mechanism has been put forward. Human skin permeability has been reported to vary (both inter- and intra-individual), and, therefore, pigskin is commonly used as an alternative model to human skin in percutaneous absorption studies aiming at developing transdermal formulations [27].

In this sense, the major goal of the research work described herein was to try to uncover the mechanism by which DES, and particularly choline geranate, enhance transdermal permeation using porcine ear skin as a skin model and curcumin as the bioactive substance to be permeated.

2. Materials and Methods

2.1. Materials

2.1.1. Cell Lineages

The 3T3 and HaCaT (immortalized human keratinocytes) cell lines used in the genotoxicity (Comet™) and MTT assays were purchased from Sigma-Aldrich (St. Louis, MO, USA). The cells were maintained at 37 °C according to the procedure described by Rocha et al. [28].

2.1.2. Porcine Ear Skin

Domestic pig ears were obtained from a local market in the region of Sorocaba (São Paulo, Brazil) and, from these, the skin was excised and used in the transdermal permeation assays.

2.1.3. Chemicals

Tap water was purified in a Master System All (model MS2000, Gehaka, São Paulo, Brazil) to a final resistivity of ca. 18.18 MΩ.cm and conductivity of 0.05 µS.cm⁻¹. Geranic acid (85% stabilized; ref. W412101-1KG-K) and choline bicarbonate (ref. C7519-500ML) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol (LiChrosolv®, CAS-No: 67-56-1) was purchased from Merck (Darmstadt, Germany).

2.2. Experimental Procedures

2.2.1. Synthesis and Characterization of DES

Synthesis of Choline Geranate Deep Eutectic Solvent (CG-DES)

CG-DES (1:2) were prepared following the methodology described in detail elsewhere [17,19]. Briefly, a ratio of choline bicarbonate to geranic acid of 1:2 was used to synthesize CG-DES. To a 250 mL round bottom flask, 18.72 g (or 0.1133 mol) of pure choline bicarbonate were added, followed by the addition of 39.58 g (or 0.2350 mol) of pure geranic acid (in excess, to ensure a maximum extent of the reaction). Twenty milliliters of methanol were then added to the mixture in order to reduce the viscosity, and magnetic stirring proceeded overnight at room temperature in an open system fashion until CO₂ production ceased. The solvent was subsequently removed (60 °C during ca. 20 min) using a rotary evaporator, the resulting CG-DES were transferred into a 50 mL Falcon tube, the headspace of which was flushed with nitrogen, and the tube was immediately capped and duly sealed with Parafilm™ (Bemis Flexible Packaging, Neenah, WI, USA).

Assessment of the Cytotoxicity Potential of Plain CG-DES via Mitochondrial Activity (MTT) Assay

Evaluation of the cytotoxicity potential of plain CG-DES to HaCaT (immortalized human keratinocyte) cell lines (assessment of cellular viability) was performed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, following the procedure described in detail elsewhere [17–19,28].

Assessment of the Genotoxicity Potential of Plain CG-DES, via the Comet™ Assay

Cell lineage 3T3 was placed in contact with the sample (250 µL CG-DES were diluted in 10 mL ultrapure water and, from the resulting solution, 300 µL were used for the test), with the negative being a plain DMEM (Dulbecco's Modified Eagle's medium, Gibco Life Technologies, São Paulo/SP, Brazil) medium in contact with the 3T3 cells, for a period of 1 h. Tests were carried out in triplicate following the procedure described in detail elsewhere [17,28].

2.2.2. The Effect of DES on Skin Permeability

Porcine Ear Skin Preparation

The ear skin was prepared according to the procedure described by Salerno et al. [29], with slight modifications [18,19]: the skin was excised from domestic pig ears (4 to 6 months old, obtained from a local market in the region of Sorocaba (São Paulo, Brazil)),

and the ears were thoroughly cleaned under tap water immediately after excision. Any hair from the external region of the ears was duly removed, the skin was carefully separated from the cartilage using a scalpel, and subcutaneous adipose tissue was carefully removed so as to produce a uniform thickness of 1 mm (controlled with a caliper rule). Although the porcine ear skin presents some anatomical differences relative to the human skin, there is a relative uniformity of the samples and ease of dissection and separation of the subcutaneous tissue [13,30]. After being dried with a paper tissue, the skin discs were immediately mounted on the Franz vertical diffusion cells [31,32].

Preparation of DES Suspensions

Suspensions of CG-DES in saline solution (0.9% NaCl, *w/w*) were prepared at different CG-DES percentages (*w/v*): 0.5%, 1.0%, 1.5%, 2.0%, and 2.5%.

In Vitro Skin Permeation Testing

The non-invasive effect of CG-DES in enhancing skin permeation was studied in a DHC-6T Transdermal System from Logan Instruments Corporation (Somerset, NJ, USA), using thawed porcine ear skin discs (0.5 mm thick $\times$ 30 mm ϕ_{ext}). The porcine ear skin discs were then clamped on top of the Franz diffusion cell (with a 15 mm ϕ_{ext} central hole, contacting the receptor fluid beneath; Figure 1). On top of these porcine ear skin discs, a cavity with $\phi_{\text{ext}} = 30$ mm was carefully filled with 2 mL ionic liquid suspension (integrating different volume percentages of CG-DES, containing or not curcumin) eliminating any air bubbles. To the receptacle of the Franz diffusion cell (possessing a small cylindrical Teflon-coated magnetic stirring bar) beneath the porcine ear skin disc, 8 mL of saline solution (0.9% NaCl, *w/w*) buffered to pH 7.0 were carefully poured until touching the lower surface of the skin ensuring the absence of air bubbles and stretching the skin so as to minimize the presence of furrows. This compartment was maintained at 32 ± 3 °C for 30 min. The CG-DES suspension (with or without curcumin) was allowed to contact the upper part of the porcine ear skin discs for a period of 12 h. When evaluating curcumin permeation, 2-mL samples were withdrawn from the receiving fluid beneath the porcine ear skin disk at predetermined time intervals over a timeframe of 6 h, and 2-mL of fresh saline solution (pH 7.0) were duly added so as to reset the volume. Each sample was then assayed spectrophotometrically for curcumin quantification at a wavelength of 421 nm using disposable plastic cuvettes (Kartell, Noviglio MI, Italy) in a UV-Vis Spectrophotometer from Agilent (model Cary 60 UV-Vis, Santa Clara, CA, USA). A calibration curve for curcumin was prepared using solutions of curcumin in buffered saline solution (0.9% NaCl, *w/w*, pH 7.0) at several concentrations in the range 0–25 $\mu\text{g/mL}$: $\text{Abs}_{421\text{ nm}} = 0.0606 \times [\text{curcumin}, \mu\text{g/mL}] + 0.0029$ ($r^2 = 0.9983$).

Skin Conservation for Histological Preparations

Following the transdermal permeation tests and separation of the section of interest of the skin discs in contact with the suspensions of CG-DES, the tissues were immediately placed in 10% formalin buffer (fixative) in order to prevent solubilization of tissue proteins. The porcine ear skin samples were dehydrated with increasing concentrations of ethanol in order to avoid a pronounced tissue retraction which would cause irreversible cellular structural lesions.

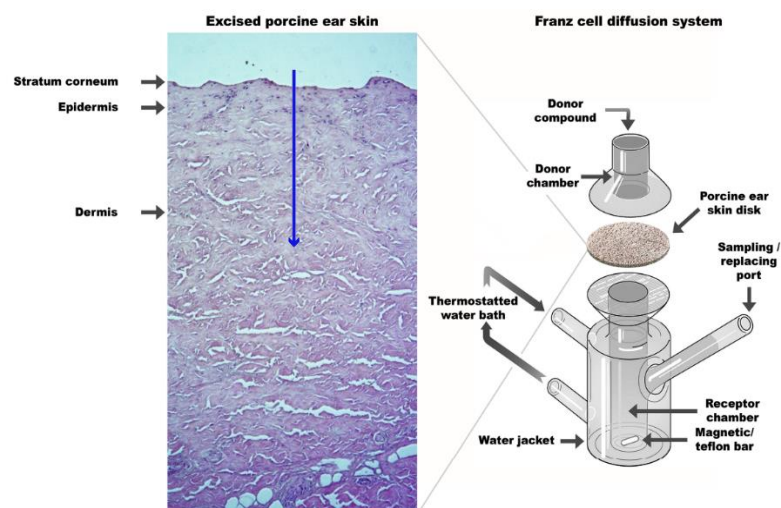


Figure 1. Schematic diagram of the static Franz diffusion cell used in the in vitro permeation tests with CG-DES showing the pathway for drug permeation (blue arrow).

2.2.3. Preparation of Porcine Ear Skin Histological Cuts

The histological analysis of the porcine ear skin discs contacted with different percentages (*v/v*) of CG-DES (0.5%, 1.0%, 1.5%, 2.0%, and 2.5%) was performed after each porcine ear skin disc had been fixed and processed. Tissue fixation was carried out in buffered formalin for 48 h time, after which the samples were washed overnight in running water, and the tissues were then stored in ethanol at 70% (*v/v*). Subsequently, the tissues were treated in an automatic tissue processor (from O Patologista[®], model PT12, Guarulhos SP, Brazil), and the pieces were embedded in histological paraffin according to the sequence [33]: (i) dehydration (passage in ethanol at 70% (*v/v*); 50 min in ethanol at 90% (*v/v*); 150 min in ethanol at 100% (*v/v*)), (ii) clarification with xylol during 120 min, and (iii) impregnation with paraffin during 120 min. The histological cuts of the porcine ear skin samples, with a thickness of four micrometers, were prepared in a rotary-manual microtome (from O Patologista[®], model MR 2014, Guarulhos, SP, Brazil) and then stained via hematoxylin and eosin staining techniques: (i) dewaxing with xylol for 30 min, (ii) hydration with ethanol at 100% (*v/v*) for 20 s, (iii) hydration with ethanol at 95% (*v/v*) for 10 s, (iv) hydration with ethanol at 70% (*v/v*) for 10 s, (v) washing with water for 2 s, (vi) staining with hematoxylin for 6 min, (vii) washing with running water for 3 min, (viii) staining with eosin for 2 min, (ix) washing with water for 2 s, (x) dehydration with ethanol at 70% (*v/v*) for 10 min, (xi) dehydration with ethanol at 100% (*v/v*) for 20 min, (xii) fixing the dye and preserving the material with xylol for 1 s, and (xiii) fixing the dye and preserving the material with xylol until the slide was assembled, and, finally, (xiv) embedded with histological resin [33,34]. The analysis of the histological sections was performed qualitatively with the aid of an optical microscope coupled to a digital camera (Biological Microscope, Model Axio Lab. A1, Zeiss[®], Oberkochen, Germany).

3. Results

In the present research work, we proposed a putative mechanistic model for the action of CG-DES in enhancing transdermal permeation of curcumin. Since intermolecular interactions between the DES, curcumin, and skin components were also likely to play a role in the transdermal delivery of curcumin, one also performed the photomicrographical study of the histological section of the porcine ear skin after the transdermal permeation of curcumin using CG-DES at 2.0% (*w/v*), and compared the results with those of the

blank experiments, i.e., without permeating any substance, and also with porcine ear skin contacted with only saline solution (0.9% NaCl, *w/w*).

Figure 2a displays the results obtained for the maximum average permeated curcumin for different amounts of CG-DES (% *w/w*), allowing us to determine the optimum concentration of CG-DES to be used in the transdermal permeation assays, whereas Figure 2b displays the results of the transdermal permeation assays of curcumin as a function of time for the optimum CG-DES concentration.

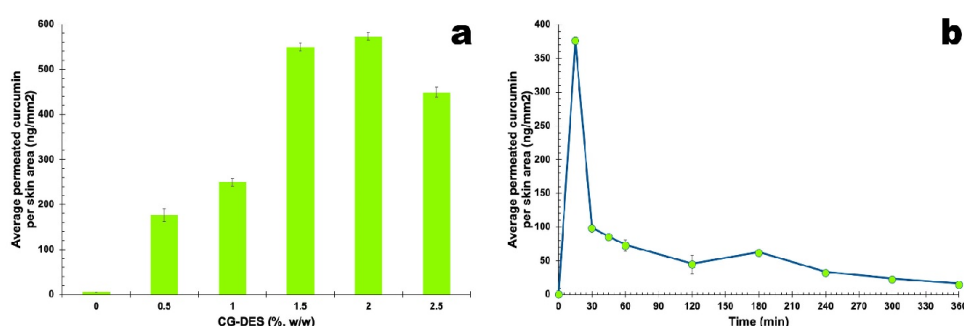


Figure 2. Results gathered from transdermal permeation assays of curcumin aided by CG-DES, as average permeated curcumin ($\text{ng}/\text{mm}^2_{\text{skin}}$), for several CG-DES concentrations (a) and during a 6 h assay using the best CG-DES concentration (viz. 2%, *w/w*) (b). All values represent the mean of three experiments and the error bars represent the standard deviations.

The results of the histological analysis performed on the porcine ear skin following the contact with CG-DES and the staining with hematoxylin and eosin are depicted in Figure 3 in the form of photomicrographs with $\times 200$ magnification.

In Figure 3, it is possible to observe that no significant morphological changes were produced, following contact of the skin with CG-DES. However, possible breakdowns in the *stratum corneum* were noticeable depending on the concentration of CG-DES used. The cell nuclei of the basal layer were most evident in the photomicrographs since the histological sections were performed, favoring visualization between the epidermis and the dermis. It was worth noting that areas of cartilage and adipose tissue were excluded from these histological cuts.

Figure 4 displays the histological cuts of porcine ear skin exposed to plain saline solution (0.9% NaCl, *w/w*; Figure 4a) and after transdermal permeation of curcumin using CG-DES at 2.0% (*w/v*; Figure 4b).

CG-DES promoted a great enhancement in the transdermal permeation of curcumin, the bioactive molecule used as a model in this work (Figure 2a). These results were in close agreement with previous findings from our research group, namely in the transdermal permeation of insulin [17] and in the transdermal permeation of bacteriophage particles to treat skin infections (either in the deeper layers of the skin and in the more superficial ones) [18,19]. CG-DES were able to solubilize amphiphilic molecules and were, therefore, an ideal solvent for the transdermal delivery of macromolecules such as the aforementioned ones.

Monti et al. [15] suggested that ionic liquids could affect transdermal permeation by changing the electric properties of the *stratum corneum* and favoring the transfer of the drug into the deeper layers of the skin; however, the exact mechanism by which this happens remains poorly understood, and, to date, no definitive proof of this putative rationale has been put forward.

Figure 5 depicts a schematic representation of the skin, displaying the skin barrier (i.e., the *stratum corneum*) and two putative transdermal permeation routes potentiated by CG-DES.

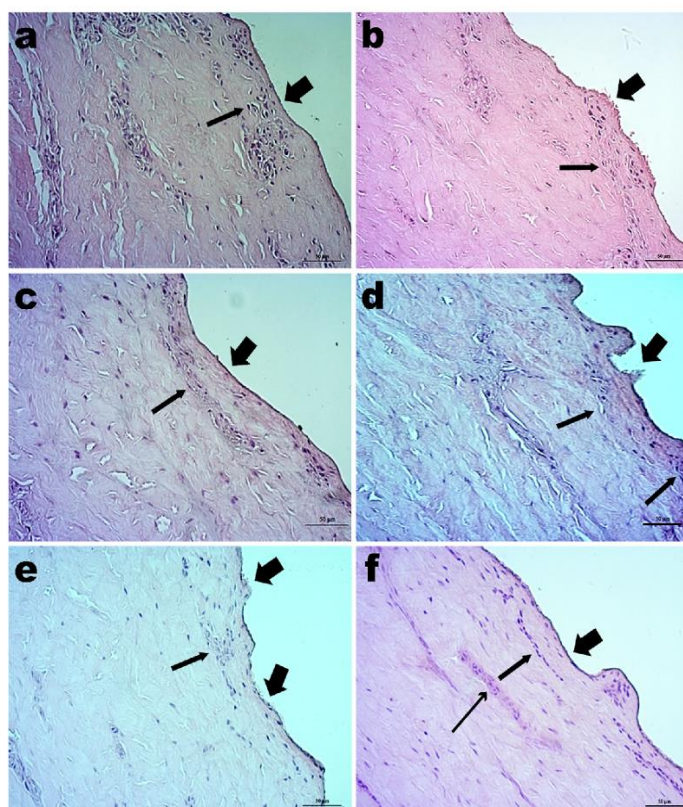


Figure 3. Photomicrographs ($\times 200$ magnification) of (a) the histological sections of plain porcine ear skin, and of porcine ear skin following contact with (b) 0.5% CG-DES, (c) 1.0% CG-DES, (d) 1.5% CG-DES, (e) 2.0% CG-DES, and (f) 2.5% CG-DES. All sections were stained with hematoxylin and eosin. For the legend of inserted (thick, thin, and skinny) arrows, please refer to the Discussion section.

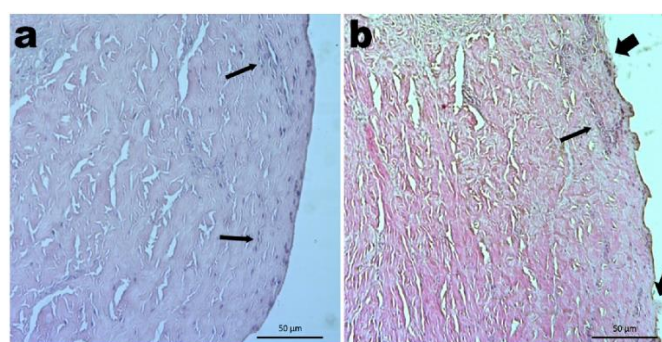


Figure 4. Photomicrographs ($\times 200$ magnification) of (a) the histological section of porcine ear skin contacted with only saline solution (0.9% NaCl, *w/w*) and (b) the histological section of the porcine ear skin after transdermal permeation of curcumin using CG-DES at 2.0% (*w/v*). All sections were stained with hematoxylin and eosin. For the legend of inserted (thick, thin, and skinny) arrows, please refer to the Discussion section.

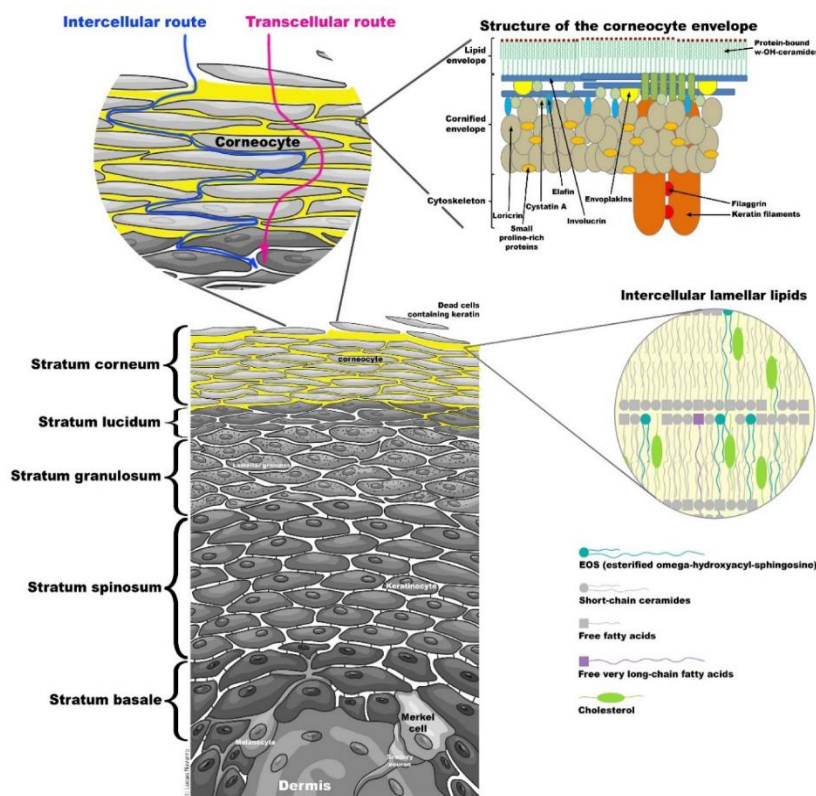


Figure 5. Schematic representation of the skin displaying the skin barrier (i.e., the *stratum corneum*, consisting of corneocytes surrounded by a multilamellar lipid matrix of ceramides, free fatty acids, and cholesterol densely packed in an approximately 1:1:1 molar ratio), the structure of the corneocyte envelope, and two putative transdermal permeation routes potentiated by CG-DES. Reproduced from [35,36], Eos, 2020.

One of the many important biological functions of ceramides is to maintain skin moisture, with a unique class of ceramides being indispensable for maintaining the natural barrier of the skin. Such class of ceramide lipids integrate linoleic acid, the most abundant essential fatty acid present in the epidermis, esterified to the w-hydroxyl group of a very-long-chain fatty acid linked at the N-acyl moiety to sphingosine. A good understanding of the structure of the skin barrier, the *stratum corneum*, is therefore essential if one intends to postulate a rationale for the mechanism of action of ionic liquids as permeation enhancers. In Figure 5, two putative routes for permeation of bioactive molecules potentiated by CG-DES have been put forward.

4. Discussion

Based on the many reported uses of ILs and DES as permeation enhancers, there was a clear interest in using these substances in transdermal drug delivery, but the associated permeation mechanism remained essentially unclear. ILs were shown to enhance the transdermal transport, either transcellularly and paracellularly; however, there were indications that ILs bypass the barrier properties of the *stratum corneum* employing mechanisms such as disruption of cellular integrity, fluidization, creation of diffusional pathways, and extraction of lipid components in the *stratum corneum* [23].

Curcumin is a phytopolyphenol pigment isolated from the plant *Curcuma longa*, commonly known as turmeric, a natural product with therapeutic potential for skin diseases such as (but not limited to) psoriasis and cancer [37–39], but it has quite low aqueous solubility, which contributes to the low absorption of curcumin from the GIT and the low oral bioavailability due to the rapid first-pass metabolism, which limits its usefulness as an oral drug but makes it an interesting molecule for administration through the skin [40–42]. In this context, the transdermal route was an important strategy for the administration of bioactive macromolecules since it avoids the first-pass biotransformation and allows the use of self-administered pharmaceutical forms, with concomitant improvement of patient compliance [43,44]. However, due to both the excellent barrier function of the *stratum corneum* and the relatively high lipophilicity and biochemical/structural degradation of curcumin, skin delivery of curcumin was challenging. Hence, it was chosen as a model biomacromolecule for the study.

In the research work described herein, the performance of a transdermal permeation enhancer (viz. CG-DES) was evaluated via in vitro permeation testing (IVPT) using porcine ear skin as the skin model. The animal models used to replace human skin in transdermal permeation studies are domestic pigs, rats, mice, guinea pigs, and snakes. However, porcine ear skin showed results comparable to those of normal human skin [45].

The synthesized CG-DES showed virtually nil genotoxic effects in 3T3 cells [17], closely agreeing with the lack of significant cytotoxic effects promoted by CG-DES, and, overall, these results were in clear agreement with results published elsewhere [19,21,45].

In the photomicrograph of Figure 3a, one can observe the histological section of plain porcine ear skin (without any contact with CG-DES) following staining with hematoxylin and eosin, evidencing the *stratum corneum* (thick arrow) without any flaking and the basal layer (thin arrow) formed by cuboid or cylindrical cells (stem-cells). In this *stratum*, in addition to keratinocytes, Merkel cells and melanocytes (cell types not identifiable in routine preparations) were also present. Right after the basal layer, one could observe the compact dermis consisting of dense connective tissue not modeled with collagen fibers, stained by eosin (pink to red), thick, and oriented in several directions. The *stratum corneum* did not present changes in either its structure and disposition of the epidermis, making it possible to observe the keratin layers with an intact structure and appearance (thick arrow). The basal lamina (thin arrow) had no nuclear or eosinophilic cytoplasm changes in its cuboid or cylindrical cells, and the dermis showed no changes in the amount and disposition of collagen fibers stained by eosin.

The photomicrograph in Figure 3b shows the histological cut of the porcine ear skin after exposure to 0.5% (*w/w*) CG-DES for 12 h suggesting a possible deconstruction of the corneal layer of the epidermis (thick arrow); however, not being observed in the basal layer nuclear or cytoplasmic changes in its cuboid or cylindrical cells (thin arrow), nor changes in the amount and disposition of collagen fibers stained by eosin in the dermal layer.

The photomicrograph in Figure 3c shows the histological section of the porcine ear skin exposed to 1.0% (*w/w*) CG-DES. The thick arrow indicated the *stratum corneum* without changes either in its structure or in the disposition of the epidermis, and the basal layer was also shown without nuclear changes or changes in the eosinophilic cytoplasm (thin arrow). The dermis layer showed no changes in the amount and disposition of collagen fibers, stained by eosin.

The photomicrograph in Figure 3d shows the histological cut of the porcine ear skin exposed to 1.5% (*w/w*) CG-DES. The contact with this concentration of CG-DES suggested a possible deconstruction of the *stratum corneum* of the skin and the layers of keratin (thick arrow), with the basal layer showing no nuclear changes and maintaining preserved the eosinophilic cytoplasm (thin arrows). The compact dermis showed no changes in the amount and disposition of collagen fibers.

The photomicrograph in Figure 3e shows the histological cut of the porcine ear skin exposed to 2.0% (*w/w*) CG-DES. This photomicrograph indicated two sites of possible deconstruction of the *stratum corneum* of the epidermis disorganizing the keratin layers

(thick arrows). The basal lamina was, however, preserved (thin arrow), with oval nuclei and eosinophilic cytoplasm showing its preserved cuboid or cylindrical cells. The dermis still showed no changes in the amount and disposition of collagen fibers.

The photomicrograph in Figure 3f shows the histological section of the porcine ear skin exposed to 2.5% (*w/w*) CG-DES. After the contact with this concentration of CG-DES, the *stratum corneum* of the epidermis did not show flaking, and the keratin layers maintained their structure, and the appearance stayed intact (thick arrow). The basal lamina (thin arrow) presented no nuclear or cytoplasmic changes maintaining preserved its cuboid or cylindrical cells. The dermis continued to show no changes in the amount of collagen fibers showing only slight compaction of these fibers (skinny arrow).

After analyzing all the histological sections shown in Figure 3, one may conclude that the contact of the porcine ear skin with various concentrations of CG-DES did not cause significant morphological changes in the skin. However, possible deconstructions were observed (although to a limited extent) in the *stratum corneum* after contact with some concentrations of CG-DES (photomicrographs b, d, and e in Figure 3). The basal layer nuclei were the most evident ones in all histological sections since the histological preparations were performed favoring visualization between the epidermis and the dermis. It was also worth noting that areas of cartilage and adipose tissue were disregarded from all histological cuts.

In Figure 2a, one can observe that the maximum average permeated curcumin for different amounts of CG-DES (% *w/w*) were higher for 2% (*w/w*) CG-DES, and this amount of CG-DES allowed the transdermal permeation of a reasonable amount of curcumin (viz. ca. $375 \text{ ng}_{\text{curcumin}}/\text{mm}^2_{\text{skin}}$) in only 15 min of contact with the skin, followed by a steady transdermal permeation of curcumin between $75 \text{ ng}_{\text{curcumin}}/\text{mm}^2_{\text{skin}}$ and $25 \text{ ng}_{\text{curcumin}}/\text{mm}^2_{\text{skin}}$ up to the 6 h timeframe of the assay (Figure 2b). Despite promoting a slight deconstruction of the *stratum corneum*, as apparent from inspecting the histological cut in the photomicrograph of Figure 3e, this amount of CG-DES was highly effective in enhancing the transdermal permeation of bioactive macromolecules [17–19] without promoting major morphological changes in the skin, as can be observed in Figure 3.

The photomicrographs in Figure 4 show the histological cuts of porcine ear skin exposed to the plain saline solution (0.9% NaCl, *w/w*; Figure 4a) and after transdermal permeation of curcumin using CG-DES at 2.0% (*w/v*; Figure 4b). In the photomicrograph of porcine ear skin exposed to plain saline solution, no deconstructions or flaking of the *stratum corneum* could be observed, and the basal layer (thin arrows) formed by cuboid or cylindrical cells (stem-cells) were well observed. In the photomicrograph of porcine ear skin after transdermal permeation of curcumin using CG-DES at 2.0% (*w/v*; Figure 4b), two sites of possible deconstruction of the *stratum corneum* of the epidermis were apparent, with disorganization of the keratin layers (thick arrows). The basal lamina was maintained, however, preserved (thin arrow), with oval nuclei and eosinophilic cytoplasm, also showing its preserved cuboid or cylindrical cells, although with slight compaction. These results were, in fact, quite similar to those of the blank experiments (i.e., without permeating curcumin, see photomicrograph in Figure 3e) using the same weight percentage of CG-DES.

As apparent from the histological cuts displayed in Figure 3, the skin maintained its morphological structure virtually intact upon contacting with CG-DES, and a possible explanation for the permeation enhancing properties of CG-DES might be that this DES promoted transdermal permeation through the skin layers via the two putative routes illustrated in Figure 5. According to Chantasart and Kevin [46], increased permeation depended strongly on the lipophilicities of the permeation enhancers. The mechanism of the action of the permeation enhancers was related to the fluidization of the intercellular lipids in the *stratum corneum*, supporting the hypothesis that the site of action of the permeation enhancer was in the semi-polar region of the intercellular lipid domain in the *stratum corneum*, promoting drug penetration across the *stratum corneum* lipoidal pathway (intercellular route, Figure 5). However, it seemed that the transdermal permeation promoted by CG-DES also occurred in a more direct way, viz the transcellular route (Figure 5).

Islam et al. [47] developed a series of microemulsions with acyclovir and ionic liquid (choline oleate) to evaluate in vitro the permeation of acyclovir through the skin. The results obtained by those researchers revealed that the acyclovir permeation was intensified by using the ionic liquid. They observed by FTIR analyses that the enhanced drug permeation occurred due to a reduction of the skin barrier function via modification and disruption of the regular arrangement of the corneocytes of the *stratum corneum*, which corroborated the results presented herein and confirmed the putative transdermal permeation routes potentiated by CG-DES that were put forward.

The molecules of CG-DES were therefore hypothesized to slip around (intercellular route, through the intercellular lamellar lipids, see Figure 5) the corneocytes that made up the *stratum corneum*, and through (transcellular route) both the intercellular lamellar lipids and the fatty compounds that made up the plasma membrane of skin cells (viz, the corneocyte lipid envelope, see Figure 5), creating small transient openings through which the bioactive molecules could permeate [22].

5. Conclusions

In this research effort, the effect of CG-DES on the skin was studied following abundant experimental evidence that this DES promoted transdermal permeation of bioactive macromolecules. The results of this study clearly showed that the use of CG-DES highly enhanced transdermal permeation, and a rationale for the mechanism of action by which CG-DES permeabilized the skin was postulated based on an in vitro skin histological study. The molecules of CG-DES were hypothesized to slip around (intercellular route, through the intercellular lamellar lipids) and through (transcellular route) the fatty compounds that made up the corneocyte lipid envelope creating small transient openings through which bioactive molecules could permeate. The use of CG-DES in topical formulations was proved by our research group to greatly increase the transdermal permeation of curcumin (which will enable advances in the application of this biomacromolecule to treat chronic skin diseases), insulin (which will enable the development of non-invasive systems for insulin administration), and bacteriophage particles (enabling the treatment of localized bacterial infections in the deeper layers of the skin). The system developed will also allow the non-invasive delivery of a wide range of bioactive macromolecules with poor water solubility in an easy fashion, with a high potential for translation into real-world applications with clear benefits to patients.

Author Contributions: All authors participated in the conception and design of the experiments, and analyzed the resulting data; R.B., E.C.S., É.A.C., and T.J.O. performed the experiments; R.M.R.-C. contributed to the analyses of the histological sections; V.M.B. and M.M.D.C.V. wrote the paper; V.M.B. supervised the work, revised the paper, and contributed with reagents and analysis tools. All authors have read and agreed to the published version of the manuscript.

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

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4.2 Characterization of commercially available turmeric for use in pharmaceutical products and food supplements.

O artigo "*Characterization of commercially available turmeric for use in pharmaceutical products and food supplements*" foi publicado na revista *Journal of the Brazilian Chemical Society*, estando disponível em http://jbcs.sbq.org.br/forthcoming_papers.

A proposta deste trabalho foi avaliar a qualidade da curcumina disponível comercialmente, uma vez que, este ativo foi escolhido para estudo como uma alternativa para o tratamento da psoríase.

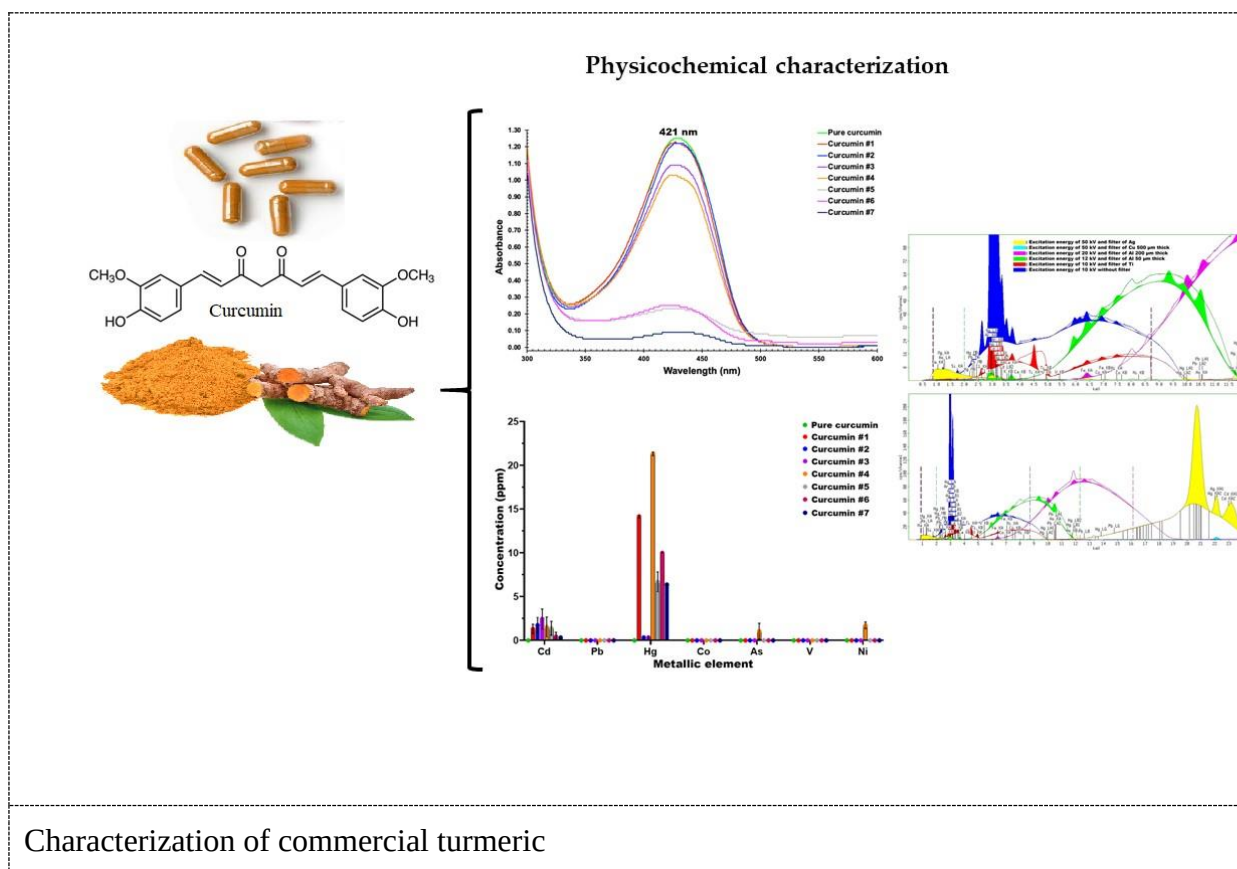
Em função de seu elevado valor comercial, a curcumina tem sido frequentemente adulterada com os mais diversos materiais e estratégias, o que pode resultar em produtos de baixa qualidade, com menor eficácia (MÉLO *et al.*, 2011). Além disto, havia a preocupação de contaminação com chumbo. Há relatos de adulteração de açafrão com o cromato de chumbo composto amarelo vibrante, tóxico e cancerígeno, com o objetivo de destacar a cor da curcumina e tornar o produto mais atraente (ERASMUS, 2021).

Assim, foram realizados testes de ponto de fusão, atividade antioxidante por DPPH, atividade antimicrobiana pelo ensaio de difusão em disco de ágar, determinação de contaminantes metálicos por Fluorescência de Raios-X com Dispersão de Energia (EDXRF), análise de espectros por Infravermelho com Transformada de Fourier (FTIR) e por Ultravioleta–Visível (UV-VIS) e determinação da concentração de curcumina por UV-VIS.

Observou-se significativa variação dos parâmetros avaliados e, como contaminante metálico tóxico de maior proeminência, mercúrio. Provavelmente, esta contaminação deva-se a contaminação ambiental.

A amostra de curcumina que apresentou as melhores características, tendo como padrão de comparação curcumina PA, foi a escolhida no preparo do gel e os ensaios deram prosseguimento.

Graphical Abstract (GA)





Characterization of Commercially Available Turmeric for Use in Pharmaceutical Products and Food Supplements

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We addressed some physicochemical and biological differences in commercial turmeric, acquired from both handling pharmacies and stores selling natural products for quality assessment. For this, it was determined the melting point, antioxidant activity by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), antimicrobial activity via the agar disk-diffusion assay, metallic contaminants by X-ray fluorescence with energy dispersion (EDXRF), spectral behaviors by Fourier transform infrared (FTIR) and ultraviolet-visible (UV-Vis) and determination of curcumin concentration by UV-Vis. The results obtained of the several commercial turmeric samples were not homogeneous, showing in fact significant differences regarding melting points, UV-Vis spectral scan profiles, and FTIR spectra, presenting toxic metals and quite low curcumin contents. This sheds light on the urge to implement adequate quality control in this type of raw material for human safe use of curcumin. Particularly, contamination with heavy metals, such as mercury, is a serious health problem, due to its high toxicity and accumulative power in the body.

Keywords: commercial turmeric, curcumin, physicochemical characterization, antimicrobial activity

Introduction

Curcumin is a compound derived from turmeric (*Curcuma longa* L.) (3 to 5%) that exhibits an extensive spectrum of biological activities.^{1,2} *Curcuma longa* L. is a plant belonging to the Zingiberaceae family, cultivated normally in south and southeast tropical Asia.^{3,4}

Curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione, is a substance with low solubility in water being estimated to be 11 ng mL⁻¹.⁵ Currently, the action of curcumin in several diseases has been re-evaluated based on the proof of its capacity anti-inflammatory, antioxidant, chemoprotective, tissue-protective, antibacterial, antifungal, antiviral, metabolism-regulating, immunomodulating, antineoplastic and anti-depressant properties.⁵⁻⁸ Thus, curcumin has been used in the treatment of arthritis, diabetes, psoriasis, gastrointestinal diseases, acute heart disease, anxiety, hyperlipidemia, liver disease, bacterial

infections, inflammation, etc.⁹⁻¹¹ However, the major problems with curcumin is its poor bioavailability, probably due to poor absorption, rapid metabolism and rapid systemic elimination. Hence, efforts have been made to improve the bioavailability of curcumin and consequent bio-efficacy,¹² such as formulating nanoparticles, liposomes, micelles, solid dispersions, emulsions, and microspheres containing curcumin.^{5,10}

This plant species is also used to produce the yellow color in food, cosmetic and in some drug preparations. Currently, interest in natural dyes, particularly for use in food, has grown due to consumer demand for healthier products, together with the evidence of the harmful effects of many artificial dyes.¹³ The most commonly used natural dyes for obtaining the yellow color is carotenoids (bixin, lutein and crocin), betalains (betaxanthins), flavonoids (carthamin), curcuminoids (curcumin) and riboflavins.¹⁴

Brazil has favorable climatic conditions for the cultivation of *C. longa*. However, it is necessary to establish more rigorous quality standards for the expansion of the market.¹⁵ The quality of plant products may vary due

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to different agricultural practices, climate change and manufacturing systems^{15,16} causing significant variations in the amount of curcumin.¹⁷ In addition, due to its many potential commercial applications, adulteration of curcumin with botanical additives and chemical substances has increased.¹⁸ Medicinal plants are still important resources for health around the world. However, the main problem with herbal medicines is that they are prone to tampering and substitution. In this way, it is extremely important to establish quality parameters for each plant species and carry out inspections.¹⁹

In this perspective, it is of greatest importance to evaluate curcumin in commercially available turmeric samples for the production of pharmaceutical products and food supplements, because such large chemical variability will result in ineffective products or even in the potential for toxicity. This way, the objective of this research was to evaluate some physicochemical and biological parameters of different samples of curcumin, in order to verify if the products that are commercially available are safe for human consumption.

Experimental

Chemicals

The samples analyzed were constituted of dry commercial turmeric extracts and were acquired in the region of Sorocaba, SP (Brazil). Commercial turmeric powder was acquired from manipulation pharmacies (samples 1-5) and local establishments selling natural products (samples 6 and 7). Water was purified in a Master System All (model MS2000, Gehaka, São Paulo, Brazil). As standard it was used curcumin from Sigma-Aldrich (Ref. No. C1386, CAS No. 1386, St. Louis, MO, USA) with a purity of 98.0%. DPPH (2,2-diphenyl-1-picrylhydrazyl) (ref. Sigma D9132-1G) was purchased from Sigma-Aldrich (St. Louis MO, USA). High performance liquid chromatography (HPLC)-grade methanol (LiChrosolv®, CAS-No. 67-56-1) was purchased from Merck (Darmstadt, Germany). L-Ascorbic acid was purchased from Sigma-Aldrich (ref. Sigma A92902, CAS No. 50-81-7, St. Louis, MO, USA). TSB (trypticase soy broth) and TSA (trypticase soy agar) were purchased from Merck KGaA (Darmstadt, Germany).

Bacterial strains

Staphylococcus aureus strains CCCD-6538, CCCD-29213; CCCD-14458; CCCD-33591; and CCCD-43300, *Pseudomonas aeruginosa* CCCD-P004 and *Escherichia*

coli CCCD-E003, from the Collection Culture Cefar Diagnóstica, were kindly donated by the Hospital of Clinics at Unicamp (Campinas, SP, Brazil).

Procedures

Melting point

The melting points of the commercial turmeric extracts and pure curcumin were obtained using a digitally micro-processed heating device from Gehaka (model PF 1500 FARMA, São Paulo, Brazil). The temperature increase occurred at rate of 1 °C min⁻¹. All the analytical procedures were done in triplicate.

Antioxidant activity by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate)

The antioxidant activity was described by Brand-Williams *et al.*²⁰ with adaptations by Duarte-Almeida *et al.*²¹

To prepare the reagent DPPH, 4 mg of 2,2-diphenyl-1-picrylhydrazyl was dissolved in 100 mL of ethanol. Curcumin samples were prepared in methanol at a concentration of 1.0 mg mL⁻¹, as positive control it was used L-ascorbic acid, as negative control it was used water and DPPH reagent, and ultrapure water was used as blank. For the assay, 500 µL of the curcumin solution (1.0 mg mL⁻¹ in methanol) were added to 3.5 mL of DPPH reagent. The same procedure was followed for ascorbic acid (1.0 mg mL⁻¹ in methanol). For the blank, 500 µL of ultrapure water were added to 3.5 mL of DPPH reagent. The samples were homogenized and kept in the dark for 25 min. Their absorbances were then read at the wavelength of 517 nm on a UV-Vis spectrophotometer from Agilent (model Cary 60 UV-Vis, Santa Clara, CA, USA). The antioxidant activity (AA) was calculated according to equation 1, where AA (%) is the percentage of antioxidant activity and Abs is the absorbance at the used wavelength.

$$AA(\%) = \{ (Abs_{sample} - Abs_{blank}) / (Abs_{ascorbic\ acid} - Abs_{blank}) \} \times 100 \quad (1)$$

Antimicrobial activity via the agar disk-diffusion assay

The antimicrobial activity of the commercial turmeric and standard curcumin samples was determined via the agar disk-diffusion technique for susceptibility to antimicrobials, according to the Clinical and Laboratory Standards Institute.²² The antimicrobial activity assays were carried out using *Staphylococcus aureus* strains (CCCD-6538, CCCD-29213, CCCD-14458, CCCD-33591, CCCD-43300); *Pseudomonas aeruginosa* (CCCD-P004) and *Escherichia coli* (CCCD-E003).

For revival of the bacterial strains, the lyophilized pellets were suspended in 50 mL TSB and incubated

at 37 °C during 24 h. Subsequently, bacterial cultures were homogenized and inoculated by spreading in Petri dishes with solidified TSA. Curcumin samples in ethanol (100 µg mL⁻¹) were impregnated in sterile filter paper discs (ca. 6.0 mm in diameter) and dried at 36 °C during 24 h to remove the solvent and produce ca. 90 µg curcumin *per* disc. The disks impregnated with curcumin were then applied over the inoculated TSA medium in the Petri plates, which were incubated at 37 °C during 24 h. The negative standard of inhibition was 30 µg tetracycline *per* sterile paper disk (Sensidisc DME, TET30, DME-Diagnósticos Microbiológicos Especializados, Araçatuba, SP, Brazil) whereas the positive standard of inhibition was a sterile paper disc impregnated with sterile saline solution (NaCl 0.9% m/m). Petri dishes were incubated at 37 ± 0.5 °C for 24 h. After this period, the presence of growth inhibition halos was observed.

Fourier transform infrared spectrophotometry (FTIR) analyses

2 mg of each sample was ground with 300 mg of KBr. This mixture was pressed in a Shimadzu brand hydraulic press (model SSP-10A, Kyoto, Japan) to obtain transparent discs. The infrared spectra were reading using a Fourier transform infrared spectrophotometer (FTIR) Shimadzu (model IR-Affinity-1, Shimadzu, Kyoto, Japan), in the wavenumber range from 4000 to 400 cm⁻¹, with a resolution of 2 cm⁻¹.

Metallic contaminants in the commercial turmeric samples by X-ray fluorescence with energy dispersion (EDXRF)

Metallic contaminants in the samples were determined in a compact EDXRF system by Malvern Panalytical (Santo Amaro, SP, Brazil), composed of a silicon detector (silicon drift diode) with an area of 25 mm² and thickness of 500 µm, protected by a beryllium window of 12.5 µm. The X-ray source used for excitation of the samples consisted of a miniature X-ray tube with an Ag anode (Amptek, Bedford MA, USA). The X-ray tube was set up to work at a voltage of 30 kV and current of 10 µA. The energy resolution of the detection system was ca. 125 eV, quoted as the full width at half maximum (FWHM) for Mn Kα 5.9 keV X-rays from a ⁵⁵Fe source. All measurements were carried out using atmospheric air, and the measuring time was set for 45 min for each sample. The Epsilon software (Malvern Panalytical, Santo Amaro, SP, Brazil) was used for signal control and data acquisition. The EDXRF system was calibrated with control samples. Tablets of approximately 5 mm high by 22 mm in diameter were produced using a spectrometric sample compaction press (PDCA-40, São Paulo, SP, Brazil) by applying a force of 15 tons.

UV-Vis spectra of curcumin samples

UV-Vis spectrophotometric scans (from 200 to 800 nm) of the commercial turmeric and standard curcumin samples in methanol (10 µg mL⁻¹) were carried out using quartz cuvettes in a UV-Vis spectrophotometer from Agilent (model Cary 60 UV-Vis, Santa Clara, CA, USA).^{23,24} The determination of curcumin concentration in the commercial turmeric samples was based on the procedure described by Rapalli *et al.*²³ and Singh and Avupati.²⁴ Stock solutions of 100 µg mL⁻¹ were prepared by dissolving 2.5 mg of each sample in 25 mL methanol. All solutions were sonicated during 15 min and filtered through Whatman filter paper No. 41. From the stock solutions, a concentration of 5 µg mL⁻¹ was produced via dilution with methanol. Standard curcumin solutions (Sigma-Aldrich, Ref. No. C1386, CAS No. 1386, St. Louis, MO, USA) in methanol were produced in concentrations ranging from 1 to 15 µg mL⁻¹ and used to obtain the calibration curve. All spectrophotometric measurements were performed at a wavelength of 421 nm.

Results and Discussion

The use of medicinal herbal products has increased over the past three decades by 80%.²⁵ Many people use herbal medicines in primary healthcare. Although therapies with medicinal herbal products have shown potential efficacy for the treatment of several diseases, many products from plant species are few and poorly controlled.²⁵ Adding to this, the growing demand for medicinal herbal products has transformed the large-scale manufacture of these products into a routine. Large scale production may result in longer storage times with concomitant possible product deterioration.²⁶ Hence, standardization of some parameters for the establishment of a consistent biological activity or simply to maintain the quality of production and manufacturing of herbal drugs is of utmost importance.²⁵ *Curcuma longa* L. is, worldwide, a plant of high medicinal and economic value. Although the key active ingredients have been studied extensively, the concerns remain about the quality of commercial extracts.²⁷

Melting point

Physical characterization is a crucial factor for assessing the quality of crude drugs. Simple methods such as, for example, melting point, are useful in this regard. The results obtained for the melting point of the commercial turmeric and standard curcumin samples are displayed in Figure 1.

There was no difference observed between the values of the melting points between samples acquired in handling pharmacies, when compared with the values of the melting

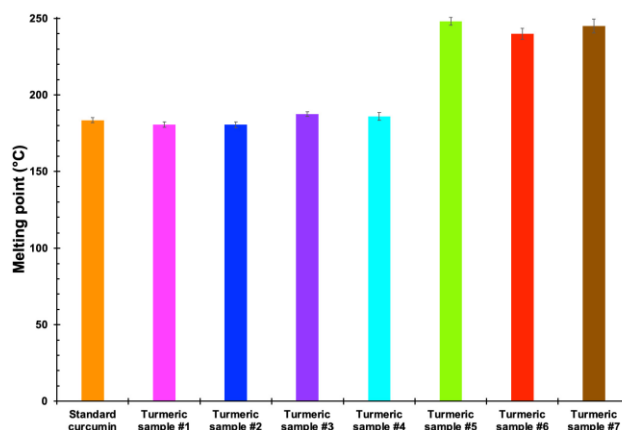


Figure 1. Average melting point of commercial turmeric and standard curcumin samples. All values represent the mean of three experiments, and the error bars represent the standard deviations.

point of the standard curcumin sample. However, in commercial turmeric samples acquired in establishments selling natural products, there was a significant difference in those values, indicating a possible contamination or adulteration of the product (Figure 1). The average value ($n = 3$) of the melting point obtained for the standard sample of curcumin (183.5 ± 1.7 °C) showed a value very close to the information provided by the manufacturer (viz. 183 °C). Bagchi *et al.*²⁸ reported a value of 180 °C, whereas Priyadarsini²⁹ reported a value of 183 °C for this physical parameter. These variations are expected since the melting point for pure chemicals or phytochemicals is constant, but crude drugs from animal or plant origin contain mixed chemicals, and that is why they are described with certain range of melting point.³⁰

Antioxidant activity

Curcumin extracts have strong antioxidant activity and this way can be important in disease prevention and health preservation.³¹ Thus, evaluating this characteristic can be a way of evaluating the quality of samples.

The antioxidant activity of the commercial turmeric and standard curcumin samples was carried out via the DPPH method. Among the *in vitro* methods the DPPH method is the most popular one due to its simplicity, speed, and low cost.³² In this method, DPPH is a free radical that becomes stable after accepting an electron or hydrogen radical. The method is based on the discoloration of a solution composed of stable DPPH radicals (violet color) by the transfer of electrons from an antioxidant compound to a free radical, DPPH•, which, when reduced, loses its purple color.^{21,33}

Table 1 displays the results obtained for the antioxidant activities of all commercial turmeric and standard curcumin samples assayed.

Table 1. Antioxidant activity (AA) of curcumin samples ($n = 3$). Pure curcumin (Sigma-Aldrich), curcumin 1 to 5 acquired in handling pharmacies, curcumin 6 and 7 acquired in natural products establishments

Sample	AA / %
Standard curcumin	93.11
Commercial turmeric sample 1	85.86
Commercial turmeric sample 2	84.03
Commercial turmeric sample 3	85.59
Commercial turmeric sample 4	84.18
Commercial turmeric sample 5	5.33
Commercial turmeric sample 6	22.23
Commercial turmeric sample 7	23.32

Determination of the antioxidant activity revealed that commercial turmeric samples 5, 6 and 7 exhibited a lower antioxidant capacity when compared to the remaining commercial turmeric samples, as displayed in Table 1. The low values of antioxidant activity of the samples obtained in bulk from establishments selling natural products (viz. 22.23 and 23.32%) can be explained in terms of a putative exposure to environmental conditions, favoring loss of antioxidant capacity. Curcumin is a substance with potent antioxidant activity, which has been demonstrated by several *in vitro* studies, suggesting that such antioxidant activity arises from either the hydroxyl group or the methylene group of the α -diketone (heptadiene-dione) portion of curcumin. The phenolic hydroxyl group is also

important for the antioxidant activity of curcumin.^{12,34,35} In free radical reactions, the curcumin hydrogen most easily removable is that of the phenol-OH group, resulting in the formation of phenoxy radicals. These radicals are stabilized by resonance through a keto-enol structure that allows an almost perfect conjugation between the two aromatic rings containing the active antioxidant centers.^{36,37}

Antimicrobial activity via the agar disk-diffusion assay

Verification of the antimicrobial activity of the commercial turmeric and standard curcumin samples was also carried out, since some studies^{33,38} have reported antimicrobial activity in products obtained from turmeric.

Several *in vitro* laboratory methods can be used to evaluate the antimicrobial activity of plant extracts. Among the most common, the disk-diffusion was selected because is a well-known procedure and provides good qualitative results.³⁹

Notwithstanding such reports on the antimicrobial activity of curcumin, no antimicrobial activity whatsoever was observed in the commercial turmeric and standard curcumin samples in the microorganisms tested, as can be observed by inspection of the results displayed in Figure 2. According to several authors,^{11,37,40} the absence of antimicrobial activity of turmeric extracts may be associated with the hydrophobic characteristic of curcuminoids, which makes it difficult for them to permeate into the environment.

Fourier transform infrared spectrophotometry (FTIR) analysis

The employment of the FTIR aimed to identify the characteristic functional groups in curcumin samples and this way check for possible tampering or falsifications. According to Lestari and Indrayanto³⁶ and Dhakal *et al.*,¹⁸ pure curcumin samples should exhibit peaks at 3511, 1630, 1601, 1510, 1429, 1278 and 1028 cm^{-1} . A long shallow peak at 3255 cm^{-1} and a sharp peak at 3507 cm^{-1} indicate

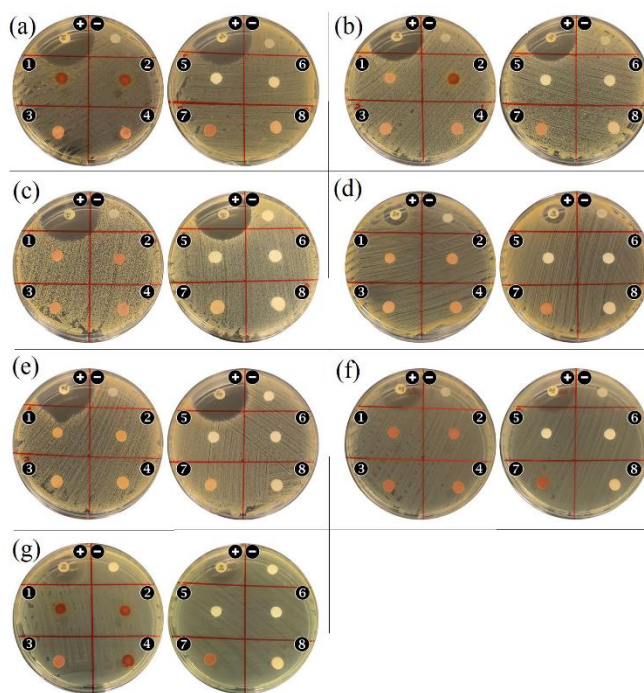


Figure 2. Results of the antimicrobial activity assays of both commercial turmeric and standard curcumin via the agar disk-diffusion methodology. (a) *Staphylococcus aureus* CCID-6538; (b) *Staphylococcus aureus* CCID-29213; (c) *Staphylococcus aureus* CCID-14458; (d) *Staphylococcus aureus* CCID-33591; (e) *Staphylococcus aureus* CCID-43300; (f) *Escherichia coli* CCID-F003; (g) *Pseudomonas aeruginosa* CCID-P004; (+) positive control (disc with 30 μg tetracycline); (-) negative control (disc with saline solution (NaCl 0.9% m/m)). (1) Standard curcumin; (2-6) handling pharmacies; (7, 8) turmeric from establishments selling natural products.

the presence of the hydroxyl functional group ($-\text{OH}$). The peak detected at 1626 cm^{-1} has a predominantly mixed character regarding the double bond between carbons ($\text{C}=\text{C}$) and the carbonyl functional group ($\text{C}=\text{O}$), whereas a strong band at 1600 cm^{-1} is attributed to the vibrations of symmetrical stretching of the aromatic ring ($\text{C}-\text{C}_{\text{ring}}$). The peak at 1504 cm^{-1} is attributed to the carbonyl functional group ($\text{C}=\text{O}$), the peak attributed to enol ($\text{C}-\text{O}$) is displayed at 1273 cm^{-1} , the peak at 1026 cm^{-1} refers to the ether functional group ($\text{C}-\text{O}-\text{C}$), and the *cis*-CH vibration of the aromatic ring appears at 714 cm^{-1} .^{3,36,41}

The peaks displayed in the standard curcumin spectrum at 466.77 , 542.00 , 574.79 , 599.86 , 713.66 , 810.1 , 856.39 , 885.33 , 962.48 , 1028.06 , 1116.78 , 1151.50 , 1182.36 , 1205.51 , 1232.51 , 1280.73 , 1313.52 , 1427.32 , 1508.33 , 1600.92 , 1627.92 and 3510.45 cm^{-1} (Figure 3a) could also be observed in samples 1, 2 and 3 (Figures 3b, 3c and 3d). For sample 3 (Figure 3d) variations in peak intensity were observed between 3000 and 3500 cm^{-1} , suggesting a stretching of the hydroxyl group ($-\text{OH}$) of primary alcohols.^{3,33} The spectrum of sample 4 (Figure 3e) shows variations in peak intensity between 466 and 810 cm^{-1} and in the range of 3500 cm^{-1} . Observing the spectra of samples 5, 6 and 7 (Figures 3f, 3g, 3h) peaks are observed at 921.97 , 1078.2 , 1155.36 , 1514.12 , 1631.78 and 2927 cm^{-1} . Significant changes can be observed in both the intensity and range of the wavenumbers relative to the standard curcumin (Figure 3a), which can be attributed to different interactions between functional groups of curcumin; these observations also reinforce the evidence of either polymorphism or pseudo-polymorphism, or even adulterations in the products.³

The results obtained in the FTIR analyses performed to the standard curcumin and commercial turmeric samples are displayed in Figure 3 in the form of normalized spectra.

Metallic contaminants by X-ray fluorescence with energy dispersion (EDXRF)

The presence of metallic elements is common in all medicinal plants, since numerous agricultural, domestic, medical, industrial and technological applications have led to extensive distribution of heavy metals all over the environment.³⁹ Attempting to avoid damage to the health, there are tolerable limits of such metals in foodstuff. Monitoring of quality control and evaluation of medicines plants is an important aspect as in the production as well as in the distribution on the pharmaceutical market.⁴² The Brazilian Pharmacopoeia (BF)⁴³ admits, for medicinal plants in general, up to 20 ppm of heavy metals, which cannot exceed the limits of 1 ppm of cadmium, 5.0 ppm of lead, 0.1 ppm of mercury and 5.0 ppm of arsenic. However, the BF indicates as methods of analysis of heavy metals only limit tests and atomic absorption. The X-ray fluorescence method has significant advantages over these two methods, such as the accuracy, minimal sample preparation and multi-element analysis.⁴⁴ However, studies using X-ray fluorescence in pharmaceutical products are scarce.

The EDXRF equipment utilized uses different energies to excite the sample and, for each excitation energy, only for some elements the concentrations are obtained. Hence, for detection of low elements, low excitation energies were used, whereas for detection of heavy metals higher

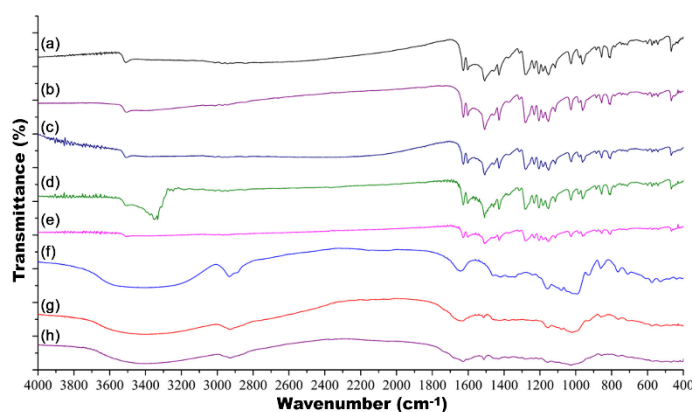


Figure 3. FTIR spectra of samples of pure curcumin and commercial turmeric. (a) Pure curcumin; (b) curcumin 1; (c) curcumin 2; (d) curcumin 3; (e) curcumin 4; (f) curcumin 5; (g) curcumin 6; (h) curcumin 7. Samples 2-6 were acquired in handling pharmacies, whereas samples 7 and 8 were acquired in establishments selling natural products.

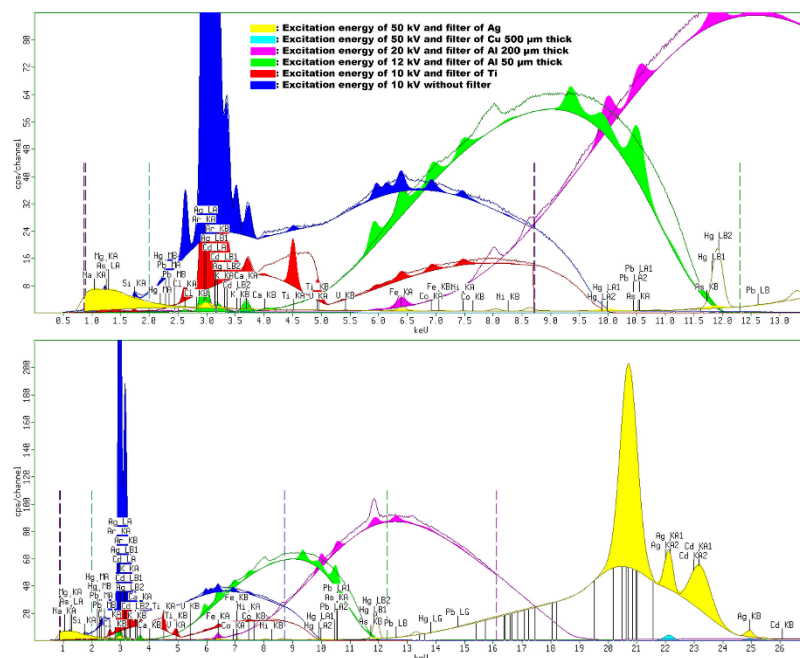


Figure 4. EDXRF spectrum of one of the turmeric samples (viz. curcumin sample 6), showing the different excitation energies used in the elemental detection.

excitation energies were used to hit the sample, allowing emission of elemental characteristic X-rays. Figure 4 displays the EDXRF spectrum of one of the turmeric samples (viz. curcumin sample 6), showing different excitation energies.

The average concentrations (and respective standard deviations) of cadmium (Cd), lead (Pb), mercury (Hg), cobalt (Co), arsenic (As), vanadium (V) and nickel (Ni) in standard curcumin and commercial turmeric samples are displayed in Figure 5.

The presence of these metallic contaminants was detected in all samples assayed (except for the standard curcumin). Seven samples indicated the presence of Hg and Cd; there was no detection of the presence of Pb, Co and V. Regarding Hg, with the exception of samples 6 and 7, all the other samples exhibited Hg above the acceptable level (≤ 0.1 ppm). Sample 4 showed the highest levels of Hg (21.33 ppm), Cd (2.45 ppm), As (1.06 ppm) and Ni (1.54 ppm).

Although metals such as copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) have physiological functions in the body, many of the metals encountered in the samples assayed have no benefits to the human physiology, such as Pb, Hg and Cd, and can even interfere with the functions

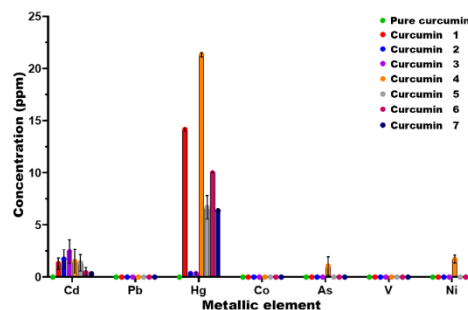


Figure 5. Average values for the concentration of metallic contaminants present in standard curcumin and commercial turmeric samples. Samples 1-5 were acquired in handling pharmacies, whereas samples 6 and 7 were acquired in establishments selling natural products. Values represent the mean of three determinations and error bars the standard deviations.

of various organs and systems in the body, such as the central nervous system, the hematopoietic system, the liver and kidneys.⁴⁰ Exposure to Hg compounds leads to toxic effects in the cardiovascular, pulmonary, urinary, gastrointestinal, neurological and skin systems, which might become fatal.⁴⁵ Environmental contamination and exposure to heavy metals, such as As, Cd, Pb and Hg, is a serious and growing global problem, being even more

serious in some developing countries, where the economy has been prioritized over environmental issues.^{46,47} Air, soil and water, are known to be polluted by heavy metals.⁴⁰ Particularly, contamination of soils by Hg is often due to the addition of this heavy metal as part of fertilizers, lime, sludges, and manures and the most of the plants that uptake Hg tend to accumulate it on the roots.⁴⁸

Based on the results obtained for the presence of metallic contaminants in the standard curcumin and commercial turmeric samples assayed, a greater control in relation to the determination of heavy metals in these products is of utmost importance if they are to be used in pharmaceutical and food applications. The consumption of plant materials or herbal drugs contaminated by heavy metals can lead to intoxication and consequent detrimental effects to the human health. Even when present at not so high concentrations, such heavy metals tend to accumulate in the body over time due to their non-biodegradable and persistent nature, which will eventually be detrimental to health.⁴⁹

UV-Vis method for determination of the amount of curcumin and spectral behavior

UV-Vis spectroscopic method has been reported to be suitable to determine concentration of curcumin and offers simplicity, rapidity and reliability. In this sense, it was used to verify the spectral behavior as well as to determine the concentration of curcumin in the samples.⁵⁰

The results obtained in the UV-Vis spectral scans performed to standard curcumin and commercial turmeric samples are displayed in Figure 6. The maximum absorption wavelength of curcumin in all samples assayed

occurred at 421 nm. These results were similar to those reported by Sharma *et al.*⁵¹ In another study by Singh and Avupati,²⁴ where the solvent used was ethyl acetate, the maximum absorption of curcumin occurred at 418 nm. Despite absorbing at the same wavelength of 421 nm, commercial turmeric samples 5, 6 and 7 exhibited much lower absorption intensity (Figure 6), which may indicate low levels of curcumin.

The spectrophotometric method used showed a good linear correlation, producing as calibration curve: $Abs_{421nm} = 0.1643 \times (\text{curcumin} / (\mu\text{g mL}^{-1})) - 0.00254$ (coefficient of determination $r^2 = 0.995$). As can be observed from inspection of the data displayed in Table 2, three of the commercial turmeric samples (*viz.* curcumin 5, 6 and 7) presented curcumin values well below the expected value, with these results corroborating the FTIR spectra (Figure 3) and the UV-Vis spectral scans (Figure 6).

Table 2. Curcumin content of the standard curcumin and commercial turmeric samples, as determined spectrophotometrically. Samples 1-5 were acquired in handling pharmacies, whereas samples 6 and 7 were acquired in establishments selling natural products

Sample	Sample amount assayed / ($\mu\text{g mL}^{-1}$)	Amount of curcumin found / ($\mu\text{g mL}^{-1}$)	Average amount of curcumin / (% m/m)
Pure curcumin	5.000	4.964	99.28
Curcumin 1	5.000	4.797	95.94
Curcumin 2	5.000	4.771	95.42
Curcumin 3	5.000	4.065	81.30
Curcumin 4	5.000	3.848	76.96
Curcumin 5	5.000	0.925	18.50
Curcumin 6	5.000	1.316	26.32
Curcumin 7	5.000	1.414	28.28

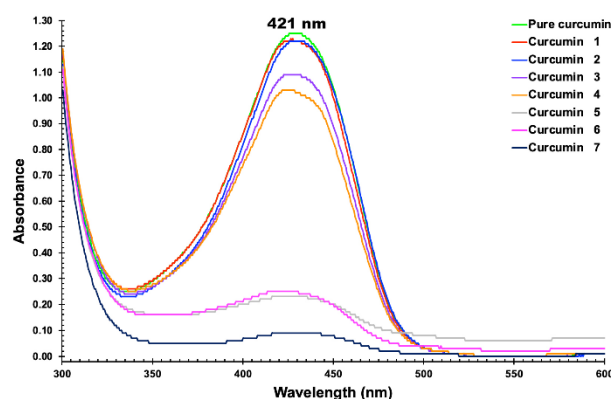


Figure 6. UV-Vis spectral scans of samples of standard curcumin and commercial turmeric samples. Samples 1-5 were acquired in handling pharmacies, whereas samples 6 and 7 were acquired in establishments selling natural products.

Commercially available saffron extracts possess three curcuminoids, 77% (m/m) of which are pure curcumin, 17% (m/m) are demethoxycurcumin and 3% (m/m) are bisdemethoxycurcumin.^{5,10,24} Evidently, different species of turmeric may present different weight percentages of these curcuminoids.¹¹ However, for medicinal purposes, it is important to correctly identify the species, in order to ensure adequate levels of curcumin.

Samples 1 to 3 were purchased from a handling pharmacy and showed good levels of curcumin (Table 2). Sample 4 was also acquired in a handling pharmacy but, although showing a lower content in curcumin, it is still within the specifications encountered in the specialty literature where amounts of 77% (m/m) of curcumin in dry extracts are reported.^{5,10,24} Regarding sample 5, despite having been acquired in a handling pharmacy, it presented curcumin values much lower than expected (Table 2). Samples 6 and 7, acquired in commercial establishments selling natural products, also presented concentrations of curcumin substantially lower in comparison to the other commercial turmeric samples (Table 2).

Conclusions

It can be concluded that the physicochemical characteristics of the several commercial turmeric samples were not homogeneous, showing in fact significant differences regarding melting points, UV-Vis spectral scan profiles and FTIR spectra, presenting toxic metals and quite low curcumin contents. The results obtained indicated that there is no adequate quality control in this type of raw material. The purchase of herbal products is a real challenge, as there is no homogeneity among commercial establishments with regard to the origin of the product, which can substantially impact negatively the performance of the commercialized products. Particularly, contamination with heavy metals, such as mercury, due to its high toxicity and accumulative power can cause serious health problems. In this way, there is a need for greater rigor in the quality control of these products, both in free trade products in stores specializing in herbs and spices, as well as in raw materials for handling pharmacies.

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Author Contributions

All authors read and agreed with the published version of the manuscript. Specifically, R.B. performed the experiments; T.H.P. analyzed the presence of metals by X-ray fluorescence; J.M.O.Jr. and V.M.B. analyzed the data obtained; R.B., V.M.B. and M.M.D.C.V. wrote the text; M.M.D.C.V. and V.M.B. were responsible for the general supervision of the work.

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4.3 Transdermal permeation of curcumin promoted by choline geranate ionic liquid: potential for the treatment of psoriasis

Este artigo foi publicado na Revista *Saudi Pharmaceutical Journal*, estando disponível *on-line* na íntegra (https://www.sciencedirect.com/science/article/pii/S1319016422000378?dgcid=rss_sd_all&utm_source=researcher_app&utm_medium=referral&utm_campaign=RESR_MRKT_Researcher_inbound)

O objetivo desta pesquisa foi determinar qual a menor e mais eficaz concentração de líquido iônico a ser utilizado no gel para a permeação da curcumina. Empregar a menor quantidade de um promotor de permeação dérmica é interessante para evitar ou minimizar irritação na pele, reduzir custos e evitar a oclusão (SOUZA *et al.*, 2021).

Os resultados obtidos mostraram que 2% (m/m) de geranato de colina presente no gel foi eficaz em romper a estrutura da pele de forma transitória, facilitando a passagem da curcumina. Assim, a porcentagem de 2% (m/m) foi definida como a mais adequada e utilizada nos géis para os demais experimentos.

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Original article

Transdermal permeation of curcumin promoted by choline geranate ionic liquid: Potential for the treatment of skin diseases

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ABSTRACT

The transdermal permeation of curcumin aided by choline and geranic acid ionic liquid (CAGE-IL) was addressed as a potential treatment for skin diseases. An in-depth analysis of the effect of CAGE-IL concentration in the enhancement of transdermal permeation of curcumin was performed, and the results were modelled via nonlinear regression analysis. The results obtained showed that a low percentage of CAGE-IL (viz. 2.0%, w/w) was effective in disrupting the skin structure in a transient fashion, facilitating the passage of curcumin dissolved in it.

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1. Introduction

Curcumin, 7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione, is a yellow-orange active compound extracted from curcuma rhizomes, especially *Curcuma longa* L., that exhibits a wide array of biological activities (Carvalho et al., 2015). *Curcuma longa* L. whose scientific synonyms are *C. domestica* Valetton and *Amomum curcuma* Jacq., is a perennial plant belonging to the *Zingiberaceae* family, cultivated in south and southeast tropical Asian countries (Guimarães et al., 2020; Siddiqui, 2015).

Curcumin is a lipophilic compound, whose water solubility at room temperature (both at acidic and neutral pH values) is estimated at 11 mg mL^{-1} . While curcumin is soluble in alkali, it is quite susceptible to self-degradation in this form (Anindya et al., 2015; Stohs et al., 2020). Curcumin is a commercially available mixture of curcuminoids, containing 72–78% of curcumin, 12–18% of demethoxycurcumin, and 3–8% of bisdemethoxycurcumin (Burapan et al., 2020; Hassanzadeh et al., 2020).

The multicomponent nature of curcumin is well documented, possessing “generally recognized as safe” (GRAS) status attributed by the Food and Drug Administration (FDA), and being used as a food additive at different levels between 20 and 500 mg kg^{-1} (EFSA, 2010; Nelson et al., 2017). Clinical reports found in the specialty literature state that curcumin does not produce any harmful effect on the human body even at dosages as high as $1000\text{--}2000 \text{ mg day}^{-1}$ (Hewlings and Kalman, 2017). However, several adverse effects have been related to the use of curcumin, including gastrointestinal and hematological adverse effects (Agrawal and Goel, 2016).

Curcuma longa has, since ancient times, been widely used in Ayurveda and traditional Chinese medicine for the medical treatment of several diseases (Kocaadam and Şanlıer, 2017). In the last few years, numerous scientific studies have rediscovered the therapeutic potential of curcumin and its derivatives due to a wide array of beneficial effects of this compound including (but not limited to) anti-inflammatory, antioxidant, chemoprotective, tissue protective, antibacterial, anti-fungal, antiviral, metabolism regulating, immuno-modulating, antineoplastic and anti-depressant properties (Rendon and Schäkel, 2019; Stohs et al., 2020). In this way, curcumin helps in the recovery from many acute and chronic diseases such as arthritis, diabetes, diabetic microangiopathy, diabetic nephropathy, psoriasis, gastrointestinal diseases, liver disease, inflammation, and others (Burapan et al., 2020; Hassanzadeh et al., 2020; Khalid et al., 2020).

Curcumin has also been reported as having potential for the topical treatment of (but not limited to) diseases such as psoriasis, iatrogenic dermatitis, wounds, and skin cancer (Vollono et al.,

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2019). However, due to its intrinsic low solubility in water, many efforts have been made aiming at increasing bioavailability in medicines for topical or systemic use. Research works have been performed employing combination of curcumin with cutaneous permeation facilitators, and encapsulation of curcumin in liposomes, micelles and nanoparticles (Jantarat et al., 2018; Nikolic et al., 2020; Vollono et al., 2019).

Among skin permeation facilitators, ionic liquids (ILs) and their deep eutectic solvents (DESs) have emerged as promising substances that effectively enhance transdermal permeation (Zakrewsky et al., 2014; Harada et al., 2018). ILs are able to solubilize amphipathic molecules and, therefore, act as perfect solvents for topical and transdermal delivery of bioactive substances. The IL molecules are hypothesized to slip through and around the fat-soluble molecules that make up skin cells, corneocytes, creating small transient openings through which bioactive macromolecules (carried by IL) can permeate (Boscarol et al., 2021). ILs and their DESs are able to transiently disrupt the skin barrier function by modifying the regular arrangement of the corneocytes of the stratum corneum (Islam et al., 2020; Boscarol et al., 2021). Choline and geranic acid ionic liquid (CAGE-IL) has been successfully used by our research group to increase skin permeation of several substances, such as insulin (Jorge et al., 2020), phage particles (Campos et al., 2020; Silva et al., 2021) and curcumin (Boscarol et al., 2021), and also by other research groups (Banerjee et al., 2017).

There is no uniformity in toxic activities exhibited by ILs. The cytotoxicity of ILs depends on their structure and varies widely, from micromolar to millimolar ranges (Egorova et al., 2017). According Musiał et al. (2021) two main factors that influence the cytotoxicity are the type of anion and the length of the alkyl chain substituent to the ion. Cytotoxicity increases with increasing alkyl chain length of the cation due to the ability of the longer alkyl chain to more easily embed in and ultimately disrupt, the cell membrane (Yoo et al., 2016). ILs with longer alkyl chains ($n > 4$) are more lipophilic than those with shorter alkyl chains. It can be assumed that the former tends to incorporate into the phospholipid bilayers of biological membranes (Musiał et al., 2021). In particular, liquid ionic choline generate has been shown to be safe to use as a skin enhancer. Compared to conventional chemical penetration enhancers such as ethanol, these compounds showed lesser toxicity to cells thereby mitigating the issue of skin irritation that is characteristic of many chemical enhancers (Banerjee et al., 2017). Other works have also demonstrated the effectiveness of CAGE-IL as antimicrobial agents in products for topical use and in higher concentrations. Its efficiency and safety have been demonstrated in human studies indicating its non-toxicity (Shevachman et al., 2020; Ko et al., 2021).

For the delivery of bioactive molecules into the dermis, one can employ gels and hydrogels, using natural biopolymers. Polysaccharide-based hydrogels have received special attention over the last few years, due to their biocompatibility, similarity to the native extracellular matrix, low toxicity and susceptibility to biodegradation by human enzymes. In addition, polysaccharide-based hydrogels can be physically cross-linked, without involving any toxic substances, which makes them attractive in the development of delivery systems for bioactive molecules (Pasqui et al., 2012; Zhu et al., 2019). A biopolysaccharide used with success is locust bean gum (LBG) (Dionísio and Grenha, 2012). LBG is a vegetable galactomannan extracted from locust bean seeds (from the carob tree, *Ceratonia siliqua*), used as a biomaterial in several areas of application such as medical, food and cosmetic, since it allows formation of barriers against water vapors and enhances mechanical properties (Pettinelli et al., 2020). For these reasons, the major goal of the research effort entertained herein was to develop and evaluate a LBG gel integrat-

ing CAGE-IL aiming at the delivery of curcumin via transdermal permeation, with potential application in the treatment of skin diseases such as psoriasis.

2. Materials and methods

2.1. Materials

2.1.1. Cell lineages

The HaCaT (immortalized human keratinocytes) cell line used in the cytotoxicity assays was purchased from Sigma-Aldrich (St. Louis, MO, USA). The cells were maintained at 37 °C according to the procedure described by Rocha et al. (2017).

2.1.2. Porcine ear skin

Domestic pig ears were obtained from a local butchery in the region of Sorocaba (São Paulo, Brazil) and, from these, the skin was excised and used in the transdermal permeation assays.

2.1.3. Chemicals

Water was purified in a Master System All (model MS2000, Gehaka, São Paulo, Brazil) to a final resistivity of ca. 18.18 MΩ.cm and conductivity of 0.05 $\mu\text{S}\cdot\text{cm}^{-1}$. Geranic acid (85% stabilized; ref. W412101-1KG-K), choline bicarbonate (ref. C7519-500ML), locust bean gum (Ref. No. G0753), xanthan gum (Ref. No. G1253), carrageenan gum (Ref. No. C1013), gellan gum (Ref. No. G1910) and curcumin (Ref. No. C1386) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol (LiChrosolv®, CAS-No: 67-56-1) was purchased from Merck (Darmstadt, Germany).

2.2. Experimental procedures

2.2.1. Synthesis of choline and geranic acid ionic liquid (CAGE-IL)

CAGE-IL was prepared according to the procedure described by Jorge et al. (2020), Silva et al. (2021) and Zakrewsky et al. (2014). To a 1000-mL round bottom flask, 48 mL of acid geranic (CAS No. 459-80-3; Sigma-Aldrich, St. Louis MO, USA), 20 mL of choline bicarbonate at 80% (w/v) (CAS No. 62-49-7; Sigma-Aldrich, St. Louis MO, USA) and 20 mL of methanol (CAS No. 67-56-1; Chenco, Brazil) were added. The mixture was magnetically stirred at room temperature, in an open system fashion, overnight, until CO₂ production ceased. The solvent was subsequently removed using a rotary evaporator (BÜCHI Labortechnik AG model R-215, Germany), at 60 °C, during ca. 20 min. The CAGE-IL prepared was transferred into a 50 mL Falcon tube, the headspace flushed with nitrogen, and the tube immediately capped and duly sealed with Parafilm™ (Bemis Flexible Packaging, Neenah WI, U.S.A.).

2.2.2. Preparation and characterization of biopolysaccharide gels

Biopolysaccharide gel with curcumin. Gel formulations integrating curcumin were prepared using locust bean gum (Ref. No. G0753; Sigma-Aldrich, St. Louis MO, USA), xanthan gum (Ref. No. G1253; Sigma-Aldrich, St. Louis MO, USA), carrageenan gum (Ref. No. C1013; Sigma-Aldrich, St. Louis MO, USA), and gellan gum (Ref. No. G1910; Sigma-Aldrich, St. Louis MO, USA) as gelling agents. For each gel, exact amounts of curcumin (Ref. No. C1386; Sigma-Aldrich, St. Louis MO, USA) (viz. 1.0, 1.5 and 2.0% (w/w)), methylparaben (0.1%, w/w) and gum (2%, w/w) were dispersed in ultrapure water and mixed under continuous magnetic stirring. The resulting gels were evaluated in order to define the best gum to be used with curcumin and CAGE-IL.

Biopolysaccharide gel with curcumin and CAGE-IL. Exact mass concentrations of curcumin (1.0%, 1.5% and 2.0% (w/w)) (Ref. No. C1386; Sigma-Aldrich, St. Louis MO, USA), methylparaben

(0.1%, w/w) and locust bean gum (2%, w/w) (Ref. No. G0753; Sigma-Aldrich, St. Louis MO, USA) were dispersed in ultrapure water under continuous magnetic stirring. Three series of biopolysaccharide gels were prepared, with six samples, each of which receiving a different amount of CAGE-IL: in the first series of biopolysaccharide gels containing 1% curcumin, CAGE-IL was added up to final concentrations of 0, 0.5, 1.0, 1.5, 2.0 and 2.5% (w/w). The same procedure was employed for the biopolysaccharide gels integrating curcumin at 1.5% (w/w) and 2.0% (w/w). In the preparation of the gels, careful homogenization was performed in order to avoid air incorporation.

Determination of biopolysaccharide gel spreadability. The effect of the gum type on the spreadability properties of the biopolysaccharide gel was evaluated. The spreadability tests were carried out using a set of glass plates of different dimensions and weights, a circular mold plate made of glass with a central hole (1.2 cm in diameter) and a support plate. The set (support plate, mold plate and glass plate) was placed on top of paper with a millimetric scale. A sample of the biopolysaccharide gel (ca. 2 g) was introduced into the hole in the mold plate and duly leveled with the help of a spatula. The mold plate was removed, and a glass plate of known weight was placed over the sample so as to spread it on the support plate. The diameters covered by the product sample were read on the millimetric scale of the paper and the average diameter was calculated. All determinations were carried out in triplicate at 25 °C. The spreadability (E_i) was then calculated using Eq. (1).

$$E_i = \frac{d^2 \times \pi}{4} \quad (1)$$

where E_i is the spreadability for a given weight of the glass plate (mm^2); d is the mean diameter (mm); and π is the pi number. Plate 1 wt = 162.41 g; Plate 2 wt = 135.26 g; Plate 3 wt = 95.23 g; Plate 4 wt = 61.47 g; Plate 5 wt = 34.65 g; Plate-mold with central orifice = 22.91 g.

Rheological analyses. The viscosity of the gel formulations was studied in a viscometer from Brookfield (model Dv-1 Prime, Middleboro MA, U.S.A.). For all gel formulation samples, one used a Brookfield spindle with reference S-28, at rotating speeds of 20, 30, 50, 60, and 100 rpm, at both 25 °C (room temperature) and 33.5 °C (the normal average temperature of the human skin surface). All analyses were carried out in triplicate. The viscometer software provides, in addition to the values of viscosity, results of shear stress as a function of shear rate, and so these results were also used for the rheological characterization of the various gel formulations produced. The shear stress was determined mathematically by using Eq. (2), where τ is the shear stress, $\dot{\gamma}$ is the shear rate, and η is the viscosity. The shear stress and shear rate were used to characterize the flow behavior of the various gels.

$$\tau = \dot{\gamma} \times \eta \quad (2)$$

The thixotropic or rheopectic behavior of the gels was evaluated on the basis of the measurements of viscosity (η) as a function of time (t).

Fourier Transform Infrared spectrophotometry (FTIR) analyses. Sample weights of ca. 2 mg were mixed with 300 mg of KBr so as to form a tablet. The FTIR spectra of samples of pure curcumin (CUR); gel containing 2% (w/w) LBG and 2% (w/w) curcumin; gel containing 2% (w/w) LBG, 2% curcumin (w/w) and 2% (w/w) DES; and gel containing 2% (w/w) LBG, 2.087% (w/w) commercial turmeric (containing 2% (w/w) curcumin) and 2% (w/w) CAGE-IL, were gathered using a Fourier Transform Infrared Spectrophotometer from Shimadzu (model IR-Affinity-1, Shimadzu, Kyoto, Japan), in the wavenumber range from 4000 cm^{-1} to 400 cm^{-1} , with a resolution of 2 cm^{-1} , and using Happ-Genzel apodization.

Mechanical analyses. Mechanical properties (hardness, compressibility, adhesiveness and cohesiveness) of the gel containing 2% (w/w) LBG and 2% (w/w) curcumin; gel containing 2% (w/w) LBG, 2% (w/w) curcumin and 2% (w/w) DES; and gel containing 2% (w/w) LBG, 2.087% (w/w) commercial turmeric (containing 2% (w/w) curcumin) and 2% (w/w) CAGE-IL, were evaluated via relationship between force (N) versus time (s) using a probe (P/10) in a texturometer from Stable Micro Systems (model TA.XT Plus Texture Analyser, Godalming, United Kingdom). Beforehand, the texturometer was calibrated with a load cell with mass of 5 kg. An aliquot of each sample was transferred to a glass beaker and kept in a water bath with temperature adjusted independently to either 25 °C or 33.5 °C. For the temperature of 25 °C, an analytical probe with 10 mm in diameter was compressed on the surface of the samples, with a force of 0.01 N. The procedure for each sample was performed in triplicate. The test speed was set to a rate of 0.5 mm s^{-1} , with a 5% strain mode. The probe's penetration depth was set to 1 mm. For the temperature of 33.5 °C, an analytical probe with a diameter of 10 mm was compressed on the surface of the samples, with a force of 0.005 N. The procedure for each sample was also performed in triplicate. The test speed was set to a rate of 0.5 mm s^{-1} , with a strain mode of 2.5%. The probe's penetration depth was also set to 1 mm.

Evaluation of the cytotoxicity potential of the biopolysaccharide gel integrating both curcumin and CAGE-IL via the agar disk-diffusion assay. The cytotoxicity potential of samples of curcumin and of LBG gel formulations was evaluated via the agar disk-diffusion methodology using cell lineage HaCaT. Cell lineage HaCaT was plated in 50 mm Petri plates, at a concentration of 1.5×10^5 cells/mL using DMEM culture medium supplemented with bovine fetal serum at 10% (w/w), during a period of 48 h at 37 °C, in an incubation chamber with an atmosphere containing 5% CO_2 (v/v). Subsequently, the liquid medium was discarded and solid "overlay" medium (Eagle medium, 2x concentrated, with agar at 1.8% (w/w) containing 0.01% (w/w) neutral red, was added. After solidification, the samples (impregnated in sterile filter paper) were placed in the center of the plates and these were incubated during 24 h at 37 °C, in an environment containing 5% (v/v) CO_2 . A disc of sterile atoxic paper was used as negative control whereas a disc of sterile latex was used as positive control, following the same procedure described above. The readings of the inoculated plaques were made macroscopically, where the presence of cytotoxicity was observed by the formation of a clear halo around the sample due to cell lysis surrounding the sample tested (Rogero et al., 2003), corresponding to the dead cells, and microscopically, for the morphological changes of the cells surrounding the sample (Rocha et al., 2017; Pusnik et al. 2016; Jorge et al., 2020; Silva et al., 2021).

2.2.3. Spectrophotometric method for quantification of curcumin

Quantification of curcumin was performed by UV-Vis spectrophotometry at a wavelength of 421 nm, using quartz cuvettes, in a UV-Vis Spectrophotometer from Agilent (model Cary 60 UV-Vis, Santa Clara, CA, USA), following the procedure described by Singh and Avupati (2017) with slight modifications. For the transdermal permeation assays, the calibration curve for quantification of curcumin was prepared using standard solutions of pure curcumin (Ref. No. C1386; Sigma-Aldrich, St. Louis MO, USA) in aqueous PEG 10,000 (Merck, Darmstadt, Germany) at 10% (w/v), at several concentrations in the range 0–25 $\mu\text{g mL}^{-1}$ ($\text{Abs}_{421\text{nm}} = 6.0571 \times [\text{curcumin}, \mu\text{g mL}^{-1}] + 0.0003$; $r^2 = 0.9983$). For the skin retention assays, the calibration curve for quantification of curcumin was prepared using standard solutions of pure curcumin (Ref. No. C1386; Sigma-Aldrich, St. Louis MO, USA) in aqueous ethanol at 50% (v/v), at several concentrations in the range 0–25 $\mu\text{g mL}^{-1}$ ($\text{Abs}_{421\text{nm}} = 1.9121 \times [\text{curcumin}, \mu\text{g mL}^{-1}] + 0.1596$; $r^2 = 0.9870$).

2.2.4. In vitro skin permeation assays

Transdermal permeation of curcumin from the biopolysaccharide gel samples was studied in a DHC-6 T Transdermal System from Logan Instruments Corp. (Somerset NJ, USA), using thawed porcine ear skin discs (0.5 mm thick $\times$ 30 mm ϕ_{ext}) as permeation membranes, following the procedure described in detail by Boscaroli et al. (2021), Jorge et al. (2020) and Campos et al. (2020). The porcine ear skin discs were prepared according to the procedure described by Salerno et al. (2010). The porcine ear skin discs were then clamped on top of the Franz diffusion cell (with a 15 mm ϕ_{ext} central hole, contacting the receptor fluid beneath). On top of these porcine ear skin discs, a cavity with $\phi_{\text{ext}} = 30$ mm was carefully filled with 2 mL ionic liquid suspension (integrating different volume percentages of CAGE-IL), eliminating any air bubbles. To the receptacle of the Franz diffusion cell (possessing a small cylindrical teflon-coated magnetic stirring bar) beneath the porcine ear skin disc, 8 mL of aqueous solution of polyethylene glycol 10,000 (10%, w/v) (PEG 10000, CAS-No. 25322-68-3, Merck, Darmstadt, Germany) were carefully poured until touching the lower surface of skin, ensuring the absence of air bubbles and stretching the skin so as to minimize the presence of furrows. This compartment was thermostatted at 32 ± 3 °C during 30 min. The ionic liquid suspension was allowed to contact with the upper part of the porcine ear skin discs for a period of 720 min. At pre-determined time intervals (viz. 0, 15, 30, 45, 60, 120, 180, 240, 300, 360, 420, 480, 540, 600, 660 and 720 min), 2 mL-samples were withdrawn from the receiving fluid beneath the porcine ear skin disc, and 2-mL of fresh aqueous PEG solution were added so as to reset the volume. Determination of the amount of curcumin in the samples was performed via spectrophotometric readings at 421 nm.

2.2.5. Evaluation of in vitro skin retention of curcumin

Extraction of the curcumin retained in the porcine ear skin discs was based on the methodology described by Sato et al. (2007). After 12 h of *in vitro* permeation, the porcine ear skin discs were removed from the diffusion apparatus and the skin area exposed to the receiving fluid was cleaned with aqueous ethanol (50% (v/v)), and cut and shredded. The skin fragments thus produced were processed with 25 mL of absolute ethanol using an UltraTurrax tissue homogenizer (model LR 5/10 from IKA Werke GmbH & Co. KG, Staufen, Germany) until total tearing of the skin. The resulting suspension was further subjected to sonication via ultrasounds to disrupt the cells, the resulting suspension was filtered into a 50 mL volumetric flask, and the volume made up with aqueous ethanol (50% (v/v)). Determination of the amount of curcumin retained in the porcine ear skin samples was carried out by spectrophotometry as described earlier.

2.2.6. Permeation parameters

The cumulative amount of curcumin permeated through the porcine skin (Q) was calculated using Eq. (3) (Mitragotri et al., 2011; Patel et al., 2009; Sato et al., 2007).

$$Q = (C_{\text{measured},t} \times V_r) + \sum_{a=0}^{n-1} C_a \times V_a \quad (3)$$

where Q (mg) is the cumulative amount of curcumin permeated after 12 h; $C_{\text{measured},t}$ is the measured curcumin concentration in the receptor fluid ($\mu\text{g/mL}$) at time t (min); V_r is the volume (mL) of the receiving solution in the diffusion cell; C_a is the concentration ($\mu\text{g/mL}$) of the sample withdrawn; and V_a is the volume (mL) of the sample withdrawn.

For the mathematical modelling of the experimental (calculated) data, nonlinear fitting of the lognormal distribution (Eq. (4)) to the experimental data was performed using the graphing

software GraphPad Prism v. 9.0 (GraphPad Software, San Diego CA, U.S.A.).

$$f(x; \mu, \sigma) = \frac{1}{x\sigma\sqrt{2\pi}} \times \exp\left\{-\frac{(\ln x - \mu)^2}{2\sigma^2}\right\}; x > 0 \quad (4)$$

where μ and σ are the average log and the standard deviations of the log, respectively, with the average being given by $(\mu + \sigma^2/2)$ and the variance being given by $\exp(2\mu + \sigma^2)(\exp(\sigma^2) - 1)$.

The total amount of curcumin permeated over 12 h (Q_{12}) was determined by calculating the area under the curve (AUC_{0-12}) in GraphPad Prism 9.0, by fitting Eq. (5).

$$AUC_{0-12} = A \times \sqrt{2\pi} \times \ln(\text{GeoSD}) \quad (5)$$

where A is related to the amplitude and area of the distribution, and GeoSD is the geometric standard deviation (dimensionless).

The maximum concentration of curcumin permeated (C_{max}) was also calculated using GraphPad Prism 9.0, through Eq. (6).

$$C_{\text{max}} = \frac{A}{\text{GeoMEAN}/\exp(0.5 \times \ln(\text{GeoSD})^2)} \quad (6)$$

where A is the amplitude, GeoMEAN is the geometric mean of the data, and GeoSD is the geometric standard deviation (dimensionless).

The curcumin flow ($\mu\text{g mm}^{-2}\text{h}^{-1}$) through the porcine ear skin matrix area as a function of time (Mitragotri et al., 2011; Arce et al., 2020) was calculated by using Eq. (7).

$$J = \frac{Q}{A \times t} \quad (7)$$

where J is the flow ($\mu\text{g}/\text{cm}^2/\text{hr}$); Q is the permeate quantity (μg); A is the porcine ear skin matrix area (cm^2) available for permeation; and t is the time (min).

2.2.7. Scanning electron microscopy analyses (SEM)

After lyophilization in a lyophilizer from ThermoFisher (model ModulyOD R23T-659559-RT, Pittsburgh PA, U.S.A.), the morphology of the biopolysaccharide matrix of the optimized gel formulation was observed in a scanning electron microscope (SEM) from JEOL (model JSM-IT200, Tokyo, Japan) working at high-vacuum, equipped with an Energy Dispersive X-ray Spectrometer (EDS) detector also from JEOL (model DRY SD™25 Detector Unit, Tokyo, Japan). Samples of the lyophilized gel were sputter-coated with a Au film (92 Å thickness) via cathodic pulverization on a carbon layer produced by evaporation in a metalizing device also from JEOL (Sputter Coater model DIH-29010SCTR Smart Coater, Tokyo, Japan). Photomicrographs were gathered using electron beams with acceleration speeds of 10.0 keV via random scanning.

3. Results

In the research effort described herein, synthesis of choline and geranic acid ionic liquid (CAGE-IL) followed the procedure described in detail by Boscaroli et al. (2021), Silva et al. (2021), Jorge et al. (2020), and Zakrewsky et al. (2016), with a product yield of 45% and presenting a density of 1.02 g mL^{-1} , totally fluid and transparent at room temperature. The biopolysaccharide gels produced, integrating both CAGE-IL and either pure or commercial curcumin, were duly characterized and used in transdermal permeation studies of the active principle, aiming at a potential application in the treatment of skin diseases such as psoriasis.

3.1. Determination of biopolysaccharide gel spreadability

The spreadability tests performed to the several biopolysaccharide gels produced indicated that the ones prepared with LBG exhibited the highest spreadabilities (Fig. 1). In addition, LBG showed a good compatibility with curcumin, ease of incorporation and homogenization, allowing to produce uniform gel preparations at all curcumin concentrations tested (Fig. 1). Hence, LBG was chosen as the gelling agent for the subsequent research work.

Regarding the results of the spreadability tests of the gel formulations prepared with LBG, CAGE-IL and curcumin (Fig. 2), one could observe that an increase in the concentration of CAGE-IL

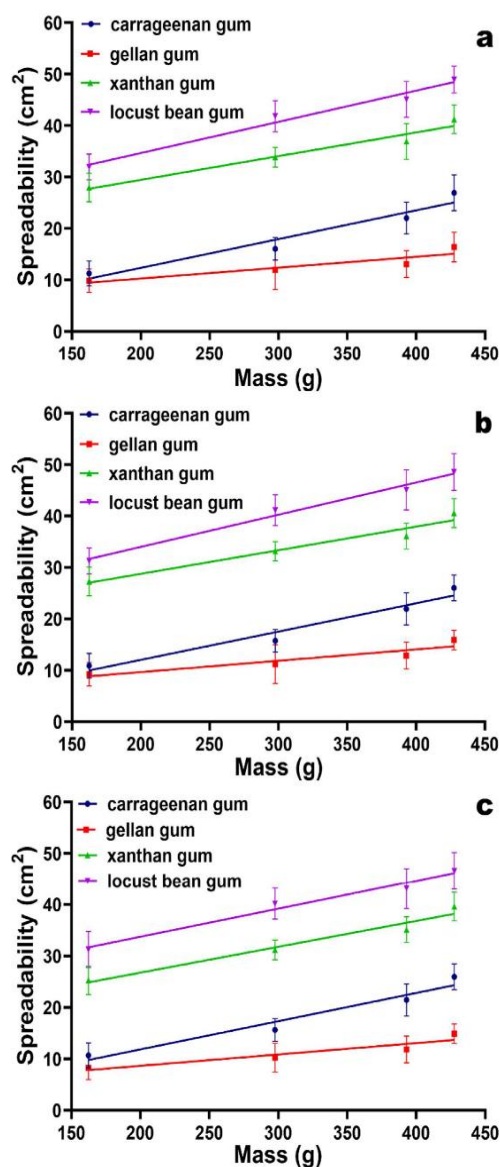


Fig. 1. Spreadability of the gel formulations prepared with the different biopolysaccharide gums and integrating pure curcumin at 1.0% (w/w) (a), 1.5% (w/w) (b) and 2.0% (w/w) (c), as a function of the weight applied. All values represent the mean of three experiments, and the error bars represent the standard deviations.

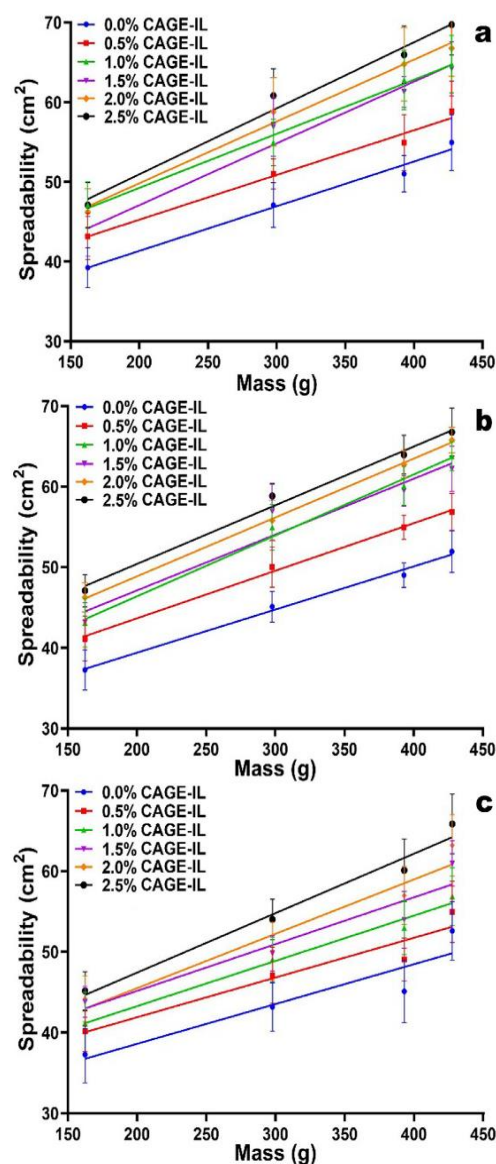


Fig. 2. Spreadability of the gel formulations prepared with LBG and variable concentrations of CAGE-IL, and integrating pure curcumin at 1.0% (w/w) (a), 1.5% (w/w) (b) and 2.0% (w/w) (c), as a function of the weight applied. All values represent the mean of three experiments, and the error bars represent the standard deviations.

resulted in a greater spreadability of the gel, probably due to the establishment of hydrogen bonding between CAGE-IL and the biopolysaccharide and also to the low viscosity of CAGE-IL.

3.2. Evaluation of the cytotoxicity potential of the biopolysaccharide gel integrating both curcumin and CAGE-IL via the agar disk-diffusion assay

To carry out these analyzes, the cell line HaCaT (primary mouse embryonic fibroblast cells) was used. The results obtained are displayed in Fig. 3.

3.3. In vitro skin permeation assays

In the IPVT assays carried out in the research effort entertained herein, an aqueous polyethylene glycol (PEG 10000) solution (10%, w/v) was used as the receiving fluid to favor dissolution of the permeated curcumin. Polyethylene glycols are polymers of condensed ethylene oxide, widely used as solvents due to their very high affinity towards water (Zhang et al., 2020).

The results obtained for the cumulative amount of curcumin permeated through the porcine ear skin epidermis, departing from gel formulations integrating different amounts of CAGE-IL, are displayed in Fig. 4.

3.4. Evaluation of in vitro skin retention of curcumin

Determination of the amount of curcumin retained in the porcine ear skin matrix (Fig. 5) following IVPT assays, via UV-Vis spectrophotometry, was performed after ethanol-mediated extraction from the skin matrix. The extraction procedure followed was feasible due to the solubility of curcumin in ethanol (Singh and Avupatil, 2017), without interference of the other components of the porcine ear skin matrix, which were probably also extracted.

3.5. Modelling of in vitro transdermal permeation of curcumin

Most mathematical models postulated for the transport of chemical substances through the *stratum corneum* layer of the skin use one-dimensional diffusion equations, and the popularity of these models lies on their mathematical simplicity. It is assumed that, under steady state conditions, the flow through a permeating membrane is proportional to the concentration gradient across a unit area of a section of the membrane (Mitragotri et al., 2011; Pettinelli et al., 2020).

The transdermal permeation profiles of curcumin integrated in the gel formulations are displayed in Fig. 6, together with non-linear fittings of a lognormal model to the experimental data.

The flow of curcumin permeated through the porcine ear skin as a function of time is displayed in Fig. 7, with a close inspection of the data in this figure allowing to observe that the flow of curcumin increased with increasing concentrations of CAGE-IL in the gel formulations, up to a concentration of CAGE-IL of 2.0% (w/w).

The transdermal permeation behavior of curcumin from commercial turmeric powder is displayed in Fig. 8.

Hence, in this study, in order to evaluate the permeation parameters one determined the cumulative amount of curcumin permeated through an area of porcine ear skin over a 12 h assay (AUC_{0-12}), the flux (J), the maximum time for permeation (t_{max}), and the maximum permeated concentration (C_{max}), for all gel formulations, and the results obtained are displayed in Table 1 for the gel formulation prepared with pure curcumin, and in Table 2 for the gel formulation prepared with raw turmeric, together with the coefficients of determination (r^2).

3.6. Fourier Transform infrared spectrophotometry (FTIR) analyses

Fig. 9 shows the FTIR spectra of pure curcumin, commercial turmeric, gel formulation integrating pure curcumin, gel formulation integrating pure curcumin and CAGE-IL, and gel formulation integrating commercial turmeric and CAGE-IL.

3.7. Rheological analyses

The viscosity of the gel formulations integrating CAGE-IL and either pure curcumin or commercial turmeric are displayed in Fig. 10a for two different temperatures, viz. 25 °C (room temperature) and 33.5 °C (the normal average temperature of the human

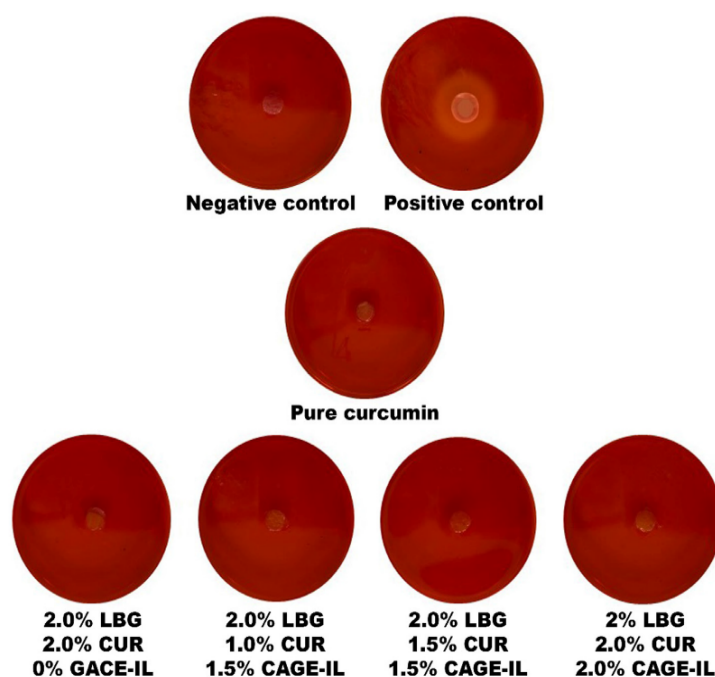


Fig. 3. Results obtained in the analysis of the potential for cytotoxicity via the agar disk-diffusion method with HaCaT cells, of pure curcumin and of the LBG gel formulations integrating both curcumin and CAGE-IL at various concentrations.

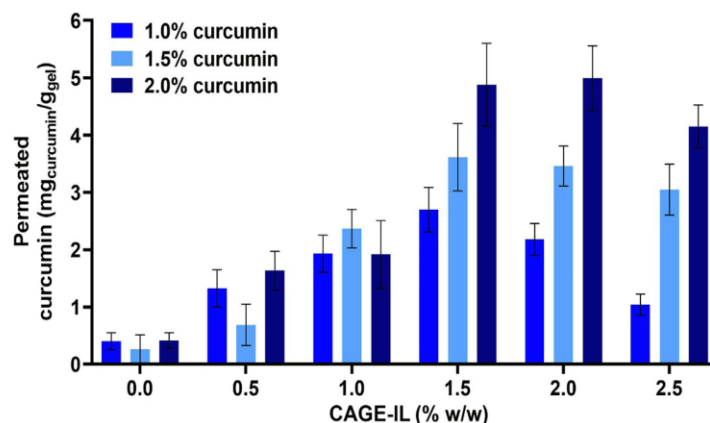


Fig. 4. Net amounts of curcumin permeated from the gel formulations containing different concentrations of curcumin and CAGE-IL. All values represent the mean of three experiments, and the error bars represent the standard deviations.

skin surface), whereas Fig. 10b displays the relationship between shear stress and shear rate allowing to determine the flow behavior of the same gel formulations at the two aforementioned temperatures.

3.8. Mechanical analyses

Evaluation of the mechanical properties of the gel formulations produced integrating pure curcumin with or without CAGE-IL and of the gel formulation integrating commercial turmeric and CAGE-IL, encompassed hardness, compressibility, adhesiveness and cohesiveness, parameters obtained from force-time curves of texture profile analysis (Fig. 11), at two different temperatures, viz. 25 °C (room temperature) and 33.5 °C (the normal average temperature of the human skin surface).

The compressibility, hardness, adhesiveness and cohesiveness of the gel formulations produced integrating pure curcumin with or without CAGE-IL and of the gel formulation integrating commercial turmeric and CAGE-IL, calculated from the force-time curves of texture profile analysis (Fig. 11), are displayed in Fig. 12 for the two different temperatures assayed, viz. 25 °C and 33.5 °C.

The biopolysaccharide matrix of the optimized gel formulation was analyzed via scanning electron microscopy, and photomicrographs of its morphology are displayed in Fig. 13 at different magnifications.

4. Discussion

There is no consensus on the definition of the best concentration of curcumin for topical treatments of skin diseases, with concentrations of curcumin ranging generally from 1% to 2% (w/w) in topical formulations. In this sense, in the research effort entertained herein, biopolysaccharide gels were produced integrating 1.0, 1.5 and 2.0% (w/w) of pure curcumin (Kim and Lio, 2020; Panahi et al., 2019; Vollono et al., 2019). The pharmaceutical form “gel” was therefore used to evaluate the effectiveness of CAGE-IL in enhancing the delivery of curcumin via the transdermal route. Usually, biopolymeric gel formulations are able to retain hydrophobic molecules and thus act as ideal carriers for bioactive molecules aiming at transdermal delivery. In addition, gel formulations are preferred over other topical semisolid preparations, since it has a longer residence time on the skin, higher viscosity, mois-

turizing effect on flaky skins due to its occlusive properties, higher bio-adhesiveness, promotes less irritation, is independent of the water solubility of the active biomolecule, is easy to apply and exhibits better release characteristics (Aiyalu et al., 2016).

Several natural biopolysaccharide gums were exploited in the study described herein, viz. locust bean gum (LBG), xanthan gum, carrageenan gum and gellan gum, aiming at developing a product with good spreadability and ability to homogeneously integrate curcumin. Besides being cheap, chemically inert, biocompatible, nontoxic, odorless and abounding, natural gums are able to increase the viscosity of a solution even at low concentrations (Mohammadinejad et al., 2020).

Another advantage observed with the use of LBG, when further adding CAGE-IL at several concentrations, was the fact that the gel formulations could be prepared without the need for adding surfactants.

A close inspection of the data in Fig. 2 allows to conclude that spreadability increases in line with the increase in the concentration of CAGE-IL, irrespective of the concentration of curcumin added to the gel.

The results obtained in the cytotoxicity assays showed the non-occurrence of cell death caused by contact with either of the gel formulations containing curcumin (CUR) and CAGE-IL, after 24 h, with the microscopy analyses confirming the integrity of the cells, thus making it possible to observe the clear halo indicative of cell death only for the positive control (in this control, the HaCaT murine cells showed changes in morphology and death (Fig. 3)).

In the evaluation of products for dermatological applications, clinical tests may be preceded, and in some cases replaced by, *in vitro* permeation testing (IVPT). *In vitro* tests allow to understand some of the phenomena that take place between application of the product and its pharmacological effect, in a practical, fast way, and without interference of any biological factors (Pettinelli et al., 2020). In IVPT, the permeation membrane is a critical factor for performing the transdermal permeation experiments. Synthetic membranes do not exhibit the anatomical and physiological features of the human skin, hence the idea is to perform transdermal permeation studies using natural membranes (either human or animal skin). Human skin is definitely the most suitable membrane for IVPT studies (Arce et al., 2020), but unfortunately it is not always accessible. Therefore, the IVPT assays were carried out using porcine ear skin as permeation membrane. Porcine ear skin has been often used as a mimicking alternative to the human skin in studies of percutaneous absorption when developing formula-

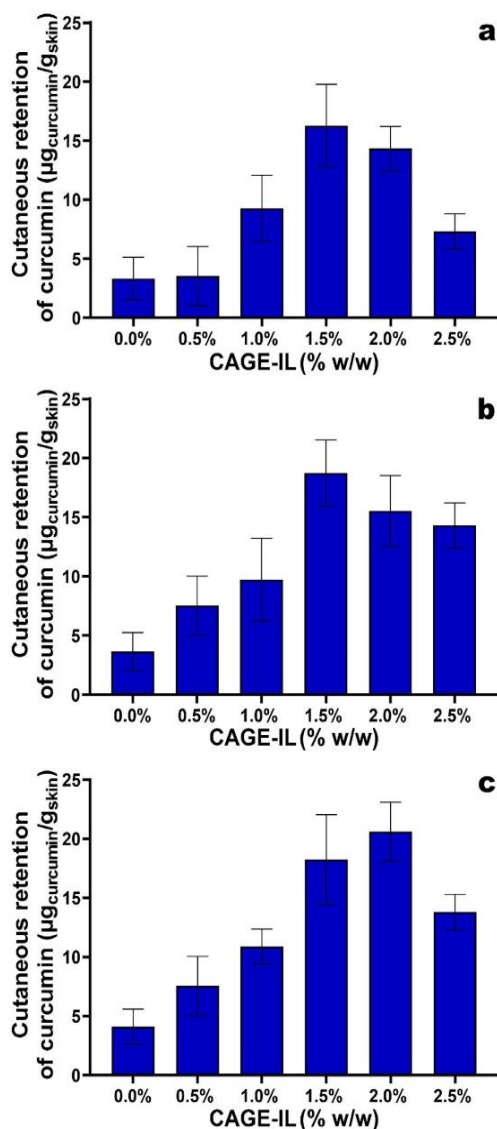


Fig. 5. Results obtained for the average amount of curcumin retained in the porcine ear skin matrix, as a function of the concentration of CAGE-IL for gel formulations integrating pure curcumin at (a) 1.0% (w/w), (b) 1.5% (w/w), and (c) 2.0% (w/w). All values represent the mean of three experiments, and the error bars represent the standard deviations.

tions for transdermal delivery of bioactive moieties (Boscaroli et al., 2021; Silva et al., 2021; Hernandes et al., 2021; Jorge et al., 2020; Campos et al., 2020; Todo, 2017; Zakrewsky et al., 2016). Several *in vitro* studies of transdermal permeation using Franz cells have indicated that porcine ear skin is the one that has flow values and permeation coefficients closest to the human skin (Ahad et al., 2021; Islam et al., 2020).

From inspection of the data in Fig. 4, one may conclude that CAGE-IL enhanced the transdermal permeation of curcumin. As can be observed from inspection of the results in Fig. 4, CAGE-IL at concentrations of 1.5% (w/w) and 2.0% (w/w) promoted the best

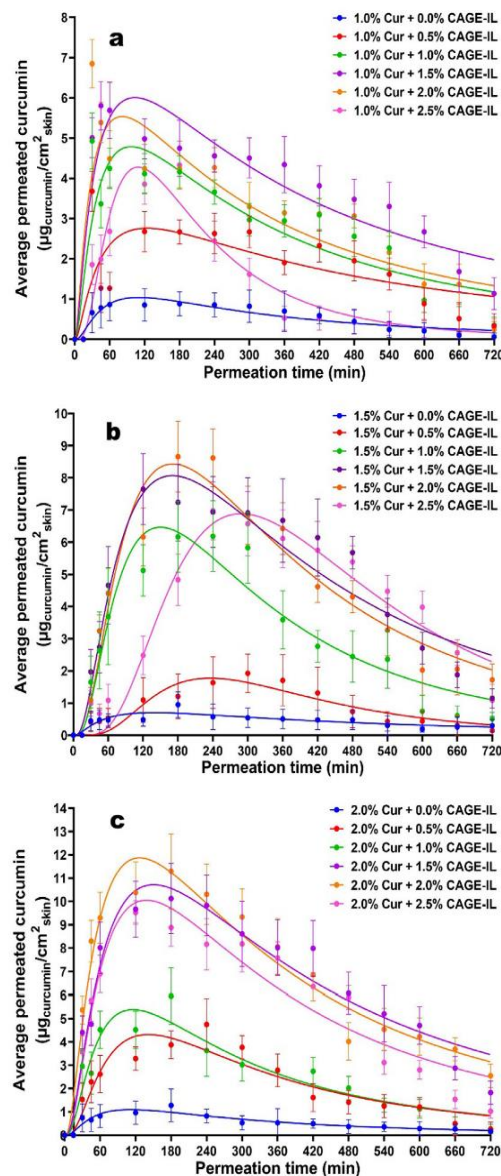


Fig. 6. Time evolution of the *in vitro* skin permeation of curcumin from the gel formulations integrating variable amounts of CAGE-IL and curcumin at (a) 1.0% (w/w), (b) 1.5% (w/w), and (c) 2.0% (w/w). Nonlinear fittings of the lognormal model are depicted by solid lines. All values represent the mean of three experiments, and the error bars represent the standard deviations.

results in terms of total permeated curcumin for all curcumin concentrations integrated in the gel. At a concentration of 2.5% (w/w), CAGE-IL promotes disruption of the *stratum corneum* and a lower amount of permeated curcumin, probably due to saturation of the skin, a result that is in close agreement with previous reports (Boscaroli et al., 2021).

According to Yousef et al. (2019) the mechanism of skin penetration enhancement of curcumin may be due to three different processes. The increase in permeation may be a function of the action of the vehicle, contact surface increase (nanoparticles) and

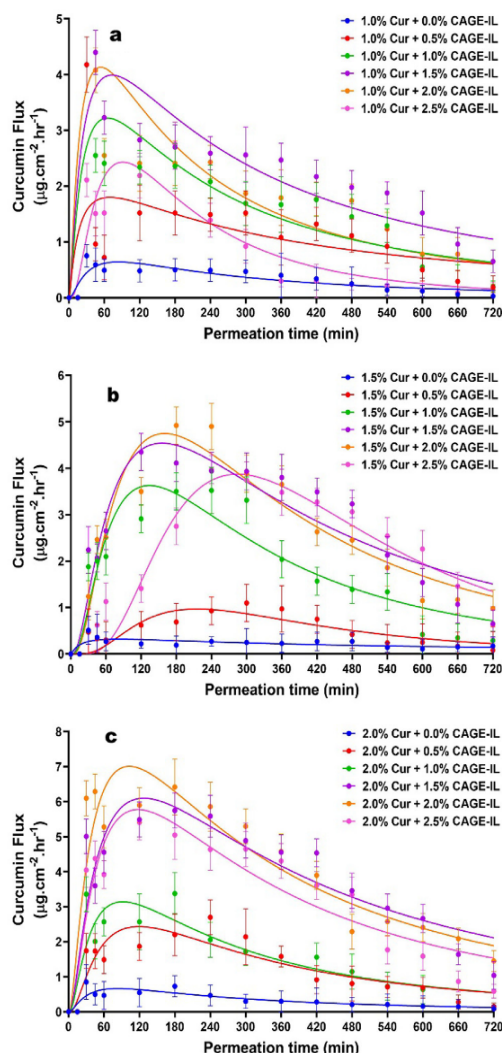


Fig. 7. Time evolution of the *in vitro* skin flow of permeated curcumin from the gel formulations integrating variable amounts of CAGE-IL and curcumin at (a) 1.0% (w/w), (b) 1.5% (w/w), and (c) 2.0% (w/w). Nonlinear fittings of the lognormal model are depicted by solid lines. All values represent the mean of three experiments, and the error bars represent the standard deviations.

by modification of the solvent nature of the stratum corneum caused by fluidisation of the stratum corneum lipids. In the work developed, there may be an increase in permeation due to the greater solubilization of curcumin by the ionic liquid and by momentary changes in the corneal extract.

As can be observed from inspection of the data displayed in Fig. 5, the amount of curcumin retained inside the skin matrix was more significant when CAGE-IL was integrated in the gel formulations at levels equal or higher than 1.5% (w/w), for all curcumin concentrations tested. In the gel formulation containing the highest concentration of curcumin (*viz.*, 2.0% (w/w)) (Fig. 5c), the best result in terms of the amount of curcumin retained within the skin matrix was obtained when CAGE-IL was integrated in the gel at a concentration of 2.0% (w/w). This result is in fact highly significant if this gel formulation is intended for the topical treatment

of psoriasis, since this very concentration of CAGE-IL allowed the highest level of transdermal permeation of curcumin (Fig. 4) for the highest level of curcumin integrated in the gel, while allowing saturation of the skin with the highest amount of curcumin (Fig. 5).

Human skin permeability values have previously been identified to follow a log-normal (or skewed non-normal) distribution (Pradal, 2020; Defraeye et al., 2020; LaCount et al., 2020; Chen et al. 2016; Purushothaman et al., 2016; Limpert et al., 2001; Williams et al., 1992), hence favoring the use of geometric means to compare between formulations. The geometric mean is a good proxy to describe permeability, when the spatial distribution of permeability for the (heterogeneous) skin matrix, with no evidence of anisotropy, is lognormal (Selvadurai and Selvadurai, 2014). The generative process leading to a lognormal distribution was explained a long time ago by Gibrat (1930), who named it “law of proportional effect”, being closely related to the power law distribution (Mitzenmacher, 2003).

As can be observed from a close inspection of the data displayed in Fig. 6, the time profile of curcumin permeated through the skin in an experimental model *in vitro* was adequately described by the aforementioned non-linear lognormal model.

The transdermal permeation behavior of curcumin was similar for all concentrations of curcumin integrated in the gel formulations (Fig. 6), with an initial higher release being observed during the first 120 min of assay and, after that, a consistent decrease in the amount of permeated curcumin until the end of the assay timeframe, most likely due to skin saturation.

From inspection of the data in Fig. 6 it is clear that for gel formulations containing 1.0% (w/w) and 1.5% (w/w) curcumin, CAGE-IL added at 1.0% (w/w), 1.5% (w/w) and 2.0% (w/w) promoted the highest amounts of permeated curcumin (Fig. 6a and 6b), indicating that the presence of the ionic liquid enhances the transdermal permeation of curcumin, results that are in close agreement with previous findings (Boscaroli et al., 2021). Notwithstanding this observation, the gel formulation integrating CAGE-IL at 2.0% (w/w) promoted the highest permeation of curcumin when it was added at 2.0% (w/w) (Fig. 6c), whereas CAGE-IL added at 2.5% (w/w) exhibited lower results for the amount of permeated curcumin. According to Boscaroli et al. (2021), Silva et al. (2021), Jorge et al. (2020), and Sidat et al. (2019), ionic liquids and their deep eutectic solvents have, in general, the ability to enhance transdermal permeation of biomacromolecules, employing mechanisms such as disruption of cellular integrity, fluidization, and creation of diffusional pathways (Boscaroli et al., 2021), and extraction of lipid components within the stratum corneum. In this sense, higher concentrations of CAGE-IL may lead to disruption and flaking of the stratum corneum, impairing permeation by altering or disturbing the pathways for active compounds to flow through (Boscaroli et al., 2021).

The transport of bio(macro)molecules through the skin, in an *in vitro* permeation study, can be considered as a steady-state process resulting from transient disruption of the stratum corneum structure, relating the amount of solute that crosses an area of membrane available for permeation. In fact, the stratum corneum behaves like a pseudo-homogeneous membrane, where its properties do not vary significantly with position and time (Mitrageoti et al., 2011; Arce et al., 2020).

The highest flow values were obtained during the first 60 min for 1% (w/w) curcumin (Fig. 7a), during the first 150 min for 1.5% (w/w) curcumin (Fig. 7b), and during the first 90 min for 2.0% (w/w) curcumin (Fig. 7c). This occurred with the different concentrations of CAGE-IL in the gel formulations. According to Boscaroli et al. (2021) and Kováčik et al. (2020), the flow of drugs and fat-soluble chemicals through the skin increases with the presence of a permeation enhancer, a statement clearly in line with the results obtained in the present research work. The results obtained

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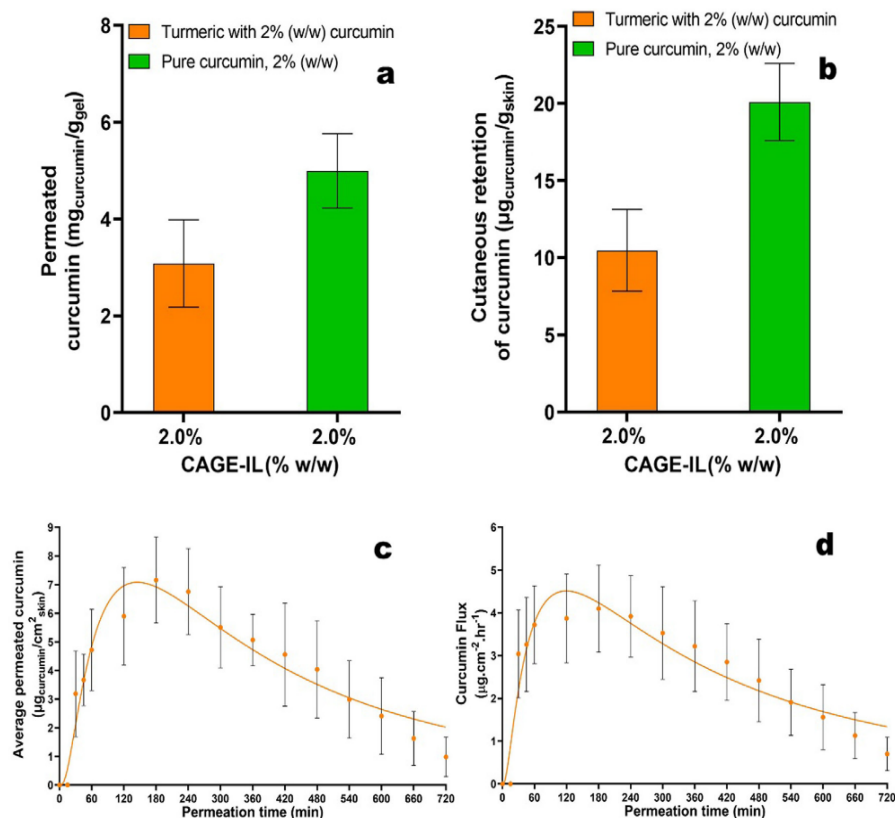


Fig. 8. Total amount of permeated curcumin from the gel formulation integrating commercial turmeric and CAGE-IL (a), average amount of curcumin retained in the porcine ear skin matrix (b), time evolution of the *in vitro* skin permeation of curcumin from the gel formulation integrating commercial turmeric and CAGE-IL (c), time evolution of the *in vitro* skin flow of permeated curcumin from the optimized gel formulation integrating commercial turmeric and CAGE-IL (d). Nonlinear fittings of the lognormal model are depicted by solid lines. All values represent the mean of three experiments, and the error bars represent the standard deviations.

Table 1

Average values of the total amount of curcumin (CUR) permeated in a 12 h assay (AUC_{0-12}), curcumin flux (J) through the skin, time to attain maximum concentration (t_{max}) and maximum concentration attained of permeated curcumin (C_{max}), for the gel formulations integrating curcumin at 1.0 % (w/w), 1.5 % (w/w) and 2.0 % (w/w) and CG-DES at 0 % (w/w), 0.5 % (w/w), 1.0 % (w/w), 1.5 % (w/w), 2.0 % (w/w), and 2.5 % (w/w). All values displayed represent the mean of three experiments and associated standard deviations.

CUR (% w/w)	CG-DES (% w/w)	AUC_{0-12} (μg)	J (μg·mm ⁻² ·h ⁻¹)	T_{max} (min)	C_{max} (μg·mL ⁻¹)	(r ²)
1.0	0.0	398.0 ± 45.5	0.32 ± 0.05	180.0 ± 28.2	0.88 ± 0.20	0.8724
	0.5	1323.0 ± 125.6	1.08 ± 0.14	30.0 ± 7.0	3.68 ± 1.20	0.5065
	1.0	1930.0 ± 322.5	1.64 ± 0.26	30.0 ± 7.0	4.93 ± 1.30	0.7624
	1.5	2695.0 ± 389.2	2.23 ± 0.52	45.0 ± 7.0	5.80 ± 1.20	0.8109
	2.0	2176.0 ± 278.6	1.98 ± 0.36	30.0 ± 7.0	6.84 ± 0.50	0.6946
	2.5	1040.0 ± 185.5	0.87 ± 0.20	180.0 ± 28.0	4.33 ± 0.50	0.9114
1.5	0.0	262.2 ± 55.1	0.21 ± 0.09	45.0 ± 7.0	0.47 ± 0.05	0.6365
	0.5	685.1 ± 360.5	0.46 ± 0.15	300.0 ± 28.0	1.92 ± 0.60	0.8216
	1.0	2368.0 ± 235.7	1.66 ± 0.27	240.0 ± 48.0	6.19 ± 0.55	0.9183
	1.5	3615.0 ± 289.5	2.45 ± 0.65	120.0 ± 48.0	7.65 ± 1.50	0.9598
	2.0	3461.0 ± 350.5	2.33 ± 0.53	180.0 ± 48.0	8.66 ± 1.40	0.9529
	2.5	3048.0 ± 345.4	1.92 ± 0.44	240.0 ± 48.0	6.98 ± 1.60	0.9493
2.0	0.0	412.0 ± 136.4	0.33 ± 0.20	180.0 ± 48.0	1.28 ± 0.35	0.8682
	0.5	1632.0 ± 338.5	1.18 ± 0.50	240.0 ± 28.0	4.75 ± 1.20	0.8672
	1.0	1918.0 ± 289.3	1.48 ± 0.50	180.0 ± 28.0	5.95 ± 1.20	0.8928
	1.5	4880.0 ± 525.1	3.48 ± 0.75	180.0 ± 28.0	10.13 ± 3.20	0.9391
	2.0	4994.0 ± 468.5	3.77 ± 0.87	180.0 ± 48.0	11.30 ± 3.30	0.9499
	2.5	4153.0 ± 372.5	3.01 ± 0.52	120.0 ± 28.0	9.52 ± 2.40	0.9226

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Table 2

Average values of the total amount of curcumin (CUR) from commercial turmeric permeated in a 12 h assay (AUC_{0-12}), curcumin flux (J) through the skin, time to attain maximum concentration (t_{max}) and maximum concentration attained of permeated curcumin (C_{max}), for the gel formulations integrating curcumin at 1.0 % (w/w), 1.5 % (w/w) and 2.0 % (w/w) and CG-DES at 0 % (w/w), 0.5 % (w/w), 1.0 % (w/w), 1.5 % (w/w), 2.0 % (w/w), and 2.5 % (w/w). All values displayed represent the mean of three experiments and associated standard deviations.

CUR (% w/w)	CG-DES (% w/w)	AUC_{0-12} (μg)	J ($\mu\text{g}\cdot\text{mm}^{-2}\cdot\text{h}^{-1}$)	T_{max} (min)	C_{max} ($\mu\text{g}\cdot\text{mL}^{-1}$)	(r^2)
2.0	2.0	3079.3 $\pm$ 603.2	2.45 $\pm$ 0.83	160.0 $\pm$ 70.0	7.16 $\pm$ 1.03	0.9307

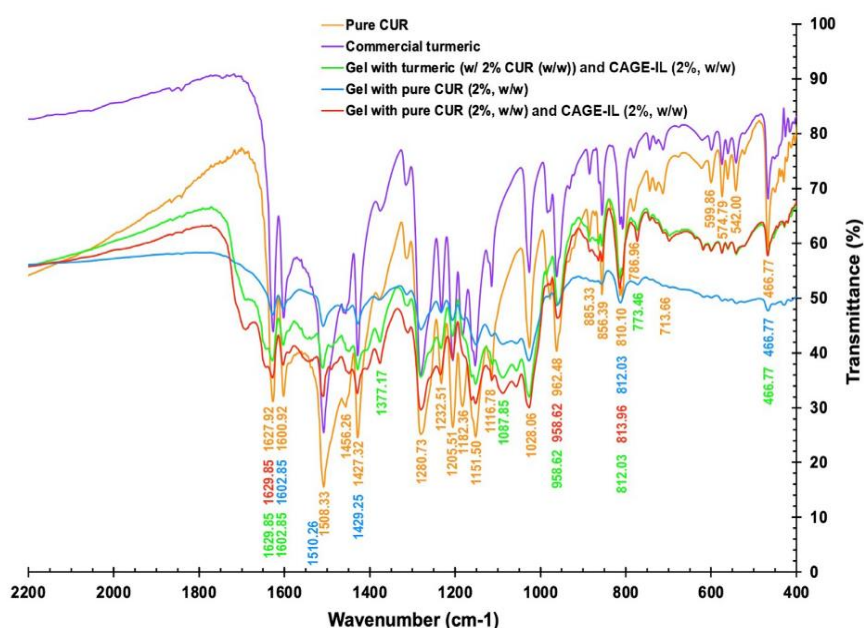


Fig. 9. FTIR spectra of samples of pure curcumin, commercial turmeric, gel formulation integrating pure curcumin, gel formulation integrating pure curcumin and CAGE-IL, and gel formulation integrating commercial turmeric and CAGE-IL.

clearly confirm the efficiency of CAGE-IL as a facilitator for the transdermal permeation of curcumin, when added at concentrations up to 2% (w/w), a result in clear agreement with previous findings (Boscaroli et al., 2021).

The transdermal permeation behavior of curcumin from commercial turmeric powder was similar for that of all gel formulations integrating pure curcumin (Fig. 8), but with a lower amount of curcumin being released when employing commercial turmeric, most likely due to interactions between curcumin and other components present in the raw turmeric extract (Fig. 8a).

From inspection of the data in Fig. 8b it is also clear that when employing raw turmeric extract (containing 2.0% (w/w) curcumin) in the gel formulation integrating CAGE-IL at 2.0% (w/w), the amount of curcumin retained in the skin was ca. half of that when using pure curcumin in the gel formulation. Notwithstanding this observation, the gel formulation integrating CAGE-IL at 2.0% (w/w) promoted permeation of curcumin from raw turmeric at acceptable levels and the skin retained more than $10 \mu\text{g}_{\text{curcumin}}/\text{g}_{\text{skin}}$.

The profile of average amount of permeated curcumin from raw turmeric integrated in the gel formulation together with CAGE-IL at 2% (w/w) during the assay timeframe (Fig. 8c) and the flow of curcumin from raw turmeric permeated through the porcine ear skin as a function of time (Fig. 8d) exhibited the same trends as those displayed in Fig. 6 and Fig. 7 for pure curcumin, respectively, allowing to conclude that raw turmeric powder is an economically

feasible alternative to pure curcumin when formulating pharmaceutical gel forms aiming at transdermal delivery of this important bioactive molecule with potential in the treatment of skin diseases such as (but not limited to) psoriasis.

A careful inspection of the data displayed in Table 1 allows to conclude that the gel formulations that allowed the highest values for permeated curcumin (viz. AUC_{0-12} , $\mu\text{g}_{\text{curcumin}}$), curcumin permeation flow (viz. J, $\mu\text{g}_{\text{curcumin}}\cdot\text{mm}^{-2}\cdot\text{h}^{-1}$) and curcumin permeation coefficient (viz. C_{max} , $\mu\text{g}\cdot\text{mL}^{-1}$), were those that integrated CAGE-IL at 1.5% (w/w) and 2.0% (w/w) (shaded areas in Table 1).

As the concentration of CAGE-IL in the gel formulation increased from 0% (w/w) to 2.0% (w/w), the transdermal permeation of curcumin was found to increase steadily. However, the use of CAGE-IL at 2.5% (w/w) did not allow an increase in the amount of permeated curcumin compared to the formulations containing CAGE-IL at 2.0% (w/w) (Table 1), with a possible explanation for this observation being that higher concentrations of CAGE-IL may lead to disruption and flaking of the *stratum corneum*, impairing permeation by altering or disturbing the pathways for active compounds to flow through (Boscaroli et al., 2021). The results obtained thus suggest that the use of curcumin at 2% (w/w) together with CAGE-IL at 2% (w/w) in the gel formulation promoted the best conditions for a maximum transdermal permeation of curcumin (Table 1). In their studies, Yousef et al. (2019) evaluated the permeation for curcumin from nanoemulsions and concluded that enhanced solubility of curcumin by

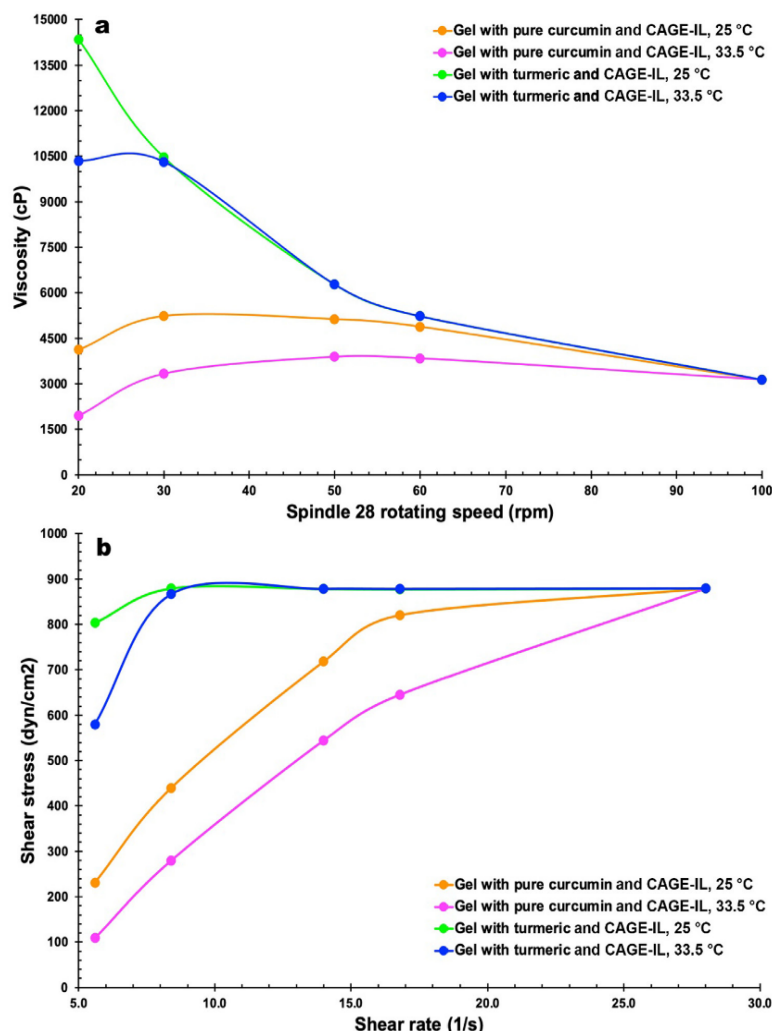


Fig. 10. Viscosity of the gel formulations integrating CAGE-IL and either pure curcumin or commercial turmeric for two different temperatures, viz. 25 °C and 33.5 °C (a) and relationship between shear stress and shear rate (b).

the employed vehicle may also favor its permeation, a conclusion that apparently seem to explain the higher amount of permeated curcumin at higher levels of CAGE-IL in the research work described herein.

A careful inspection of the data displayed in Table 2 allows to conclude that the optimized gel formulation containing turmeric (integrating 2% (w/w) curcumin) and 2.0% (w/w) CAGE-IL allowed relatively high values for permeated curcumin (viz. AUC_{0-12} , $\mu g_{\text{curcumin}} \text{ mm}^{-2} \text{ h}^{-1}$), curcumin permeation flow (viz. J , $\mu g_{\text{curcumin}} \text{ mm}^{-2} \text{ h}^{-1}$) and curcumin permeation coefficient (viz. C_{max} , $\mu g \text{ mL}^{-1}$), for a CAGE-IL concentration of 2.0% (w/w).

The analysis by infrared spectrophotometry with Fourier transform allows identification of functional groups present in a material. Each particular functional group absorbs at a characteristic frequency of radiation in the infrared spectrum, hence a plot of intensity versus frequency of radiation (infrared spectrum) allows to characterize the functional groups of a given material

(Cienfuegos and Vaitsman, 2000). The FTIR analysis were carried out for samples of pure curcumin, commercial turmeric, gel formulation integrating pure curcumin, gel formulation integrating pure curcumin and CAGE-IL, and gel formulation integrating commercial turmeric and CAGE-IL (Fig. 9), to further elucidate the interaction of curcumin with LBG and CAGE-IL in the gel formulations. Curcumin showed its signature peaks at 1627.92 cm^{-1} (aromatic moiety $\text{C}=\text{C}$ stretching and $\text{C}=\text{O}$ bond stretching of the conjugated ketone), 1116.78 cm^{-1} (benzene ring stretching vibrations), 1508.33 cm^{-1} ($\text{C}=\text{O}$ and $\text{C}=\text{C}$ vibrations), 1456.26 cm^{-1} (attributable to the group $-\text{CH}_2$), 1427.32 cm^{-1} (olefinic $-\text{CH}_2$ bending vibrations and stretch of the $\text{C}=\text{C}$ bond of the aromatic and aliphatic ring), 1280.73 cm^{-1} (aromatic $\text{C}-\text{O}$ of enol stretching vibrations), 1028.06 cm^{-1} (stretching vibrations of the bond $\text{C}-\text{O}-\text{C}$ of the ether) (Yallapu et al., 2010; Chen et al. 2015; Darandale and Vavia, 2013; Gangwar et al., 2012; Li et al., 2015). The same exact peaks appear in the spectrum of commercial turmeric.

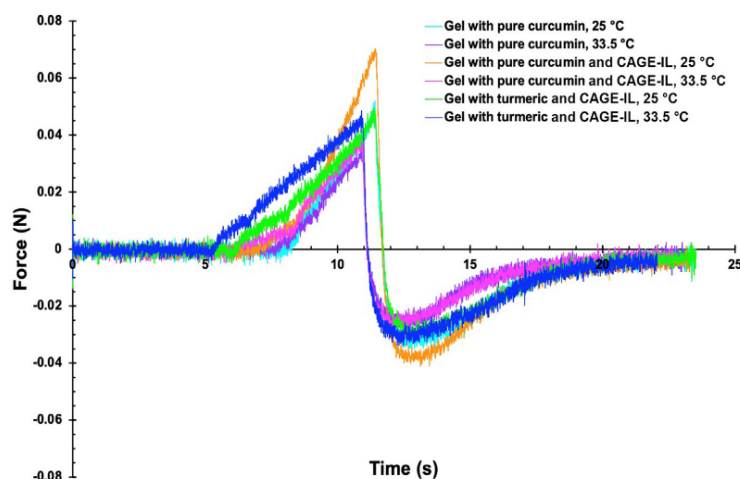


Fig. 11. Force-time curve of texture profile analysis of the gel formulations produced integrating pure curcumin with or without CAGE-IL and of the gel formulation integrating commercial turmeric and CAGE-IL, at 25 °C and 33.5 °C.

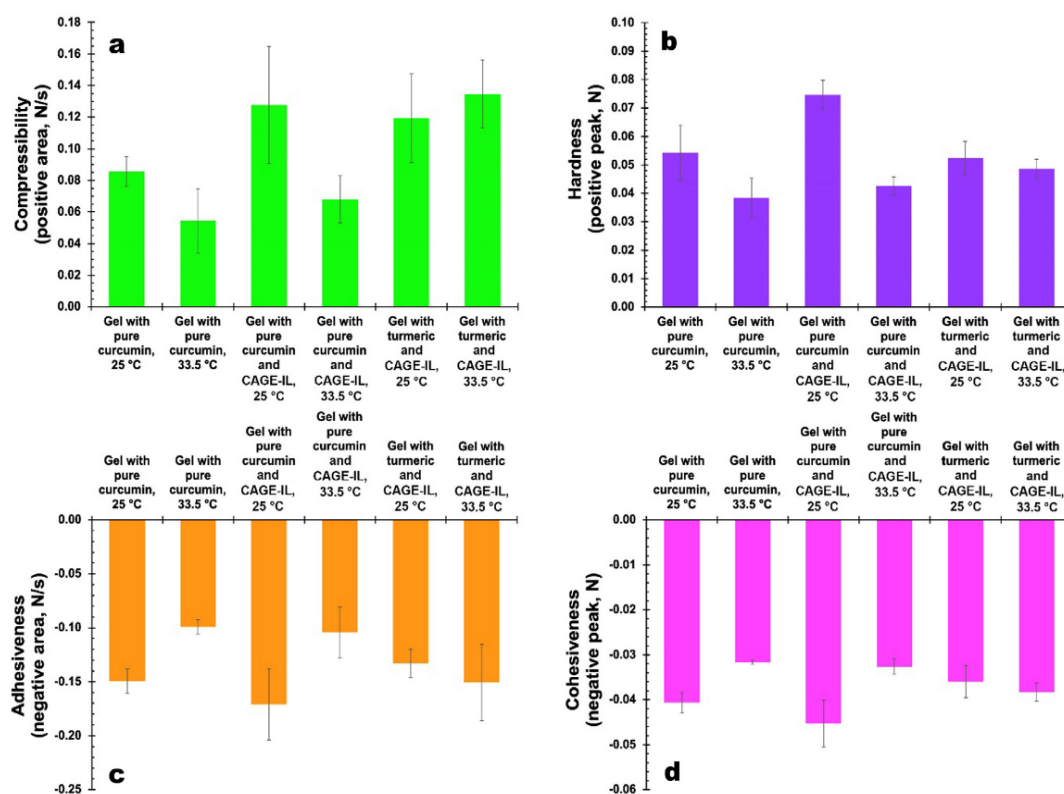


Fig. 12. Results obtained for the compressibility (a), hardness (b), adhesiveness (c) and cohesiveness (d) of the gel formulations produced integrating pure curcumin with or without CAGE-IL and of the gel formulation integrating commercial turmeric and CAGE-IL, at the two different temperatures assayed.

As observed in Fig. 9, the FTIR spectrum of curcumin indicates the presence of C–H bands at 713.66 cm^{-1} , 786.96 cm^{-1} , and 810.10 cm^{-1} , respectively. The peak at 1600.92 cm^{-1} corresponded to C=C stretching in the benzene ring. Moreover, the peak at 1508.33 cm^{-1} indicates the presence of ethylene group in cur-

cumin (Nam et al., 2007). Additionally, the bands in the region of $962.48\text{--}810.10\text{ cm}^{-1}$ belonged to C–H out-of-plane bending and aromatic stretching.

The band around 1028.06 cm^{-1} is assigned to C–O stretching and a band at 1377.17 cm^{-1} is attributable to CH_2 scissoring vibra-

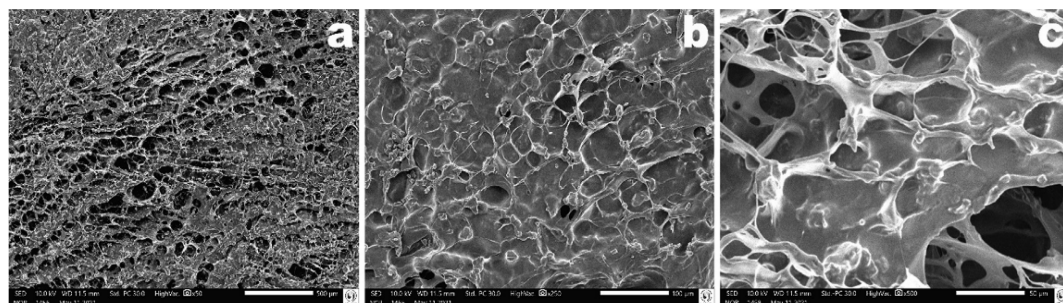


Fig. 13. Results obtained from the biopolysaccharide matrix morphology analysis of the optimized gel formulation via scanning electron microscopy at different magnifications, viz. x50 (a), x250 (b) and x500 (c).

tion in the component LBG of the gel formulations (Giri et al., 2015). Moreover, the bands in the region of $1350\text{--}1450\text{ cm}^{-1}$ show the symmetrical deformations of the CH_2 and COH groups in the LBG component of the gel formulations (Bashardoust et al., 2013).

The stretching bands and peaks of C-H at 713.66 cm^{-1} , 786.96 cm^{-1} and 810.10 cm^{-1} , C-O bands at 1602.85 cm^{-1} and 1629.85 cm^{-1} , and the ethylene group at 1510.26 cm^{-1} confirmed the presence of curcumin in our fabricated LBG/ CAGE-IL gels, a result that is in close agreement with previous findings by Virk et al. (2019) and Qiao and Duan (2020). The LBG gel formulations with curcumin exhibited peaks at 1629.85 cm^{-1} (aromatic moiety C=C stretching), 1602.85 cm^{-1} (benzene ring stretching vibrations) and 1510.26 cm^{-1} (C=O and C=C vibrations), which are the characteristic peaks of curcumin, indicating that curcumin retained its characteristics in the gel formulations.

The characteristic peaks of curcumin, LBG and CAGE-IL were present in the FTIR spectra of the gel formulations. It should be noted that in the spectrum of LBG-curcumin gels, the C=O stretching of Cur shifted from 1627.92 cm^{-1} to 1629.85 cm^{-1} ; meanwhile, the C=C stretching in benzene ring at 1600.92 cm^{-1} and 1508.33 cm^{-1} was shifted to 1602.85 cm^{-1} and 1510.26 cm^{-1} , respectively. These results demonstrated that hydrogen bonding between LBG/ CAGE-IL and curcumin occurred in the gel formulations containing curcumin. In brief, LBG, CAGE-IL and Cur were compounded together excellently. These same types of interactions were reported by Qiao and Duan (2020).

Comparing the spectra of plain pure curcumin and commercial turmeric (Fig. 9) with the spectra of the gel formulations integrating curcumin and CAGE-IL, the same characteristic peaks can be observed with only minor variations in peak intensity, which clearly suggests that the chemical aspect of curcumin was preserved during incorporation into the biopolysaccharide gels.

While the results displayed in Fig. 9 suggest that the spectra of the gel formulations manifest themselves as a sum of the curcumin and LBG biopolymer spectra, indicating that no significant interaction occurred between curcumin, LBG and CAGE-IL, the FTIR spectra displayed in Fig. 9 indicates full compatibility between LBG, CAGE-IL and curcumin, with all the functional group frequencies being present.

The rheological characteristics of semi-solid products are important properties to be considered in the development of pharmaceutical formulations for topical use. Rheology has been a subject of great and growing importance for the cosmetic and pharmaceutical industries, as the rheological characteristics can influence the physical stability of the system, the spreadability, the sensory characteristics and the intended purposes of use. In the applied research work described here, gels prepared with concentrations of 2.5% (w/w) locust bean gum, 2.0% (w/w) CAGE-IL and 2.0% (w/w) of either pure curcumin or commercial turmeric

were evaluated in terms of their viscosities at two different temperatures ($25\text{ }^{\circ}\text{C}$ (room temperature) and $33.5\text{ }^{\circ}\text{C}$ (the normal average temperature of the human skin surface)). As can be observed from inspection of the data displayed in Fig. 10a, the gel formulations prepared with either pure curcumin or commercial turmeric exhibited quite different viscosities for low rotating speeds, irrespective of the temperature under scrutiny, with the former gel displaying much lower viscosity than the later. For higher rotating speeds of the spindle employed, both gel formulations exhibited the same viscosity irrespective of the temperature.

An inspection of the data displayed in Fig. 10a allows to conclude that both types of gel formulations displayed a non-linear plastic flow behavior. The gel formulation prepared with pure curcumin exhibiting a pseudoplastic (shear thinning) with yield (Herschel-Bulkley) behavior at both temperatures assayed, and the gel formulation prepared with commercial turmeric exhibiting a plastic behavior at both temperatures (Fig. 10b).

The compressibility, hardness, adhesiveness and cohesiveness of the gel formulations produced integrating pure curcumin with or without CAGE-IL and of the gel formulation integrating commercial turmeric and CAGE-IL, calculated from the force-time curves of texture profile analysis displayed in Fig. 11 for the two different temperatures assayed, viz. $25\text{ }^{\circ}\text{C}$ and $33.5\text{ }^{\circ}\text{C}$. According to the results (Fig. 12), the best results in terms of (higher) compressibility and (lower) hardness were obtained at $33.5\text{ }^{\circ}\text{C}$ for the gel prepared with CAGE-IL and commercial turmeric (Fig. 12a and b). At the temperature commonly encountered in the surface of the human skin, viz. $33.5\text{ }^{\circ}\text{C}$, the gel prepared with commercial turmeric also displayed the best results in terms of adhesiveness and cohesiveness (Fig. 12c and d). While “in-the-packaging” features such as lower compressibility and higher hardness together with lower adhesiveness and lower cohesiveness are desired at room temperature, those “out-of-the-packaging” features are better reversed at the intended temperature of use, viz. $33.5\text{ }^{\circ}\text{C}$, for skin applications. As can be observed from inspection of the data in Fig. 12, the gel that performed better in terms of these mechanical features was the one integrating CAGE-IL and commercial turmeric. The ability of the gel to stick on the surface of the skin is an especially important attribute, since its adhesiveness and cohesiveness are important characteristics for skin applications. These mechanical properties of the gels are mainly related with the biopolysaccharide's ability to form bonds in polymer chains, leading to resistance to their separation when subject to mechanical forces (Yang, 2012; Gong and Hong, 2012). In addition, the results obtained in the microstructural and morphological analysis of the gel matrix showed (Fig. 13) a highly porous and entangled network made of biopolysaccharide, which clearly favored release and availability of the entrapped curcumin for transdermal permeation aided by the CAGE-IL integrated in the formulation. The major goal

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of the research work undertaken was to develop a gel formulation based on biopolysaccharides and integrating CAGE-IL and curcumin from commercial turmeric, suitable for application on the skin aiming at the delivery of curcumin via transdermal permeation. In our perspective, the gel applied directly to the skin should strongly adhere, allowing release of curcumin and easy removal of the spent gel from the skin by running water. Thus, compressibility, hardness, adhesiveness and cohesiveness were the mechanical resistance parameters evaluated, whereas resistance to traction, relaxation and resilience were not at all considered important in the research work developed.

5. Conclusions

In this research effort, the effect of CAGE-IL as a permeation enhancer of curcumin was studied, following abounding experimental evidence that this ionic liquid promotes transdermal permeation of bioactive macromolecules. The results of this study clearly show that the use of CAGE-IL highly enhances transdermal permeation of curcumin, with high potential in the treatment of skin diseases such as (but not limited to) psoriasis. The gel formulated with 2% (w/w) CAGE-IL and integrating 2% (w/w) of either pure curcumin or commercial turmeric allowed transdermal permeation of curcumin in reasonable amounts and concomitant skin retention of this bioactive molecule. The formulation integrating 2% (w/w) turmeric showed the best results in terms of mechanical properties, with a high potential for translation into "real world" applications with clear benefits to patients.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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4.4 Gel de alfarroba e curcumina com geranato de colina: ensaios de estabilidade

Este tópico teve como foco verificar a estabilidade do gel desenvolvido. Ensaio de estabilidade de produtos farmacêuticos abrangem um conjunto de procedimentos como o objetivo de avaliar a qualidade, eficácia e segurança de uma formulação farmacêutica. A estabilidade química é um dos aspectos de importância na determinação da estabilidade farmacêutica, uma vez que, pode-se determinar quaisquer incompatibilidades fármaco-excipientes na formulação, permite-se selecionar as condições de armazenamento e de acondicionamento adequadas para o produto desenvolvido (VAZ, 2016).

Neste sentido, foi realizado ensaio de estabilidade preliminar como um teste preditivo para verificar o equilíbrio da formulação; estudo da compatibilidade físico-química entre os componentes do gel por calorimetria de varredura diferencial (DSC) e, por fim, foram feitos testes de estabilidade acelerada.

O produto apresentou-se estável durante os 180 dias de armazenamento.

GEL DE ALFARROBA E CURCUMINA COM GERANATO DE COLINA: ENSAIOS DE ESTABILIDADE

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RESUMO

A curcumina, um composto fitoquímico, apresenta várias propriedades biológicas e tem sido utilizada no tratamento da psoríase. Para facilitar sua permeação transdérmica, permeadores cutâneos, como o líquido iônico geranato de colina têm sido usados. A goma de alfarroba, galactomanano vegetal extraído de sementes de alfarroba, por sua capacidade em formar géis estáveis também encontra uso em aplicações farmacêuticas. Para garantir a qualidade, a eficácia e, a segurança de produtos farmacêuticos estudos de estabilidade devem ser realizados. Assim, este estudo buscou-se desenvolver e avaliar a estabilidade de um gel de curcumina e alfarroba com geranato de colina como facilitador de permeação transdérmica. Os estudos de estabilidade foram realizados durante seis meses, com análises nos intervalos de 0 (24h), 30, 60, 90, e 180 dias. As amostras foram mantidas a $40\pm 2^{\circ}\text{C}$, com $75\pm 5\%$ de umidade relativa (UR). Os parâmetros analisados foram: características morfológicas, pH e espalhabilidade. As amostras mostraram-se estáveis, com características físico-químicas adequadas, atendendo às especificações de qualidade para aplicação cutânea.

Palavras-chave: Curcumina. Geranato de colina, Goma de alfarroba, Ensaio de estabilidade.

ABSTRACT

Curcumin, a phytochemical compound, has several biological properties and has been used in the treatment of psoriasis. To facilitate its transdermal permeation, *permeation* enhancers as the ionic liquid choline geranate have been used. Locust bean gum, a vegetable galactomannan extracted from locust bean seeds, for its ability to form stable gels also finds use in pharmaceutical applications. To ensure the quality, efficacy, and safety of pharmaceuticals stability studies must be carried out. Thus, this study aimed to develop and evaluate the stability of a curcumin and locust bean gel with choline geranate, as a facilitator of transdermal permeation. Stability studies were performed for six months, with analyzes at intervals of 0 (24h), 30, 60, 90, and 180 days. The samples were kept at $40\pm 2^{\circ}\text{C}$, with $75\pm 5\%$ relative humidity (HR). The parameters analyzed were: morphological characteristics, pH and spreadability. The samples were shown to be stable, with adequate physicochemical characteristics, meeting the quality specifications for cutaneous application.

Keywords: Curcumin. Choline geranate, Locust bean gum. Stability testing.

1 INTRODUÇÃO

A curcumina é um composto ativo derivado da espécie vegetal cúrcuma (*Curcuma longa* L.) que apresenta uma grande variedade de atividades biológicas, como antioxidante, anti-inflamatória, antitumoral, antimicrobiana, antiparasitária, entre outras (HUSSAIN et al., 2017). A curcumina é uma molécula simétrica, também conhecida como diferuloilmetano. O nome IUPAC (International Union of Pure and Applied Chemistry) deste composto é (1E-6E) -1,7-bis(4-hidroxi-3-metoxifenil) -1,6-heptadieno-3,5-diona. A fórmula química da curcumina é $\text{C}_{21}\text{H}_{20}\text{O}_6$ e massa molecular de $368,385\text{g mol}^{-1}$. (RATHORE et al., 2020). A curcumina é o componente principal dos rizomas da cúrcuma e, comercialmente, pode ser adquirida como uma mistura complexa formada por curcumina (~77%); desmetoxicurcumina (~17%); e bisdesmetoxicurcumina (~3%) (SUETH-SANTIAGO et al., 2015). Estes

curcuminóides são análogos estruturalmente, diferenciando-se apenas pela quantidade de grupos metoxila (OCH_3) em sua estrutura química como pode ser visto na Figura 1 (BURAPAN et al., 2020).

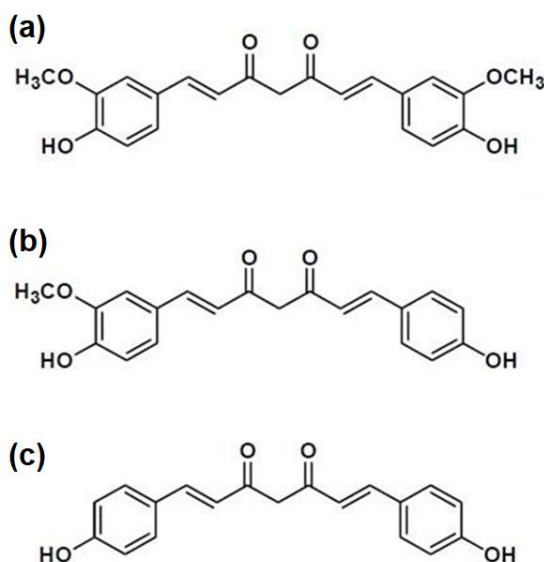


Figura 1. Fórmulas estruturais dos curcuminóides. **(a)** fórmula estrutural da curcumina, **(b)** fórmula estrutural da demetoxicurcumina, **(c)** fórmula estrutural da bisdemetoxicurcumina.

Estudos avaliaram o potencial terapêutico da curcumina no tratamento da psoríase, com resultados positivos (GOPINATH; KARTHIKEYAN, 2017; KUNNUMAKKARA et al., 2017; PANAHI et al., 2019). A psoríase é uma doença inflamatória crônica autoimune e não transmissível, afetando a pele e, ocasionalmente, as articulações. A psoríase é caracterizada por lesões vermelhas e escamosas presentes, frequentemente, nos cotovelos, joelhos, couro cabeludo, mãos e pés (DUTTA; CHAWLA; KUMAR, 2018).

A curcumina pode minimizar o estresse oxidativo das lesões psoriáticas; reduzir a proliferação de células psoriáticas, pela regulação negativa de citocinas pró-inflamatórias e; inibir canais de potássio expressos nas células T, que parecem estar envolvidos no aparecimento da psoríase (CAMPANI et al., 2020; NARDO et al., 2018). A curcumina tem baixa solubilidade aquosa e baixa biodisponibilidade oral devido ao rápido metabolismo de primeira passagem, limitando assim sua utilidade como medicamento oral, mas a torna uma molécula interessante para a

administração tópica (VIGATO et al., 2019). A aplicação pela via transdérmica de moléculas bioativas oferece uma opção de administração indolor e ao mesmo tempo, evita o efeito de primeira passagem do metabolismo. Entretanto, superar a barreira da pele ainda é um desafio (CHENG et al., 2020; TANNER et al., 2019). Assim, com o objetivo de melhorar o transporte de fármacos, através da camada córnea, tem sido utilizadas substâncias facilitadoras da permeação cutânea, como os líquidos iônicos (JORGE et al., 2020; TODKE; SHAH, 2018).

Os líquidos iônicos constituem sais em estado líquido preparados a partir de materiais pouco onerosos e com *status* GRAS (*Generally Recognized As Safe*). Os líquidos iônicos aumentam a permeabilidade de drogas via barreira cutânea. Estes compostos são capazes de deslizar através dos ácidos graxos, que constituem as células da pele, criando pequenas lacunas transitórias, através das quais moléculas bioativas, transportadas pelo líquido iônico podem permear (ZAKREWSKY et al.; 2016; KO et al., 2021). Os líquidos iônicos preparados à base de bicarbonato de colina e ácido gerânico na razão molar 1:2 (Figura 2), têm sido utilizados para melhorar a entrega transdérmica de várias moléculas, de pequena a elevada massa molecular (BOSCARIOL et al., 2021; IBSEN et al., 2018; TANNER et al., 2019; JORGE et al., 2020).

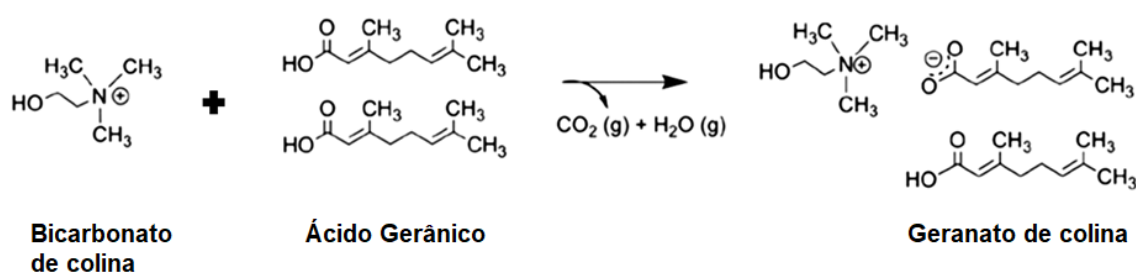


Figura 2. Síntese do líquido iônico de geranato de colina. Reação química entre bicarbonato de colina e ácido gerânico na razão molar 1:2.

Entre as várias formas farmacêuticas tópicas disponíveis os géis e hidrogéis são muito populares e, possuem ótima aceitação pelos pacientes pela facilidade de aplicação (WANG et al., 2019). Os géis à base de polissacarídeos naturais podem ser produzidos, sem a presença de substâncias tóxicas, o que os torna interessantes no desenvolvimento de sistemas de entrega de moléculas bioativas (MITURA;

SIONKOWSKA; JAISWAL, 2020). Neste sentido, um biopolissacarídeo que vem despertando atenção é a goma de alfarroba, um galactomanano vegetal extraído de sementes de alfarroba (da alfarrobeira, *Ceratonia siliqua*) (MOSTAFAVI et al., 2016). A goma de alfarroba apresenta uma estrutura química linear constituída por uma estrutura principal de β (1-4)-manose com unidades D-galactopiranosil ligadas por meio de ligações α (1-6) como ramo lateral, na proporção de 4:1 manose/galactose (YUAN et al., 2021) (Figura 3). Este polímero apresenta uma série de características atrativas para aplicações biofarmacêuticas, entre as quais sua alta capacidade gelificante, biodegradabilidade, baixa toxicidade e disponibilidade a baixo custo (DIONÍSIO; GRENHA, 2012).

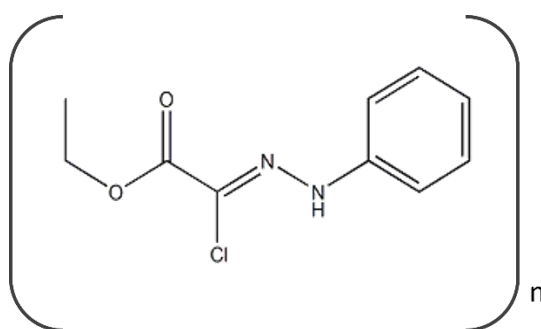


Figura 3. Estrutura química da goma alfarroba

Para garantir a qualidade, eficácia e segurança de produtos farmacêuticos são realizados estudos de estabilidade, uma vez que fornecem dados importantes para determinar a vida útil e condições de estocagem (BHAGYASHREE et al., 2015; BODINI, 2017; ENUMO et al., 2020). Além disto, são procedimentos de rotina e exigidos para a produção de medicamentos (BRASIL, 2019). Estudos de estabilidade da formulação tem por finalidade avaliar o comportamento dos medicamentos em função do tempo e a influência de várias condições e fatores, sendo levado em consideração tanto o fármaco, quanto a mistura de excipientes ou veículos utilizados, assim como a interação entre ambos, face às condições as quais estão submetidos (ROCHA, 2017). A estabilidade das formulações pode ser alterada por fatores extrínsecos e intrínsecos com sérias consequências para a qualidade

dos produtos farmacêuticos. Destacam-se como fatores extrínsecos causadores de degradação como temperatura, luz, oxigênio, umidade, material de condicionamento e microrganismos. Fatores intrínsecos são aqueles relacionados com a interação entre os componentes da formulação capazes de gerar alteração do valor do pH, da viscosidade, das características organolépticas, separação de fases, redução do teor da substância ativa, entre outras (HUYNH-BA; DONG, 2020).

Pelos fatos expostos objetivou-se, neste estudo, desenvolver e avaliar a estabilidade de um gel de alfarroba com curcumina associado ao líquido iônico geranato de colina, com potencial para aplicação no tratamento tópico da psoríase.

2 PARTE EXPERIMENTAL

Neste trabalho foram utilizados reagentes de grau analítico e/ou grau farmacêutico e água purificada em ultrapurificador (modelo MS2000, Gehaka, São Paulo/SP, Brasil) com resistividade de $18,2 \text{ M}\Omega\cdot\text{cm}^{-1}$ e condutividade de $0,05 \text{ }\mu\text{S}\cdot\text{cm}^{-1}$.

2.1 Síntese do líquido iônico de geranato de colina (LI)

O geranato de colina foi preparado de acordo com o procedimento descrito por Jorge et al. (2020), Banerjee et al. (2017) e Zakrewsky et al. (2016). Num balão volumétrico de 1000 mL, 48mL de ácido gerânico (CAS No. 459-80-3; Sigma-Aldrich, St. Louis MO, EUA), 20mL de bicarbonato de colina em 80% (m/v) (CAS No. 62-49-7; Sigma-Aldrich, St. Louis MO, EUA) e 20mL de metanol (CAS No. 67-56-1; Chenco, Brasil) foram adicionados. A mistura foi agitada magneticamente à temperatura ambiente, em sistema aberto, *overnight*, até que a produção de CO_2 cessasse. O solvente foi, na sequência, removido usando um evaporador rotativo (Buchi Labortechnik AG modelo R-215, Alemanha), a 60°C , durante de cerca de 20 minutos. O LI preparado foi transferido para um tubo Falcon de 50mL, o *headspace* lavado com nitrogênio, e o tubo tampado e selado com Parafilm™ (Bemis Flexible Packaging, Neenah WI, EUA).

2.2 Preparo dos géis de curcumina pura (Sigma-Aldrich) ou curcumina comercial e líquido iônico.

Os géis foram preparados pela dispersão de goma alfarroba (2%, m/m) (Ref. No. G0753; Sigma-Aldrich, St. Louis MO, EUA) em água ultrapura. A suspensão foi aquecida a 80 °C e agitada até a solubilização da goma utilizando agitador mecânico com aquecimento (modelo TE 0181, Tecnal, Piracicaba/SP, Brasil). (KAWANO et al., 2017). Na sequência, foi adicionado metilparabeno (0,1%, m/m). Após resfriamento, a temperatura ambiente, foi acrescentada a curcumina pura (pureza $\geq 95,0\%$ (Ref. No. C1386; Sigma-Aldrich, St. Louis MO, EUA) ou a curcumina comercial (pureza 95% CAS: 458-37-7; Lote: CJH-A-915549, China) na concentração de 2% (m/m) previamente solubilizada em geranato de colina na concentração de 2% (m/m), sob agitação manual e temperatura ambiente. As amostras foram armazenadas em frascos estéreis (radiação ionizante) de polipropileno (marca Cralplast, Cotia/SP, Brasil) de volume 80mL, opacos e com tampa em rosca.

2.3 Estudos de estabilidade

Os estudos de estabilidade foram realizados durante seis meses, com análises em 0 (24h), 30, 60, 90 e 180 dias (ANVISA, 2015). As amostras de gel foram mantidas em uma câmara climática (modelo AL5100, AmericanLab, USA) a $40\pm 2^\circ\text{C}$, com $75\pm 5\%$ de umidade relativa (UR). Os parâmetros analisados foram características morfológicas, pH e espalhabilidade. Para a realização de cada teste, foram retiradas amostras de cerca de 10g dos frascos originais, nos tempos determinados.

Características morfológicas

As amostras dos géis produzidos foram analisadas visualmente quanto às possíveis alterações de cor, odor, homogeneidade e existência de separação de fases.

Espalhabilidade

Os ensaios de espalhabilidade foram realizados utilizando um conjunto de placas de vidro de diferentes dimensões e pesos, uma placa de molde circular de vidro com

um orifício central (1,2 cm de diâmetro) e uma placa de suporte. O conjunto (placa de suporte, placa de molde e placa de vidro) foi colocado sobre papel em escala milimétrica. Uma amostra de cada gel formulado (2g) foi introduzida no orifício da placa do molde e devidamente nivelada com o auxílio de uma espátula. A placa de molde foi removida e uma placa de vidro de peso conhecido foi colocada sobre a amostra de modo a espalhá-la na placa de suporte. Os diâmetros cobertos pela amostra do produto foram lidos na escala milimétrica do papel e o diâmetro médio foi calculado. Todas as determinações foram realizadas em triplicado a 25°C. A espalhabilidade (E_i) foi então calculada usando a Equação (1).

$$E_i = \frac{d^2 \times \pi}{4} \quad (1)$$

Onde: $E(i)$ = espalhabilidade da amostra para uma determinada massa da placa de vidro (mm^2); d = diâmetro médio (mm); $\pi = 3,14$

Placa 1=162,41g; Placa 2 =135,26g; Placa 3 = 95,23g; Placa 4 = 61,47g; Placa 5 = 34,65g; Molde de placa com orifício central = 22,91 g.

pH

As medidas de pH foram realizadas em pHmetro (Analisador-modelo 300, São Paulo, Brasil), previamente calibrado com soluções tampão de pH 4,0 e 7,0. Para a execução da medida do pH uma amostra de 1g de cada hidrogel foi disperso em 9 mL de água purificada previamente neutralizada. As medidas foram realizadas em triplicata.

2.4 Teste de Centrifugação

Cerca de 5 g de amostra foram acondicionadas em tubos Falcon e centrifugadas a 3000 rpm, durante 30 min em centrífuga (modelo Universal-320, Hettich, Alemanha). Em seguida, foram inspecionadas visualmente quanto ao seu aspecto, presença de precipitação e separação de fases (ANVISA, 2015).

2.5 Potencial Zeta (ZP), Espalhamento dispersivo da luz (DLS) e Índice de polidispersividade (PDI)

Através de equipamento específico (NanoBrook, modelo 90 PlusPALS, Brookhaven, USA) foram avaliadas as características das partículas de curcumina formadas no processo de dissolução em geranato de colina. Foram preparadas diluições das amostras (1:400) empregando-se 50µL das soluções de curcumina em 20mL de água ultrapura (GLASSER et al., 2016).

2.6 Análise estatística

As formulações foram preparadas e analisadas em triplicata, seus resultados foram expressos como média $\pm$ desvio padrão. Os dados foram avaliados estatisticamente através de teste *t* ou de análise de variância (ANOVA). Todos os testes foram conduzidos utilizando o software GraphPad Prism 8.0 (San Diego, EUA). Valores de $p < 0,05$ foram considerados como indicativos de significância estatística.

3 RESULTADOS E DISCUSSÃO

Géis são formados como uma rede espacial contínua de moléculas conectadas entre si pela reticulação de moléculas poliméricas ou pequenas moléculas via ligação covalente/não-covalente e, com comportamento reológico semelhante a um sólido (HAN et al., 2021). Os géis são formulações consideradas oficinais, onde seu preparo segue as especificações da Farmacopeia Brasileira ou o Formulário Nacional. A etapa mais criteriosa na sua preparação refere-se à hidratação do polímero, para se evitar a formação de grumos não desejados ou incorporação de ar (MELO et al., 2018). A goma de alfarroba, para sua completa dissolução, necessita de aquecimento. Neste sentido, e seguindo as determinações de Mudgil; Barak; Khatkar, (2014), uma solução contendo água e goma de alfarroba foi aquecida a 70° C por cerca de 2 h até a completa dissolução.

Na sequência, incorporou-se uma solução de curcumina e líquido iônico. A curcumina por ser uma molécula hidrofílica, com baixa solubilidade em água

(particularmente em ambiente ácido ou neutro, onde permanece totalmente protonada) necessita, antes de sua incorporação em uma matriz aquosa, ser dissolvida em algum solvente ou substância hidrofóbica (ZHANG; ZHANG; MCCLEMENTS, 2020). Alguns autores empregam o etanol (PATEL et al., 2008), complexos com ciclodextrina (VANDANA; PAWAR, 2019), polietilenoglicol (PEG), entre outros (TORKY et al., 2018) para a solubilização da curcumina. O uso do geranato de colina, nesta pesquisa, também permitiu a solubilização da curcumina, o que pode ser comprovado pelo tamanho das partículas obtidas por DLS e pelo potencial Zeta.

A análise por DLS foi realizada com o objetivo de se averiguar o tamanho das partículas de curcumina após a dissolução no geranato de colina. Esta técnica mensura a distribuição de tamanho de partículas em escala nanométrica, com precisão, confiável e de fácil reprodução, necessitando de pouca quantidade de amostra (GLASSER et al., 2016; MASSIMINO; YOSHIOKA, 2016). Através dessa técnica também se obtém o índice de polidispersividade (PDI), que é o índice de distribuição de tamanho, representando a semelhança entre as partículas. Um valor de PDI com proximidade a 1 sinaliza que os tamanhos das partículas são diferentes (DANAEI et al., 2018; RAPOSO; CONCEIÇÃO; BARROS, 2020). As análises de DLS e de PDI das amostras de solução de curcumina revelaram que a curcumina comercial dissolvida no líquido iônico apresentou valor médio de tamanho de partículas de $134,33 \pm 2,6$ nm e PDI igual a $0,26 \pm 0,005$; já a curcumina Sigma-Aldrich apresentou valor médio de tamanho de partículas de $95,140 \pm 2,8$ nm e PDI igual a $0,243 \pm 0,008$ como ilustrado na Figura 4.

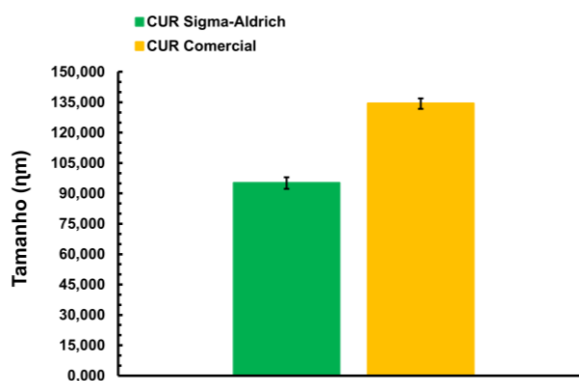


Figura 4. Valores de espalhamento dispersivo da luz (DLS) para as amostras de curcumina dissolvidas em geranato de colina (média $\pm$ desvio padrão, $n=3$).

Esses resultados mostram diferenças entre dissolução no geranato de colina entre as amostras de curcumina. A curcumina comercial apresentou partículas maiores em relação a Sigma-Aldrich, indicando diferenças na pureza e entre os produtos. O líquido iônico geranato de colina demonstrou ser eficaz na solubilização de moléculas insolúveis em meio aquoso. Mesmo havendo diferenças no tamanho das partículas entre as amostras de curcumina, estes resultados são semelhantes e até melhores que outros estudos que utilizaram sistemas nanoestruturados para a veiculação de curcumina em formulações magistrais (MASSIMINO; YOSHIOKA 2016; NIKOLIC et al., 2020; RAPOSO; CONCEIÇÃO; BARROS, 2020), indicando a eficácia do líquido iônico na solubilização da curcumina. Em relação ao teste de índice polidispersividade, as amostras de curcumina apresentaram um valor baixo, quando dissolvidas no geranato de colina, sinalizando tamanhos de partículas homogêneos.

O potencial Zeta é a medida da carga de superfície da partícula em meio específico, utilizada para analisar e prever as interações das partículas em suspensão. A manipulação dessas cargas de superfície constitui uma estratégia para melhorar a estabilidade de formulações magistrais (BALCÃO; VILA, 2015; GLASSER et al., 2016).

As partículas presentes na suspensão que apresentam um valor elevado, positivo ou negativo, tendem a se repelir, assim não há a tendência de flocular, ou seja, o sistema é estável. Partículas com valores de potencial Zeta igual a $\pm 30\text{mV}$ ou maiores, são considerados estáveis (INSTRUMENTS, 2013). Os resultados obtidos indicaram que a amostra de curcumina comercial dissolvida no geranato de colina apresentou um valor médio de potencial Zeta em $-34,97\text{mV}$ e a amostra de curcumina Sigma-Aldrich dissolvida no geranato de colina obteve um valor médio de $-59,69\text{mV}$, conforme ilustra a Figura 5. Estes valores indicam que partículas estáveis foram formadas na solução.

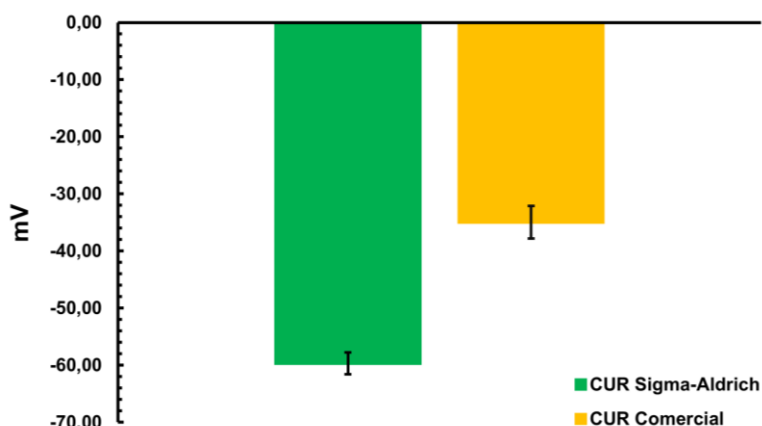


Figura 5. Valores do Potencial Zeta para as amostras de curcumina dissolvidas em líquido iônico de geranato de colina (média $\pm$ desvio padrão, $n=3$).

As amostras de curcumina solubilizaram-se bem no líquido iônico geranato de colina, o que é um resultado muito importante, uma vez que a curcumina é insolúvel em meio aquoso (BURAPAN et al., 2020; KOCAADAM; ŞANLIER, 2017; KOTHA; LUTHRIA, 2019). O interessante, foi a não necessidade de uso de surfactantes como Twen 80 ou lauril sulfato de sódio que são, normalmente, usados em formulações farmacêuticas para conferir estabilidade e prevenir os fenômenos de coalescência (KAPOOR et al., 2019). Neste sentido, para verificar e confirmar a estabilidade preliminar do gel foi realizado testes de centrifugação.

Testes de centrifugação são importantes pois antecipam sinais de instabilidade na formulação por provocar um estresse ao simular um aumento na gravidade e portanto, aceleram a mobilidade das partículas (ANVISA, 2015). Através da inspeção da Figura 6 é possível constatar, que nenhuma das formulações apresentou alterações visíveis. Assim, pela ausência de separação de fases, floculação e coalescência, os géis desenvolvidos nesse estudo mostraram-se estáveis frente a este teste e, na sequência, foram feitos os ensaios de DSC e de estabilidade acelerada por 180 dias de armazenamento a $40\pm2^{\circ}\text{C}$ e $75\pm5\%$ UR.

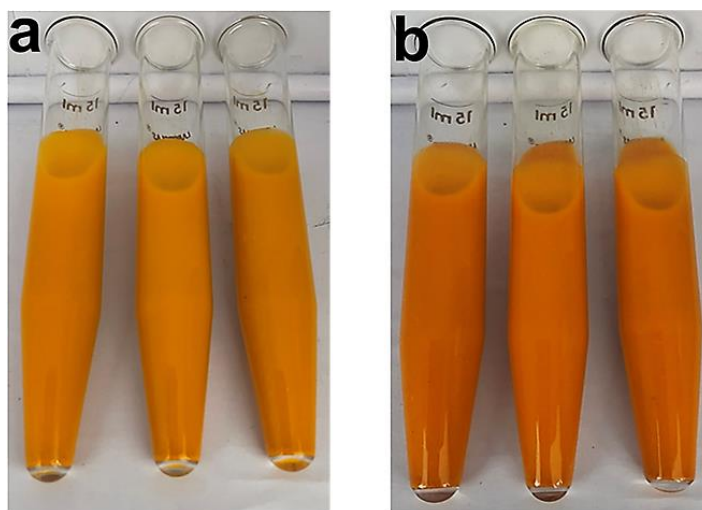


Figura 6. Imagens representativas dos géis de curcumina formulados submetidos ao teste de centrifugação. **(a)** gel de curcumina Sigma-Aldrich, **(b)** gel de curcumina comercial.

Os resultados do estudo de estabilidade de formulação a respeito das características macroscópicas (aspecto geral, cor, odor e separação de fases) indicaram que não houve alterações significativas durante 180 dias de armazenamento. A Figura 7 ilustra o aspecto geral e a pigmentação das formulações.

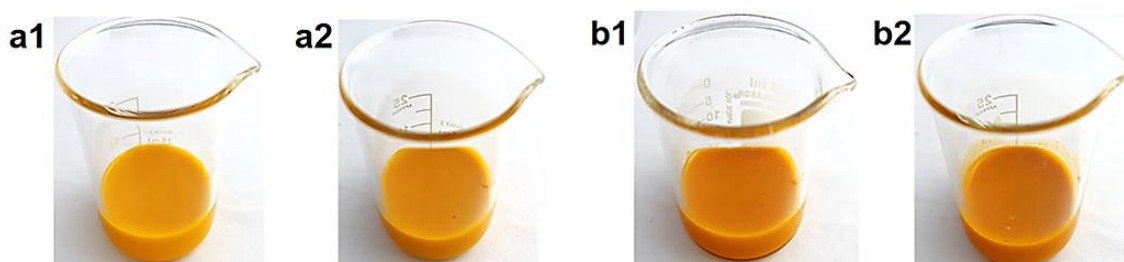


Figura 7. Características morfológicas das formulações após 180 dias de teste ($40\pm 2^{\circ}\text{C}$ e $75\pm 5\%$ de umidade relativa (UR)). **(a)** gel curcumina Sigma-Aldrich, **(1)** 24 horas, **(2)** 180 dias. **(b)** gel curcumina comercial, **(1)** 24 horas, **(2)** 180 dias.

A avaliação da espalhabilidade no desenvolvimento de formulações semissólidas é um teste importante, pois verifica-se a capacidade da formulação espalhar-se sobre uma superfície e, portanto, relaciona-se com a aplicação no local desejado. Ambas as formulações apresentaram um aumento da espalhabilidade após 15 dias de exposição nas condições estabelecidas. Após 120 dias, não houve alterações.

Provavelmente, a presença do geranato de colina, em função de sua característica oleosa, pode ter contribuído para o aumento da espalhabilidade (HERNANDES et al., 2021), com alterações na tensão residual estática e, no comportamento de pseudoplástico da goma alfarroba (YUAN et al., 2021). A Figura 8 ilustra os resultados obtidos dos perfis de espalhabilidade dos géis produzidos. Houve diferença estatística nos resultados das formulações ($p < 0,05$), talvez devido a diferença de pureza entre as amostras de curcumina utilizadas. Entretanto, não se poderia afirmar que o uso da curcumina comercial seria prejudicial para o produto.

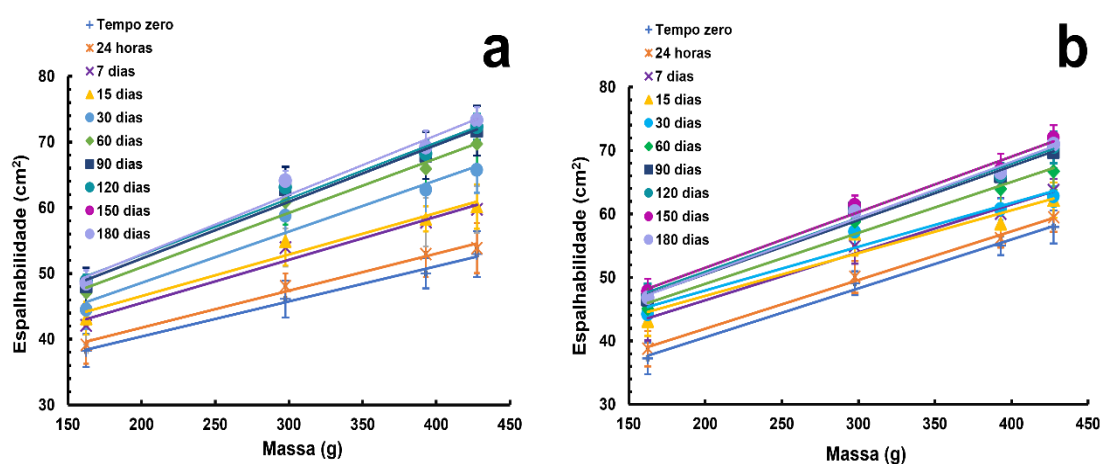


Figura 8. Valores de espalhabilidade dos géis formulados em função da massa (g) aplicada ($40 \pm 2^\circ\text{C}$; $75 \pm 5\%$ UR) (média $\pm$ desvio padrão, $n=3$) ($p < 0,05$). **(a)** gel de curcumina Sigma-Aldrich (2%, m/m). **(b)** gel de curcumina comercial (2%, m/m).

Em relação aos valores de pH, as amostras de gel não apresentaram mudanças ao longo de 180 dias, permanecendo constantes, entre 5,8 e 6,0. Os produtos apresentam pH adequado para produtos tópicos, uma vez que, pH “normal” da superfície da pele da maioria das partes do corpo é ácido e na faixa de pH 4,1-5,8 (PROKSCH, 2018). Além disto, a faixa de pH dos géis mantêm a estabilidade da curcumina, já que a curcumina mantêm-se estável numa faixa de pH entre 3 e 7 em função de um equilíbrio ceto-enólico (SUETH-SANTIAGO et al., 2015).

4 CONCLUSÃO

Neste trabalho foram desenvolvidas duas formulações de géis de alfarroba com curcumina, associados ao líquido iônico geranato de colina. As formulações mostraram-se estáveis durante os 180 dias de armazenamento, com características físico-químicas adequadas, atendendo às especificações de qualidade para a aplicação cutânea. A goma de alfarroba permitiu a formação de um gel estável e com características adequadas para uso tópico. O líquido iônico, utilizado como um facilitador de permeação cutânea, também se mostrou adequado para a solubilização da curcumina. Pelos resultados dos testes de DLS e potencial Zeta, houve a produção de partículas na escala nanométrica. Neste sentido, pode-se concluir que as formulações foram elaboradas de forma simples, com uso de poucos compostos e com potencial de aplicação clínica.

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4.5 Transdermal permeation assays of curcumin aided by CAGE-IL: *in vivo* control of psoriasis

Este artigo foi publicado na revista *Pharmaceutics* em edição especial *Curcumin in Biomedical Applications* (https://www.mdpi.com/journal/pharmaceutics/special_issues/curcumin_ba).

Este trabalho teve como foco comprovar em ensaios *in vivo* a eficácia da curcumina carregada por líquido iônico no tratamento da psoríase. O ensaio foi aprovado pelo Comitê de Ética no Uso de Animais da Universidade de Sorocaba (Protocolo nº 199/2021) estando disponibilizado no Anexo E.

Até o momento, os modelos animais ainda são ferramentas indispensáveis na pesquisa da psoríase, embora seja um desafio, uma vez que, esta doença não ocorre naturalmente em animais de laboratório. Foi utilizado indução de dermatite após aplicação tópica de imiquimode (quimioterápico tópico) em orelhas de camundongos, pois é o modelo mais utilizado em ensaios envolvendo animais no estudo da psoríase (SCHÖN; MANZKE; ERPENBECK, 2021). Embora a aplicação de imiquimode não produza exatamente a psoríase humana, o tratamento com imiquimode na pele de camundongos pode exercer padrões específicos de expressão de citocinas, alterações histopatológicas e infiltrados celulares semelhantes ao observado em pacientes com psoríase (HORVÁTH *et al.*, 2019).

Os resultados comprovaram a eficácia do gel de curcumina e geranto de colina no tratamento da psoríase.



Article

Transdermal Permeation Assays of Curcumin Aided by CAGE-IL: In Vivo Control of Psoriasis

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Abstract: Psoriasis is a clinically heterogeneous skin disease with an important genetic component, whose pathophysiology is not yet fully understood and for which there is still no cure. Hence, alternative therapies have been evaluated, using plant species such as turmeric (*Curcuma longa* Linn.) in topical preparations. However, the stratum corneum is a barrier to be overcome, and ionic liquids have emerged as potential substances that promote skin permeation. Thus, the main objective of this research was to evaluate a biopolysaccharide hydrogel formulation integrating curcumin with choline and geranic acid ionic liquid (CAGE-IL) as a facilitator of skin transdermal permeation, in the treatment of chemically induced psoriasis in mice. The developed gel containing curcumin and CAGE-IL showed a high potential for applications in the treatment of psoriasis, reversing the histological manifestations of psoriasis to a state very close to that of normal skin.

Keywords: choline and geranic acid ionic liquid; transdermal permeation; curcumin; psoriasis; in vivo assays

1. Introduction

Psoriasis is a relatively common skin disease, being, from a pathological point of view, chronic dermatitis with rapid uncontrolled proliferation of epithelial cells on the surface, hyperemia and dense lymphocytic infiltration [1]. Essentially, the disease can start at any stage of life and persist for a long time, with permanent or periodic eruptions [2,3]. It is recognized as a genetically modulated disease [4] that results in a chronic, autoimmune, standard inflammatory condition [1] characterized by thick, red, scaly patches or plaques distributed throughout the body. The condition is usually governed by components of the patient's immune system [5,6]. A variety of topical and systemic drug therapies exist, and treatment regimens must be optimized for achieving optimal compliance and benefit [7–9]. However, despite the availability of different topical agents and systemic therapeutic options, none of the treatments provide excellent clinical results without the risk of side effects [10,11].

More recently, studies point to curcumin (CUR) as a potential asset in the therapy of psoriasis [12–16]. However, curcumin is practically insoluble in water and has low availability, so for use in topical preparations it is necessary to use substances that favor its permeation. Transdermal permeation aims to transport active pharmaceutical ingredients (APIs) in the skin tissue to the deeper layers, with clear therapeutic advantages compared to giving APIs orally or parenterally [17,18]. However, the stratum corneum is a natural protective barrier, with a low permeation rate. Thus, the development of

permeation-promoting systems that promote a transient change in the structure of the skin, enabling more efficient bioaccessibility and permeability of bioactive molecules, is of extreme importance [14,19,20]. Ionic liquids have emerged as facilitators of skin permeation for protein entities of interest [19–21]. Some ionic liquids such as choline and geranic acid ionic liquid (CAGE-IL) have the ability to cause transient alterations in the intercellular and intracellular lipids and the protein organization of the stratum corneum, allowing the penetration of bioactive molecules carried by these liquids [19,20,22,23]. In addition, ionic liquids can be incorporated into drug delivery systems, among which we can highlight hydrogels [24].

For psoriasis research trials, there are several categories of experimental animal models that can be used, among which the “direct induction” type is most viable for drug development and screening. Imiquimod (IMQ) is a ligand for Toll-like 7/8 receptors that activates macrophages, monocytes and dendritic cells and, when administered directly to the skin of mice, induces skin damage similar to psoriasis [25,26]. The agonist effect of imiquimod on the Toll-like receptor causes dermal dendritic cells to express cytokines such as interferon- α (IFN- α), tumor necrosis factor- α (TNF- α) and the interleukins IL-1, IL-6 and IL-8 [27]. This innate immune response can lead to adverse events such as redness, swelling and a burning sensation [28,29]. The IMQ-treated mouse model is the most widely explored animal model for studying psoriasis *in vivo*. It is a rapid and convenient model, which allows the elucidation of the mechanisms underlying the disease and the evaluation of new therapies against psoriasis [30]. In general, three days after application of imiquimod, mouse skin begins to resemble human skin with psoriasis, in terms of erythema and skin thickening, skin desquamation, epidermal changes (acanthosis, parakeratosis) and neoangiogenesis, as well as showing an inflammatory infiltrate consisting of T cells, neutrophils and dendritic cells [29,31]. However, there are differences in both the mechanism and the clinical features between imiquimod-induced psoriasis in mice and human psoriasis. In view of its acute and severe characteristics in the progression of the disease, the use of this animal model is restricted to the development of preclinical medications [29,32]. To treat the lesions developed by the experimental model, low-cost topical treatments based on natural products such as curcumin have attracted scientific attention [14,19,33,34].

Ionic liquids can transiently disrupt the skin barrier function by modifying the disposition of the corneocytes of the stratum corneum [19,33,35]. The biological outcomes of ionic liquids, including transdermal delivery, antibacterial activity and cytotoxicity are fully related to the chemical properties and their constituent cations and anions [35,36]. Compared to traditional organic solvents with small alkyl chains, such as ethanol, they are less toxic to cells, thus minimizing problems associated with solvent-induced skin irritation [37]. In particular, the ionic liquid of choline and geranic acid (CAGE-IL) has been successfully used by our research group to increase the skin permeation of several substances such as insulin [20], bacteriophage particles [24,38] and curcumin [19,33]. It has also been used by other research groups [39]. Therefore, CAGE-IL was chosen as a facilitator of dermal permeation in this study.

The objective of this research was to develop and evaluate *in vivo* a locust bean gum (LBG) gel integrating CAGE-IL, aiming at the transdermal permeation of curcumin for the treatment of imiquimod-induced psoriasis using a mouse model.

2. Materials and Methods

2.1. Materials

2.1.1. Test Animals

A total of 40 mice of the BALB/c strain (20 males and 20 females), aged 7–9 weeks, with average weights of ca. 25 g, were acquired from the Anilab bioterium (Paulínia/SP, Brazil) and used in the *in vivo* assays. All animals were maintained in the bioterium of the University of Sorocaba (Sorocaba/SP, Brazil). All procedures were performed in the vivarium of the University of Sorocaba, where the animals were kept in cages with a maximum number of 2 to 3 animals, grouped by gender, kept under constant temperature

conditions of 24 ± 1 °C, with a night–day cycle of 12:12 h and relative air humidity between 60 and 70%. Wood shavings were placed in the cages, and the animals were fed standard rodent chow and water ad libitum. All animals underwent an acclimatization period of 7 d prior to the beginning of the experiments. The acquisition of the animals and the experimental procedures performed were approved by the Ethics Committee on the Use of Animals of the University of Sorocaba (CEUA-UNISO), with approval resolution CEUA-UNISO no. 199/2021.

2.1.2. Chemicals

Water (ultra)purification was performed in a Master System All (model MS2000, Gehaka, São Paulo, Brazil). Geranic acid (85% stabilized; cat.# W412101-1KG-K), choline bicarbonate (cat.# C7519-500ML), locust bean gum (LGB) (cat.# No. G0753) and curcumin (CUR) (cat.# No. C1386) were acquired from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol (LiChrosolv®, CAS No: 67-56-1) was purchased from Merck (Darmstadt, Germany). Imiquimod cream (50 mg/g) (Modik®) was acquired from Germed Farmacêutica LTDA. (Hortolândia/SP, Brazil).

2.2. Experimental Procedures

2.2.1. Synthesis of Choline and Geranic Acid Ionic Liquid (CAGE-IL)

Choline and Geranic acid Ionic Liquid (CAGE-IL) was obtained in accordance with the procedures described in [20,22,36]. In brief, 48 mL of geranic acid (CAS No. 459-80-3; Sigma-Aldrich, St. Louis, MO, USA), 20 mL of choline bicarbonate at 80% (*w/v*) (CAS No. 62-49-7; Sigma-Aldrich, St. Louis, MO, USA) and 20 mL of methanol (CAS No. 67-56-1; Chemco Indústria e Comércio Ltda., Hortolândia/SP, Brazil) were added to a 1000 mL round-bottom flask and mixed until CO₂ production ceased. The solvent was removed in a rotary evaporator (BÜCHI Labortechnik GmbH, model R-215, Essen, Germany) at 60 °C, for ca. 20 min. The prepared CAGE-IL was transferred to a 50 mL Falcon tube and nitrogen bubbled to scavenge any dissolved oxygen, thereby preventing oxidation. The tube was capped and sealed with Parafilm™ (Bemis Flexible Packaging, Neenah, WI, USA).

2.2.2. Preparation and Characterization of Biopolysaccharide Gels

Biopolysaccharide gel with curcumin. Gel formulations integrating curcumin were prepared using locust bean gum (LGB) (Ref. No. G0753; Sigma-Aldrich, St. Louis, MO, USA) as a gelling agent. For each gel, precise amounts of curcumin (CUR) (Ref. No. C1386; Sigma-Aldrich, St. Louis, MO, USA) with 95% purity (2.087%, *w/w*), methylparaben (0.1%, *w/w*) and LBG (2%, *w/w*) were dispersed in ultrapure water and mixed under continuous magnetic stirring.

Biopolysaccharide gel with curcumin and CAGE-IL. Precise mass concentrations of curcumin (CUR) (Ref. No. C1386; Sigma-Aldrich, St. Louis, MO, USA) with 95% purity (2.087%, *w/w*), methylparaben (0.1%, *w/w*) and locust bean gum (LGB) (2%, *w/w*) (Ref. No. G0753; Sigma-Aldrich, St. Louis, MO, USA) were dispersed in ultrapure water under continuous magnetic stirring. CAGE-IL was then added up to a final concentration of 2% (*w/w*). In the preparation of the gels, careful homogenization was performed to avoid air incorporation.

The percentage of curcumin utilized (2%, *w/w*) was established based on the work of Patel et al. [39], and the most adequate percentage of CAGE-IL (2%, *w/w*) was selected on the basis of previous studies [19,33]. The percentage of LGB used was experimentally determined by testing weight percentages from 2% to 3%, as indicated by its gelling capacity [23,40], with the percentage of 2% (*w/w*) being determined as the most suitable for the intended purpose. Table 1 displays the details of all gel formulations prepared.

Full physicochemical characterization of the gel formulations formed the subject of a previous study [33], and in this study, thermal characterization was additionally performed in order to study the influence of the various components of the gel formulations on thermal stability.

Table 1. Detailed composition of all gel formulations prepared.

Compound	Function in the Formulation	Formulation					
		{LBG} Gel	{LBG + CUR} Gel	{LBG + CUR + CAGE – IL} Gel	{LBG} Gel	{LBG + CUR} Gel	{LBG + CUR + CAGE – IL} Gel
		% (w/w)					Mass (g)
Locust bean gum (LBG)	Gel-forming agent	2	2	2	2.000	2.000	2.000
Curcumin (CUR)	Bioactive compound	—	2	2	2.000	2.000	2.000
Choline and geranic acid ionic liquid (CAGE-IL)	Skin permeation enhancer	—	—	2	—	—	2.000
Methylparaben	Preservative, antifungal	0.1	0.1	0.1	0.100	0.100	0.100
Ultrapure water	Solvent	up to 100	up to 100	up to 100	up to 100	up to 100	up to 100

LBG gel: gel formulation with locust bean gum; {LBG + CUR} gel: gel formulation with locust bean gum and curcumin; {LBG + CUR + CAGE – IL} gel: gel formulation with locust bean gum, curcumin and choline and geranic acid ionic liquid.

Thermal characterization of biopolysaccharide gels via differential scanning calorimetry (DSC) analyses. Thermal characterization of samples of pure curcumin (CUR); CAGE-IL; gel containing 2% (*w/w*) LBG and 2% (*w/w*) CUR; gel containing 2% (*w/w*) LBG, 2% (*w/w*) CUR and 2% (*w/w*) CAGE-IL; and gel containing 2% (*w/w*) LBG, 2.087% (*w/w*) commercial turmeric (containing 2% (*w/w*) CUR) and 2% (*w/w*) CAGE-IL, was accomplished via differential scanning calorimetry (DSC) analyses in a microcalorimeter from Shimadzu (model DSC-60, Kyoto, Japan) coupled with a TA 60W thermal analyzer also from Shimadzu, following the procedure described in detail in [20,41], using sample weights from ca. 1.24 mg to ca. 2.55 mg.

2.2.3. Experimental Design and Induction of Psoriasis

After an acclimatization period of one week, the mice were randomly divided into eight groups: G1, G2, G3, G4, G5, G6, G7 and G8, and placed in individual cages, with five animals (10 ears, *n* = 10) per group. In group G1 (3 males + 2 females), there was no application, characterizing this group as the absolute control group; group G2 (2 males + 3 females) received LBG gel (2%, *w/w*) in the ears for 20 consecutive days; group G3 (3 males + 2 females) received LBG gel (2%, *w/w*) with CUR (2%, *w/w*) in the ears for 20 consecutive days; group G4 (2 males + 3 females) received LBG gel (2%, *w/w*) with CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*) in the ears for 20 consecutive days. Applications in groups G2 to G4 occurred in the right ear, keeping the left ear as a negative control. In groups G5 to G8, all animals received imiquimod (IMQ) (commercially available in the form of a 5% (*w/w*) cream) at a dose of 25 mg/day, which corresponded to 1.25 mg of the active component, on the inner surface of both ears once a day for 10 consecutive days [42]. After this period, the G5 group (2 males + 3 females) did not receive any further treatment, characterizing this group as the IMQ control group. Group G6 (2 males + 3 females) received treatment with LBG gel (2%, *w/w*); group G7 (3 males + 3 females) received treatment with LBG gel (2%, *w/w*) with CUR (2%, *w/w*) at a dose of 0.5 mg/day; and the G8 group (3 males + 2 females) received treatment with LBG gel (2%, *w/w*) with CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*) at a dose of 0.5 mg/day [43]. Groups G6 to G8 received treatments in both ears.

Mice received topical IMQ cream on the epidermis on the dorsal and ventral surfaces once a day for 10 consecutive days. The IMQ cream was chosen because of its direct action, as it can be applied directly to the animal's epidermis. In general, mouse skin begins to resemble human skin with psoriasis within three days of application of IMQ [25,29].

2.2.4. Assessment of Inflammation Severity

The procedure for monitoring and recording the severity of the inflammation of the skin of the animals' ears was performed using a scoring system based on the Psoriasis Area Severity Index (PASI) [27,44,45]. Observations of erythema and peeling of the skin were independently recorded by assigning values on a scale of 0 to 4 as follows: 0, none; 1, light; 2, moderate; 3, intense; 4, very intense. The evolution of the thickness of the ears was also monitored using a pachymeter (Digimess Instrumentos de Precisão, Ltda—São Paulo/SP, Brazil) throughout all the experiments, performing measurements in triplicate in all groups of animals.

2.2.5. Mouse Euthanasia

All mice were euthanized 28 days after the start of treatment(s), which was a period of time sufficient for the appearance of inflammatory lesions and scales in the animals treated with IMQ [46]. The animals were euthanized by applying ketamine (100 mg/kg) associated with xylasin (900 mg/kg) [47], after which the ears were surgically removed for histological analysis.

2.2.6. Preparation of Histological Cuts

Histological analysis of the skin of the mice ears was performed after fixation. The ears were placed in buffered formalin for 8 h. Then, the samples were washed with ultrapure water and the tissues stored in 70% (v/v) ethanol. Afterwards, the tissues were treated in an automatic tissue processor (O Patologista®, model PT12, Guarulhos/SP, Brazil), and the pieces were fixed in paraffin in the sequence [27,45]: (i) dehydration (passage through 70% (v/v) ethanol; 50 min in 90% (v/v) ethanol; 150 min in 100% (v/v) ethanol), (ii) clarification with xylene for 120 min and (iii) paraffin impregnation for 120 min. The histological sections were prepared in a manual rotary microtome (O Patologista®, model MR 2014, Guarulhos/SP, Brazil) and were subsequently stained with hematoxylin and eosin ((i) dewaxing with xylene for 30 min, (ii) hydration with 100% (v/v) ethanol for 20 s, (iii) hydration with 95% (v/v) ethanol for 10 s, (iv) hydration with 70% (v/v) ethanol for 10 s, (v) washing with ultrapure water for 2 s, (vi) staining with hematoxylin for 10 min, (vii) washing with running water for 3 min, (viii) staining with eosin for 3 min, (ix) washing with ultrapure water for 2 s, (x) dehydration with 70% (v/v) ethanol for 10 min, (xi) dehydration with ethanol at 100% (v/v) for 20 min, (xii) dye fixation and material preservation with xylene for 1 s, (xiii) dye fixation and preservation of material with xylene until the slide was mounted and finally (xiv) assembly with histological resin) [48–50]. The analysis of the histological sections was performed using an optical microscope with a digital camera (biological microscope, model Axio Lab.A1, Zeiss®, Oberkochen, Germany).

2.2.7. Statistical Analyses

The experimental results were subjected to extensive descriptive statistical analysis using the Statistica™ software (version 10, StatSoft Inc., Hamburg, Germany). Data variability was evaluated using the ANOVA test: a non-parametric test used to compare three or more independent samples. A significance level of 0.05 was adopted for the mean and standard deviation. Having rejected the null hypothesis ($p < 0.05$), indicating a difference between at least two groups of animals, the Tukey post hoc test was applied ($p < 0.001$). For skin thickness data, a one-way ANOVA test was performed followed by Tukey's test. For the score data (erythema and desquamation), Kruskal–Wallis and Tukey tests were performed.

3. Results

The choline and geranic acid ionic liquid (CAGE-IL) was synthesized according to the procedures described in detail in [19,20,33], integrated as permeation enhancer in a locust bean gum (LBG) biopolysaccharide gel containing curcumin (CUR) and used in the *in vivo* transdermal permeation assays for controlling imiquimod (IMQ)-induced psoriasis in mice.

3.1. Thermal Characterization of Biopolysaccharide Gels via Differential Scanning Calorimetry (DSC) Analyses

The DSC thermograms of samples of CAGE-IL, pure CUR, commercial turmeric, LBG gel formulation integrating pure CUR, LBG gel formulation integrating pure CUR and CAGE-IL and LBG gel formulation integrating commercial turmeric and CAGE-IL are displayed in Figure 1.

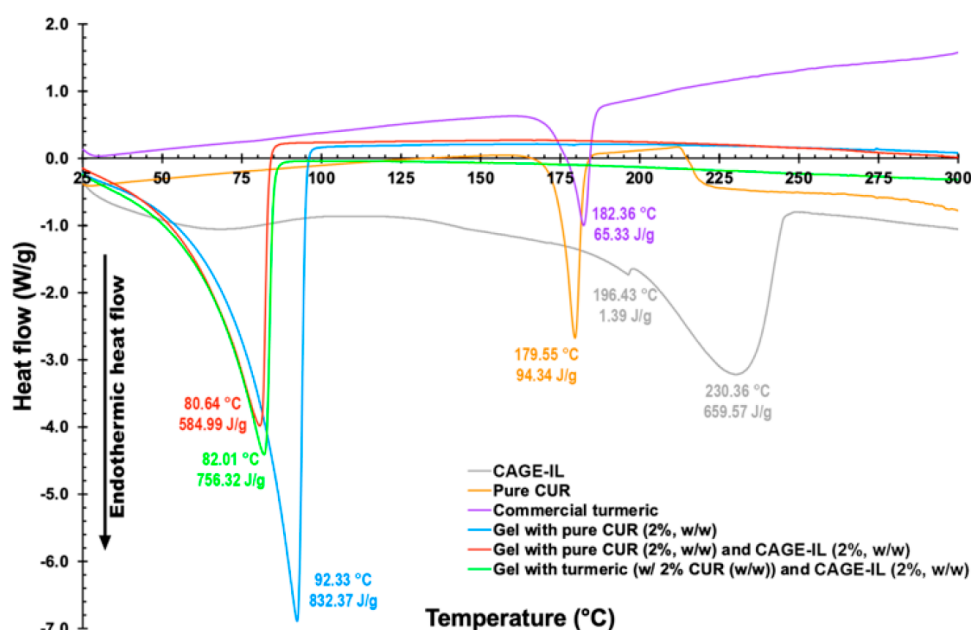


Figure 1. Differential scanning calorimetry thermograms of CAGE-IL (grey line), pure CUR (orange line), commercial turmeric (purple line), LBG gel formulation integrating pure CUR (blue line), LBG gel formulation integrating pure CUR and CAGE-IL (red line) and LBG gel formulation integrating commercial turmeric and CAGE-IL (green line).

No significant differences were observed (see Figure 1), as the endothermic events displayed by the gel formulations integrating CUR (blue line in Figure 1) or CUR and CAGE-IL (red line in Figure 1) were quite similar, with the addition of CAGE-IL to the gel formulation having the effect of displacing the endothermic peak from 92.33 °C to 80.64 °C, with a concomitant decrease in the melting enthalpy from 832.37 J/g to 584.99 J/g (Figure 1).

3.2. Macroscopic/Microscopic Analysis of Mouse Ears

During the *in vivo* experiments, macroscopic changes were observed in the lesions formed by the application of IMQ cream on the epidermis of the mouse ears. Normal/docile behavior of the animals was noted due to the fact that the minimal manipulation necessary for the application of the cream did not generate any type of stress to the animal. After applying the treatments proposed here, it was possible to observe macroscopically the regression of lesions caused by IMQ in the epidermis of the animals' ears, with a more evident effect observed after treatment with the gel containing CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*), classifying the topical treatment as the gold standard for lesions in the epidermis. No significant weight changes were observed in the animals during the experiments, and no animals were lost.

3.2.1. Control Groups

The mice in the control group (G1) did not receive any type of application or treatment during the experiments. For 20 days, mice from groups G2, G3 and G4 received the application of LBG gel (2%, *w/w*), LBG gel (2%, *w/w*) with CUR (2%, *w/w*) and LBG gel (2%, *w/w*) with CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*), respectively. In these groups, the formation of erythema and the desquamation of the skin of the ears were observed. The monitoring of the evolution of the thickness of the ears also indicated no change during the entire period of the experiments, in either male rats (Figure 2) or female rats (Figure 3), demonstrating the safety of the use of the formulations.

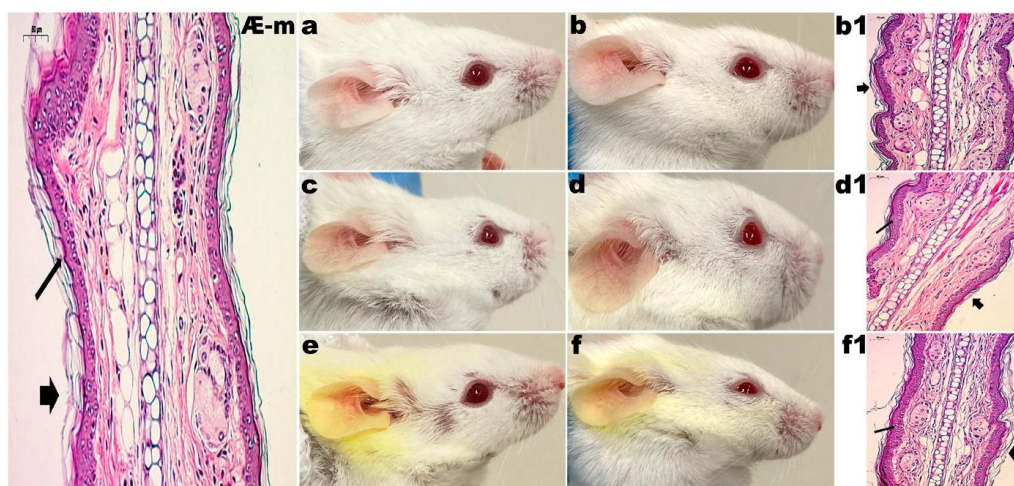


Figure 2. Results obtained after application of the formulations in the ears of male mice. (a) (G2) 10 days of LBG (2%, *w/w*) gel application; (b) (G2) 20 days of LBG (2%, *w/w*) gel application; (c) (G3) 10 days of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*); (d) (G3) 20 days of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*); (e) (G4) 10 days of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*); (f) (G4) 20 days of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*); (a-m) (G1) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin of male absolute control; (b1) (G2) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin after 20 d of application of LBG gel (2%, *w/w*); (d1) (G3) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin after 20 d of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*); (f1) (G4) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin after 20 d of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*). The histological sections were stained with hematoxylin and eosin. For the significance of the inserted (thick, thin and arrowhead) arrows, please refer to the Discussion section. Scale bar represents 50 μ m. Note: the yellowish color observed in the fur of the animals is due to the use of the gel with CUR and CAGE-IL, with the ionic liquid promoting a greater solubility of CUR and consequently dying the fur.

The choline and geranic acid ionic liquid (CAGE-IL) in the LBG gel formulation (2%, *w/w*) containing CUR (2%, *w/w*) applied to the G4 group promoted a very noticeable deposition of CUR in the ears of both male and female mice (Figures 2e,f and 3e,f), which was not observed in the animals of the G3 group, for which the LBG gel formulation (2%, *w/w*) applied did not contain CAGE-IL (Figures 2c,d and 3c,d).

Figure 4 displays the evolution of the thickness of the ears of the animals in the control groups, over 20 d of application of LBG gel (2%, *w/w*), LBG gel (2%, *w/w*) with CUR (2%, *w/w*) and LBG gel (2%, *w/w*) with CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*).

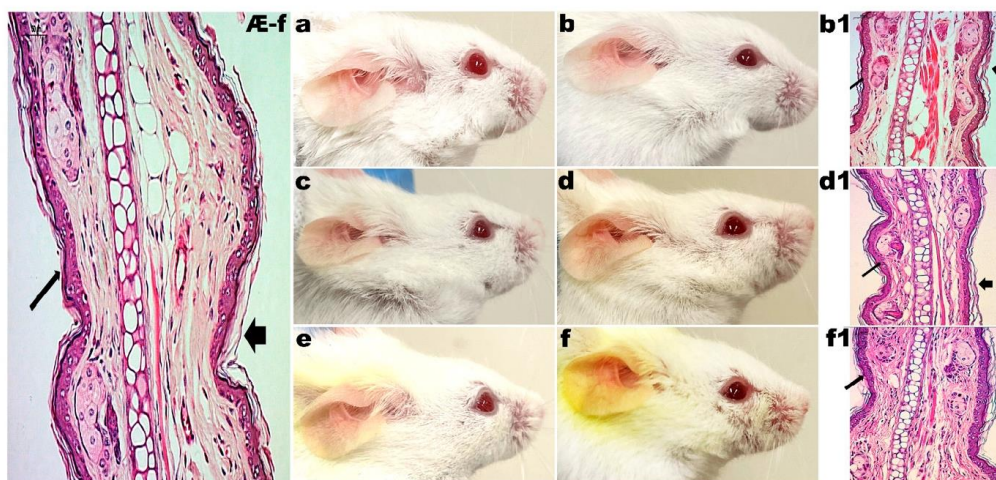


Figure 3. Results obtained after application of the formulations in the ears of female mice. (a) (G2) 10 days of LBG (2%, *w/w*) gel application; (b) (G2) 20 days of LBG (2%, *w/w*) gel application; (c) (G3) 10 days of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*); (d) (G3) 20 days of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*); (e) (G4) 10 days of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*); (f) (G4) 20 days of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*); (A–f) (G1) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin of female absolute control; (b1) (G2) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin after 20 d of application of LBG gel (2%, *w/w*); (d1) (G3) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin after 20 d of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*); (f1) (G4) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin after 20 d of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*). The histological sections were stained with hematoxylin and eosin. For the significance of inserted (thick, thin and arrowhead) arrows, please refer to the Discussion section. Scale bar represents 50 μm . Note: the yellowish color observed in the fur of the animals is due to the use of the gel with CUR and CAGE-IL, with the ionic liquid promoting a greater solubility of CUR and consequently dying the fur.

3.2.2. Induced Psoriasis Groups

The animals in groups G5, G6, G7 and G8 were subjected to the psoriasis induction model via application of IMQ over 10 d, with the aim of developing symptoms similar to those of human psoriasis. After this period of time, groups G6, G7 and G8 received treatment in the form of a gel formulation for 10 consecutive days, once a day, in both ears, with the animals of the G6 group being treated with LBG gel (2%, *w/w*), the animals of the G7 group treated with LBG gel (2%, *w/w*) containing CUR (2%, *w/w*) and the animals of the G8 group treated with LBG gel (2%, *w/w*) containing CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*). The animals in the G5 group did not receive any treatment, characterizing this group as the control group for IMQ-induced psoriasis, where a gradual remission of the lesions caused by the natural process of the mouse body's response was observed. The application of the IMQ cream produced the characteristics of psoriasis inflammation, erythema and scaling of the skin in all groups of animals, both male and female, as expected. The manifestations of psoriatic lesions and the results of the various treatments applied

to the epidermis of the ears of the mice can be visualized in Figures 5 and 6 for male and female mice, respectively.

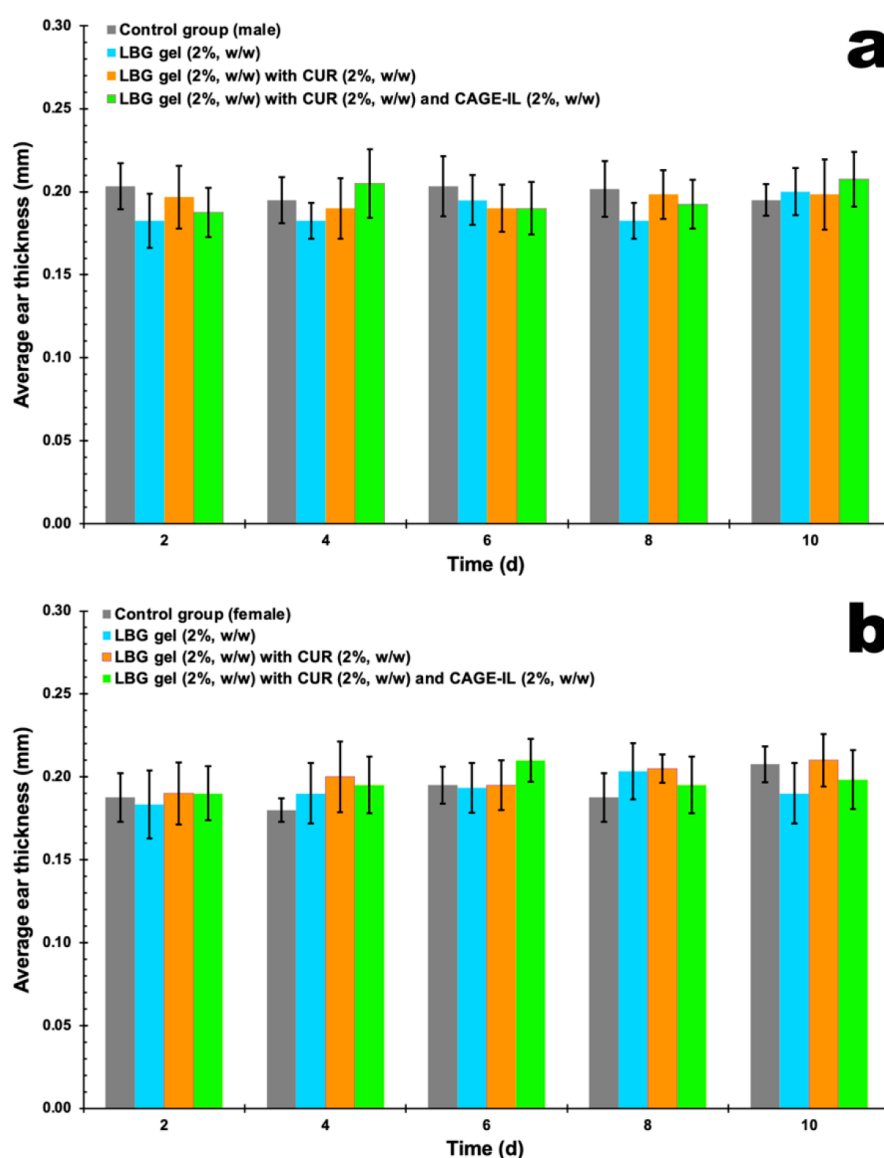


Figure 4. Results obtained for the evolution of the thickness of the ears of the animals in the control groups, during 20 d of application of LBG gel (2%, w/w), LBG gel (2%, w/w) with CUR (2%, w/w) and LBG gel (2%, w/w) with CUR (2%, w/w) and CAGE-IL (2%, w/w). (a) Male mice ($p > 0.05$) and (b) female mice ($p > 0.05$). The results displayed are the average of three determinations, and the error bars represent the standard deviations.

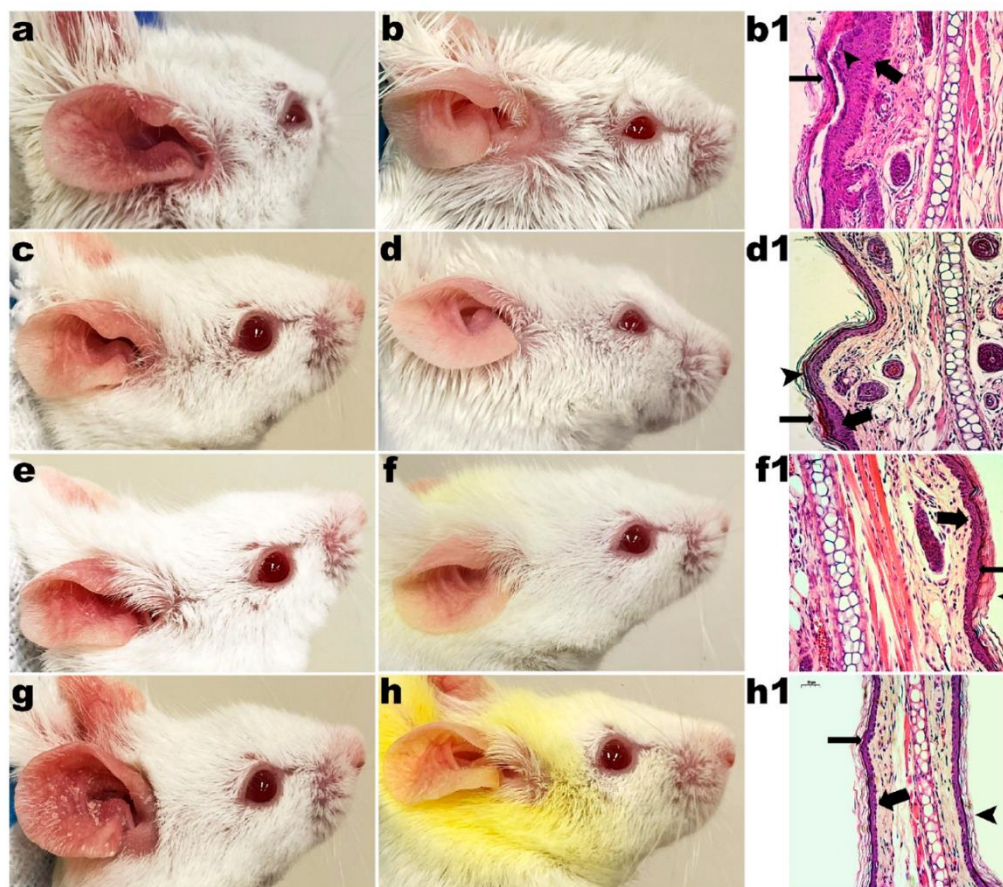


Figure 5. Results obtained after IMQ-induced psoriasis and application of the formulations in the ears of male mice. (a) (G5) 10 days of application of IMQ; (b) (G5) natural recovery of the mouse ears without treatment; (c) (G6) 10 days of application of IMQ; (d) (G6) 10 days of application of LBG (2%, w/w) gel; (e) (G7) 10 days of application of IMQ; (f) (G7) 10 days of application of LBG gel (2%, w/w) with CUR (2%, w/w); (g) (G8) 10 days of application of IMQ; (h) (G8) 10 days of application of LBG gel (2%, w/w) with CUR (2%, w/w) and CAGE-IL (2%, w/w); (b1) (G5) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin after 20 d of natural recovery of IMQ-induced psoriasis; (d1) (G6) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin with IMQ-induced psoriasis after 20 d of application of LBG gel (2%, w/w); (f1) (G7) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin with IMQ-induced psoriasis after 20 d of application of LBG gel (2%, w/w) with CUR (2%, w/w); (h1) (G8) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin with IMQ-induced psoriasis after 20 d of application of LBG gel (2%, w/w) with CUR (2%, w/w) and CAGE-IL (2%, w/w). All histological sections were stained with hematoxylin and eosin. For the significance of inserted (thick, thin and arrowhead) arrows, please refer to the Discussion section. Scale bar represents 50 μ m. Note: the yellowish color observed in the fur of the animals is due to the use of the gel with CUR and CAGE-IL, with the ionic liquid promoting a greater solubility of CUR and consequently dying the fur.

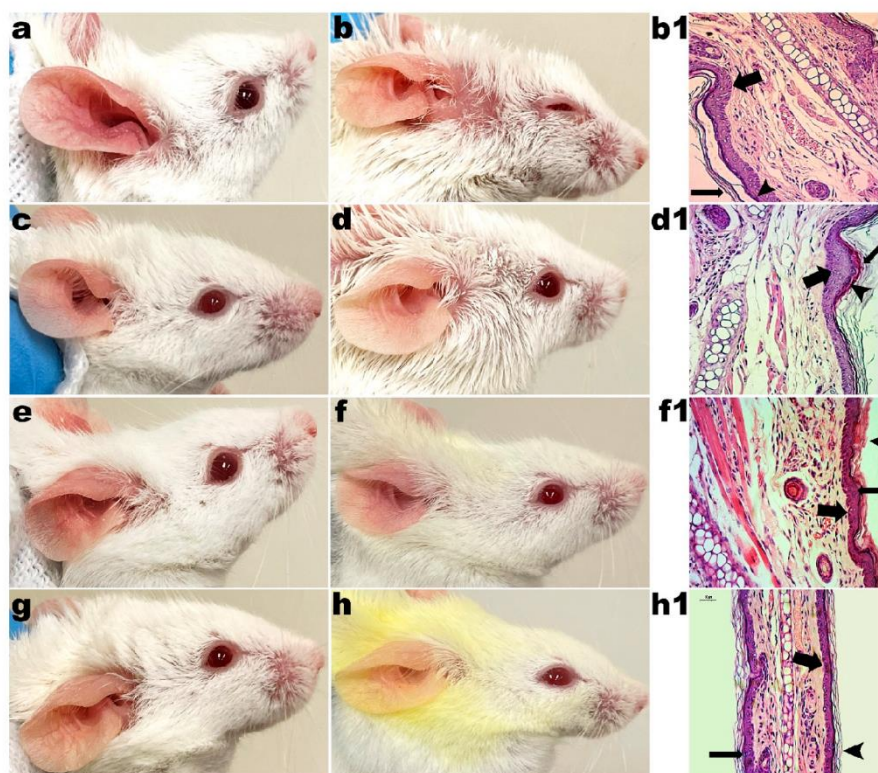


Figure 6. Results obtained after IMQ-induced psoriasis and application of the formulations in the ears of female mice. (a) (G5) 10 days of application of IMQ; (b) (G5) natural recovery of the mouse ears without treatment; (c) (G6) 10 days of application of IMQ; (d) (G6) 10 days of application of LBG gel (2%, *w/w*) gel; (e) (G7) 10 days of application of IMQ; (f) (G7) 10 days of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*); (g) (G8) 10 days of application of IMQ; (h) (G8) 10 days of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*); (b1) (G5) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin after 20 d of natural recovery of IMQ-induced psoriasis; (d1) (G6) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin with IMQ-induced psoriasis after 20 d of application of LBG gel (2%, *w/w*); (f1) (G7) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin with IMQ-induced psoriasis after 20 d of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*); (h1) (G8) photomicrograph ($\times 200$ magnification) of histological section of mouse ear skin with IMQ-induced psoriasis after 20 d of application of LBG gel (2%, *w/w*) with CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*). All histological sections were stained with hematoxylin and eosin. For the significance of inserted (thick, thin and arrowhead) arrows, please refer to the Discussion section. Scale bar represents 50 μm . Note: the yellowish color observed in the fur of the animals is due to the use of the gel with CUR and CAGE-IL, with the ionic liquid promoting a greater solubility of CUR and consequently dying the fur.

3.3. Treatment of Psoriasis

To measure the severity of inflammation of the epidermis of the ear, the Psoriasis Area Severity Index (PASI) scoring system was used, with erythema and scaling of the skin being independently rated on a scale of 0 to 4. Figure 7 presents the results obtained for the

evolution of erythema and skin desquamation induced by imiquimod (IMQ) in male and female mice.

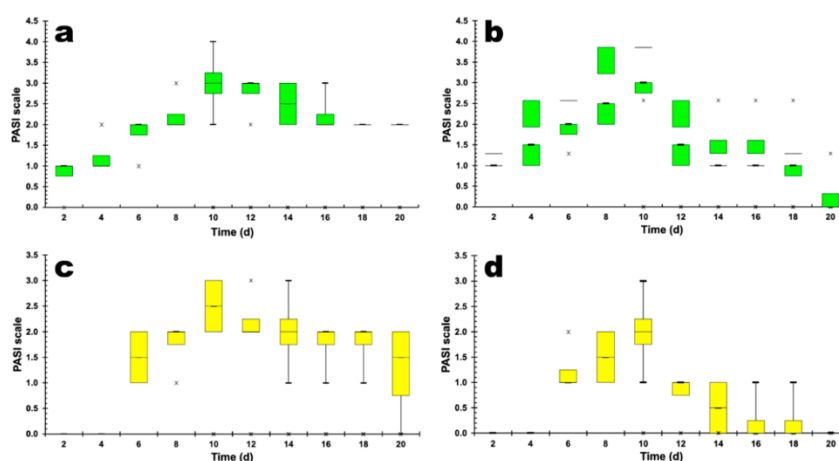


Figure 7. Boxplot graphs of the evolution of erythema (a,b) and ear skin peeling (c,d) in male (a,c) ($p < 0.001$) and female (b,d) ($p < 0.001$) mice. Legend: (*) represent outlier points off the average values.

Figure 8 presents the results obtained for the evolution of erythema and ear skin peeling induced by imiquimod (IMQ) and the comparison of the remission obtained via application of the treatments with the LBG gel formulations.

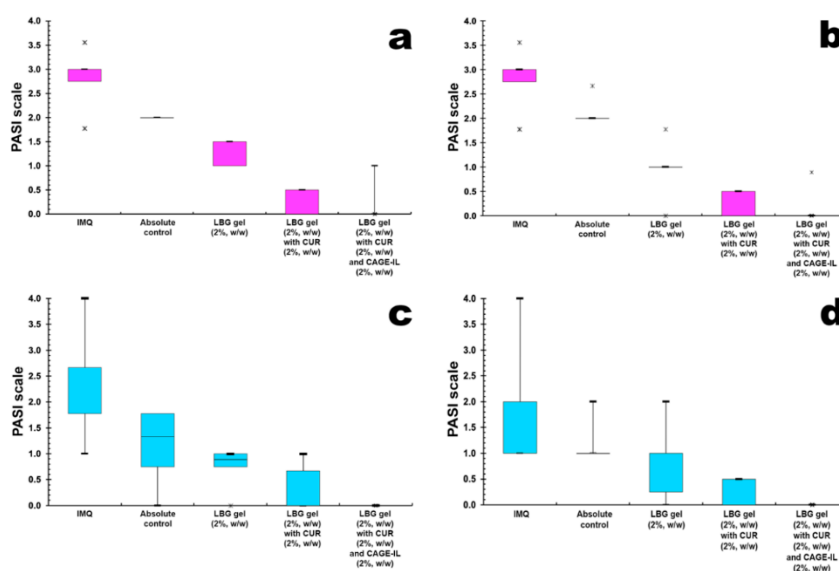


Figure 8. Boxplot graphs of the evolution of erythema (a,b) and ear skin peeling (c,d) induced by imiquimod (IMQ) and the comparison of the remission obtained via application of the treatments with the LBG gel formulations in male (a,c) ($p < 0.001$) and female (b,d) ($p < 0.001$) mice. Legend: (*) represent outlier points off the average values.

3.4. Assessment of Mouse Ear Epidermis Thickness

Control groups for the formulated gels were exposed to treatments for 20 consecutive days in order to verify the safety of using the developed formulations. For the groups of animals with induced psoriasis, the animals (males and females) were exposed to IMQ for 10 consecutive days, after which the animals were treated with the formulated gels for another 10 consecutive days, with only the control group being exposed to IMQ without the application of any treatment (following the natural course of the mouse organism). The results for the increase in the thickness of the epidermis of the skin of the mouse ears and the remission after the applied treatments are displayed in Figure 9a for the male mice and in Figure 9b for the female mice, for a test period of 20 days. All ear thickness measurements were performed using a pachymeter (Digimess Instrumentos de Precisão Ltd., São Paulo/SP, Brazil).

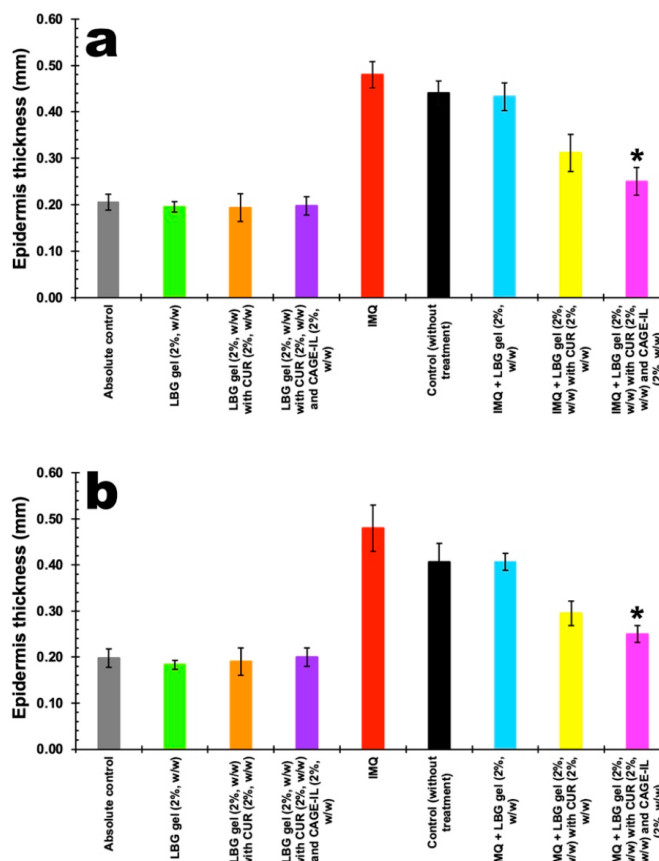


Figure 9. Results obtained for the thickness of the epidermis of the ears of the animals in the control and treatment groups, after 20 d of application of LBG gel (2%, w/w), LBG gel (2%, w/w) with CUR (2%, w/w) and LBG gel (2%, w/w) with CUR (2%, w/w) and CAGE-IL (2%, w/w), and after 10 d following induction of psoriasis with IMQ. (a) Male mice ($p > 0.05$) and (b) female mice ($p > 0.05$). The results displayed are the averages of three determinations and the error bars represent the standard deviations. The data were statistically analyzed via one-way ANOVA, and * represents a statistically significant result compared with the control group (without treatment, black columns).

4. Discussion

The gel formulations produced basically consisted of water and locust bean gum (LBG). LBG was dissolved in water and heated at 70 °C for 2 h for complete dissolution [51]. CUR, as a hydrophobic molecule with low solubility in water (particularly in an acidic or neutral environment, where it remains fully protonated), must be dissolved in some solvent or hydrophobic substance before its incorporation into an aqueous matrix [52]. Thus, the CAGE-IL, in addition to enhancing permeation, aided in increasing CUR dissolution. The percentage chosen for the ionic liquid was established as the most adequate in previous studies [19,33].

Among the various topical pharmaceutical forms available, gels and hydrogels are very popular and have high acceptance by patients, due to their ease of application. Gels based on natural polysaccharides can be produced without the presence of toxic substances, which makes them interesting for the development of delivery systems for bioactive molecules [53]. A biopolysaccharide that has attracted attention is LBG, a plant galactomannan extracted from locust bean seeds (from the carob tree, *Ceratonia siliqua*) [54,55]. This polymer has a series of attractive characteristics for biopharmaceutical applications, including its high gelling capacity, biodegradability, low toxicity and low cost [55]. Due to these characteristics, it was chosen as the main component in the gel formulation.

The gel developed presented suitable characteristics for dermal applications, as pointed out in a previous study [33], and DSC analysis was carried out in order to evaluate the thermal stability of the components integrated into the gel formulation. DSC is a thermal analysis method for measuring how the physical properties of a sample change with temperature over time. During a linear increase in temperature, DSC measures the amount of heat that is liberated or absorbed by the sample on the basis of a temperature difference between the sample and a reference material [56]. Hence, the three formulations tested were studied via differential calorimetry to verify whether any of the added component(s) could change the thermal stability, causing a loss of stability of the gel formulations. No significant differences were observed (see Figure 1), as the endothermic events displayed by the gel formulations integrating CUR (blue line in Figure 1) or CUR and CAGE-IL (red line in Figure 1) were quite similar, with the addition of CAGE-IL to the gel formulation having the effect of a displacing the endothermic peak towards a lower temperature, with a concomitant decrease in the melting enthalpy of 29.7% (Figure 1). Thus, the thermal stabilities of the gel formulations with CUR, with or without CAGE-IL, were similar.

One can observe very similar thermal events for the three gel formulations, and the ratios of the melting enthalpies of the gel without CAGE-IL and the gels with CAGE-IL were of the same order of magnitude ($1.42\times$ and $1.10\times$, respectively, for the LBG gels integrating pure CUR and commercial turmeric). Integrating CAGE-IL into the LBG formulations promoted a displacement in the major endothermic event from 92.33 °C to ca. 81–82 °C (Figure 1), thereby improving the plasticity of the LBG gel formulation at lower temperatures. The major endothermic event in pure CUR was the sharp peak at 179.55 °C (with an associated melting enthalpy of 94.34 J/g), whereas the major endothermic event in CAGE-IL was the sharp peak at 230.36 °C (with an associated melting enthalpy of 659.57 J/g) (Figure 1). Regarding the gel samples, the major endothermic event in the gel integrating pure CUR was the sharp peak at 92.33 °C (with an associated melting enthalpy of 832.37 J/g), and the major endothermic event in the gel integrating pure CUR and CAGE-IL was the sharp peak at 80.64 °C (with an associated melting enthalpy of 584.99 J/g). The effect of integrating CAGE-IL in the gel formulation was to substantially decrease the melting temperature of the gel formulation, with the gel formulation integrating pure CUR displaying a peak temperature displaced to lower temperatures, not far away from the melting point of water, since the gel formulations contained ca. 94% ultrapure water. As is apparent from inspection of the data obtained (Figure 1), the gel formulation integrating CAGE-IL and either pure CUR or commercial turmeric exhibited relatively high melting temperatures, allowing us to conclude that these gel formulations are stable for the intended application, since the temperature at the surface of the skin rarely exceeds 33.5 °C.

The animal model used to study psoriasis-like inflammation in mice with topical application of IMQ onto the depilated backs or ears of mice is the most popular model. This model has several advantages, the first one being that it is cheap and very easy to perform. Although applied topically, IMQ may induce weight loss and systemic cytokine elevation in mice, most likely due to ingestion (via licking) of the cream [57]. In the experiments performed, the animals were healthy, without noticeable problems related to their weight. The animal model used was thus considered adequate for the intended purpose. According to [57], 5% IMQ cream applied daily to the depilated backs or ears of mice for 5 to 7 days induces the development of an inflammatory response in mice similar to human psoriasis.

Both males and females were included in the animal trials with the aim of verifying whether there were differences in the induction of psoriasis by IMQ, as pointed out in [58]. In addition, the inclusion of female mice was thought to be interesting from the point of view of checking for changes in response due to estrous cycle variability [59].

It was observed that the gel formulations did not produce any signs of inflammation or peeling of the skin of the ears during the twenty days of application (male mice, Figure 2b,d,f; female mice, Figure 3b,d,f), thus showing very good compatibility with the epidermis of the skin of the animals' ears. The application of the formulations in the respective groups also promoted no significant changes in the thickness of the ears in either male (Figure 4a) or female mice (Figure 4b), and the statistical analysis of the results obtained did not reveal any differences between the control groups ($p > 0.05$). In Figures 2, 3, 5 and 6 one can observe that, in the groups of animals where the LBG gel with CUR and CAGE-IL was applied (photomicrographs *e* and *f* in Figures 2 and 3, and photomicrographs *f* and *h* in Figures 5 and 6), a yellowish coloration formed in the ears and in the nearby fur of the mice. This was probably a result of the greater solubilization of CUR by CAGE-IL, together with a greater transdermal permeation of CUR into the deeper layers of the skin. According to [60], the use of ionic liquids and deep eutectic solvents in biomedical applications has grown significantly due to their peculiar characteristics such as the ability to solubilize drugs.

Figure 7 shows the evolution of the severity of the inflammation of the epidermis (erythema) of the animals' ears, induced by IMQ in male (Figure 7a) and female (Figure 7b) mice. After the 2nd day of IMQ application, the male mice's ears showed a slight formation of erythema (mean value equal to 1), and on the 10th day of application they already had psoriasis (Figure 7a) (mean value equal to 3). A possible explanation might be a difference in response in male and female mice depending on the cyclic hormonal changes across the ovulatory cycle in females, introducing variability in the measures in comparison to males [59].

The erythema remained intense until the 16th day after the application of IMQ, remaining a mild manifestation until the 20th day of follow-up. The boxplot plot in Figure 7b shows the evolution of erythema in the ears of female mice after the application of IMQ. After the 2nd day of IMQ application, a slight formation of erythema was observed in the ears of female mice (mean value equal to 1) and on the 10th day of application they already presented psoriasis (mean value equal to 3). The erythema remained intense until the 16th day, and at the 20th day of follow-up the presence of erythema in the ears of female mice was not observed.

The boxplot plot in Figure 7c shows the evolution of epidermis desquamation for male mice after the application of IMQ. Peeling of the skin of the ears began on the 6th day after the application of IMQ, with observation of a mild to moderate peeling intensity (mean value equal to 1.5). On the 10th day after the application of IMQ, the peeling became intense (average value equal to 2.5). The desquamation of the skin of the ears of male mice (Figure 7c) continued to manifest itself until the 18th day after the application of IMQ (average value equal to 2.0), and on the 20th day, desquamation was still observed but with only slight intensity.

The boxplot plot in Figure 7d shows the evolution of epidermis desquamation in the female mouse ears after the application of IMQ. The peeling of the skin of the ears started on the 8th day after the application of IMQ, and a peeling of the skin of the ears of the

female mice of light to moderate intensity (mean value equal to 1.5) was observed. On the 10th day after the application of IMQ, a slight increase in the PASI score was observed, with moderate intensity (mean value equal to 2.0). The desquamation of the skin of the ears of female mice appeared at slight intensity up to the 18th day after application of IMQ, and by the 20th day no more desquamation was observed.

By inspecting the median values of the boxplot graphs in Figure 8a,b, one can observe that the groups of animals exposed to IMQ showed erythema values close to 4, both in male mice (Figure 8a, $p > 0.05$) and in female mice (Figure 8b, $p > 0.05$), reaching the maximum lesion value according to the PASI scale. The LBG gel (2%, *w/w*) containing CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*) was more effective compared to the other gel formulations ($p < 0.001$) in the treatment of erythema for both male (Figure 8a) and female (Figure 8b) mice.

The peeling of the skin caused by induction of psoriasis and its remission by the treatments (gel formulations) applied was also monitored, and the data recorded on the PASI scale. The boxplot graphs in Figure 8c,d show the results for the mean skin desquamation of the ears of male mice (Figure 8c) and female mice (Figure 8d) caused by the application of IMQ and the comparison of the remission obtained with treatment using the formulated gels. By inspecting the median values of the graphs, one can observe that the male mice belonging to the groups exposed to IMQ showed median values for skin desquamation of the ears ranging from 2 to 2.5 (Figure 8c), indicating moderate intensity of desquamation according to the PASI scale ($p > 0.05$), while the female mice belonging to the groups exposed to IMQ showed median values for peeling of the skin of the ears ranging from 1 to 2.0 (Figure 8d), also indicating moderate intensity of peeling according to the PASI scale ($p > 0.05$).

The LBG gel (2%, *w/w*) containing CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*) was more effective than the other gel formulations ($p < 0.001$) in the treatment of desquamation of the skin of the ears for both male mice (Figure 8c) and female mice (Figure 8d).

Thus, according to the results obtained in this research, the LBG gel (2%, *w/w*) containing CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*) showed high potential for the treatment of psoriasis, as it promoted the remission of both erythema and scaling of the skin produced in the animal model of induction of psoriasis with IMQ.

In the absolute control group (G1, male mice, Figure 2E-m; G1 female mice, Figure 3A-e-f), the granular layer was present and there were no nuclei in the stratum corneum (thin arrow). Cornified cells in the section had a loose reticulated appearance typical of orthokeratosis (thick arrow). In group G2, after 20 d of application of LBG gel (2%, *w/w*) in male mice ears (Figure 2b1) and in female mice ears (Figure 3b1), there were no changes in the granular layer, which was present, and there were no nuclei in the stratum corneum (thin arrow). Cornified cells in section had a loose reticulated appearance typical of orthokeratosis (thick arrow). In group G3, after 20 d of application of LBG gel (2%, *w/w*) containing CUR (2%, *w/w*) in male mice ears (Figure 2d1) and in female mice ears (Figure 3d1), there were no changes in the granular layer, which was present, and there were no nuclei in the stratum corneum (thin arrow). Cornified cells in section had a loose reticulated appearance typical of orthokeratosis (thick arrow). In group G4, after 20 d of application of LBG gel (2%, *w/w*) containing CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*) in male mice ears (Figure 2f1) and in female mice ears (Figure 3f1), there were no changes in the granular layer, which was present, and there were no nuclei in the stratum corneum (thin arrow). Cornified cells in section had a loose reticulated appearance typical of orthokeratosis (thick arrow).

In the IMQ control group (G5, male mice, Figure 5b1; G5 female mice, Figure 6b1), the presence of considerable thickening of the epidermis due to an increase in the cells of the spinous layer (acanthosis) was observed (thick arrow). The maturation of epidermal cells was accelerated, with parakeratosis being noted, due to the presence of nuclei in the stratum corneum and the absence of the granular layer, and therefore the absence of keratohyalin granules (thin arrow). Changes had occurred in the granular layer, which was not present, and there were nuclei in the stratum corneum (arrowhead). In group

G6, after 10 d of application of LBG gel (2%, *w/w*) in male mice ears (Figure 5d1) and in female mice ears (Figure 6d1), application of the LBG gel (2%, *w/w*) had slightly decreased acanthosis (thick arrow), as well as parakeratosis; the presence of nuclei in the stratum corneum and an absence of the granular layer, and therefore an absence of keratohyalin granules (thin arrow), were also observed. Changes had occurred in the granular layer, which was not present, and there were nuclei in the stratum corneum (arrowhead). In group G7, after 10 d of application of LBG gel (2%, *w/w*) containing CUR (2%, *w/w*) in male mice ears (Figure 5f1) and in female mice ears (Figure 6f1), application of the LBG gel (2%, *w/w*) containing CUR (2%, *w/w*) promoted a reduction in acanthosis (thick arrow), with a reduction in parakeratosis and the presence of few nuclei in the stratum corneum (thin arrow). Cornified cells returned to a loose reticulated appearance (arrowhead). In group G8, after 10 d of application of LBG gel (2%, *w/w*) containing CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*) in male mice ears (Figure 5h1) and in female mice ears (Figure 6h1), application of the LBG gel (2%, *w/w*) containing CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*) promoted considerable reduction of acanthosis (thick arrow). There was a reduction in parakeratosis, and few nuclei were present in the stratum corneum (thin arrow). Cornified cells in section again showed a loose reticulated appearance, indicating the formation of orthokeratosis (arrowhead). The LBG gel (2%, *w/w*) containing CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*) thus showed high potential for the treatment of psoriasis, reversing the histological manifestations of psoriasis to a state very close to normal.

By inspecting the results shown in Figure 9, it can be seen that after 20 d of application of the formulated gels, there was no change in the thickness of the epidermis of the ears of the mice, in either males or females, revealing that the gels are safe to use. The one-way ANOVA statistical analysis performed on the experimental data showed that there were no differences between the groups where gels were applied and the absolute control group ($p > 0.05$), for either male mice (Figure 9a) or female mice (Figure 9b). The groups of animals subjected to the application of IMQ showed a considerable increase in the thickness of the epidermis of the ears, observed in both male mice and female mice, with no statistical differences ($p > 0.05$) between them. Treatment with LBG gel (2%, *w/w*) had the same effect as the natural response of the mice organisms, irrespective of gender. Compared with the other treatments, treatment for 10 consecutive days with LBG gel (2%, *w/w*) containing CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*) significantly reduced the thickness of the epidermis of the ears in both male (Figure 9a) and female (Figure 9b) mice compared to the untreated psoriasis group ($p < 0.001$). Thus, treatment for 10 consecutive days with LBG gel (2%, *w/w*) containing CUR (2%, *w/w*) and CAGE-IL (2%, *w/w*) was shown to produce a much better remission of the thickness of the epidermis of the skin of the ears of the mice, irrespective of gender, with results very close to those of the absolute control group.

In previous studies by our research group [19,33] the LBG gel formulation containing CUR at 2% (*w/w*) and CAGE-IL at 2% (*w/w*) promoted the best results in terms of the amount of CUR retained within the skin matrix. This result is highly significant if this gel formulation is intended to be used in the topical treatment of psoriasis, since at 2% (*w/w*), CAGE-IL allows the highest level of transdermal permeation of CUR [19,33] while allowing saturation of the skin with the highest amount of CUR.

According to Boscardiol et al. [19,33], CAGE-IL has, in general, the ability to increase transdermal permeation of biomacromolecules, using mechanisms such as transient disruption of the intercellular and intracellular lipids and protein organization of the stratum corneum, fluidization and the creation of diffusional pathways [19].

It is known that psoriasis is a chronic, complex and multifactorial inflammatory autoimmune skin disease, with affected individuals experiencing significant social and psychological problems with a negative impact on their quality of life. It affects both men and women around the world [61], with women being more impacted, experiencing fluctuations in disease activity due to hormonal changes and with the stages of a journey towards motherhood [62,63]. These aspects of the manifestations of psoriasis motivated the research described herein, where the induction and manifestation of psoriasis was studied

in an animal model with male and female mice. The initial statistical analysis reported in this research showed that both males and females responded to the psoriasis induction procedures, and as early as the third day, the female mice of groups G5 ($p = 0.0470$), G6 ($p = 0.0030$), G7 ($p = 0.0090$) and G8 ($p = 0.0090$) treated with IMQ already presented erythema, whereas in the male mice, the manifestation of erythema was observed on the fourth day in groups G5 ($p = 0.0002$), G6 ($p = 0.0002$), G7 ($p = 0.0001$) and G8 ($p = 0.0001$). Regarding the treatment with the formulated gels, the male mice, on the fourth day, in groups G7 ($p = 0.9770$) and G8 ($p = 0.9770$), showed improvement in relation to the control group G5 for desquamation, whereas the female mice in groups G7 ($p = 0.9830$) and G8 ($p = 0.9820$) also presented similar results compared to the control group G5. Ear thickness reduction results were observed in male mice in groups G7 ($p = 0.0860$) and G8 ($p = 0.0867$), but the thickness did not return to normal values compared to group G1. Female mice showed a reduction in the thickness of the ears to the normal range in groups G7 ($p = 0.2200$) and G8 ($p = 0.2230$) compared to group G1.

A second statistical analysis was performed: the Tukey HSD test with unequal n (a generalization of Tukey's test to the case of unequal samples sizes), without separating male and female mice, and considering each group of 5 mice, with $n = 10$ for the ears. The statistical analysis revealed that after 10 days of induction of psoriasis by IMQ, groups G5, G6, G7 and G8 (all with p -value = 0.00123) already presented all the manifestations of psoriasis, and that for the formulated gels, groups G2, G3 and G4 did not develop any signs of psoriasis compared to group G1 ($p = 1.0000$), proving that the gel formulations were non-toxic. On the second day of treatment, groups G7 ($p = 0.0297$) and G8 ($p = 0.000149$) both showed improved erythema compared with group G5. Group G6 ($p = 0.0193$) showed improved psoriasis erythema only on the eighth day of treatment. On the fourth day after initiating treatment, group G8 ($p = 0.0924$) showed a reduction in desquamation compared to the control, returning to normality.

In groups G2 ($p = 0.9370$), G3 ($p = 0.9900$) and G4 ($p = 1.000$) no changes were observed in ear thickness. On the second day of treatment, group G8 ($p = 0.000123$) already showed an improvement in ear thickness, with a significant reduction compared to group G5. On the fourth day, group G7 ($p = 0.000126$) also showed a decrease in ear thickness. Group G6 ($p = 1.000$) did not show an improvement in ear thickness, even after 10 days of treatment.

Finally, it was observed that performing a statistical analysis after randomly dividing male and female mice between the groups, with a sample of 2 mice ($n = 4$ ears) or 3 mice ($n = 6$ ears), produced results that were comparable to analyzing a sample of 5 mice ($n = 10$ ears). The division of the mice into males and females was important for the study of possible variations in the manifestation of psoriasis between genders.

5. Conclusions

Approximately 80% of patients with mild to moderate psoriasis can be treated with topical therapies. The advantages of topical treatments over traditional systemic treatments include availability, lower costs and lack of safety issues. Furthermore, topical therapies can empower patients to take control of the psoriatic disease. However, to increase the effectiveness of bioactive molecules, it is often necessary to increase the skin permeability to the biomolecules. Curcumin is a good example. Ionic liquids, in particular choline and geranic acid ionic liquid (CAGE-IL), have shown great promise in drug delivery applications and have been used to enhance the transdermal delivery of several molecules. CAGE-IL has the potential to become a powerful excipient to improve the pharmacokinetic profiles of many pharmaceutically active molecules in transdermal applications. The effect of an LBG gel formulation containing curcumin at 2% (w/w) and CAGE-IL at 2% (w/w) in controlling psoriasis was studied in vivo using a mouse model with psoriasis induced by imiquimod. The LBG gel (2%, w/w) containing curcumin (2%, w/w) and CAGE-IL (2%, w/w) showed a high potential for applications in the treatment of psoriasis, reversing the histological manifestations of psoriasis to a state very close to normal, with clear benefits to patients suffering from psoriasis. From the results obtained in this study, it can be said

that the association between curcumin and CAGE-IL in pharmaceutical formulations is successful for the transdermal delivery of curcumin in the topical treatment of psoriasis.

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5 Considerações finais e sugestão para trabalhos futuros

Ao se completar as etapas de trabalho idealizadas no projeto de pesquisa, pode-se concluir que os objetivos propostos foram alcançados.

Ficou evidenciado que o geranato de colina foi capaz de promover a permeação transdérmica de macromoléculas bioativas, como a curcumina por modificação transitória do estrato córneo.

Também se comprovou a não uniformidade de características físico-químicas em amostras comerciais de curcumina. As amostras apresentaram diferenças significativas quanto aos pontos de fusão, perfis de varredura espectrais (UV-Vis e FTIR) e reduzidos teores de ativo. Além disto, observou-se a presença de metais pesados como o mercúrio, em algumas amostras. Os resultados indicaram que não há controle de qualidade adequado neste tipo de matéria-prima, o que pode impactar negativamente no desempenho dos produtos comercializados.

As formulações desenvolvidas mostraram-se estáveis e atenderam às especificações de qualidade para aplicação cutânea. A goma de alfarroba permitiu a formação de um gel estável e com características adequadas para uso tópico. O líquido iônico, utilizado como um facilitador de permeação cutânea, também se mostrou adequado para a solubilização da curcumina produzindo partículas na escala nanométrica. O gel sintetizado com 2% (m/m) de CG-DES e integrando 2% (m/m) de curcumina pura ou cúrcuma comercial favoreceu a permeação transdérmica de curcumina e a retenção cutânea desta molécula. A formulação com 2% (m/m) de cúrcuma apresentou os melhores resultados em termos de propriedades mecânicas.

O ensaio *in vivo* comprovou a eficácia da curcumina no tratamento da psoríase e, que o uso de geranato de colina favoreceu a permeação do ativo.

Assim, pelos resultados obtidos pode-se afirmar que os objetivos da pesquisa foram atendidos com a formulação de um produto com alto potencial de aplicação comercial para o tratamento da psoríase leve a moderada.

Evidentemente, outros ensaios devem ser realizados visando otimização da formulação, a fim de amenizar manchas amarelas e possíveis odores na pele, após

a aplicação do gel. Neste sentido, estudos utilizando outras formas farmacêuticas, uso de aromatizantes e sistemas nanoestruturados poderiam ser propostos.

A determinação de biomarcadores associados a psoríase (citocinas pró-inflamatórias, indicadores de estresse oxidativo, etc.) poderia ser realizada, em amostras de pele dos animais, para comprovação da eficácia do gel. Para avaliação de qualquer indício de toxicidade, dosagens bioquímicas, no plasma dos camundongos, também poderiam ser realizadas em trabalhos futuros. Além disto, um estudo clínico precisaria ser planejado para avaliar a eficácia e segurança do produto desenvolvido para o uso em seres humanos.

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ANEXO A

Certificado e Resumo do I Congresso Brasileiro Interdisciplinar em Ciência e Tecnologia (CoBICET) - Um Mundo em Constante Transformação.

Evento on-line, 31 de agosto a 04 de setembro de 2020



Resumo apresentado no I Congresso Brasileiro Interdisciplinar em Ciência e Tecnologia (CoBICET) - Um Mundo em Constante Transformação.
Evento on-line, 31 de agosto de setembro de 2020

Anais do I CoBICET – Resumo expandido
 Congresso Brasileiro Interdisciplinar em Ciência e Tecnologia
 Evento online – 31 de agosto a 04 de setembro de 2020



**AValiação DA PERMEação CUTânea DE CURCUMINA
 CARREADA POR Líquido Iônico**

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Resumo: A curcumina apresenta excelentes propriedades antioxidantes e anti-inflamatórias. Pela sua baixa solubilidade o uso em formulações tópicas é um desafio. Os líquidos iônicos, como o geranato de colina, emergiram como potenciais facilitadores da permeação cutânea. O objetivo, deste trabalho, foi avaliar o uso de geranto de colina na permeação da curcumina. Pelos resultados obtidos o geranato de colina promoveu a permeação da curcumina sendo proporção de 2% a mais adequada.

Palavras-chave: curcumina, geranato de colina, líquido iônico, permeação cutânea

INTRODUÇÃO

Curcumina é o principal bioativo extraído do rizoma da *Curcuma longa* Linn (*Zingiberaceae*), popularmente conhecida como açafrão. Trata-se de um polifenol de fórmula química $C_{21}H_{20}O_6$, massa molar de 368,38 g mol⁻¹ e, com ponto de fusão de 183°C, sendo praticamente insolúvel em água. A curcumina em testes *in vitro* tem demonstrando excelente atividade antioxidante, eficácia antiparasitária, efeitos antiespasmódicos e anti-inflamatórios, bem como potencial atividade anticâncer (Basnete Škalko-Basnet, 2011; Gonçalves *et al.*, 2014; Modasiya; Patel, 2017). Contudo, a curcumina é praticamente insolúvel em água e apresenta baixa disponibilidade (Modasiya e Patel, 2012). Assim, para o uso deste ativo em preparações tópicas é necessário utilizar substâncias que favorecem sua permeação. Os líquidos iônicos surgem como proposta inovadora de aumento de permeação cutânea de diversos ativos. Os líquidos iônicos são essencialmente sais em estado líquido que podem ser preparados a partir de materiais pouco dispendiosos e com *status* GRAS (*Generally Recognized As Safe*). Possuem propriedades ideais, tais como baixa pressões de vapor à temperatura ambiente, perfis de solubilidade extensos e variáveis, alta estabilidade térmica, não inflamável, polaridade adaptável, perfis químicos inertes, pontos de fusão baixos e viscosidades variáveis (Moniruzzaman *et al.*, 2010). Alguns líquidos iônicos têm a capacidade de provocar alterações momentâneas na organização lipídica e proteica inter- e intracelular do estrato córneo, permitindo a permeação de moléculas bioativas (Zakrewsky *et al.*, 2014). Neste sentido, justifica-se o trabalho que visou avaliar o uso do

líquido iônico geranto de colina como facilitador da permeação cutânea da curcumina.

MATERIAL E MÉTODOS

Síntese de geranato de colina: Em um balão de fundo redondo, adicionou-se 48 mL de ácido gerânico (0,2266 mol); 20 mL de bicarbonato de colina a 80% (m/m) (0,1133 mol); e 20 mL de metanol. Agitou-se por uma noite. O solvente foi removido à 60 °C por 20 minutos (Zakrewsky *et al.*, 2014).

Proporção entre curcumina e líquido iônico: Foram utilizadas diferentes proporções de líquido iônico para a dissolução de curcumina, sendo: 0%; 0,5%; 1%; 1,5%; 2% e 3%.

Permeação cutânea *in vitro* da curcumina com líquido iônico: Utilizou-se células de difusão vertical (células de Franz) e pele de orelha suína como membrana (Salerno *et al.*, 2010). As amostras foram coletadas ao longo de 12 horas (0, 15, 30, 45, 60, 120, 180, 240, 300, 420, 540, 660 e 720 min).

Doseamento da curcumina: O doseamento foi feito por espectrofotometria em $\lambda = 421\text{nm}$ (Ashwinder e Avupati, 2017).

RESULTADOS E DISCUSSÃO

O geranato de colina foi sintetizado com rendimento de 45%. O produto apresentou fluidez e transparência à temperatura ambiente, em concordância com o trabalho de Zakrewsky *et al.* (2014).

Para verificação da melhor proporção entre curcumina e líquido iônico foram feitas diluições. Observou-se que a partir da 2% de líquido iônico a dissolução de curcumina foi mais eficiente (Figura 1). Os líquidos iônicos são substâncias que estão sendo utilizadas como solventes de fármacos e drogas pouco solúveis com sucesso (Banerjee *et al.*,

2017; Shi *et al.*, 2020), como também observado com a curcumina neste trabalho.

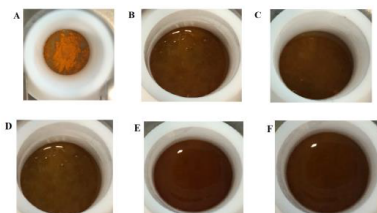


Figura 1. Solubilização de curcumina nas diferentes porcentagens de líquido iônico: (A) 0%; (B) 0,5%; (C) 1%; (D) 1,5%; (E) 2% e (F) 3%.

O método de dosagem de curcumina apresentou coeficiente de correção de 0,9983, com equação ($A = 6,0571C + 0,0003$) mostrando-se linear (Brasil, 2017) e adequado para a finalidade pretendida, permitindo a determinação da concentração liberada no processo de permeação cutânea).

O perfil de liberação de cada amostra testada está mostrado na Figura 2, e observa-se maior permeação de curcumina para a amostra com 2% de líquido iônico, totalizando $139,80 \mu\text{g.mL}^{-1}$.

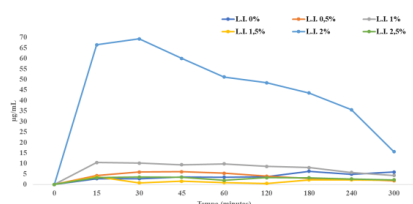


Figura 2. Curva de permeação cutânea para curcumina.

O efeito dos intensificadores de permeação está relacionado ao seu caráter iônico e sua propriedade de agir como surfactantes catiônicos. Em associação com outras substâncias pode favorecer a permeação das mesmas (Kumar *et al.*, 2018). Os resultados indicaram que o líquido iônico geranato de colina promove a permeação da curcumina, sendo a proporção de 2% a mais adequada.

CONCLUSÃO

O geranato de colina mostrou-se promissor como facilitador na permeação de curcumina, podendo ser utilizado como um carreador de este ativo no desenvolvimento de formulações tópicas.

AGRADECIMENTOS

Agradecemos à Uniso pelas bolsas concedidas aos alunos Audrey Hernandez, Erica Silva, Thais Oliveira e Rodrigo Boscariol. Agradecemos também à Fundação de Amparo à Pesquisa do Estado de São

Paulo (FAPESP, São Paulo, Brasil) pelos financiamentos concedidos (FAPESP Refs. No. 2016/08884-3 (Projeto PneumoPhageColor), 2016/12234-4 (Projeto TransAppIL) e 2018/05522-9 (Projeto PsaPhageKill, na forma de Bolsa BPE concedida a VMB)). Este trabalho também recebeu suporte do CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico, na forma de Bolsas Produtividade em Pesquisa (PQ) concedidas a VMB (Refs. No. 306113/2014-7 e 308208/2017-0).

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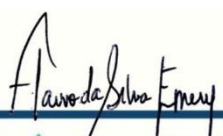
ANEXO B

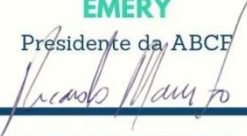
Certificado e Resumo do V Congresso da Associação Brasileira de Ciências Farmacêuticas (ABCF). Evento on-line, 1-3 de outubro de 2020**CERTIFICADO**

CERTIFICAMOS QUE

Rodrigo Boscarior

realizou uma apresentação do trabalho "DESENVOLVIMENTO DE GEL DE CURCUMINA E GERANATO DE COLINA PARA TRATAMENTO DE PSORÍASE", exposta no V congresso da Associação Brasileira de Ciências Farmacêuticas que ocorreu entre 1 a 3 de outubro de 2020, digitalmente, e gerou este certificado no total de 1 horas.


FLÁVIO DA SILVA EMERY
Presidente da ABCF


RICARDO NEVES MARRETO
Presidente do V Congresso ABCF

Outros autores: Audrey Stefani Naufal Hernandez, Erica Cristina Santos Silva, Thais Jardim Oliveira, Liliam Katsue Harada, Edjane Consorte Cinto, Victor Manuel Cardoso Figueiredo Balcão, Marta Maria Duarte Carvalho Vila.



Resumo apresentado no V Congresso da Associação Brasileira de Ciências Farmacêuticas (ABCF). Evento on-line, 1-3 de outubro de 2020.

DESENVOLVIMENTO DE GEL DE CURCUMINA E GERANATO DE COLINA PARA TRATAMENTO DE PSORÍASE

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A psoríase é uma doença inflamatória crônica da pele com forte predisposição genética, traços patogênicos autoimunes, com curso recidivante e prevalência mundial variável. A curcumina é o principal bioativo extraído do rizoma da *Curcuma longa* e apresenta propriedades antioxidantes, anti-inflamatórias e antibacterianas. Devido a estas propriedades, a curcumina tem sido usada para o tratamento de várias afecções da pele, como a psoríase. No entanto, a permeação pelo estrato córneo é dificultada por sua baixa solubilidade e absorção. Na tentativa de melhorar a permeação cutânea, substâncias facilitadoras da permeação tópica, como os líquidos iônicos, têm sido estudadas. Objetivou-se o desenvolvimento de um gel de curcumina e líquido iônico (geranato de colina) para veiculação de curcumina visando o tratamento da psoríase. A síntese do líquido iônico efetivou-se na reação entre bicarbonato de colina e ácido gerânico [1:2]. Foram avaliadas diferentes proporções de geranato de colina (0%; 0.5%; 1%; 1.5%; 2% e 2.5%) visando definir a menor concentração de líquido iônico capaz de permear o ativo. Os estudos de permeação cutânea *in vitro* foram realizados utilizando células de difusão de Franz e pele de orelha suína como membrana. O doseamento da curcumina permeada foi feito por espectrofotometria em comprimento de onda de 421nm. O geranato de colina foi sintetizado com rendimento de 45%. O produto apresentou fluidez e transparência à temperatura ambiente. O método de dosagem de curcumina apresentou coeficiente de correção de 0.9983, com equação ($A = 6,0571C + 0,0003$) mostrando-se linear e adequado para a finalidade pretendida. Observou-se uma maior permeação de curcumina para a amostra com 2% de líquido iônico, sendo esta proporção utilizada para os próximos experimentos.

ANEXO C

Certificado do V International Workshop on Rational Use of Medicines / III Workknowledge of Evidence-Informed Policy / III Symposium ISPE BrazInt RIG/ I Symposium of ISPE Brazilian Student Chapters.
Evento on-line, 1-5 de março de 2021.



Poster apresentado no V International Workshop on Rational Use of Medicines
/ III Workknowledge of Evidence-Informed Policy / III Symposium ISPE BrazInt
RIG/ I Symposium of ISPE Brazilian Student Chapters.
Evento on-line, 1-5 Mar 2021.



V International Workshop on Rational Use of Medicines
III Workknowledge of Evidence-Informed Policy
III Symposium ISPE Brazilian International RIG
I Symposium of ISPE Brazilian Student Chapters



DEVELOPMENT OF CURCUMIN GEL AND CHOLINE GERANATE FOR PSORIASIS TREATMENT

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²UNIVERSITY OF SOROCABA

CONTEXT

Psoriasis is a chronic inflammatory skin disease with a strong genetic predisposition, autoimmune pathogenic traits, with recurrent course and variable worldwide prevalence. Curcumin is the main bioactive extracted from the rhizome of *Curcuma longa* and has antioxidant, anti-inflammatory and antibacterial properties. Due to these properties, curcumin has been used to treat various skin conditions, such as psoriasis. However, permeation by the stratum corneum is hampered by its low solubility and absorption. In an attempt to improve skin permeation, substances that facilitate topical permeation, such as ionic liquids, have been studied.

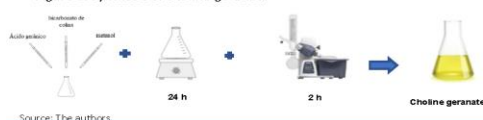
OBJECTIVES

To develop a curcumin gel and ionic liquid (choline geranate) for the transmission of curcumin to treat psoriasis.

METHODS

This is a pre-clinical experimental study in vitro, the synthesis of the ionic liquid was performed in the reaction between choline bicarbonate and geranic acid [1: 2]. Different proportions of choline geranate (0%; 0.5%; 1%; 1.5%; 2% and 2.5% w/w) were evaluated in order to define the lowest concentration of ionic liquid capable of permeating the asset. In vitro skin permeation studies were performed using Franz diffusion cells and porcine ear skin as a membrane. The measurement of permeate curcumin was performed by spectrophotometry at $\lambda = 421\text{nm}$. Choline geranate was synthesized in 45% yield.

Figure 1: Synthesis of choline geranate

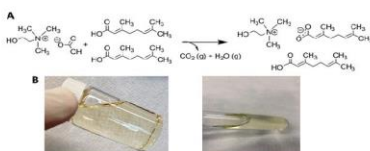


Source: The authors.

RESULTS

- 45% yield on the synthesis of choline geranate, the product showed fluidity and transparency at room temperature.

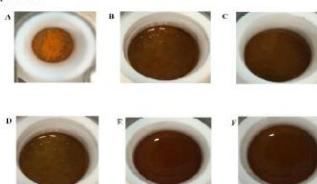
Figure 2: Esterification reaction between (A) choline bicarbonate and geranic acid to produce choline geranate. (B) comparison between the fluidity and transparency of the choline geranate produced by Zakrewsky et al. (2014) and that produced in this work.



Source: The authors.

- From 2% ionic liquid, dissolving curcumin was more efficient.
- Ionic liquids are substances that are being used as solvents for drugs and poorly soluble drugs, as also observed with curcumin in this work.

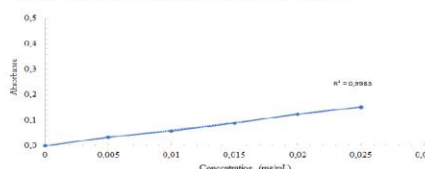
Figure 3: Solubilization of curcumin in different percentages of ionic liquid: (A) 0%; (B) 0.5%; (C) 1%; (D) 1.5%; (E) 2% and (F) 2.5%.



Source: The authors.

- The curcumin dosage method showed a correction coefficient of 0.9983, with an equation ($A = 6.0571C + 0.0003$) being linear.

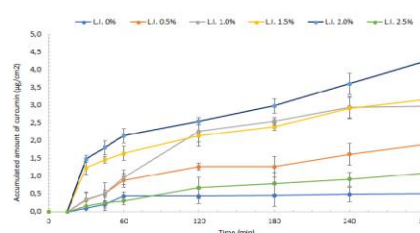
Figure 4: Curcumin calibration curve ($\lambda = 421\text{nm}$).



Source: The authors.

- The results indicated that the choline geranate ionic liquid promoted the permeation of curcumin, the proportion of 2% being the most adequate.

Figure 5: Cutaneous permeation curve for curcumin.



Source: The authors.

CONCLUSIONS

Choline geranate has shown promise as a facilitator in the permeation of curcumin, and can be used as a carrier of this asset in the development of topical formulations.



ANEXO D

Certificados e resumos do 13th International Congress of Pharmaceutical Sciences (CIFARP)

Evento on-line, Ribeirão Preto, SP, Brasil 3-6 de novembro de 2021.



CERTIFICATE

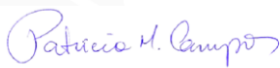
This is to certify that

Rodrigo Boscariol, Denicezar Angelo Baldo, Matthieu Tubino, José Martins de Oliveira Jr., Victor Manuel Cardoso Figueiredo Balcão, Marta Maria Duarte Carvalho Vila for presenting the Poster Entitled

Transdermal permeation of curcumin promoted by choline geranate deep eutectic solvent: potential for the treatment of psoriasis

during the 13th International Congress of Pharmaceutical Sciences (13th CIFARP) held on November 3-6, 2021.

Ribeirão Preto, November 06, 2021.



Prof. Dr. Patricia Maria Berardo Gonçalves Maia Campos
President of the 13th CIFARP 2021



Prof. Dr. Osvaldo de Freitas
Director of the School of Pharmaceutical Sciences
of Ribeirão Preto-USP

Rector Valter Aguiar, PhD Vice-Rector Antonio Carlos Hernandez, PhD	Dean Osvaldo de Freitas, PhD Vice-Dean Maria Regina Tonquetti, PhD	Organizing Committee Patricia Maria Berardo Gonçalves Maia Campos, PhD Paulo César Vieira, PhD Andriela Machado Leopoldino, PhD Fabiana Rossi Veraldo, PhD Fabiana Testa Moura de Carvalho Vicentini, PhD Jones Augusto Rizzato Paschoal, PhD Leonardo Régis Leira Pereira, PhD Ludmila Maria Greggi Antunes, PhD	Scientific Committee Alessandra Vincenzi Jager, PhD Ana Patricia Yatsuda Matsui, PhD Clea Mara Marzocchi Machado, PhD Fabiana Testa Moura de Carvalho Vicentini, PhD Heliana Maria Debonis, PhD Jairo Kempp Bastos, PhD Leonardo Régis Leira Pereira, PhD Lorena Rigo Gaspar Cordero, PhD Ludmila Maria Greggi Antunes, PhD Rose Mary Juresten Georgetto Nadi, PhD Sérgio Alira Uyemura, PhD	POWERED BY 
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CERTIFICATE

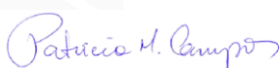
This is to certify that

Rodrigo Boscariol, Thais Hora Paulino, Matthieu Tubino, José M. de Oliveira Jr., Victor Manuel Cardoso Figueiredo Balcão, Marta Maria Duarte Carvalho Vila for presenting the Poster Entitled

Physicochemical characterization of commercially available turmeric for use in pharmaceutical products and food supplements

during the 13th International Congress of Pharmaceutical Sciences (13th CIFARP) held on November 3-6, 2021.

Ribeirão Preto, November 06, 2021.



Prof. Dr. Patricia Maria Berardo Gonçalves Maia Campos
President of the 13th CIFARP 2021



Prof. Dr. Osvaldo de Freitas
Director of the School of Pharmaceutical Sciences
of Ribeirão Preto-USP

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Resumo apresentado no 13th International Congress of Pharmaceutical Sciences (CIFARP).

Evento on-line, Ribeirão Preto, SP, Brasil 3-6 de novembro de 2021.



Transdermal permeation of curcumin promoted by choline geranate deep eutectic solvent: potential for the treatment of psoriasis

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Keywords: Choline geranate deep eutectic solvent; Transdermal permeation; Curcumin; Psoriasis

ABSTRACT

Curcumin, 7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione, is an active compound extracted from curcuma rhizomes, especially *Curcuma longa* L. Curcumin has been reported as having potential for the topical treatment of (but not limited to) diseases such as psoriasis and skin cancer [1,5]. Ionic liquids (ILs) and their deep eutectic solvents (DES) have emerged as promising substances that effectively enhance transdermal permeation [2,3,4,6]. In this research, we addressed the transdermal permeation of curcumin aided by choline geranate deep eutectic solvent (CG-DES) as a potential treatment for psoriasis. An analysis of the effect of CG-DES concentration in the enhancement of transdermal permeation of curcumin was performed and duly modelled via nonlinear regression analysis. The results showed that a low percentage of CG-DES (viz. 2.0%, w/w) was effective in disrupting the skin structure in a transient fashion, facilitating the passage of curcumin dissolved in it.

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Physicochemical characterization of commercially available turmeric for use in pharmaceutical products and food supplements

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Keywords: Commercial turmeric; Curcumin; Physicochemical characterization; Antimicrobial activity

ABSTRACT

Curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione, is an oil-soluble compound, insoluble in water at room temperature at both acidic and neutral pH values [1,2]. It is derived from turmeric (*Curcuma longa* L.), exhibiting a wide spectrum of biological activities [6]. Studies have rediscovered the therapeutic potential of curcumin with a range of beneficial effects including anti-inflammatory, antioxidant, antimicrobial, antiviral, immuno-modulating, antineoplastic properties [1,2,3]. This research, addressed the physicochemical differences in commercial turmeric, acquired from both handling pharmacies and stores selling natural products. The results obtained for the physicochemical characteristics of the several commercial turmeric samples were not homogeneous, showing in fact significant differences regarding melting points, UV-Vis spectral scan profiles and FTIR spectra [4], presenting toxic metals [5] and quite low curcumin contents. This sheds light on the urge to implement an adequate quality control in this type of raw material.

ACKNOWLEDGEMENTS

Project funding by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, São Paulo, Brazil) (FAPESP Refs. No. 2016/08884-3 (Project PneumoPhageColor) and 2016/12234-4 (Project TransApplL)), is hereby gratefully acknowledged. Funding for Victor M. Balcão through a BPE grant from FAPESP (São Paulo, Brazil) (Ref. No. 2018/05522-9, Project PsaPhageKill) is hereby gratefully acknowledged. This work also received support from CNPq, National Council for Scientific and Technological Development Brazil, in the form of Research Productivity (PQ) fellowships granted to Victor M. Balcão (Refs. No. 306113/2014-7 and 308208/2017-0). The authors have no conflicts of interest whatsoever to declare.

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Anexo E**UNIVERSIDADE DE SOROCABA****COMISSÃO DE ÉTICA NO USO DE ANIMAIS****CEUA-UNISO****PARECER**

Protocolo nº 199/2021
Interessado (a): Rodrigo Boscariol
Orientador (a): o mesmo
Título do Projeto: Desenvolvimento de sistema transdérmico para permeação cutânea de curcumina carregada por líquido iônico para tratamento de psoríase.

Apresentado à Comissão de Ética no Uso de Animais - CEUA para análise, segundo a Lei No. 11.794, de 8 de outubro de 2008, que regulamenta o inciso VII do parágrafo 1º do artigo 225 da Constituição Federal, foi considerado:

☒ **APROVADO.**

☐ **APROVADO com RECOMENDAÇÃO**, devendo o proponente encaminhar as modificações sugeridas em anexo para complementação do protocolo;

☐ **COM PENDÊNCIA**, devendo o proponente readequar os itens do protocolo;

☐ **REPROVADO**

Manifestação do Parecerista:

--

Nome: Denise Grotto

Coordenador da CEUA-Uniso

Assinatura:



Data: 30/03/2021