UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA

Jéssica Franco Dalenogare

ATIVIDADE CICATRIZANTE DO CUBIU *(Solanum sessiliflorum)* E DO CAMPO MAGNÉTICO

Santa Maria, RS
2022

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Tese apresentada ao Programa de Pós-Graduação em Farmacologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito para a obtenção do título de Doutor em Farmacologia.

Orientadora: Prof.ª Dr.ª Liliane de Freitas Bauermann
Coorientadora: Prof.ª Dr.ª Michele Rorato Sagrillo

Santa Maria, RS
2022

This study was financied in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001

Dalenogare, Jéssica
ATIVIDADE CICATRIZANTE DO CUBIU (Solanum sessiliflorum) E DO CAMPO MAGNÉTICO / Jéssica Dalenogare.- 2022.
111 p.; 30 cm

Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências da Saúde, Programa de Pós Graduação em Farmacologia, RS, 2022

1. Cicatrização de pele I. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

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Santa Maria, RS
2022

Agradecimentos

Agradeço primeiramente a Deus, por ter me dado a permissão de chegar até aqui, e por toda a força concedida na concretização desse sonho. Além disso, agradeço a Ele por todas as pessoas que cruzaram meu caminho e que estão aqui citadas, todas muito especiais.

Aos meus pais e irmão, minha base, pelo amor incondicional, por tudo o que que sou. Pai e mãe, obrigada pelas oportunidades e pelo privilégio de poder estudar. Amo vocês!

A toda minha família, pelo apoio e suporte para a realização deste e tantos outros sonhos.

A minha orientadora, Profª Liliane de Freitas Bauermann, o meu sincero agradecimento pela orientação, confiança, carinho, amizade e por todos os ensinamentos durante essa fase de amadurecimento pessoal e profissional.

A minha co-orientadora Profª Michele Rorato Sagrillo por todo auxílio, amizade, orientação e ensinamentos transmitidos.

Aos professores que compõem a banca de defesa desta tese, por aceitarem participar, proporcionando discussões e sugestões que servirão para crescimento, aprendizado e incentivo à pesquisa.

Aos queridos amigos e colegas de laboratório e formação, pelo auxílio nos experimentos, pela partilha de conhecimentos, disponibilidade, companheirismo, amizade, dedicação e pelos momentos de alegria e descontração.

Aos professores e funcionários do Programa de Pós-Graduação em Farmacologia que contribuíram para minha formação.

A todas as pessoas que, direta ou indiretamente, contribuíram para a execução desta Tese de doutorado.

A Universidade Federal de Santa Maria e ao Programa de Pós-Graduação em Farmacologia pela oportunidade de realização deste curso.

Por fim, agradeço à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão da bolsa de estudos.

“Conheça todas as teorias, domine todas as técnicas, mas ao tocar uma alma humana, seja apenas outra alma humana.”

Carl Jung

RESUMO

ATIVIDADE CICATRIZANTE DO CUBIU (*Solanum sessiliflorum*) E DO CAMPO MAGNÉTICO

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A cicatrização de pele é um processo complexo e distinto, no qual tratamentos que visem o seu favorecimento e aceleração, como a utilização terapêutica de campos magnéticos, são benéficos. Entretanto, a atuação dos campos magnéticos no balanço oxidativo e no perfil inflamatório sistêmico, ainda é controversa na literatura. Visto isso, é sugerido a utilização dos mesmos em conjunto a antioxidantes. Dentre estes, destacam-se os antioxidantes naturais, oriundos de plantas, como a solanácea herbácea *Solanum sessiliflorum* (cubiu). O cubiu é amplamente utilizado na medicina tradicional não só como antioxidante, mas também, com alegação de propriedades hipoglicêmicas, hipolipidêmicas, no combate a diabetes e para cicatrização de pele. Entretanto, apesar do uso popular, têm-se poucos estudos investigando a segurança para sua utilização quanto a toxicidade, elucidando sua real atuação no perfil oxidativo, assim como, ainda não há dados que confirmem seu efeito anti-inflamatório e cicatrizante. Dessa forma, o presente estudo tem por objetivo investigar a atividade farmacológica do cubiu (toxicidade, atividade antimicrobiana, antioxidante, anti-inflamatória) e sua ação cicatrizante frente a feridas cutâneas e em associação ao campo magnético. Para isso, foi conduzido um primeiro estudo *in vitro* investigando a atividade antimicrobiana do extrato de cubiu frente as cepas de *Aeromonas caviae*, *Pseudomonas aeruginosa* (PA01) e *Sphingomonas paucimobilis*, e também na inibição e destruição do biofilme de PA01. Ainda, nesse estudo foi avaliada a atividade cicatrizante do extrato de cubiu, atividade anti-inflamatória e o perfil de segurança, utilizando células de fibroblastos humanos HFF1 e células mononucleares de sangue periférico humano. Neste estudo, foi constatado que o cubiu apresenta atividade cicatrizante, tendo a modulação da resposta inflamatória como mecanismo de ação, agindo como anti-inflamatório, além de demonstrar ser antimicrobiano conta as cepas testadas, inibir e destruir o biofilme de PA01 e demonstrar segurança para o uso. A partir disso, o próximo estudo da continuidade a investigação da atividade cicatrizante do cubiu frente a um processo de cicatrização de pele e em associação ao campo magnético, utilizando ratos *Wistar.* Primeiramente, é conduzido um protocolo que avalia a toxicididade de uma curva dose resposta do cubiu *in vivo*, assim determinando a dose ideal. Como sequência, é apresentado o estudo de cicatrização de pele onde avalia-se as atividades antioxidantes e anti-inflamatórias dos dois tratamentos isolados e em associação (cubiu e campo magnético). Nesse estudo foi possível evidenciar que o cubiu não apresentou toxicidade em nenhuma das concentrações testadas, e que ambos os tratamentos atuam como antioxidantes e anti-inflamatórios frente ao processo de cicatrização de pele, tendo sua resposta aumentada quando utilizados em associação. Em conclusão, a partir da triagem farmacológica realizada, este é o primeiro estudo a comprovar que o extrato de cubiu é um importante composto contra doenças de pele, promovendo a cicatrização de feridas cutâneas, atividade antimicrobiana e anti-inflamatória.

Palavras-chave: Maná-cubiu, cocona, cicatrização de pele, reparo tecidual.

ABSTRACT

WOUND HEALING ACTIVITY BY CUBIU (Solanum sessiliflorum) AND MAGNETIC FIELD

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Skin healing is a complex and distinct process, in which treatments aimed at favoring and accelerating it, such as the therapeutic use of magnetic fields, are beneficial. However, the role of magnetic fields in the oxidative balance and in the systemic inflammatory profile is still controversial in the literature. Given this, it is suggested to use them together with antioxidants. Among these, natural antioxidants from plants, such as the herbaceous nightshade *Solanum sessiliflorum* (cubiu), stand out. Cubiu is widely used in traditional medicine not only as an antioxidant, but also with claims of hypoglycemic and hypolipidemic properties, in the fight against diabetes and for skin healing. However, despite its popular use, there are few studies investigating the safety of its use in terms of toxicity, elucidating its real action in the oxidative profile, as well as, there are still no data to confirm its anti-inflammatory and healing effect. Thus, the present study aims to investigate the pharmacological activity of cubiu (toxicity, antimicrobial, antioxidant, anti-inflammatory activity) and its healing action against skin wounds and in association with the magnetic field. For this, a first in vitro study was conducted investigating the antimicrobial activity of cubiu extract against the strains of *Aeromonas caviae, Pseudomonas aeruginosa* (PA01) *and Sphingomonas paucimobilis*, as well as the inhibition and destruction of the PA01 biofilm. Furthermore, this study evaluated the healing activity of cubiu extract, anti-inflammatory activity and safety profile, using human fibroblast cells HFF1 and human peripheral blood mononuclear cells. In this study, it was found that cubiu has healing activity, with the modulation of the inflammatory response as a mechanism of action, acting as an anti-inflammatory, in addition to demonstrating that it is antimicrobial against the strains tested, inhibiting and destroying the PA01 biofilm and demonstrating safety for the use. From this, the next study will continue the investigation of the healing activity of the cubiu in the face of a skin healing process and in association with the magnetic field, using Wistar rats. First, a protocol is conducted that evaluates the toxicity of a dose-response curve of cubiu in vivo, thus determining the ideal dose. As a sequence, the study of skin healing is presented, where the antioxidant and anti-inflammatory activities of the two treatments alone and in association (cubiu and magnetic field) are evaluated. In this study, it was possible to show that cubiu did not present toxicity at any of the concentrations tested, and that both treatments act as antioxidants and anti-inflammatory agents against the skin healing process, with an increased response when used in association. In conclusion, from the pharmacological screening carried out, this is the first study to prove that cubiu extract is an important compound against skin diseases, promoting skin wound healing, antimicrobial and anti-inflammatory activity.

Keywords: Maná-cubiu, cocona, skin wound healing, tissue repair.

LISTA DE ILUSTRAÇÕES

REVISÃO BIBLIOGRÁFICA

MANUSCRITO 1

MANUSCRITO 2

ANEXOS

LISTA DE TABELAS

MANUSCRITO 1

MANUSCRITO 2

LISTA DE ABREVIAÇÕES E SIGLAS

PDGF – Fator de crescimento derivado de plaquetas

TGF-β – Fator de crescimento transformante - β

$O_2^{\bullet-}$ – Radical ânion superóxido

H_2O_2 – Peróxido de hidrogênio

TNF – Fator de necrose tumoral

IFNs – Interferons

CSFs – Fatores estimuladores de colônia

ILs – Interleucinas

FGF-2 – Fator de crescimento de fibroblasto 2

TBARS – Substâncias que reagem ao ácido tiobarbitúrico / Substances that react to thiobarbituric acid

NPSH – Conteúdo de grupos tióis não proteicos / Content of non-protein thiol groups

SOD – Superoxido dismutase / Superoxide dismutase

CAT – Catalase

GST – Glutationa S- transferase / Glutathione S-transferase

PDA – matrix of photodiodes

TGO – Transaminase glutâmica oxalacética / glutamic oxalacetic transaminase

TGP – Transaminase glutâmica pirúvica / Pyruvic glutamic transaminase

GGT – Gama glutamil transpeptidase / Glutamyl transpeptidase range

IL-1 – Interleucina 1 / Interleukin 1

IL-6 – Interleucina 6 / Interleukin 6

IL-10 – Interleucina 10 / Interleukin 10

TNF-α – Fator de necrose tumoral - α / Tumor necrosis factor-α

MPO – Mieloperoxidase / myeloperoxidase

PMSF – Phenylmethylsulfonyl fluoride

EROs / ROS – Espécies reativas de oxigênio / Reactive oxygen species

RNS - Reactive Nitrogen Species

LPO – Lipoperoxidação / Lipoperoxidation

MDA – Malondialdeído / Malondialdehyde

GSH – Glutationa / Glutathione

DMEM – Dulbecco's Modified Eagle Medium

SFB – Soro fetal bovino

MTT – Ensaio do Brometo de 3-[4,5-dimetil-tiazol-2-il]-2,5-difeniltetrazólio

IP – Iodeto de Propídeo

FGF-1 – Fator de crescimento de fibroblasto -1

KGF – Fator de crescimento de queratinócito

DCFH-DA – Diclorofluoresceína diacetato

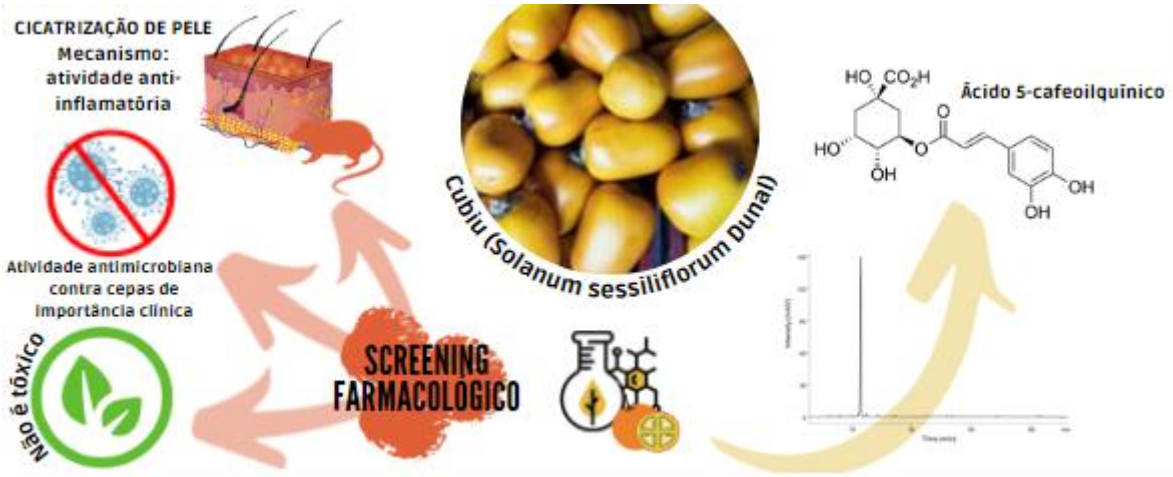
OH• – Radical hidroxil

SUMÁRIO

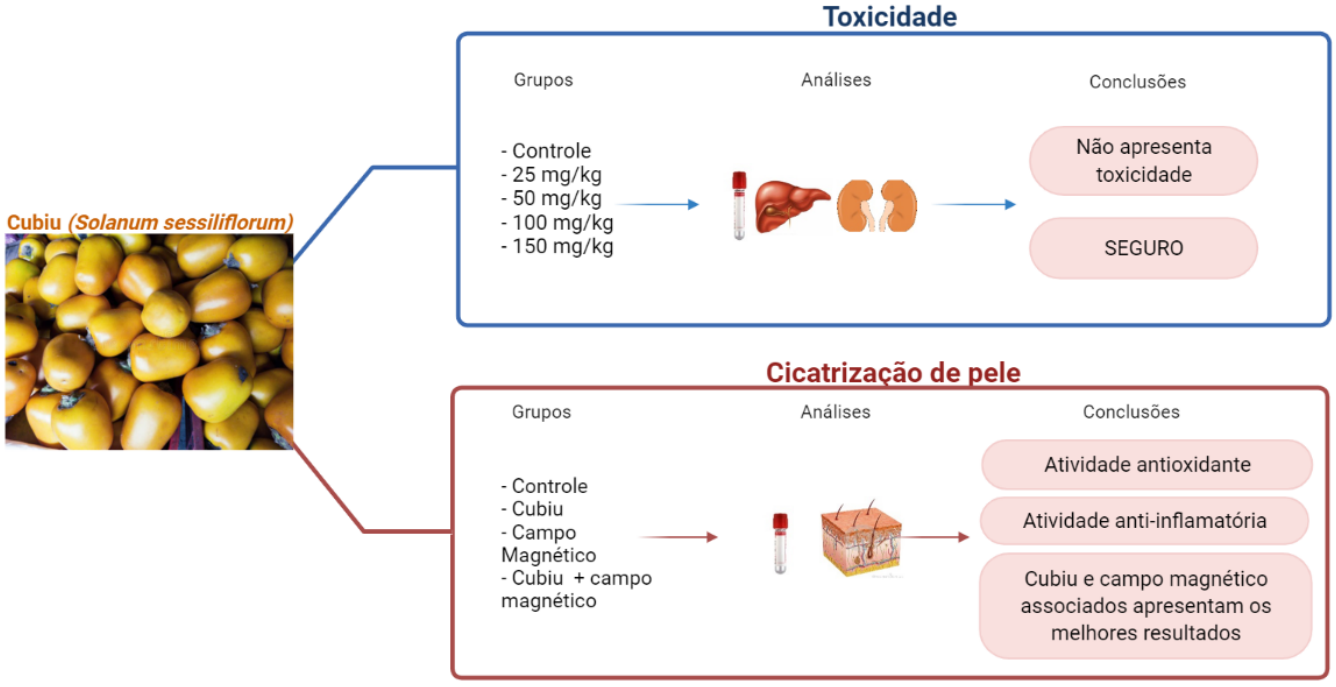
APRESENTAÇÃO

A tese ora apresentada encontra-se na forma de dois artigos científicos: o primeiro mostra o potencial farmacológico do cubiu, evidenciando sua segurança, atividade antimicrobiana, anti-inflamatória e cicatrizante *in vitro*; enquanto o segundo demonstra a avaliação da toxicidade do cubiu e sua ação antioxidante e anti-inflamatória frente a um processo de cicatrização de pele *in vivo*, isolado e associado ao campo magnético. Os esquemas a seguir sintetizam os dois estudos que compõem esta tese.

Artigo 1 – estudo *in vitro*



Artigo 2 – estudo *in vivo*



1 INTRODUÇÃO

A pele é o maior órgão do corpo humano em extensão, desempenhando atividades imprescindíveis para sobrevivência do indivíduo, atuando na regulação térmica, como barreira de proteção contra agentes externos e micro-organismos, assim como, barreira para traumas físicos (STUNOVA; VISTEJNOVA, 2018). O rompimento das estruturas normais da pele, caracteriza uma lesão, sobre a qual, a pele tem capacidade de reparo, denominado processo de cicatrização (KIMURA; TSUJI, 2021). Esse processo é complexo, contendo fases de reparo distintas e sobrepostas. Diante da importância e complexidade da cicatrização tecidual, tratamentos que visem o seu favorecimento e aceleração, são benéficos. Entres estes, destacam-se os campos magnéticos ou também chamada radiação magnética (CARRUTHERS; CARRUTHERS, 2012).

O organismo é movido através de uma constante formação de energia biomagnética, através dos fatores bioquímicos, os fluidos celulares, atividade física e mental, assim como, da própria atividade celular. Devido a isso, é justificável a aplicação de campos magnéticos em tratamentos para a saúde, por ter como uma consequência, trocas com o potencial biomagnético do organismo, e assim produzir efeitos benéficos em processos fisiológicos, como no tratamento de cicatrização de pele. Dessa forma, os campos magnéticos agem principalmente mediante controle do fluxo sanguíneo, controle humoral e propriedades anti-inflamatórias (ÁLVARES; GARCÍA; BLANCO, 2007; VARANI et al., 2017; SHANG et al., 2019).

Entretanto, esses benefícios que podem ser proporcionados pelos campos magnéticos são dependentes de seus parâmetros de aplicação. Em relação ao balanço oxidativo, a atuação dos campos magnéticos é bastante controversa na literatura, relatando que o mesmo pode atuar protegendo contra dados oxidativos (GLINKA et al., 2018), assim como, é visto que em outros parâmetros os campos podem ser responsáveis por ocasionar esses danos e também aumentar o estado inflamatório em outros tecidos (ORTIZ et al., 2016). Assim, o tempo de exposição e a intensidade se mostram determinantes para estabelecer os efeitos produzidos pelos campos magnéticos (CANSEVEM et al., 2008).

Dessa forma, é sugerido a utilização de campos magnéticos associados a antioxidantes (CEYHAN et al., 2012; GHODBANE et al., 2015). Entre os antioxidantes, encontram-se os antioxidantes naturais, oriundos de plantas (MORAES et al., 2020). Destas, destaca-se a planta *Solanum sessiliflorum*, conhecida popularmente como

cubiu (TAUCHEN et al., 2016; VARGAS-ARANAet al.,2021). O cubiu é uma solanácea herbácea originária da bacia Amazônica, atualmente distribuído por toda a Amazônia brasileira, equatoriana, colombiana e venezuelana. O fruto é bastante nutritivo, rico em ferro, niacina, ácido cítrico e pectina. Assim, o cubiu possui compostos que são considerados auxiliares na promoção da saúde, como suas fibras e minerais, exercendo também ação antioxidante (COLODEL et al., 2017; SERENO et al., 2018; MONTAGNER et al., 2020).

Ainda, o cubiu é comumente utilizado pelas populações da Amazônia como medicamento e cosmético. Dessa forma, na medicina tradicional, tem sido utilizado com alegação de propriedades hipoglicêmicas, hipolipidêmicas e cicatrizantes (SILVA FILHO et al., 2005). Assim como também, para combate ao diabetes (AGUDELO et al., 2015). Entretanto, é pouco elucidado na literatura científica sua real atuação no perfil oxidativo, e não há dados até o presente momento que comprovem sua atividade anti-inflamatória e cicatrizante. Assim, o presente estudo visa investigar a atividade farmacológica do cubiu (toxicidade, atividade antimicrobiana, antioxidante, anti-inflamatória) e sua ação cicatrizante frente a feridas cutâneas e em associação ao campo magnético.

1.1 OBJETIVOS

O presente estudo tem por objetivo investigar a atividade farmacológica do cubiu (toxicidade, atividade antimicrobiana, antioxidante, anti-inflamatória) e sua ação cicatrizante frente a feridas cutâneas de forma isolada e em associação ao campo magnético.

1.1.1 **Objetivos específicos**

In vitro:

- Identificar e quantificar a presença de metabólitos secundários no extrato de cubiu;

- Avaliar a atividade antimicrobiana do extrato de cubiu, assim como, sua atividade na prevenção e destruição de biofilme bacteriano;

- Avaliar o perfil de segurança do extrato de cubiu;

- Avaliar a atividade anti-inflamatória do extrato de cubiu;

- Avaliar a atividade cicatrizante do extrato de cubiu.

In vivo:

- Avaliar a toxicidade do extrato de cubiu frente a uma curva dose-resposta;

- Avaliar a atividade antioxidante e anti-inflamatória do cubiu e do campo magnético (isolados e em associação) frente ao processo de cicatrização de pele.

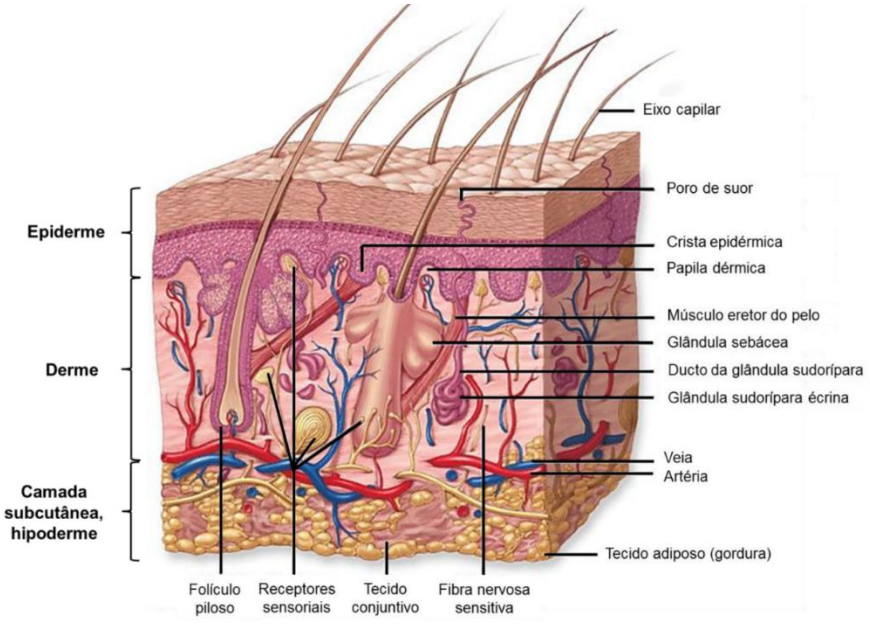
2 REVISÃO BIBLIOGRÁFICA

2.1 PELE

A pele é considerada o maior órgão do corpo humano em extensão, sendo responsável pela proteção e revestimento externo do organismo. Dentre suas funções, a pele atua como barreira para agentes físicos e químicos, exercendo também, proteção contra radiação ultravioleta e regulação térmica. Ainda, a pele atua como um importante componente periférico do sistema imune, desencadeando resposta imune primária quando frente a um patógeno (FROHM et al., 2014; BRAGAZZI et al., 2019).

Assim, a pele é um órgão complexo com diversos tipos celulares, tendo sua estrutura dividida em duas camadas: epiderme e derme (Figura 1). A hipoderme ou tecido subcutâneo está logo abaixo da derme, não sendo propriamente uma das camadas da pele, porém devido a sua íntima relação anatômica com a mesma, alguns autores ainda a consideram como constituinte da pele (LOSQUADRO, 2017).

Figura 1- Estrutura da pele



Fonte: Adaptado de MESCHER, 2010.

A epiderme é a camada mais externa, dessa forma exercendo atividade como proteção contra traumas mecânicos, entrada de agentes estranhos que sejam patogênicos, e impedir a perda de água. A morfologia da epiderme é classificada como um epitélio estratificado pavimentoso queratinizado que se constitui por cinco camadas: camada basal, camada espinhosa, camada granulosa, camada lúcida e camada córnea (TOBIN, 2017).

Diversos tipos celulares compõe a epiderme, sendo que os principais são os queratinócitos. Estes, começam sua diferenciação na camada basal e vão migrando através das sucessivas camadas. Ao se aproximarem da superfície, na última subcamada, conhecida como extrato córneo, os queratinócitos têm sua morfologia alterada, não apresentando mais núcleo, assim sofrendo apoptose e descamando, sendo substituídos por novas células que já vinham migrando e se diferenciando. Devido a esse constante processo de renovação, a epiderme apresenta-se como uma estrutura dinâmica (BARONI et al., 2012; DAS; OLMSTED, 2016).

Outra característica da epiderme é a ausência de vascularização, dessa forma, a mesma encontra-se apoiada a derme, através de uma lamina basal, sendo então, a derme responsável pelo aporte sanguíneo da epiderme. Assim, dentre as funções desempenhadas pela derme, está o suprimento de nutrientes necessários para a epiderme, assim como, a termo regulação (MAKRANTONAKI; ZOUBOULIS, 2007).

A derme, por sua vez, é dividida em duas camadas. A primeira destas é a camada papilar, mais superficial e frouxa, caracterizada por fibras colágenas e elásticas mais finas e desorganizadas. Mais profundamente, encontra-se a segunda camada, a qual é denominada camada reticular. Esta apresenta-se mais densa, possui fibras elásticas mais espessas e entrelaçadas com as fibras de colágeno, as quais são grossas e com boa organização. Na derme, as principais células são os fibroblastos, responsáveis por sintetizar colágeno, elastina, proteoglicanos, glicosaminoglicanos, fibronectina e outras proteínas que compõem a matriz extracelular, sendo essas as responsáveis por conferir resistência e elasticidade para a pele (STUNOVA; VISTEJNOVA, 2018).

Além disso, as células presentes na derme possuem um papel indispensável em processos inflamatórios e cicatriciais, dentre essas, destacam-se os macrófagos. Dessa forma, as células residentes da derme são

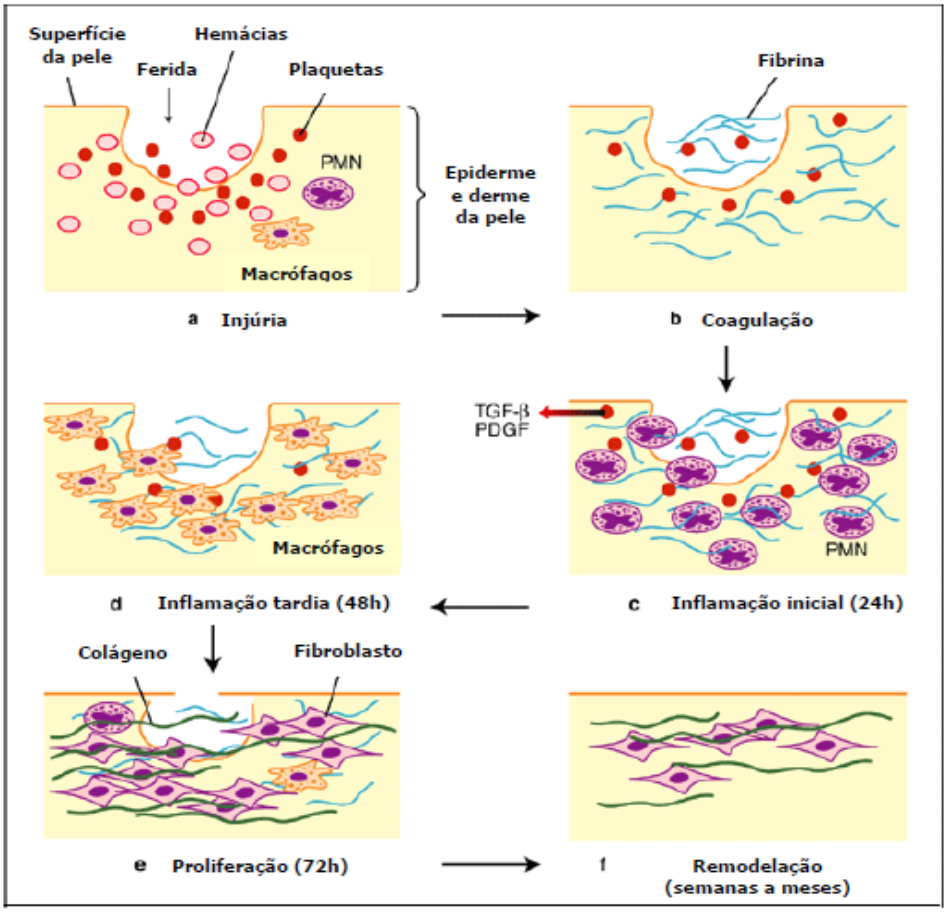
especializadas em produzir substâncias desencadeadoras de uma resposta inflamatória, as quais são acionadas quando necessário, com a finalidade de restabelecer a fisiologia normal da pele frente a uma lesão tecidual. Após ocorrer tal lesão, inicia-se uma sequência de eventos para o reparo tecidual, reestabelecendo assim, a estrutura e função protetora da pele, sendo esse mecanismo denominado de cicatrização (FREINKEL; WOODLEY, 2001; CALIXTO et al., 2004; KIMURA; TSUJI, 2021).

2.2 CICATRIZAÇÃO

A lesão de pele é caracterizada pelo rompimento de sua estrutura normal. Essa lesão pode ocorrer através de agentes físicos, químicos ou biológicos. Porém, a pele possui uma capacidade de autorregeneração, atuando na cicatrização desse ferimento, fator imprescindível para a sobrevivência (LEITE et al., 2012). Esse processo de cicatrização é bastante complexo e dinâmico envolvendo fenômenos bioquímicos. Entretanto, frente a algumas alterações locais e sistêmicas, o processo de cicatrização pode ser retardado ou prejudicado. Entre esses fatores encontram-se infecções, necrose, corpos estranhos e localização do ferimento, assim como também, fatores genéticos, idade, doenças como diabetes e uso de alguns medicamentos como corticoides (RUSHTON, 2007; BORGES, 2008; KIMURA; TSUJI, 2021).

O processo de cicatrização da pele envolve uma série de eventos que representam a tentativa de reestabelecer a estrutura anatômica e funcional normal do tecido lesionado. Esse processo vai ocorrer em três fases distintas, complexas e sobrepostas, as quais são denominadas: fase inflamatória, fase proliferativa e fase de remodelação tecidual (CHANDIKA; KO; JUNG, 2015; KIYA; KUBO, 2019), representadas na figura 2.

Figura 2- Representação esquemática das fases da cicatrização de feridas cutâneas



Fonte: Adaptado de BEANES et al., 2003.

2.2.1 Fase Inflamatória

A primeira fase do processo de cicatrização é a fase inflamatória, tendo duração média de três dias. Logo após o dano tecidual, inciam-se as respostas hemostáticas, provocando a formação do coágulo e ativação plaquetária, originando uma matriz provisória para a migração celular, selando o ferimento (LAUREANO; RODRIGUES, 2011).

As plaquetas ativam uma reação vasoconstritora, impedindo a perda de sangue. Ocorre a coagulação do agregado plaquetário com fibrina, a qual é depositada no local do ferimento través da conversão do fibrinogênio. Ainda, as plaquetas liberam fatores quimiotáxicos, incluindo o fator de crescimento

derivado de plaquetas (PDGF) e o fator de crescimento transformante (TGF-β), que atraem leucócitos polimorfonucleares para a ferida, sinalizando o início da inflamação e realizando a defesa contra microrganismos (GONZALEZ et al., 2016).

Logo após o ferimento, já é iniciada também a resposta celular, quando os leucócitos polimorfonucleares, entre eles os neutrófilos, começam a invadir a periferia da injuria. Muitos desses leucócitos sofrem lise, liberando assim seus grânulos citoplasmáticos que promovem a degradação enzimática e preparação para a fagocitose realizada pelos macrófagos. Após 24 horas do ferimento inicial, encontram-se presentes aguns monocitos, porém, com 48 horas após o ferimento, o número de macrófagos aumenta consideravelmente, sendo estes o principal tipo celular atuante na resposta inflamatória. Juntos, os neutrófilos e macrófagos fazem o desbridamento da ferida, liberam fatores de crescimento e iniciam a reorganização da matriz extracelular (GONZALEZ et al., 2016; RODERO; KHOSROTEHRANI, 2010).

Em conjunto à fagocitose e a ativação de neutrófilos é conduzida a liberação de espécies reativas de oxigênio (ROS) e proteases. Os neutrófilos contêm várias enzimas hidrolíticas e moléculas tóxicas em seus grânulos, podendo também gerar oxidantes como o radical ânion superóxido ($O_2^{\bullet-}$) e peróxido de hidrogênio (H_2O_2). Além disso, a mieloperoxidase, enzima presente nos neutrófilos, pode gerar ácido hipoclorídrico, um agente de oxidação altamente reativo (TOUMI; BEST, 2006).

Dessa forma, inúmeros mediadores inflamatórios estão presentes no decorrer da resposta da inflamação, entre eles prostaglandinas, bradicinina, serotonina, histamina, leucotrienos, substância P, tromboxanos, fator de ativação plaquetários, espécies ativas de oxigênio e nitrogênio, fator de necrose tumoral (TNF), assim como também, outras citocinas. Estas últimas, as citocinas, são um grupo muito diversificado de peptídeos e glicopeptídeos que coordenam e ativam respostas celulares imunes, entre eles encontram-se: interferons (IFNs), fatores estimuladores de colônia (CSFs), quimiocinas, o fator de crescimento transformante beta (TGFβ) e Interleucinas (ILs) (LAUREANO; RODRIGUES, 2011; STUNOVA; VISTEJNOVA, 2018).

As ILs podem atuar de forma pró-inflamatória (IL 1, IL 6), e também, anti-inflamatória (IL 10). Ainda, destaca-se a atuação dos TNF, os quais exercem

papel imprescindível na inflamação, como o TNF-α, produzidos pelos macrófagos. (TANSEY; SZYMKOWSKI, 2009). Neste contexto, além dos macrófagos, evidencia-se também a importância dos tipos celulares queratinócitos e fibroblastos, os quais também participam da produção de vários mediadores pró-inflamatórios, como as citocinas (BURBACH et al, 2000).

Essa cascata de eventos fisiológicos é essencial para que ocorra o processo de cicatrização do ferimento, entretanto, se nessa fase inicial os eventos ocorrerem de forma exacerbada, irão acabar por ocasionar efeitos deletérios para o tecido. Dessa forma, um tempo menor e adequado de fase inflamatória implica em uma cicatrização eficaz e com tempo reduzido (DE MACEDO; SANTOS, 2006; TIAN et al., 2007).

2.2.2 Fase proliferativa

A segunda etapa do processo de cicatrização é denominada fase proliferativa ou fase de proliferação, a qual ocorre após aproximadamente três dias do ferimento inicial. Os fibroblastos são ativados, estimulando assim a síntese de colágeno. Em conjunto se dá o início ao processo de re-epitelização, o qual ocorre através da proliferação de queratinócitos, e ainda, o início do processo de angiogênese (LANDÉN; LI; STAHLE, 2016).

A angiogênese é a formação de vasos sanguíneos que se dá no local da lesão, sendo imprescindível para manter o aporte de nutrientes e oxigênio para as células metabolicamente ativas. Dessa forma, a fase proliferativa culmina na formação do tecido de granulação, o qual é denso em fibroblastos, granulócitos, macrófagos, altamente vascularizados e apresentando feixes de colágeno tipo III fracamente ligados (LAUREANO; RODRIGUES, 2011; LANDÉN; LI; STAHLE, 2016).

A partir da síntese de colágeno e outras macromoléculas pelos fibroblastos, a força tensil do ferimento começa a ser gradualmente aumentada, devido ao acúmulo desse colágeno. Posteriormente, os fibroblastos diferenciam-se em miofibroblastos, responsáveis por promover a contração da ferida (LAUREANO; RODRIGUES, 2011; GONZALEZ et al., 2016).

2.2.3 Fase de remodelação

A terceira etapa é a fase de remodelação ou remodelamento. Com o decorrer do processo de cicatrização, a vascularização é gradualmente reduzida, através da diminuição capilar e do fim do processo de formação de novos vasos sanguíneos, além disso, muitos dos vasos neoformados são reabsorvidos (GONZALEZ et al., 2016).

Nessa fase, também e evidenciado o desaparecimento da maioria das células do tecido de granulação, através da apoptose de fibroblasto de células endoteliais. Ainda, a deposição do colágeno se dá de forma acentuada. Através desses processos, é originado a cicatriz ou tecido cicatricial. Esse tecido cicatricial começa a sofrer retração, tendo também a substituição do colágeno tipo III (gerado na fase proliferativa) por colágeno tipo I, o qual é mais resistente (REINKE; SORG, 2012).

Considera-se como resolução total de um ferimento, quando a maturação e remodelagem da matriz extracelular estão concluídos. Entretanto, esse é um processo lento, podendo levar meses ou anos. Isso se dá porque embora a taxa de síntese de colágeno desacelere após cerca de três semanas, a reticulação e reorganização do colágeno ocorrem por meses após a lesão. Após a resolução total do ferimento, tem-se a cicatriz madura, a qual possui apenas 70% da resistência de uma pele íntegra (STUNOVA; VISTEJNOVA, 2018).

2.2.4 Espécies reativas no processo de cicatrização

Considerando o processo cicatricial como um todo, é imprescindível ressaltar a participação das espécies reativas, visto que estas podem estar envolvidas em todas as fases do processo de cicatrização, principalmente no que diz respeito a sinalização celular. Além disso, as ROS desempenham importante papel nas condições inflamatórias, como por exemplo, o radical ânion superóxido, que tem sua produção aumentada durante o processo cicatricial,

exercendo assim ação antimicrobiana, protegendo contra micro-organismo invasores (WLASCHEK; SCHARFFETTER-KOCHANEK, 2005; PARK; LIM, 2011).

Dessa forma, quando em baixas concentrações, as ROS e seus subprodutos, tais como os metabolitos da oxidação de proteínas e lipídios, estarão envolvidos nas vias de transdução de sinal intracelular e regulação de expressão gênica. Assim, podendo modular a resposta anti-inflamatória, crescimento celular, diferenciação, proliferação e a resposta ao estresse (HALLIWELL; GUTTERIDGE, 1999; HALLIWELL, 2001; NAKASHIMA et al, 2003).

Com isso, as ROS são responsáveis por regular o processo de cicatrização. Entretanto, conforme já descrito por Sies (1991) altas concentrações de ROS devem ser evitadas pelo organismo, visto que a reatividade destes radicais traz consequências celulares deletérias. Podem provocar a oxidação de biomoléculas o rompimento da homeostase celular. Dessa forma, deve se evitar o excesso destas, visto que, podem levar ao estresse oxidativo durante o processo de reparo tecidual, com isso gerar danos celulares, e assim, retardar esse processo de cicatrização de ferimentos (SCHÄFER; WERNER, 2008).

Visto a complexidade do processo de cicatrização, a utilização de terapias que visam auxiliar na resolução efetiva do ferimento, são de extrema relevância, entre elas está o uso de campos magnéticos (MATIC et al., 2009).

2.3 CAMPOS MAGNÉTICOS

Os campos magnéticos, ou também conhecidos por radiação magnética, são utilizados nos tratamentos de enferminades, principalmente no que diz respeito a reparação tecidual. Os mesmos, aceleram e potencializam o processo de cicatrização, pois promovem trocas com o potencial biomagnético do organismo, exercendo controle do fluxo sanguíneo, controle humoral e propriedades anti-inflamatórias (ÁLVARES; GARCÍA; BLANCO, 2007; SHANG et al., 2019).

A melhora na cicatrização obtida a partir da utilização de campos magnéticos é fundamentada por estudos que demonstram menores quantidades de citocinas inflamatórias a partir da exposição a esse campo, demonstrando assim seu efeito anti-inflamatório (WERNER; GROSE 2003; VARANI et al., 2017; PATRUNO 2018,). Além disso, também é evidenciado que os campos magnéticos promovem aumento do fluxo sanguíneo e a ativação de monofosfato de guanosina cíclico, resultando na liberação de fatores de crescimento, entre estes, o fator de crescimento de fibroblasto-2 (FGF-2), assim, estimulando a angiogênese. Através desse conjunto de fatores, o processo de cicatrização de pele é acelerado (CALLAGAN et al., 2008; CARRUTHERS; CARRUTHERS, 2012).

Strauch e colaboradores (2007), demonstraram que a partir da exposição a um campo magnético pulsado de 0,1 mT, 30 min por dia, é possível acelerar o processo de cicatrização. Ainda, evidenciam que há um aumento na força de tração do tecido cicatrizado, sendo este um fator de alta relevância clínica, devido a frequente necessidade da mobilização precoce de pacientes no leito de hospitais.

Bertolino e colaboradores (2006), constataram quem a partir de um campo magnético de 160 mT, continuamente durante 15 dias, foi possível acelerar o fechamento da ferida, além de ter uma atuação benéfica nos parâmetros histológicos, com maior infiltração de fibroblastos na fase proliferativa entre estes, melhorando assim a cicatrização. Ainda, outro estudo demonstra que com uma exposição de 30 minutos por dia, a um campo magnético alternado de 30Hz e 800 mT, também foi capaz de melhorar o fechamento da ferida nos ratos expostos (MATIC et al., 2009).

Quando expostos queratinócitos humanos (HaCaT) a um campo magnético sinusoidal de 2 mT (50 Hz) por 96h, é evidenciado um aumento na proliferação celular e modificações estruturais desses queratinócitos. Constatando assim, que a exposição de tecidos lesionados a campos magnéticos, irá beneficiar a regeneração da pele (MANNI et al., 2002).

Entretanto, quanto a atuação dos campos magnéticos no perfil oxidativo, a literatura é bastante controversa. Estudos relatam que estes podem atuar promovendo o estresse oxidativo, por vezes mantendo a homeostase redox, ou ainda, atuar como antioxidantes. Essa diferença se dá a partir dos parâmetros

de campo magnético utilizados. Ou seja, a frequência, intensidade e duração da exposição ao campo, são determinantes para o efeito que os mesmos irão causar no balanço redox (CANSEVEM et al., 2008).

Dessa forma, diversos estudos apontam que os campos magnéticos podem acabar gerando estresse oxidativo em diferentes tecidos (SHALABY; SHAWKI, 2006; HASHISH et al., 2008; EMRE et al., 2011). Contudo, foi demonstrado que a exposição de ratos a um campo magnético pulsado de 2 mT não ocasionou danos oxidativos, visto que, os biomarcadores oxidativos analisados: Substâncias que reagem ao ácido tiobarbitúrico (TBARS), superóxido dismutade (SOD), glutationa peroxidase (GPx), catalase (CAT) e glicose-6-fosfato desidrogenase; não demonstraram diferença entre os grupos, permanecendo com os parâmetros normais e sem danos (ERASLAN et al., 2007).

Ainda, um estudo *in vitro* com cultura de fibroblastos expostos a um campo magnético estático, evidenciou que o tratamento com o campo magnético não causou estresse oxidativo nos fibroblastos investigados, e que inclusive apresentou uma leve atividade antioxidante (GLINKA et al., 2018).

Glinka e colaboradores (2013) demonstrou que um campo magnético de 10 mT e frequência de 40 Hz, em 6 dias de tratamento, promoveu um aumento na atividade da glutationa S-tranferase (GST), ainda, em 14 dias de tratamento, uma diminuição nos níveis de TBARS e aumento na atividade da GPx no fígado de ratos. Dessa forma, concluindo que o tratamento promoveu ativação do sistema antioxidante enzimático e redução da lipoperoxidação (LPO).

Em concordância, Cansevan e colaboradores (2008), evidenciou que a exposição a campo magnético de baixa frequência de 50 HZ em diferentes intensidades (1, 2 e 3 mT 4-8h) levou a um aumento nos níveis de GSH no tecido cardíaco de ratos e uma diminuição na atividade da mieloperoxidase. Ainda, é reforçado pelo estudo citado que diferentes intensidades de campo, e o tempo de exposição, podem modificar o balaço redox também de forma negativa. Os níveis de malondialdeído (MDA), assim como, as ações enzimáticas, apresentaram-se elevados ou diminuídos, dependendo dos parâmetros utilizados.

Dentre os parâmetros, o tempo de exposição mostra-se determinante na resposta ao balanço oxidativo. A exposição a um campo magnético de 40 Hz, 7

mT, 30 min por dia, durante 14 dias, demonstrou não afetar o balanço oxidativo, mantendo inalterados os níveis de TBARS, glutationa (GSH), peróxido de hidrogênio, total de grupos sulfidrila livres no coração, e também, a capacidade antioxidante total do plasma de ratos. Entretanto, a exposição ao mesmo campo durante 60 min gerou estresse oxidativo (GORAKA; CIEJKA; PIECHOTA, 2010).

Visto que as ROS estão envolvidas no processo de cicatrização (WLASCHEK; SCHARFFETTER-KOCHANEK, 2005), e também que, quando em quadro de estresse oxidativo, estão relacionadas à patogênese de ferimentos de difícil cicatrização (SCHÄFER; WERNER, 2008), além de diversos outros prejuízos a macromoléculas de diferentes tecidos (SIES, 1991), é imprescindível a determinação de um tratamento com campos magnéticos, que seja capaz de auxiliar na cicatrização da pele e em conjunto não ocasionar danos oxidativos.

Ainda, devido a possibilidade de os campos magnéticos causarem estresse oxidativo em diversos tecidos, é sugerido a utilização dos mesmo em conjunto com antioxidantes. Desta forma, possibilitando a manutenção da homeostase redox e garantindo a estabilidade e segurança para o tratamento (CEYHAN et al., 2012; GHODBANE et al.,2015).

Esses antioxidantes podem ser oriundos de plantas, sendo classificados como antioxidantes naturais, como no caso a *Solanum sessiliflorum*, que tem o seu fruto conhecido como cubiu (RODRIGUES et al. 2013; MONTAGNER et al., 2020).

2.4 CUBIU (*Solanum sessiliflorum*)

O cubiu (*Solanum sessiliflorum*) é uma solanácea herbácea originária da bacia Amazônica, atualmente distribuido por toda a Amazônia brasileira, equatoriana, colombiana e venezuelana (Figura 3). O fruto é bastante nutritivo, tendo em sua composição ferro, niacina, ácido cítrico e, ainda, rico em pectina (COLODEL et al., 2017). Os nomes locais e tradicionais dessa planta são numerosos, sendo conhecida como “maná” na Amazônia, “tomate de índio” no estado de Pernambuco, “cocona” na Colômbia e na Venezuela, “topiro” no Peru e na Venezuela, além de “orinoco apple” ou “peachtomato” nos países de língua

inglesa. Assim, o cubiu possui compostos que são considerados auxiliares na promoção da sáude, como suas fibras e também minerais com ação antioxidante (PIRES et al., 2006; SERENO et al., 2018).

Figura 3- Cubiu

Fonte: INPA, disponível em: https://www.inpa.gov.br/cpca/areas/cubiu.html

Para a agroindústria moderna, o cubiu tem se constituido uma importante matéria-prima por ser uma planta fácil de ser cultivada e muito produtiva. Podendo também ser utilizado de inúmeras formas para a alimentação como sucos, doces, geléias (ARGOTE et al., 2013). Ainda, o cubiu é comumente utilizado pelas populações da Amazônia como medicamento e cosmético, tendo também por suas folhas e raízes uma utilidade medicamentosa popular. Dessa forma, na medicina tradicional, tem sido utilizado com alegação de propriedades hipoglicêmicas e hipolipidêmicas (VARGAS-ARANA et al., 2021). Além disso, o

cubiu é muito utilizado por populações locais, para cicatrizar ferimentos de pele (SILVA FILHO et al., 2005).

Os frutos são sub-globulosos, do tipo baga, vermelho-alaranjados quando maduros, de polpa ácida com sementes achatadas e apresentam-se em tamanhos que podem variar de 30 a 400g. Na Amazônia, ocorre a floração nos meses de setembro a novembro, tendo seu início quatro ou cinco meses após a germinação e sua produção de frutos sete meses após a semeadura (STEFANELLO et al., 2010).

Sua produção pode variar entre 40 a 100 toneladas por hectare e sua colheita pode ser realizada ao longo do ano (SOUZA et al., 2008). A planta é um arbusto ereto e ramificado, podendo crescer de 1 a 2m de altura (AGUDELO et al., 2016). Considerando a excelente adaptação da planta a região amazônica e o fato de ser um fruto anual, outro fator relevante é que sua produção pode ser realizada com pouco ou nenhum insumo, possibilitando sua comercialização por preços acessíveis (SILVA FILHO et al., 2005).

Além de ser cultivada em diferentes tipos de solos amazônicos, devido a sua crescente valorização, o cubiu tem sido levado a outras localidades como a zona da mata pernambucana e em distintas localidades da região sudeste e sul. No entanto, ainda são pouco conhecidos os efeitos que as condições edafo-climáticas da região ocasionam nos frutos, nos constituintes químicos e em seu tamanho e massa, assim limitando, em parte, a exploração comercial (STEFANELLO et al., 2010).

Um estudo realizado por Rodrigues e colaboradores (2013) caracterizou a presença de carotenoides e composto fenólicos no cubiu por cromatografia líquida de alta eficiência acoplada a detectores de arranjo de diodos e de espectrometria de massa (HPLC-DAD-MSN). Os principais carotenoides encontrados foram β-caroteno e luteina. Além disso, foi encontrado o ácido 5-cafeoilquínico, o qual foi o principal composto fenólico caracterizado, representando mais de 78% dos compostos fenólicos presentes. Ainda, relatam grande capacidade de sequestro de espécies reativas a partir do cubiu, evidenciando atividade antioxidante do mesmo.

Mascato e colaboradores (2015), chegam a conclusões distintas. Avaliam que no extrato hidroalcóolico de cubiu são presentes alcaloides, ácidos orgânicos, fenóis, glicosídeos flavonoides e cumarinas, enquanto que no extrato

aquoso encontram-se presentes antocianinas, gomas, taninos e mucilagem, grupos amino, ácidos voláteis e fixos. Entretanto, relatam menor capacidade de sequestro de espécies reativas. Ainda, é salientado no estudo a necessidade de demais pesquisas a fim de elucidar o potencial antioxidante do cubiu. Além disso, Hernandes et al. (2014) reportou a partir de um estudo *in vivo* utilizando ratos, que a poupa do cubiu demonstrou segurança para utilização por não apresentar efeitos genotóxicos nem citotóxicos.

Com base no uso tradicional do cubiu e na literatura apresentada, é plausível pressupor que a sua utilização isolada e em conjunto com campo magnético, possa vir a proporcionar efeitos benéficos na manutenção do equilíbrio redox e modulação do perfil inflamatório na cicatrização de pele.

3 MANUSCRITO

Os itens *materiais e métodos e resultados*, que fazem parte desta tese, estão apresentados sob a forma dos dois manuscritos a seguir.

O manuscrito 1 está submetidos a revista Fitoterapia (anexo B), em edições para o aceite. O manuscrito 2 está publicado na revista Evidence-based Complementary and Alternative Medicine, DOI: 10.1155/2022/7562569 (anexo A). Ambos os manuscritos estão redigidos conforme as normas das respectivas revistas.

3.1 MANUSCRITO 1

Phytochemical characterization, pharmacological properties and toxicity of Amazonian fruit cubiu *(Solanum sessiliflorum Dunal)*

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ABSTRACT

Cubiu or cocona (*Solanum sessiliflorum*) is a tropical fruit native to the Amazon region and used by local communities for skin medicinal and cosmetic purposes, despite the lack of data regarding the effectiveness and safety for these purposes. Based on this scenario, this study aimed to evaluate the pharmacological properties and safety of cubiu extract. The cubiu antimicrobial capacity was determined against strains of *Aeromonas caviae*, *Pseudomonas aeruginosa*, and *Sphingomonas paucimobilis*. Human peripheral blood mononuclear cells were used for the investigation of the anti-inflammatory potential. Human fibroblast cells were used to evaluate wound healing action (scratch assay) and also inflammatory parameters. The safety of cubiu extract was investigated using different methods (hemolysis, coagulation, cell viability, and genotoxicity tests). The 5-caffeoylquinic acid is the major compound in cubiu extract (84%). Cubiu extract was effective against the three bacterial strains tested and inhibited and destroyed the biofilm formed by *Pseudomonas aeruginosa*. Also, its increased anti-inflammatory cytokine levels, decreased pro-inflammatory cytokine levels, accelerated the wound healing process and showed no toxicity, maintained the hemocompatibility parameters in the biological range, and improved cell viability. From the pharmacological screening carried out in the present study, cubiu extract showed promise for use in skin diseases, promoting skin wound healing with antimicrobial and anti-inflammatory activities.

Keywords: *Solanum sessiliflorum*; Chlorogenic acid; Antimicrobial; Anti-inflammatory; Skin wound healing; Cubiu.

1 INTRODUCTION

Solanum sessiliflorum Dunal is a tropical plant of the Solanaceae family and native to the Amazon region. Its fruit, the cubiu or cocona, has a high amount of 5-caffeoylquinic acid [1,2], a compound belonging to the chlorogenic acid family that turns this fruit into a matter of great importance.

The biological actions already described for cubiu are associated with hypoglycemic, hypolipidemic, and antioxidant properties and are commonly used to treat diabetes, hypertension, and several other pathologies [3]. Furthermore, cubiu is popularly used to treat skin diseases, especially infections, and improve the wound healing process [1,4,5]. However, there is still no scientific data to confirm the safety and efficacy of using this fruit for such purposes, especially related to wound healing. Additionally, regarding wound healing, it is known that for a treatment to be effective, it must have antimicrobial and anti-inflammatory activities as its action mechanisms, which improve and accelerate the skin wound healing process and keep the skin intact and without infection [6,7].

Thus, this study aimed to determine cubiu extract pharmacological potential by investigating its antimicrobial and anti-inflammatory properties, wound healing effects, and safety.

2 METHODOLOGY

2.1 Cubiu extract preparation

This study is part of a project previously authorized by the Brazilian Ministry of the Environment to assess the components of genetic patrimony in the country (no. 010547/2013-4) and following Brazilian legislation (no. 2186-16). Cubiu

samples (~20 kg) were commercially acquired in the Municipal Market Adolfo Lisboa of Manaus (Amazonas State, Brazil). A botanic specialist Eduardo Vellez Marin (CRBio 09112-03) confirmed the fruits to be *Solanum sessiliflorum* Dunal. The cubiu samples were registered in the Genetic Heritage Management Council (CGEN, process no. A6723EB).

To obtain the cubiu extract, the fresh fruits (147 ± 38 g) were washed, peeled, and the pulp with small seeds was triturated using a mixer (particles ≤ 3mm) for approximately 5 min and placed into sealed amber glass containers with 70% absolute ethanol (Neon, São Paulo, SP, Brazil), where they remained for seven days and the solvent was changed three times. After extraction, the product obtained was filtered, evaporated, and then lyophilized[8]. The use of fresh fruit followed institutional, national, and international guidelines and legislation.

2.2 Cubiu extract phytochemical characterization

The phytochemical characterization of the cubiu extract was carried out by detecting phenolic and carotenoid compounds by high-performance liquid chromatography with a diode array detector (HPLC-DAD) according to the methodology validated by Quatrin et al. [9] and Rosso and Mercadante [10], respectively. The chromatograms for phenolic quantification purposes were obtained in a range from 280 nm to 360 nm, and 450 nm for carotenoids. All compounds were identified by comparing with the retention time of authentic standards and the spectral data obtained from UV-visible absorption spectra.

Stock solutions of phenolic compounds and carotenoid standard references were prepared in the initial mobile phase and diluted in eight equidistant points within the concentration range of LOQ-60 mg L^{-1}. The calibration curves of the

standards compounds are as follows: gallic acid: y = 79089x + 81326 (r = 0.998); chlorogenic acid (5-caffeoylquinic acid): y = 90494x + 497491 (r = 0.997); quercetin: y = 64434x - 373423 (r = 0.895), and (all-E)-β-carotene y = 19095x - 6503.8 (r = 0.999). The limits of detection (LOD) and quantification (LOQ) for gallic acid, chlorogenic acid, quercetin, and (all-E)-β-carotene were 0.012 and 0.037 ppm, 0.099 and 0.330 ppm, 0.146 and 0.444, and 0.089 and 0.25 ppm, respectively. Phenolic compounds belonging to the class of hydroxybenzoate derivatives were quantified as equivalent to gallic acid, those belonging to the class of hydroxycinnamate derivatives were quantified as equivalent to chlorogenic acid, those belonging to the class of flavonoids were quantified as equivalent to quercetin, and those belonging to the class of carotenoids were quantified as equivalent to (all-E)-β-carotene. Results were expressed as mg per 100 g of dry sample weight (mean ± standard deviation).

2.3 Antimicrobial activity

2.3.1 Microbial strains and inoculum preparation

The antibacterial activity was evaluated against standard strains of *Aeromonas caviae* (ATCC 15468), *Pseudomonas aeruginosa* (PA01), and *Sphingomonas paucimobilis* (CCT 7809). These strains belong to the Laboratory of Oral Microbiology (LAPEMICRO) bacterial collection of the Federal University of Santa Maria (UFSM), which permitted us to obtain the strains. The inoculum was prepared on Mueller-Hinton agar after bacterial growth (24 h and 35 °C ± 2). Colonies were suspended in sterile saline solution (0.85%) and adjusted to 0.5 on the McFarland scale (1 to 2 × 10^8 colony-forming units). For the broth microdilution assay, the standardized inoculum was diluted in a 1:10 ratio.

All tests of the antimicrobial activity of cubiu extract were performed in triplicate on three different days to ensure data reliability. Positive and negative growth controls were performed in all tests, had as a positive control the Mueller-Hinton agar and the bacteria that were studied, and as negative control the the Mueller-Hinton agar and cubiu extract.

2.3.2 Minimum inhibitory concentration and minimum bactericidal concentration determination

The lyophilized cubiu extract was diluted in Mueller Hinton broth to perform the antimicrobial and antibiofilm activity tests. Cubiu extract activity was evaluated on a dose-response curve at the concentrations of 0.01, 0.03, 0.1, 0.3, 1, 3, 10, and 30 mg/mL. The assays followed the standard protocols for each microbial group according to the Clinical and Laboratory Standards Institute (CLSI) M7-A9 (2015) [11].

The minimum inhibitory concentration (MIC) was determined by the broth microdilution method using sterile 96-well plates. The plates were prepared and incubated at 37 °C and, after the incubation period, the presence/absence of growth was observed. After the MIC reading, 10 µL were removed from the wells without growth, seeded, and reincubated at 37 °C. The absence of growth characterized the minimum bactericidal concentration (MBC).

2.3.3 Biofilm formation inhibition and biofilm destruction

Knowing that PA01 is a multidrug-resistant biofilm-producing bacterium [12], , biofilm formation inhibition and biofilm destruction were analyzed by the crystal violet assay using 96-well microtiter plates, according to Antunes et al. [13]. The

crystal violet retained in the adhered cells was dissolved with 200 µL of 95% absolute alcohol for 10 min, and bacterial growth was quantified by measuring the optical density at 570 nm with a spectrophotometer (Spectra-max M2e Multimode Microplate Reader, Molecular Devices, USA). The cubiu extract treatment was tested at the concentration obtained in the MIC and MBC determination against PA01 (Table 1) and in the half of this concentration. The results are demonstrated as absorbance values.

2.4 Anti-inflammatory potential of cubiu extract

Blood mononuclear cells (PBMC) were used to evaluate the anti-inflammatory activity of cubiu extract, which was tested at concentrations of 10 and 30 mg/mL since these were the concentrations chosen according to the concentration-effect curve of the antimicrobial tests. The extract was diluted in a PBMC culture medium, and all treatments and assays were performed in triplicate.

2.4.1 PBMC isolation

The PBMCs derived from discarded total peripheral blood samples from healthy adults, with no identification data, were obtained from the Laboratory of Clinical Analysis of the Franciscan University (LEAC-UFN). This experimental protocol was approved by the Ethics Committee on Human Beings of the Franciscan University (CAAE no. 31211214.4.0000.5306); the ethics committee released this study from the presentation of informed consent as these are discarded samples and have no identification data. All experiments were performed under institutional, national, and international guidelines and legislation, and this study was conducted as per the 1964 Helsinki Declaration and its later amendments.

Blood samples were initially processed to isolate the PBMCs using a density gradient difference protocol based on Ficcol Histopaque-1077® reagent (Sigma-Aldrich St Louis, MO, USA). After placing the blood in the reagent (1:1 v/v), the samples were centrifuged for 30 min at room temperature. The PBMCs were plated in 96-well plates with RPMI-1640 cell medium (Sigma-Aldrich St Louis, MO, USA) containing 10% fetal bovine serum and supplemented with 1% antibiotics. The cells were cultured at a concentration of $2x10^5$ cells/mL per well [14].

2.4.2 Evaluation of cubiu extract anti-inflammatory potential

The protocol was performed according to Maczynski et al. [15]. The PBMCs were plated in 96-well plates with 50 µL of phytohemagglutinin (PHA), a natural agent inducer of inflammatory responses [15], and incubated at 37 °C with 5% CO_2 for 48 h. The cells were then washed with PBS (1X; Gibco), and the treatments with cubiu extract were carried out and incubated once again for another 24 h. After this period, the plates were centrifuged, and the supernatant was removed for inflammatory cytokine levels determination and cell viability evaluation.

Interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (TNF-α) levels were evaluated using a human ELISA kit (eBioscience, San Diego, USA) according to the manufacturer's instructions. The results are expressed in pg/mL.

Cell viability evaluation was performed using the MTT assay as described in item 2.6.1.

As a negative control, cells that were untreated or stimulated by PHA were used, and untreated and PHA-stimulated cells were used as a positive control.

2.5 *In vitro* wound healing test

2.5.1 Cell culture

The HFF-1 cell line (ATCC®, CRL-2468TM) was obtained commercially from the Rio de Janeiro Cell Bank. The cells were thawed and maintained in polystyrene bottles in culture medium according to the American Type Culture Collection (ATCC). They contained 10% fetal bovine serum (Invitrogen) inactivated at 56 °C for 1 h, 100 U/mL penicillin (Invitrogen), and 100 U/mL streptomycin (Invitrogen) at 37 °C in a humid atmosphere containing 5% CO_2. Weekly replicates were performed in laminar flow for each bottle to receive 5 mL of medium with a fixed amount of cells at the time of the replicate ($2x10^5$ cells/mL). After obtaining a satisfactory confluence for the experimental trials, the cells were seeded in 96-well plates ($2x10^5$ cells/mL per well), and the dilutions were made in a culture medium specific for the cell line applied.

2.5.2 Scratch assay

96-well plates were used, a line was drawn in the middle of each plate well using a permanent marker to standardize the scratch and measurements before plating the cells, thus establishing a visual field in the well for analyses. The cells were plated, and after confluence/adhesion, the culture medium was removed. With the help of a needle, a continuous scratch was then made on the medial surface of each well, and this procedure led to a rupture of the contact between the cells and their removal from a certain region of the plate, thus forming a mechanical lesion in the cell monolayer. All wells were scratched (treatments with cubiu extract, positive control, and negative control), as a negative control, were used

cells untreated and not exposed to hydrogen peroxide were used, and as a positive control were used cells untreated and exposed to hydrogen peroxide. The wells were washed with PBS (Gibco) to remove the debrided cells according to the method of Seeliger et al. [16], and the treatment with cubiu extract (10 or 30 mg/mL) were performed. Thereafter, the proliferation of adjacent cells towards the free space in the plate was recorded at 48 h using photographic images.

To analyze the skin wound-healing process images was used a script developed in the Python programming language, according to Rossato et al.[17], which quantifies the number of cells present in the samples and establishes the percentage of wound healing. The results are demonstrated as a percentage of the total cells.

To investigate the anti-inflammatory action of cubiu extract during the wound healing process, the supernatants were collected to quantify IL-1, IL-6, IL-10, TNF-α, and INF-γ levels using a human ELISA kit (eBioscience, San Diego, USA) according to the manufacturer's instructions. The results are expressed in pg/mL.

2.6 Safety tests

PBMC were used for toxicity assays and erythrocytes for the hemocompatibility tests, that was tested at concentrations of 10 and 30 mg/mL. The PBMC and erythrocytes were derived from discarded total peripheral blood samples from healthy adults and isolated as described in item 2.4.1. The extract was diluted in a cells culture medium, and all treatments and assays were performed in triplicate.

Hydrogen peroxide (H_2O_2) at 100 µM was used as a positive control group, while just the cell in the culture medium was used as a negative control group.

2.6.1 Toxicity tests

Cell viability evaluation using MTT assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed on PBMCs as described by Fukui et al. [18]. PBMC were seeded in 96-well plates (2×10^5 cells/well) and treated with cubiu extract (10 or 30 mg/mL) or culture medium (negative control) for 24 h. After, 20 µL of MTT solution (0.01 M and pH 7.4) at 5 mg/mL diluted in PBS (1X phosphate buffer saline) was added to each well. Afterward, the plates were protected from light and incubated in an incubator at 37 °C for 2 h. Subsequently, the supernatant was removed from the wells, and the cells were resuspended in 150 µL of dimethyl sulfoxide (DMSO). Absorbance was read on a microplate reader at 560 nm. The results were expressed as a percentage of the negative control.

Fluorimetric DNA quantification assay using PicoGreen reagent

The free DNA in the medium was quantified using the PicoGreen reagent (Invitrogen Life Technologies, Carlsbad, USA) and performed according to Sagrillo et al. [19]. The PicoGreen reagent was added to PBMC in 96-well plates (2×10^5 cells/well) and incubated in an oven at 37 °C for 5 min. The fluorescence was read on a spectrofluorometer (480 nm excitation and 520 nm emission). The results were expressed as a percentage of the negative control.

Reactive oxygen species quantification - 2'-7'dichlorofluorescin diacetate assay

Reactive oxygen species (ROS) were quantified as described by Esposti et al. [20] through the 2'-7'dichlorofluorescin diacetate assay. The cubiu extract treatments (50 µL), Tris buffer (60 µL), and 2',7'-dichlorofluorescein diacetate reagent (10 µL; DCFH-DA) were added to PBMC in 96-well plates (2×10^5 cells/well). The plates were protected from light and incubated in an oven at 37 °C for 1 h. Fluorescence reading was carried out on an ELISA reader (488 nm excitation and 525 nm emission). The results were expressed as a percentage of the negative control.

2.6.2 Hemocompatibility tests

Hemolytic activity

Hemocompatibility tests were performed according to Souza Filho et al. [21]. Blood was added with a phosphate-buffered saline (PBS) 1X solution (1:1 v/v) and centrifuged for 15 min at 1000 rpm, and the supernatant was discarded (procedure performed three times). Afterward, 50 µL of washed red blood cells and 10 µL of treatment were added to microtubes containing 1 mL of PBS 1X at different pH (pH 7.2 to simulate sepsis due to metabolic acidosis, pH 7.4 to simulate the body's normal pH, and pH 7.5 to simulate alkalinity). The control groups were as follows: NC (red cells + 0.9% sodium chloride), PC (red cells + distilled water), and surfactants (red cells + surfactant mixture, Tween 60 + Span 60) at the same concentrations of the treatments. The tubes were incubated at room temperature and under rotation for 1 h. Subsequently, they were centrifuged for 15 min at 1000 rpm, followed by transferring 200 µL of the supernatant to a 96-well plate and read on an ELISA reader at 540 nm. The results were expressed as a percentage of the negative control.

Coagulation test

The coagulation test was carried out according to Souza Filho et al. [21]. Total blood levels were collected in a citrate tube and centrifuged for 10 min at 2500 rpm. Then, 225 µL of plasma was placed in 96-well plates with 25 µL of treatments and incubated at 37 °C for 30 min. Two independent experiments were performed in duplicate with different donors. Readings were carried out on the coagulometer Quick Timer II (Drake) that had been previously calibrated according to the manufacturer's recommendations for tests of hemostasis prothrombin time (PT; Labtest - Lot no.: 4008) and activated partial thromboplastin time (aPTT; Labtest - Lot no.: 4006). For the normal aPTT value, the range between 25 and 35 s was used as a reference, while the range between 11 and 15 s was used for the baseline PT values [22,23].

2.7 Statistical analysis

The GraphPad Prism (version 5.0; GraphPad Software, La Jolla, USA) software was used for statistical analyses and to create the figures. Data were expressed as mean ± standard error. The homogeneity of variances was verified with Levene's test, and treatments were compared using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test; $p<0.05$ was considered significant.

3. RESULTS

3.1 Cubiu extract characterization

The cubiu extract used in this study had its composition evaluated by HPLC-DAD, and the chromatographic profile is shown in Fig. 1. Due to the polarity characteristics of the solvent (ethanol 70%) used during the extraction process, only phenolic compounds were found in the lyophilized extract. However, traces of lutein were observed, but in quantities below the LOQ. The total phenolic compound content in the cubiu extract was 456.31 ± 19.78 mg/100 g of lyophilized extract. The phenolic compounds found in the extract belong to the phenolic acid class, being the hydroxybenzoic class with 11.61 ± 3.50 mg/100 g and hydroxycinnamic with 444.70 ± 16.28 mg/100 g. The major compound identified was 5-caffeoylquinic acid, which had a concentration of 392.92 ± 13.25 mg/100 g of extract, corresponding to 84% of the compounds quantified.

3.2 Cubiu extract antimicrobial and antibiofilm activity

Initially, the antimicrobial potential of cubiu extract was investigated from a curve with concentrations range of 0.01 - 30 mg/mL against *A. caviae*, *S. paucimobilis*, and *P. aeruginosa* (PA01), which are the bacteria that affect various tissues (e.g., the skin) and cause mild and severe infections in hospitals [24–26]. According to the results presented in Table 1, the cubiu treatment acted as a bacteriostatic and bactericide against all strains tested.

Knowing that *P. aeruginosa* (PA01) is a multidrug-resistant biofilm-producing bacterium [12], the analysis of biofilm inhibition and destruction was also performed. It was possible to observe that all tested concentrations of the cubiu extract inhibited biofilm formation and destroyed the previously formed biofilm (Fig. 2). The treatment with cubiu extract showed a decrease of 36.53 and 35.06% on the inhibited biofilm formation when treated with 15 and 30 mg/mL,

respectively (Fig. 2a) compared with the positive control. Moreover, the destruction of biofilm previously formed showed a decrease of 49.64 and 42.17% when treated with 15 and 30 mg/mL of cubiu extract, respectively (Fig. 2b) compared with the positive control.

3.3 Anti-inflammatory potential of cubiu extract

The anti-inflammatory potential of cubiu extract was tested against the inflammation process induced by PHA, a natural agent inducer of inflammatory responses. PHA was able to induce the inflammatory process, as can be seen in Fig 3 by the increased levels of IL-1, IL-6, TNF-α, and INF-γ levels when compared to the control without the addition of PHA (negative control).

The results showed that anti-inflammatory activity at 10 and 30 mg/mL led to a sharp drop in IL-1, IL-6, TNF-α, and INF-γ levels compared to the positive control, as well as an increase in IL-10 levels when compared to the positive control (Figs. 3a, 3b, 3c, 3d, and 3e, respectively). Furthermore, the MTT assay revealed that the treatments with 10 and 30 mg/mL of cubiu extract had a reduction compared to the positive control, bringing cell viability to basal levels (Fig. 3f).

3.4 *In vitro* wound skin wound healing - scratch assay

The cubiu extract (10 and 30 mg/mL) proved to have skin wound healing properties compared to the negative and positive control groups (Fig. 4a). Moreover, the samples in both concentrations acted as anti-inflammatory agents during the skin wound-healing process due to the significant decrease in pro-inflammatory cytokine levels (IL-1, IL-6, TNF-α, and INF-β) and increased anti-

inflammatory cytokines (IL-10) when compared to the positive control (Figs. 4b, 4c, 4d, 4e, and 4f, respectively).

3.5 Safety tests

The concentrations of cubiu extract that have been shown to have a positive effect on wound healing were evaluated for their safety. Toxicity tests have shown that cubiu extract has no toxic effects at the concentrations tested. Cell viability evaluation by MTT assay demonstrated that the cubiu extract treatments at doses of 10 and 30 mg/mL did not affect the cell viability (Fig. 5a). The results for detecting double-stranded DNA damage in cell culture, by fluorimetric DNA quantification assay using PicoGreen reagent, demonstrated that no DNA damage was observed at any of the tested concentrations (Fig. 5b). Furthermore, cubiu extract *per se* does not cause an overproduction of ROS (Fig. 5c), evaluated through the 2'-7'dichlorofluorescin diacetate assay. On the other hand, the exposure of cells to H_2O_2 100 µM (positive control group) reduced cell viability and increased ROS levels, as expected (Fig. 5a and 5c, respectively).

The hemocompatibility tests demonstrated that the hemolytic activity was not altered by any cubiu extract concentration at pH 7.2 (acidosis), pH 7.4 (physiological), and pH 7.5 (alkalosis), as illustrated in Figs. 6a, 6b, and 6c, respectively. Furthermore, were no results outside the biological range in the PT (Fig. 6d) and aPTT (Fig 6e) coagulation tests.

4 DISCUSSION

This study aimed to investigate the pharmacological potential of the cubiu extract, focusing on skin wound healing. The cubiu is the fruit of *S. sessiliflorum* and is

native to the Amazon rainforests of Brazil, Ecuador, Colombia and Peru. It is rich in fiber, minerals, iron, niacin, citric acid, and pectin [27], and the presence of phenolic compounds stands out in its composition, including chlorogenic acids, more specifically 5-caffeoylquinic acid as the major compound [2]. In agreement, we also found phenolic compounds in the cubiu extract, with 5-caffeoylquinic acid being the major compound. Hence, it is seen that Solanaceae family plants have caffeoylquinic acids in their composition to which are attributed to several of their biological activities that benefit human health [28].

The skin wound healing process occurs in three distinct and overlapping phases. The first phase is the inflammatory phase, marked by the presence of inflammatory cells and mediators. The next phase is known as proliferation, marked by the presence of fibroblasts and synthesis of the extracellular matrix, and the last phase is tissue remodeling, marked by the reorganization of the extracellular matrix and maturation of collagen fibers [6,29]. Infection and other factors can affect wound healing and contribute to the pathogenesis of chronic wounds.

Skin wound infection can lead to the alteration of the processes along the wound healing pathway. Bacteria produce inflammatory mediators that prolong the inflammatory phase and inhibit the epithelialization phase of skin wound healing process. Additionally, bacteria in an infected wound cause cell death, which will lead to a further increase in local inflammation response and prolongation of acute inflammatory phase [6]. In view of this, the antimicrobial activity of the cubiu extract was investigated and was observed that fruit extract of *S. sessiliflorum* have bacteriostatic and bactericidal activity against *A. caviae*, *S. paucimobilis*, and *P. aeruginosa* (PA01), which are bacteria of clinical relevance that affect the

skin (and also other tissues) and cause mild and severe infections [24–26]. Furthermore, knowing that PA01 is a multidrug-resistant biofilm-producing bacterium [12], we also tested the cubiu extract effectiveness in inhibiting biofilm formation and destroying this biofilm when previously formed by PA01. We evidenced that the cubiu extract could effectively inhibit biofilm formation by PA01 and destroy the biofilm previously formed by this bacterium at all concentrations tested. Biofilm is mainly associated with prolonged infections and is an important mechanism used by microorganisms to survive antibiotic treatments. Additionally, the biofilm formed by PA01 is closely related to infections in chronic skin wounds [12,30].

Various conventional antimicrobial agents have become less effective against microorganisms as they have become increasingly resistant, making it indispensable to search for new alternatives with antimicrobial activity. Thus, research with medicinal plants is a crucial alternative to treat different infectious diseases, as many of these plants have bioactive components with antimicrobial properties [31,32]. Therefore, plant extracts and their constituents are being used to combat numerous resistance mechanisms of microorganisms [33,34]. Given this scenario, we can highlight the potential use of the cubiu extract as a bacteriostatic and bactericidal agent to prevent and treat bacterial biofilms, and the antimicrobial potential exerted by this fruit can be related to its phytochemical composition. Among the bioactive substances, phenolic compounds are highly relevant concerning antimicrobial properties [35]. In a similar study, Rodrigues et al. [36] corroborate our findings by demonstrating that pitanga (*Eugenia uniflora* L.) extract was effective against bacterial biofilm, attributing this result to the presence of phenolic compounds in the fruit extract.

Evidence suggests that the possible mechanism of antimicrobial compounds is related to the cascade of reactions involving the bacterial cell [37,38]. These reactions may involve the antioxidant capacity of phenolic compounds, which may be important in the antimicrobial mode of action since these natural compounds can reduce the production of essential metabolites for microorganism survival under stress conditions. Hence, it is plausible that the cubiu extract attached and incorporated itself into the biofilm structure, impairing signaling pathways and inducing cell membrane disruption [39,40].

When talking about skin wound healing, it is essential to emphasize the crucial role of cytokines, these are essential mediators to conduct the inflammatory response to injured regions, actively participating in the wound healing process. They might be pro-inflammatory and responsible for manifesting and propagating inflammation like IL-1, IL-6, TNF-α, and INFg; or yet, they might be anti-inflammatory, like IL-10, acting as an inhibitor of inflammation [29]. However, the exacerbated production of pro-inflammatory cytokine at the lesion may retard repair, and also induce harmful systemic manifestations, such as hemodynamic instability and metabolic disturbs. Thus, after severe injuries or infections, the persistence of these cytokines could lead to lesions at target organs, leading to multiple organ failure and even death. Anti-inflammatory cytokines can minimize this impairment [41].

Knowing this, the next step of the present investigation was to evaluate whether the cubiu extract had anti-inflammatory potential. Thus, by using PBMCs (simulating a systemic response) we aimed to verify the anti-inflammatory properties of the cubiu extract against PHA, a natural agent inducer of inflammatory responses [15]. It was possible to evidence that the cubiu extract

(10 and 30 mg/mL) has anti-inflammatory activity, restoring cell viability, decreasing pro-inflammatory cytokine levels (IL-1, IL-6, TNF-a, and INF-g), and increasing anti-inflammatory cytokine levels (IL-10).

The phenolic compounds present in Solanaceae family plants provide anti-inflammatory properties [28], which may be related to your ability to inhibit enzymes such as prostaglandin synthetase, lipoxygenase, and cyclooxygenase, which are involved in the inflammatory process. Moreover, it is also known that chlorogenic acid, which is present in the composition of the cubiu, interferes with the response of leukocytes to chemokines, also preventing the interaction with adhesion molecules involved in cell migration during the inflammatory process [42].

Since cubiu extract has antimicrobial properties against bacterial strains that affect the skin, as well as anti-inflammatory activity, we tested whether it would be effective for wound healing in the scratch assay using HFF-1 cells. The scratch assay demonstrated the skin wound healing effect of the cubiu extract. The results of the present investigation agree with the reports of the popular use of cubiu to treat skin wounds [4,5], until then without scientific proof. Thus, this study is a pioneer to prove the beneficial effect of the cubiu extract to improved skin wound healing and this positive action may be related to the phytochemical composition of the extract used. It is known that phenolic substances present in natural extracts have skin wound healing properties [43,44].

Knowing that exacerbated production of pro-inflammatory cytokine at the lesion may retard repair, was tested whether the mechanism of action of cubiu extract in wound healing would be through the control of local inflammation. The anti-inflammatory activity of cubiu was also evidenced in the scratch assay using HFF-

1 cells, as both extract concentrations decreased pro-inflammatory cytokine levels (IL-1, IL-6, TNF-a, and INF-g) and increased anti-inflammatory cytokine levels (IL-10) in the wound healing process.

It is well known that wound healing treatments operate mainly at the first stage of healing, the inflammatory phase, contributing to repair due to anti-inflammatory properties and preventing infections. Thereby, the treatments that improve skin wound healing should promote antimicrobial and anti-inflammatory activities, since infections and prolonged inflammatory processes delay the healing process [6,7]. As for the cubiu extract, we proved that all these indispensable properties that enhance skin wound healing are found in it, therefore justifying the significant skin wound healing potential of this Amazonian fruit at the concentrations tested. Knowing that concentrations of 10 and 30 mg/mL of cubiu extract have promising biological activities, it is vital to verify if these concentrations are safe to use. Therefore, we followed up on the toxicity study of the cubiu extract using PBMC, and our findings revealed that it did not present toxicity due to maintaining hemolysis and blood coagulation patterns within the biological range. In addition, the extract did not cause alterations in cell viability or damage to double-stranded DNA and does not overproduce ROS, demonstrating safety for the use of cubiu extract. In agreement, Hernandes et al. [45] reported that cubiu does not present cytotoxic or genotoxic effects, further echoing that it is safe to consume.

Thereby, this is the first study to prove that the cubiu extract is an important compound against skin diseases, promoting skin wound healing and antimicrobial and anti-inflammatory activities.

5 CONCLUSIONS

Considering the focus of this study, we sought to demonstrate the phytochemical composition of the cubiu extract and increase knowledge about the plant due to its importance in folk medicine. Additionally, we evaluated its toxicity, pharmacological properties in skin wound healing, antimicrobial activity against strains that affect the skin, and anti-inflammatory activity. From the pharmacological screening carried out in the present study, cubiu extract showed promise for use in skin diseases, promoting skin wound healing, antimicrobial and anti-inflammatory activities, and it's safe to use.

Acknowledgments

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) [grant number 88882.428091/2019-01 and Edital nº 11/2009 – Pró-Equipamentos], and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS, RS, Brazil)

Declarations

All authors declare that there are no funding sources, employment, or personal financial competing interests that could influence the position presented in this manuscript. In addition, authors are not aware of any institutional competing interest of any nature or kind.

Author contribution: All authors participated in the conception, design, study, and interpretation of cited references, analysis of the results wrote and review of the manuscript. LFB guided and supervised all stages of the research. JFD designed

the research, participated in all experimental stages, and wrote the manuscript. GFFSM was responsible for obtaining and preparing the fruits and hydroalcoholic extract. TE and SS performed the phytochemical analysis of the extract and wrote the manuscript. RCV, VSM, and CMV participated in the analysis and results of microbiology. MRS Performed the analyses and results regarding peripheral blood mononuclear cells and human fibroblasts. MMFD performed the inflammatory cytokine analysis. All authors read and approved the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Figure 1 - Phytochemical chromatographic profile of the cubiu extract

“a” corresponds to cubiu extract chromatogram, and “b” corresponds to analytical standard of 5-caffeoylquinic acid. Chromatograms were acquired at 320 nm.

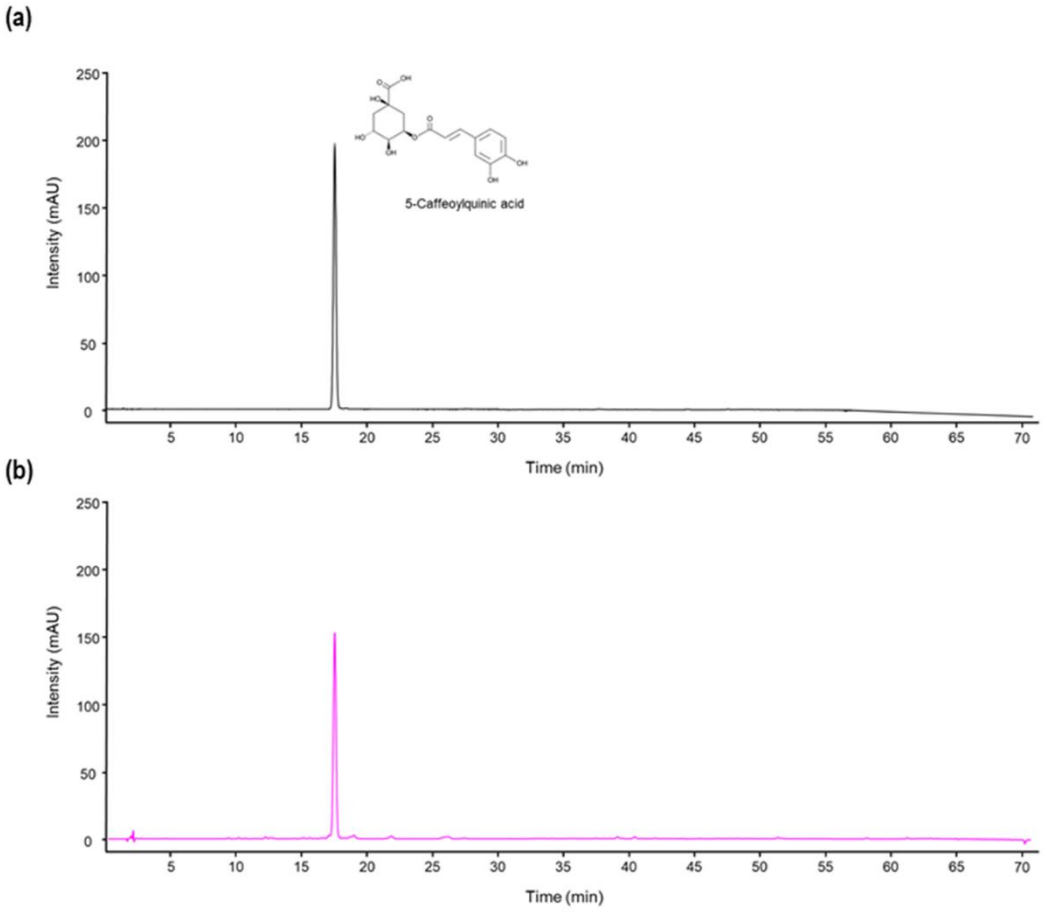

Figure 2 - Action of cubiu extract against *P. aeruginosa* (PA01) biofilms.

“a” corresponds to the inhibition of *P. aeruginosa* (PA01) biofilm formation, while “b” corresponds to the destruction of the *P. aeruginosa* (PA01) biofilm. The results are demonstrated as absorbance values. Data were expressed as mean ± standard error (SE). Values with $p < 0.05$ were considered statistically significant. “*” indicates a difference of negative control (NC), and “#” indicates a difference of positive control (PC).

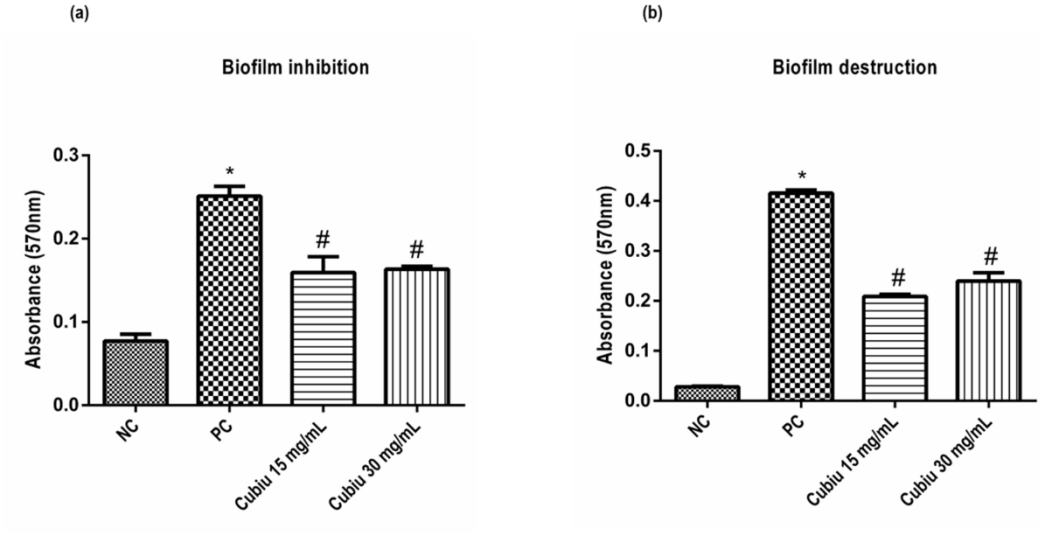

Figure 3 - Evaluation of cubiu anti-inflammatory activity.

The pro-inflammatory cytokines IL-1, IL-6, TNF-a, and INF-g are demonstrated as the letters “b,” “c,” “d,” and “e,” respectively. The anti-inflammatory cytokine IL-10 is demonstrated as the letter “f”. Interleukins results are expressed in pg/ml. “f” corresponds to the cellular viability by MTT assay (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazoline bromide). Data were expressed as mean ± standard error (SE). Values with $p < 0.05$ were considered statistically significant. “*” indicates a difference of negative control (NC), and “#” indicates a difference of positive control (PC).

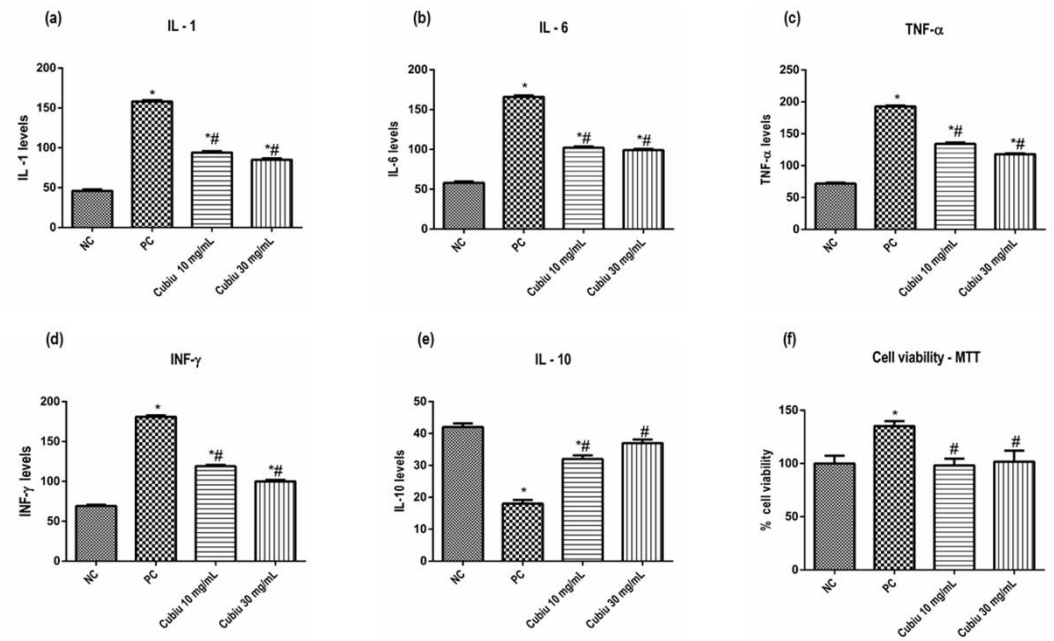

Figure 4 - *In vitro* skin wound healing - scratch assay.

“a” corresponds to skin wound healing activity; the results are demonstrated in % of cells. The pro-inflammatory cytokines IL-1, IL-6, TNF-α, and INF-g are demonstrated as the letters “b,” “c,” “d,” and “e,” respectively. The anti-inflammatory cytokine IL-10 is demonstrated as the letter “f.” The results are expressed in pg/ml. Data were expressed as mean ± standard error (SE). Values with $p < 0.05$ were considered statistically significant. “*” indicates a difference of negative control (NC), and “#” indicates a difference of positive control (PC).

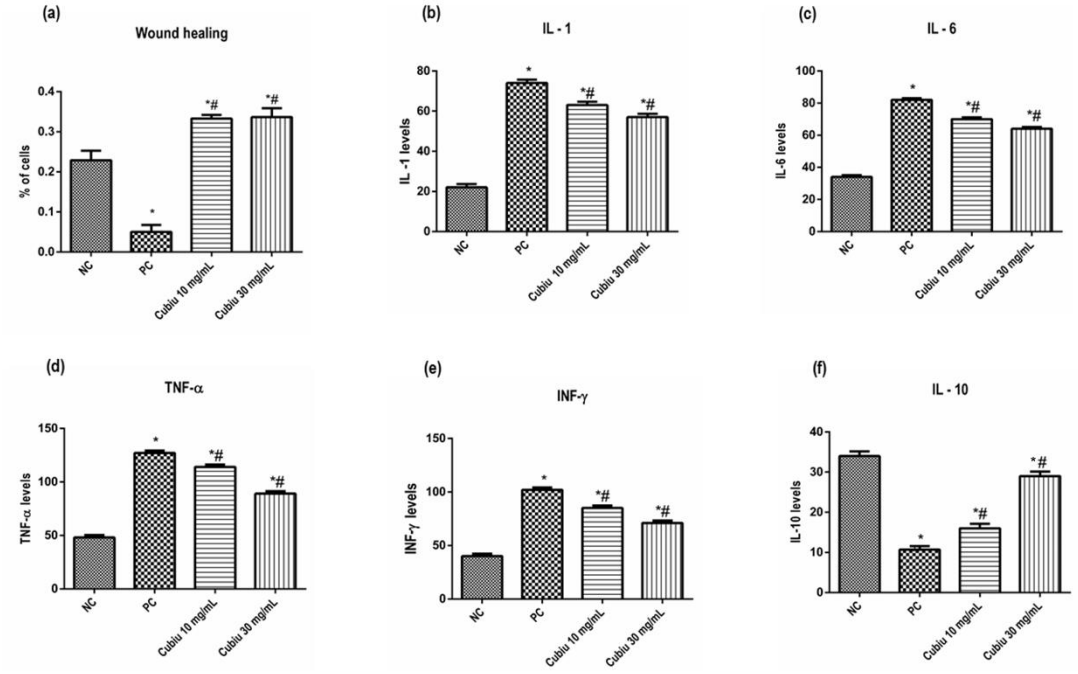

Figure 5 - Toxicity assays.

“a” corresponds to cell viability by MTT assay [3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazoline bromide]. “b” corresponds to the quantification quantity of free dsDNA (PicoGreen assay). “c” corresponds to the quantification of total levels of reactive oxygen species by 2',7'-dichlorofluorescein diacetate (DCF) assay. The data are presented as % of the untreated control group. Data were expressed as mean ± standard error (SE). Values with $p < 0.05$ were considered statistically significant. “*” indicates a difference of negative control (NC), and “#” indicates a difference of positive control (PC).

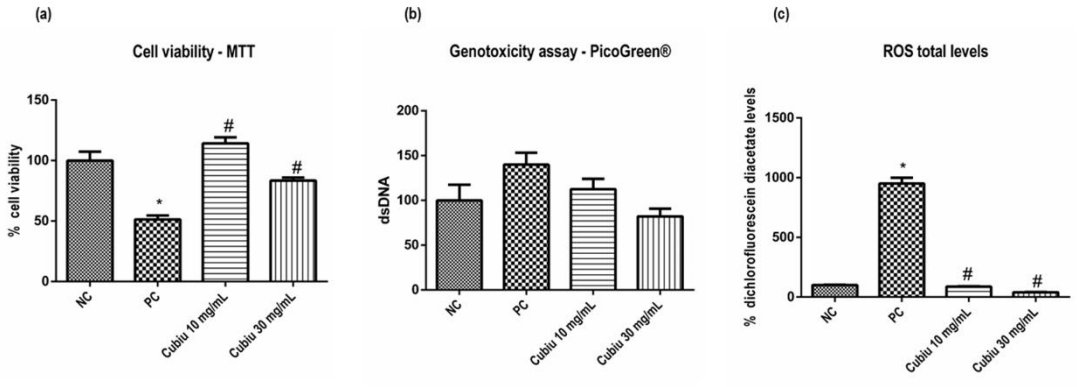

Figure 6 - Hemocompatibility assays.

The hemolytic activity at pH 7.2, 7.4, and 7.5 are represented as the letters “a,” “b,” and “c,” respectively. The results are expressed as a percentage of the negative control. For the coagulation tests, the prothrombin time test (PT) is reported as the letter “d,” and the activated partial thromboplastin time test (aPTT) in the letter “e.” For baseline PT values, the interval between 11 and 15 s was used, while for aPTT, the interval between 25 and 35 s was used as reference. Data were expressed as mean ± standard error (SE). Values with $p < 0.05$ were considered statistically significant. “*” indicates a difference of negative control (NC), and “#” indicates a difference of positive control (PC).

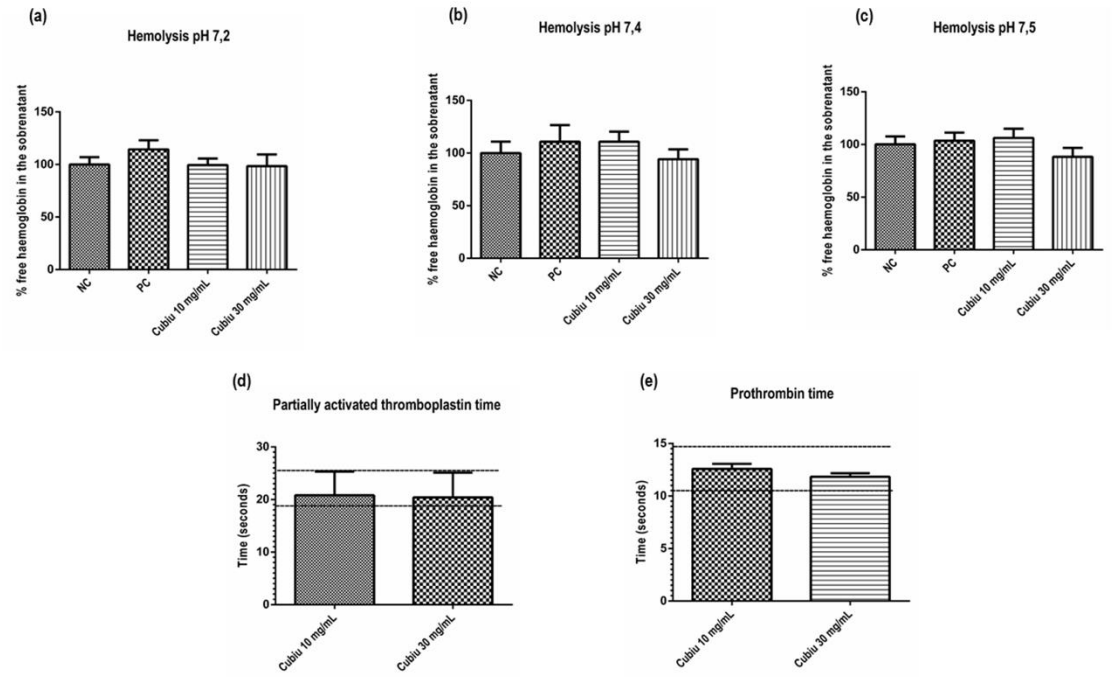

Table 1 – *In vitro* susceptibility assay of the cubiu extract against standard strains.

Microorganism	**Cubiu** (*Solanum sessiliflorum)*	
	MIC	**MBC**
Aeromonas caviae (ATCC 15468)	10 mg/mL	30 mg/mL
Pseudomonas aeruginosa PA01	30 mg/mL	30 mg/mL
*Sphingomonas paucimobilis (*ATCC 7809)	10 mg/mL	10 mg/mL

ATCC: American Type Culture Collection.

3.2 MANUSCRITO 2

Toxicity, anti-inflammatory and antioxidant activity of cubiu (*Solanum sessiliflorum*) and its interaction with magnetic field in the skin wound healing

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Abstract

Cubiu, an Amazonian fruit, is widely used as food and popular treatment for pathologies that present an inflammatory pattern, such as skin wound healing. However, there is still no confirmation in the scientific literature about the safety profile, as well as the anti-inflammatory, antioxidant and healing actions of cubiu. This study is divided into two experimental protocols using Wistar rats. Thus, the first objective (protocol 1) of this study was to evaluate the toxicity of an oral administration of cubiu extract at different doses for 28 days. The macroscopic and microscopic analysis of the liver and kidney was performed, and the following analysis were determined in the plasma: glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, gamma-glutamyl transpeptidase, glucose, triglycerides, total cholesterol, urea, creatinine and uric acid. After, we conducted the second protocol aimed to establish the potential antioxidant and anti-inflammatory capacity of cubiu and its interaction with magnetic field in skin wound healing. On days 3, 7 and 14 of treatment, skin and blood samples were collected, and was analyzed: the oxidative stress biomarkers (reactive substances to thiobarbituric acid, non-protein thiols, superoxide dismutase, catalase, and glutathione S-transferase), myeloperoxidase enzymatic activity and cytokines levels (interleukin 1, interleukin 6, interleukin 10, and tumor necrosis factor-alpha). The cubiu has shown to be safe and non-toxic. Both cubiu and magnetic field promoted decreased levels of pro-inflammatory and pro-oxidant biomarkers (interleukin 1, interleukin 6, tumor necrosis factor-alpha and reactive substances to thiobarbituric acid), as well as increased levels of anti-inflammatory and antioxidant biomarkers (interleukin 10, non-protein thiols and superoxide dismutase), with greater potential when treatments are used in association. Thus, cubiu promotes antioxidant and anti-inflammatory action in skin wound healing, while also improving results of the conventional treatment for skin healing (magnetic field) when used in association.

Keywords: Pulsed magnetic field, *Solanum sessiliflorum* Dunal, cocona, topiro, maná.

1. Introduction

Solanum sessiliflorum (Dunal) is a Solanaceae native of the Amazon Basin, currently found throughout the Brazilian, Ecuadorian, Colombian and Venezuelan Amazonian territory. Its fruit, called cubiu in Portuguese, also known as “apple/peach tomato”, is widely used as food, it can be used in numerous ways such as juices, sweets, jellies and consumed *in natura* [1]. Furthermore, cubiu is very nutritious, rich in iron, niacin, citric acid, and pectin. Thus, cubiu presents compounds considered adjuvants for health promotion, such as fibers, minerals and bioactive compounds like phenolic acids [2].

Amazonian people commonly employ cubiu as a remedy or cosmetic. In their traditional medicine system, cubiu is used because of its hypoglycemic, hypolipidemic and anti-oxidant properties to treat diabetes and skin wound healing [3–5]. However, no studies have investigated the anti-inflammatory properties of cubiu, considering the inflammatory profile of the pathologies traditionally treated with this plant. Moreover, the *in vivo* antioxidant potential of cubiu is scarcely approached in scientific literature. Cubiu also could be a suitable alternative for use in combination with magnetic fields. Therapy with magnetic fields is used for skin wound healing, but its systemic performance in the oxidative and inflammatory profile is controversial in the scientific literature. Considering these data, an investigation of magnetic fields associated with antioxidants was suggested [6,7]. Among these substances, there are natural antioxidants, primarily contained in plants. As previously mentioned, cubiu is commonly used for its antioxidant properties [8], and also popularly used for skin wound healing. Therefore, we believe to be pertinent to elucidate the effects of cubiu combined with magnetic field therapy.

Thus, the first objective of the present study was to evaluate the oral toxicity of cubiu. After completing this objective, the potential antioxidant and anti-inflammatory capacity of cubiu and its interaction with magnetic fields in an *in vivo* model of skin healing was analyzed.

2. Materials and methods

2.1 Cubiu extract preparation

Cubiu samples were purchased in the Municipal Market Adolfo Lisboa – Manaus city, Amazonas, Brazil. Botanic specialist Eduardo Vellez Marin (CRBio 09112-03) confirmed the fruits to be *Solanum sessiliflorum* Dunal. This research is part of a project previously authorized by the Brazil Environmental Ministry to assess the components of genetic patrimony in national territory (n° 010547/2013-4) according to Brazilian legislation (n° 2186-16). The cubiu samples were registered in the Management of Genetic Patrimony Council, Brazil (CGEN, process number A6723EB).

The fresh fruits of cubiu weighed 147 ± 38 g. The preparation of the cubiu extract included washing and peeling the fruits, as well as grinding the pulp with small seeds in a mixer for 5 min and then extracting with 70% absolute ethanol (Neon, commercial-03467; São Paulo, SP, Brazil). After extraction, the product obtained was filtered, evaporated, lyophilized and was stored in a -20 degrees freezer [9]. Subsequently, the lyophilized cubiu extract was diluted daily in saline and administered via oral gavage for oral administration. The use of fresh fruit followed institutional, national and international guidelines and legislation.

2.2 Experimental animals

Male Wistar rats (90 days of life, weighing 150 g) were obtained from the Central Animal Facility of the Federal University of Santa Maria (UFSM), and were maintained during the experimental protocol at the Animal Facility of the Physiology Department (UFSM), under controlled environmental conditions (23°C ± 1), 12-hour light/dark cycle, food and water provided *ad libitum*. This research was conducted following the Animal Research: Reporting of *in vivo* Experiments (ARRIVE guidelines) and the internationally accepted guidelines on animal welfare (EEC Directive 1986; 86/609 / EEC), and in agreement with national and institutional rules. The experimental protocol was approved by the Ethics Committee on Animal Use of the Federal University of Santa Maria, registration nº 119/2013.

2.3 Protocol 1: Toxicity evaluation of cubiu extract by dose-response curve

A curve was proposed for different doses of the cubiu extract diluted in saline and administrated by oral gavage for 28 days (n = 3 animals per group, total = 12

animals). This allowed verifying the toxicity of the cubiu extract and the most adequate or beneficial dose for Protocol 2. Tested doses of cubiu extract were 25 mg/kg, 50 mg/kg, 100 mg/kg and 150 mg/kg per rat body weight. This protocol had a control group that received only saline only. The toxicity was performed according to OECD guideline 407 (OECD, 2008) [10], with slight modifications.

2.3.1 Biochemical analyses

Plasma analyses of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), gamma-glutamyl transpeptidase (GGT), glucose, triglycerides, total cholesterol, urea, creatinine and uric acid were performed using a commercial kit (Bioclin®), according to manufacturer's instructions. These analyses were processed in an automatic biochemical analyzer (Mindray BS-120®). The results were expressed in UI/L (GOT, GPT); U/L (GAMA GT); mg/dL (triglycerides, total cholesterol, urea, creatinine, uric acid); and g/dL (glucose).

2.3.2 Morphological analyses

Organs were evaluated macroscopically regarding preservation of organ architecture, presence of hemorrhage and any aspects associated with degeneration, in addition to, shape, size, color and general appearance. After the euthanasia, the samples were fixed in 10% buffered formalin and embedded in histological paraffin for light microscopic examination after removal. Sections (6-μm thick) were stained using the hematoxylin and eosin method. Two independent observers performed double-blind analysis. For liver samples, following criteria were evaluated: presence of inflammatory infiltrate, cellular degeneration or necrosis, congestion or dilatation of the central vein or liver sinusoids and presence of sinusoidal vacuolization. For kidney samples, evaluated criteria were dilatation of the Bowman's capsule, dilatation of subcapsular space, dilatation of proximal and distal convoluted tubules, glomerular and tubular degeneration [11].

2.3.3 Oxidative status

Evaluation of oxidative status was conducted in homogenized hepatic and renal tissue. To homogenize tissue samples, 9 mL of sodium phosphate buffer 0,3M

(KCl 140 mM, pH 7.4) were used per gram of tissue. Phenylmethylsulfonyl fluoride (PMSF) was added at the concentration of 100 nM diluted with isopropanol, 10 µL of PMSF per mL of buffer. Homogenization was performed for 60 seconds in Ultra-Turrax at 0°C to 4°C. Centrifugation of homogenate was carried in refrigerated equipment for 10 minutes at 3000 rpm (1100*g*).

2.3.3.1 Total protein determination

Protein content in the homogenate was evaluated using the method described by Lowry et al [12]. Results were expressed in mg/mL.

2.3.3.2 Thiobarbituric acid reactive substances (TBARS) determination

TBARS method was carried in accordance with Buege and Aust [13]. Results were expressed as nmoles of TBARS/mg of protein.

2.3.3.3 Quantification of non-protein sulfhydryl groups

Quantification of nonprotein sulfhydryl (NPSH) was accomplished following Ellman [14]. Individual absorbance values were interpolated with glutathione standard curve and expressed as µg of glutathione/g of protein.

2.3.3.4 Antioxidant enzymatic activities

Catalase (CAT) activity was verified through the method described by Boveris and Chance [15] and results were expressed as nmoles/mg of protein. Superoxide dismutase (SOD) activity was evaluated as described by Boveris and Cadenas [16]. Results were expressed as units of SOD/mg of protein. Enzymatic activity of glutathione S-transferase (GST) was performed according to Habig et al. [17] and results were expressed in µmoles/min per mg of protein.

2.4 Protocol 2: Experimental wound healing design

At the beginning of the experimental protocol (day 0), to induce wound healing process along with inflammation and oxidative unbalance, a patch of skin of 1 cm^2 was removed from all animals. Each animal was anesthetized with the association of ketamine (75 mg/kg) and xylazine (10 mg/kg) administrated intraperitoneally [18]. After that, animals were immobilized at a surgical table and procedure was conducted using surgical scissors and tweezers. Wounds were

cut circularly in the middle of the upper back of each animal, about 20 mm from the base of the skull, and normalized [19]. After this surgical procedure, total animals (n = 72) were separated into four groups:

Control group: animals that did not receive any treatment, only saline by oral gavage and simulation of exposition to the magnetic field (n= 18).

Group treated with cubiu: animals treated with the ingestion by oral gavage of 50 mg/kg of cubiu extract (n= 18).

Group treated with magnetic field: animals treated by the exposition to the magnetic field (n= 18).

Group treated with magnetic field + cubiu: animals treated with the ingestion by oral gavage of 50 mg/kg of cubiu extract and exposition to the magnetic field (n= 18).

The 50 mg/kg cubiu extract dose used in the experiment was chosen according to results of toxicity studies. The pulsed magnetic field used in this experiment was a pulsed one, with a flux density of 2 mT, constant intervals between pulses (1,5 sec), frequency of 60 Hz and 30 minutes of exposition per day, was chosen based on the literature [20–22]. Treatments were performed once daily. On days 3, 7, and 14 after surgery,the animals (n = 6 per group) were euthanized to proceed with the collection of blood and skin samples [23,24]. Figure 1 Schematic demonstrates the experimental procedures of protocol 1 and 2.

2.5.1 Inflammatory cytokines

Skin and plasma determination of interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 10 (IL-10) and tumor necrosis factor-alpha (TNF-α) were evaluated using a ELISA kit (eBIOSCIENCE, San Diego, EUA), according to the manufacturer’s instructions. Hemocysteine levels were assayed using an Immulite analyzer (Diagnostic Products Corporation, Los Angeles, California). Results are expressed in pg/ml.

2.5.2 Leukocyte infiltration marker

Skin samples were collected to evaluate the myeloperoxidase activity (MPO) to estimate inflammatory cell infiltration in the skin after injury. The activity of this enzyme was used as a marker of neutrophil infiltration [25,26]. First, tissue samples were homogenized with a motor-driven homogenizer in 300 µL of

acetate buffer (8 mM, pH 5.4) containing 0.5% HTAB (45 s at 0 °C). The homogenate was then centrifuged at 11000 × g at 4 °C for 20 min, and supernatants were stored at −4 °C. To evaluate MPO activity, 10 µL of supernatant was incubated with 200 µL of acetate buffer (8 mM, pH 5.4) and 20 µLof TMB (18.4 mM) at 37 °C for 3 min. The reaction was stopped with 30 µlL of acetic acid in a cold bath, and the enzyme activity value was assessed colorimetrically at 630 nm using a microplate reader. Results were expressed as optical density (OD)/mg of protein.

2.5.3 Determination of oxidative status

Fresh homogenized hepatic and dermal tissues (skin) were made as described in item 2.4.3.

TBARS and NPSH levels and SOD, CAT and GST activities were determined as described from item 2.4.3.1 to item 2.4.3.4.

2.6 Statistical analysis

For the first protocol, homogeneity of variances among the different tested concentrations was verified with Levene's test and One-way ANOVA, followed by Tukey's post hoc test. For the second protocol, homogeneity of variances was evaluated in the same way, and a three-way ANOVA was performed followed by Tukey. The software used for this analysis was Software Statistica® 7.0. The significance level (α) adopted was ≤0.05.

3 Results

3.2 Oral toxicity evaluation of cubiu extract

Initially, cubiu extract daily oral administration toxicity was evaluated through a dose-response curve for 28 days. No changes in GOT, GPT, GGT, glucose, triglycerides, total cholesterol, urea and uric acid levels were observed between the groups that received different doses of cubiu extract in comparison to the control group (Table 1). Regarding creatinine levels, animals treated with cubiu extract at a dose of 100 mg/kg had their levels increased when compared to the animals treated with 25 mg/kg of cubiu extract.

The second objective of the experiments conducted in Protocol 1 was to determine, among the studied doses, the one with the greatest beneficial potential in the face of oxidative status. Table 2 shows the oxidative/antioxidant biomarkers in the liver, in which no change was observed in levels of TBARS, NPSH, and GST between the groups that received the different doses of cubiu extract compared control group. As for the enzymatic activity of SOD, an increase was observed in the groups treated with 25 mg/kg, 50 mg/kg and 150 mg/kg of cubiu extract compared to the control group. In addition, the group treated with 100 mg/kg of cubiu extract presented reduced SOD activity compared to the groups treated with 25 mg/kg and 150 mg/kg of cubiu extract. The enzymatic activity of CAT was increased in the group treated with 25 mg/kg of cubiu extract compared to the control group and compared to the groups treated with 100 mg/kg and 150 mg/kg of cubiu extract.

For the renal biomarkers of oxidative stress (Table 2), it was evident that enzymatic activity of SOD was increased in the groups treated with 25 mg/kg, 50 mg/kg and 100 mg/kg of cubiu extract compared to the control group. Furthermore, the enzymatic activity of CAT was increased in the group treated with 50 mg/kg of cubiu extract compared to the control group.

The morphological analysis showed no macroscopic or histological changes in hepatic and renal structures between the groups that received the different doses of cubiu extract (25, 50, 100 and 150 mg/kg) compared to the control group (Figure 2).

Following the presented data, the dose of cubiu extract chosen for continuity of the other experimental protocols was 50 mg/kg.

3.3 Inflammatory biomarkers in the skin and plasma

The results of inflammatory markers in the skin were expressed in Table 3 and in the plasma in Table 4. Levels of pro-inflammatory cytokines IL-1 and IL-6, in the skin and plasma, were decreased in all experimental groups on the 3rd day in comparison to the control group of the respective time. Also, on 3rd day the TNF-α levels decreased in the skin and plasma in groups treated with magnetic field and with magnetic field + cubiu. On days 7 and 14, the group treated with magnetic field and the group treated with magnetic field + cubiu had decreased IL-1, IL-6, and TNF-α skin and plasma levels when compared to the control group.

Additionally, on the 14th day, the group treated with cubiu had decreased IL-6 plasma levels when compared to the control group.

Furthermore, among the treatments, it was observed that in the skin, on day 3, the group treated with magnetic field and cubiu combination significantly reduced IL-1, IL-6 and TNF-α levels when compared to the group treated with cubiu or magnetic field separately. On the 7th day, the group treated with magnetic field + cubiu presented a reduction in IL-1 levels when compared to the group treated only with cubiu. On day 14, the group treated with the magnetic field and cubiu combination had reduced levels of IL-1 when compared to the groups treated with cubiu or magnetic field separately, and the group treated with magnetic field + cubiu presented a decrease in IL-6 and TNF-α levels when compared to the group treated only with cubiu. Additionally, IL-6 levels were reduced in the group treated with magnetic field compared to the group treated with cubiu.

In the plasma, among the treatments, it was observed that, on the 3rd day, the group treated with the magnetic field and cubiu combination had decreased levels of IL-1, IL-6 and TNF-α when compared to the group treated with cubiu or with magnetic field separately. On days 7 and 14, the group treated with magnetic field + cubiu presented a decrease in IL-1 and Il-6 levels compared to the group treated only with cubiu. Also on the 14th day, TNF-α levels decreased in the group treated with magnetic field + cubiu compared to the group treated only with cubiu.

IL-10 anti-inflammatory cytokine levels in the skin and plasma, were increased in the group treated with magnetic field and the group treated with magnetic field + cubiu on the 3rd day in comparison to the control group of the respective time. On the 7th day, the group treated with magnetic field + cubiu presented increased levels of IL-10 in comparison to the control group. On day 14, all treatments (cubiu, magnetic field, magnetic field + cubiu) had higher levels of IL-10 when compared to the control group. Furthermore, among the treatments it was observed that in the skin and plasma, on day 3, the group treated with magnetic field + cubiu had increased IL-10 levels in comparison to the group treated only with cubiu. On day 7, the group treated with the magnetic field and cubiu combination had increased IL-10 levels compared to the group treated with cubiu or with magnetic field separately.

Regarding the changes in IL-1, IL-6 and TNF-α pro-inflammatory cytokines skin and plasma levels over time, reductions were generally observed in all

experimental groups. In relation to IL-10 anti-inflammatory cytokine skin and plasma levels over time, increases were generally observed in all experimental groups, corresponding to what was physiologically expected.

3.4 Leukocyte infiltration markers in the skin

Results in myeloperoxidase activity are shown in Figure 3. On the 3rd day, it was evident that all treatments (cubiu, magnetic field, magnetic field + cubiu) decreased the myeloperoxidase enzymatic activity in comparison to the control group. In addition, over time, a decrease in enzymatic activity of myeloperoxidase was observed in the control group. The group treated with cubiu, and the group treated with magnetic field + cubiu presented a decrease in myeloperoxidase activity on the 14th day compared to the 3rd day. Also, the magnetic field group demonstrated a reduction in myeloperoxidase activity on the 14th day compared to the 7th day.

3.5 Oxidative status in the skin

Table 5 shows the results concerning oxidative status biomarkers in the skin. It was observed that the levels of TBARS were reduced in all treatments (cubiu, magnetic field, magnetic field + cubiu) compared to the control group on the 3rd day of treatment. On the 14th day, the group treated with magnetic field had reduced TBARS levels when compared to the control group.

NPSH levels were increased in the group treated with magnetic field + cubiu on the 14th day compared to the control group. In addition, changes over time were observed, the control group and the group treated with cubiu had reduced NPSH levels on day 7 when compared to respective the group on days 3 and 14. The group treated with magnetic field and the group treated with magnetic field + cubiu increased NPSH levels on the 14th day compared to the 3rd day and 7th day.

As for SOD activity, it was observed that the group treated with magnetic field + cubiu had its activity increased on the 14th day in relation to the control group. Also, changes were observed over time. In the control group, there was a reduction in SOD activity on day 7 compared to day 3, a reduction in the group treated with magnetic field on day 7 in comparison to days 3 and 14, and an increase in the group treated with magnetic field + cubiu on the 14th day compared to the 7th day.

CAT activity increased in the group treated with cubiu and the group treated with magnetic field + cubiu on the 14th day compared to the 7th day. Enzyme GST activity demonstrated an increase on the 14th day compared to the 3rd and 7th days in all treated groups (cubiu, magnetic field, magnetic field + cubiu), as well as in the control group.

4 Discussion

Cubiu is widely used for food and traditional medicine [1,2]. Therefore, it is important to check the safety of cubiu toxicity. Experimental results regarding the toxicity of daily oral administration of cubiu extract evaluated through a dose-response curve for 28 days demonstrated the safety of cubiu extract. Biochemical parameters indicated that the extract did not present toxicity at any dose evaluated in this study. Furthermore, macroscopic and microscopic (histology) assessment of liver and kidney, considered indispensable for toxicological analysis, evidenced that tested doses preserved the normal structure of these organs. In agreement with our results, Hernandes et al. [27] have reported safeuse of cubiu, due to the absence of genotoxic and cytotoxic effects.

The dose of 50 mg/kg was chosen for the second protocol in this study; it did not present toxicity, and it revealed benefits to oxidative status, increasing the enzymatic activity of SOD in the liver, and increasing enzymatic activity of SOD and CAT in the kidney.

After choosing cubiu extract dose, we followed a standardized wound healing model in the second protocol, also operating as an induction model for inflammatory and oxidative unbalance in the second protocol. This choice finds legitimacy in literature [19]. Magnetic fields have testified wound healing effects [28,29], and *Solanum sessiliflorum* has its popular use as a wound healing, but there is no scientific confirmation [30].

In a wound-healing model, it is an important to conduct evaluations at the 3rd, 7th and 14th day of treatment after injury induction. This strategy is very important to estimate the effect of treatments at each of the different phases of the wound healing process, divided into three distinguished but overlapping stages [31]. The first phase is the inflammatory phase, marked by the presence of inflammatory cells and mediators, occurring 0-3 days after initial injury. The next phase is

known as proliferation, characterized by the presence of fibroblasts and synthesis of the extracellular matrix. This phase takes place from the 3rd to the 7th day. The last phase is tissue remodeling, marked by the reorganization of the extracellular matrix and maturation of collagen fibers. Didactically, remodeling happens from 7th to 14th days, but it can persist for months [31,32].

It is well known that wound healing treatments operate mainly at the first stage of healing, the inflammatory phase, contributing to repair due to anti-inflammatory and antioxidant properties of drugs [29]. Even though magnetic fields are described in the literature as a treatment for skin wound healing, their contribution to systemic inflammatory and oxidant profile is still controversial. Depending on the application parameters such as time and intensity, magnetic fields can be pro-oxidant or antioxidant, as well as be anti-inflammatory or promote inflammation in other tissues [33–36]. Considering that, it has been suggested that the systemic use of antioxidant substances along with magnetic fields should be investigated [6,7]. Therefore, we analyzed the action of these treatments not only on skin but also on plasma, to evaluate the response towards the systemic inflammatory profile.

Observing the results from all treatments proposed on the inflammatory profile, it was evidenced that all treatments, isolated (cubiu or magnetic field) and combined (magnetic field + cubiu), demonstrated anti-inflammatory activity, and the anti-inflammatory action is increased with the combination of cubiu and magnetic field. Treatments were able to reduce the levels of pro-inflammatory cytokines and increase the levels of anti-inflammatory cytokine (IL-10), both on skin and plasma. Inflammatory cytokines are mediators present during the wound healing process. They can act by increasing the spread of the inflammatory process, as is the case of IL-1, IL-6 and TNF-α; and they can also act by reducing and controlling the inflammatory process, as is the case of IL-10 [32].

Thus, an increase in the levels of pro-inflammatory cytokines during healing ends up delaying this healing process, in addition to causing damage at the systemic level, as is the case of serious injuries or infections, in which there may be hemodynamic and metabolic changes, organ damage and even compromised life. In this context, anti-inflammatory cytokines act by containing and controlling the inflammatory process. [37].

The action of the magnetic field in the reduction of pro-inflammatory cytokines is justified by its action mechanism that acts on the modulation of adenosine and its Receptors A_{2A} and A_3ARs, increasing the expression of these, which are capable of suppressing high levels of pro-inflammatory cytokines [38].

Also, the anti-inflammatory activity of cubiu can be based on the bioactive compounds present in its composition. Rodrigues et al. [39] demonstrated through HPLC-MS that cubiu extract was constituted mainly of phenolic compounds, such as chlorogenic acid, specially 5-caffeoylquinic acid. Also, other species of Solanum, such as Solanum lycocarpumhas phenolic compounds, include cafeooilchinic acids in its composition, to which antioxidant and anti-inflammatory capacity exerted by Solanaceae were attributed [40]. The anti-inflammatory activity exerted by phenolic compounds has as its mechanism of action the ability to inhibit enzymes that are related to inflammation, such as prostaglandin synthetase, lipoxygenase, and cyclooxygenase [41]. In addition, chlorogenic acid interferes with the leukocyte response to chemokines, preventing their interaction with the adhesion molecules involved in cell migration, thus inhibiting cell adhesion and controlling and reducing the inflammatory process [42].

In relation to cell migration, this research also explored the effect of treatment towards the enzymatic activity of myeloperoxidase. This enzyme is known as a biomarker for neutrophil infiltrations [43]. Our results demonstrated that all treatments reduced the activity of myeloperoxidase on the 3rd day, which corresponds to the inflammatory phase of wound healing process. It indicates that treatments promoted a reduction in neutrophilic infiltration, reinforcing anti-inflammatory properties of magnetic field and cubiu. This corroborates previous results of the present study regarding the determination of cytokines, which demonstrated reduced levels of pro-inflammatory cytokines and increased levels of anti-inflammatory cytokines for treated groups.

During wound healing process, intimately related to inflammation, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are released, which play a defensive role, especially against potential microbial invasion, and act on cell signaling in the healing process. However, when the excessive formation of reactive species overcomes the body’s antioxidant capacity, a condition known as oxidative stress is generated. Oxidative stress can lead to damage on the cell

membrane, lipids, proteins and DNA, and may culminate in cellular death [44–46]. Also, oxidative stress acts on the propagation of the inflammatory response because the production of inflammatory enzymes such as cyclooxygenase (COX) and lipoxygenase is stimulated from lipid peroxidation and induces leukocytes to release pro-inflammatory cytokines [47]. Thus, it is well described how oxidative stress is closely related to the pathogenesis of non-healing wounds. Therefore, excess in reactive species must be avoided, because it slows down the healing process [48,49].

Results demonstrate that treatments proved to maintain oxidative balance with safety, manifesting a clear antioxidant potential. That becomes evident with the reduction of TBARS (biomarker for lipoperoxidation) in the skin, promoted by all treatments on day 3. The decrease of these substances in inflammatory phase is very positive, considering the great influence of reactive species in this period [49]. Thus, all treatments showed protection against lipoperoxidation.

On day 14, treatment with magnetic field reduced TBARS and treatment with magnetic field + cubiu increased NPSH and SOD levels in the skin. Therefore, treatments positively improved the antioxidant defense system. Differences throughout time are expected as part of the natural wound healing process, both for oxidative and inflammatory profile, as the quantification of analyses oscillates towards balance. Again, the combination of magnetic field + cubiu revealed positive results. It is known that magnetic fields promote exchanges with the biomagnetic potential of the organism, thus being able to activate enzymatic reactions [50,51]. The action of magnetic fields in oxidative balance can occur through the cellular signaling mechanism, such as the extracellular signal-regulated kinase pathways [52]. Also, the antioxidant activity of cubiu can be attributed to the presence of phenolic compounds in its composition, which are known for their antioxidant and chelant properties [53].

Thereby, the results from this study allow us to affirm that cubiu can be used safely, not demonstrating toxicity at the researched doses and during the period of administration. We evidenced that isolated (only magnetic field or cubiu) or combined treatments (magnetic field + cubiu) manifested anti-inflammatory and antioxidant properties, improving all stages of the wound healing process, especially the inflammatory phase. The association of the treatments has better results than when used alone.

5 Conclusion

This research revealed that cubiu extract demonstrated safety and absence of toxicity at the administrated doses during the time of treatment adopted in our protocol. It also enlightened that the proposed treatments provided decreased levels of pro-inflammatory cytokines increased levels of anti-inflammatory cytokines, and reduced activity of myeloperoxidase. Concerning stress biomarkers, treatments reduced TBARS levels, increased enzymatic activity of SOD and NPSH levels. Therefore, cubiu promotes antioxidant and anti-inflammatory action in skin wound healing and increases the results of conventional treatment for skin healing (magnetic field) when used in association.

Future perspectives: After the conclusions obtained in this study, we now intend to develop a nanostructured topical application of the cubiu, and thus test its performance in skin wound healing.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Conflict of interest: The authors declare no competing interests.

Funding Statement: This work was supported by CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, under Grant 88882.428091/2019-01.

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Table 1- Biochemical parameters of rats after 28-day treatment with cubiu extract

	Control	25 mg/kg	50 mg/kg	100 mg/kg	150 mg/kg
GOT	151.02 ± 36.00	179.83 ± 16.72	159.75 ± 31.11	151.90 ± 15.14	172.27 ± 11.71
GPT	132.98 ± 9.04	108.83 ± 12.30	147.82 ± 7.63	117.26 ± 17.12	123.96 ± 16.06
GAMA GT	28.73 ± 3.19	25.14 ± 1.56	34.83 ± 5.97	34.47 ± 3.29	24.77 ± 5.53
Glucose	100.54 ± 12.64	140.35 ± 17.12	121.79 ± 8.91	141.43 ± 9.01	105.22 ± 20.75
Triglycerides	125.92 ± 14.37	108.33 ± 28.09	110.18 ± 21.35	108.33 ± 23.29	78.70 ± 2.44
Total cholesterol	87.61 ± 21.96	88.57 ± 24.41	58.09 ± 11.23	116.18 ± 9.94	73.33 ± 20.88
Urea	52.06 ± 3.07	44.95 ± 3.69	43.24 ± 4.93	46.37 ± 2.05	44.38 ± 3.84
Creatinine	0.57 ± 0.004	0.53 ± 0.06^{D}	0.55 ± 0.02	0.73 ± 0.05^{B}	0.60 ± 0.01
Uric acid	1.43 ± 0.31	0.79 ± 0.13	1.17 ± 0.40	1.46 ± 0.40	0.95 ± 0.24

Values are represented as the mean ± standard error. * Indicates significant differences from the control group ($P < 0.05$). Different capital letters indicate significant differences between treatments ($P < 0.05$). The capital letters “B” corresponds to the group 50 mg/kg and letter “D” corresponds to the group 150 mg/kg. GOT- glutamic oxaloacetic transaminase (UI/L), GPT - glutamic pyruvic transaminase (UI/L), GAMA GT- gamma glutamyl transpeptidase (U/L), glucose (g/dL), triglycerides (mg/dL), total cholesterol (mg/dL), urea (mg/dL), creatinine (mg/dL) and uric acid (mg/dL).

Table 2- Biomarkers of oxidative status in the liver and kidney after 28-day treatment with cubiu extract

	LIVER					KIDNEY				
	Control	25	50	100	150	Control	25	50	100	150
TBARS	0.91 ± 0.24	0.83 ± 0.21	0.81 ± 0.13	0.80 ± 0.12	1.20 ± 0.13	0.72 ± 0.04	1.71 ± 0.51	1.40 ± 0.37	1.30 ± 0.37	0.74 ± 0.08
NPSH	4.06 ± 0.14	3.99 ± 0.71	3.32 ± 0.44	2.87 ± 0.16	4.10 ± 0.59	1.81 ± 0.20	2.07 ± 0.21	2.06 ± 0.26	1.83 ± 0.26	1.61 ± 0.19
SOD	4.58 ± 0.41	9.29 ± 0.31^{*C}	8.03 ± 0.63*	6.64 ± 0.39^{AD}	9.78 ± 1.06^{*C}	5.34 ± 0.21	7.69 ± 0.53*	7.17 ± 0.14*	8.02 ± 0.77*	6.47 ± 0.41
CAT	3.14 ± 0.33	5.56 ± 0.81^{*CD}	3.61 ± 0.51	3.47 ± 0.45^{A}	2.96 ± 0.22^{A}	1.14 ± 0.07	1.27 ± 0.20	1.78 ± 0.16*	1.67 ± 0.21	1.31 ± 0.10
GST	6.94 ± 1.12	11.50 ± 1.23	9.06 ± 1.30	6.67 ± 1.00	8.65 ± 2.93	1.16 ± 0.09	1.25 ± 0.22	1.08 ± 0.12	1.34 ± 0.24	1.17 ± 0.04

Values are represented as the mean ± standard error. * Indicates significant differences from the control group ($P < 0.05$). Different capital letters indicate significant differences between treatments ($P < 0.05$). The capital letters “A” corresponds to the group 25 mg/kg, “B” corresponds to the group 50 mg/kg, “C” corresponds to the group 100 mg/kg, and letter “D” corresponds to the group 150 mg/kg. TBARS- Thiobarbituric acid reactive substances (µmol/mg protein), SPSH- Non-protein thiols (nmol/mg protein), SOD - Superoxide dismutase (SOD units/mg protein), CAT - Catalase (ρmol/mg protein, GST- Glutathione S-transferase (ρmol/min/mg protein).

Table 3 - Inflammatory biomarkers in the skin

Time	Treatments	IL-1	IL-6	TNF-α	IL-10
3° day	Control	90.67 ± 0.84$^{++\ +++}$	99.17 ± 1.14$^{++\ +++}$	105.33 ± 1.69$^{++\ +++}$	20.25 ± 1.22$^{++\ +++}$
	Cubiu	84.00 ± 0.36*^{bcC}	89.67 ± 0.67*^{bcC}	101.67 ± 1.26^{bcC}	26.70 ± 1.65^{cC}
	Magnetic field	82.83 ± 0.60*^{bcC}	89.00 ± 1.39*^{bcC}	95.67 ± 1.28*^{bcC}	30.28 ± 2.05*^{c}
	Magnetic field + cubiu	71.33 ± 0.88*^{ABc}	75.83 ± 1.40*^{ABc}	82.83 ± 1.58*^{ABc}	36.01 ± 1.64*^{Ac}
7° day	Control	76.40 ± 1.50$^{+\ +++}$	81.33 ± 0.84$^{+\ +++}$	88.17 ± 0.48$^{+\ +++}$	33.15 ± 0.90$^{+\ +++}$
	Cubiu	74.33 ± 1.11^{acC}	79.33 ± 1.65^{ac}	85.00 ± 1.15^{ac}	31.89 ± 0.82^{cC}
	Magnetic field	70.50 ± 0.84*^{ac}	73.50 ± 1.34*^{ac}	79.67 ± 0.84*^{ac}	31.35 ± 1.05^{cC}
	Magnetic field + cubiu	68.20 ± 1.77*^{Ac}	73.17 ± 1.60*^{c}	80.67 ± 1.58*^{c}	40.49 ± 1.77*^{ABc}
14° day	Control	58.80 ± 1.24$^{+\ ++}$	70.50 ± 0.72$^{+\ ++}$	77.00 ± 1.24$^{+\ ++}$	47.12 ± 1.37$^{+\ ++}$
	Cubiu	55.20 ± 1.24^{abC}	64.75 ± 2.50^{abBC}	72.17 ± 1.74^{abC}	55.54 ± 1.26*^{ab}
	Magnetic field	53.00 ± 0.51*^{abC}	57.20 ± 1.46*^{aAb}	67.17 ± 1.64*^{ab}	60.20 ± 0.73*^{ab}
	Magnetic field + cubiu	46.50 ± 1.19*^{aAbB}	53.17 ± 1.01*^{aAb}	62.83 ± 1.64*^{aAb}	60.92 ± 0.95*^{ab}

Values are represented as the mean ± standard error. * Indicates significant differences from the control group at the same time ($P < 0.05$). Different capital letters indicate significant differences between treatments at the same time ($P < 0.05$). Different lowercase letters indicate significant differences between times at the same treatment ($P< 0.05$). The capital letters “A” corresponds to the group cubiu, “B” corresponds to the group MF, “C” corresponds to the group MF + cubiu. The lowercase letters “a” corresponds to the time 3rd day of respective group, “b” corresponds to the time 7th day of respective group and “c” corresponds to the time 14th day of respective group. To demonstrate significant difference between the control groups of different times are used $^{+}$ for the control 3rd day, $^{++}$ for the control 7th day and $^{+++}$ for the control 14th day.

Table 4- Inflammatory biomarkers in the plasma

Time	Treatments	IL-1	IL-6	TNF-α	IL-10
3° day	Control	150.33 ± 1.43$^{++\ +++}$	165.50 ± 2.08$^{++\ +++}$	175.67 ± 2.88$^{++\ +++}$	18.83 ± 1.14$^{++\ +++}$
	Cubiu	139.33 ± 0.71*^{bcC}	149.33 ± 1.23*^{bcC}	170.00 ± 2.07^{bcC}	24.83 ± 1.54^{cC}
	Magnetic field	137.17 ± 1.01*^{bcC}	148.00 ± 1.98*^{bcC}	159.50 ± 2.39*^{bcC}	28.17 ± 1.90*^{c}
	Magnetic field + cubiu	117.67 ± 1.89*^{ABc}	128.60 ± 1.69*^{ABc}	139.17 ± 2.50*^{ABc}	33.50 ± 1.52*^{Ac}
7° day	Control	127.50 ± 2.17$^{+\ +++}$	135.83 ± 1.40$^{+\ +++}$	147.50 ± 0.76$^{+\ +++}$	30.83 ± 0.83$^{+\ +++}$
	Cubiu	123.00 ± 1.57^{acC}	132.00 ± 2.76^{acC}	141.67 ± 1.54^{ac}	29.67 ± 0.76^{cC}
	Magnetic field	115.33 ± 1.80*^{ac}	124.60 ± 1.72*^{ac}	133.50 ± 1.73*^{ac}	29.17 ± 0.98^{cC}
	Magnetic field + cubiu	112.50 ± 2.95*^{Ac}	121.67 ± 2.69*^{Ac}	134.67 ± 2.64*^{c}	37.67 ± 1.65*^{ABc}
14° day	Control	99.50 ± 2.26$^{+\ ++}$	117.83 ± 1.11$^{+\ ++}$	128.17 ± 1.94$^{+\ ++}$	43.83 ± 1.28$^{+\ ++}$
	Cubiu	91.50 ± 1.84^{abC}	106.00 ± 3.99*^{abC}	120.50 ± 2.88^{abC}	51.67 ± 1.17*^{ab}
	Magnetic field	87.83 ± 1.01*^{ab}	95.40 ± 2.52*^{ab}	112.00 ± 2.65*^{ab}	56.00 ± 0.68*^{ab}
	Magnetic field + cubiu	80.17 ± 2.85*^{aAb}	88.67 ± 1.73*^{aAb}	105.00 ± 2.65*^{aAb}	56.67 ± 0.88*^{ab}

Values are represented as the mean ± standard error. * Indicates significant differences from the control group at the same time ($P < 0.05$). Different capital letters indicate significant differences between treatments at the same time ($P < 0.05$). Different lowercase letters indicate significant differences between times at the same treatment ($P< 0.05$). The capital letters “A” corresponds to the group cubiu, “B” corresponds to the group MF, “C” corresponds to the group MF + cubiu. The lowercase letters “a” corresponds to the time 3rd day of respective group, “b” corresponds to the time 7th day of respective group and “c” corresponds to the time 14th day of respective group. To demonstrate significant difference between the control groups of different times are used $^{+}$ for the control 3rd day, $^{++}$ for the control 7th day and $^{+++}$ for the control 14th day.

Table 5- Biomarkers of oxidative stress in the skin

Time	Treatments	TBARS	NPSH	SOD	CAT	GST
3° day	Control	2.80 ± 0.16	16.7 ± 1.55^{++}	4.93 ± 0.25^{++}	4.48 ± 0.46	2.19 ± 0.23^{+++}
	Cubiu	1.78 ± 0.07*	16.1 ± 1.09^{b}	4.19 ± 0.29	4.56 ± 0.62	1.82 ± 0.44^{c}
	Magnetic field	1.73 ± 0.16*	14.7 ± 0.94^{c}	4.42 ± 0.37^{b}	4.43 ± 0.53	0.85 ± 0.06^{c}
	Magnetic field + cubiu	1.81 ± 0.12*	15.8 ± 1.23^{bc}	3.93 ± 0.40	3.34 ± 0.37	1.17 ± 0.38^{c}
7° day	Control	2.17 ± 0.11	10.9 ± 0.44$^{+\ +++}$	2.91 ± 0.10^{+}	2.84 ± 0.21	1.79 ± 0.15^{+++}
	Cubiu	2.02 ± 0.12	10.7 ± 0.79^{ac}	3.40 ± 0.25	2.90 ± 0.24^{c}	2.35 ± 0.52^{c}
	Magnetic field	1.52 ± 0.12	10.4 ± 0.32^{c}	2.86 ± 0.23^{ac}	3.13 ± 0.40	1.57 ± 0.25^{c}
	Magnetic field + cubiu	1.93 ± 0.15	10.7 ± 0.70^{ac}	2.76 ± 0.24^{c}	3.16 ± 0.30^{c}	1.55 ± 0.07^{c}
14° day	Control	2.81 ± 0.07	17.2 ± 1.10^{++}	3.77 ± 0.24	3.95 ± 0.32	5.25 ± 0.44$^{+\ ++}$
	Cubiu	2.36 ± 0.08	18.1 ± 1.28^{b}	3.92 ± 0.14	4.77 ± 0.31^{b}	4.70 ± 0.35^{ab}
	Magnetic field	1.52 ± 0.12*	21.3 ± 1.70^{ab}	4.88 ± 0.39^{b}	3.93 ± 0.42	4.14 ± 0.19^{ab}
	Magnetic field + cubiu	2.39 ± 0.17	23.0 ± 1.24*^{ab}	5.43 ± 0.42*^{b}	5.00 ± 0.43^{b}	5.31 ± 0.26^{ab}

Values are represented as the mean ± standard error. * Indicates significant differences from the control group at the same time ($P < 0.05$). Different capital letters indicate significant differences between treatments at the same time ($P < 0.05$). Different lowercase letters indicate significant differences between times at the same treatment ($P< 0.05$). The capital letters “A” corresponds to the group cubiu, “B” corresponds to the group MR, “C” corresponds to the group MR + cubiu. The lowercase letters “a” corresponds to the time 3rd day of respective group, “b” corresponds to the time 7th day of respective group and “c” corresponds to the time 14th day of respective group. To demonstrate significant difference between the control groups of different times are used $^{+}$ for the control 3rd day, $^{++}$ for the control 7th day and $^{+++}$ for the control 14th day. TBARS- Thiobarbituric acid reactive substances (µmol/mg protein), SPSH- Non-protein thiols (nmol/mg protein), SOD - Superoxide dismutase (SOD units/mg protein), CAT - Catalase (ρmol/mg protein, GST- Glutathione S-transferase (ρmol/min/mg protein).

Figures

Figure 1 - Schematic demonstration of the experimental procedures of protocols 1 and 2.

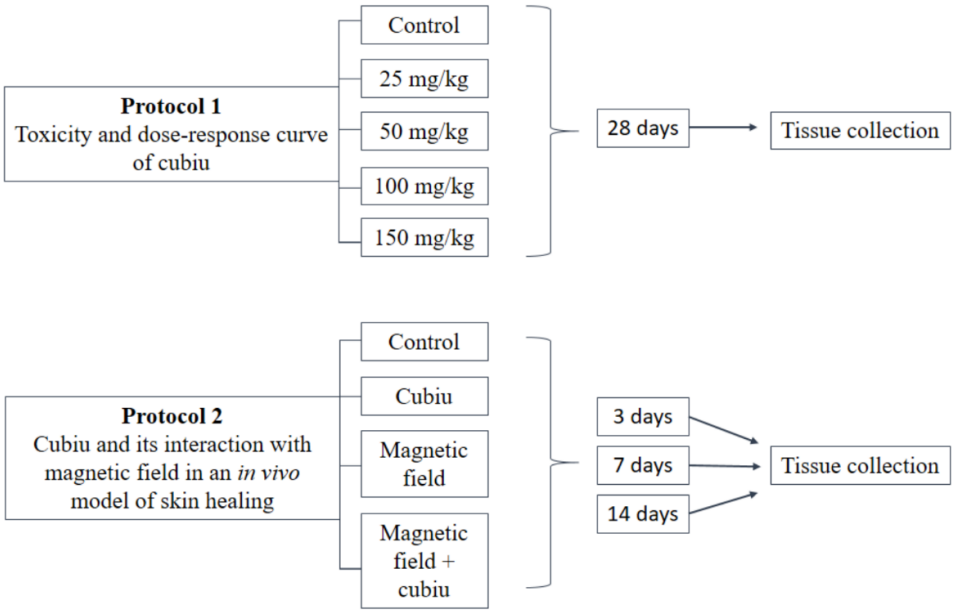


Figure 2 – Photomicrography of liver (L) and photomicrography of kidney (K) of the Wistar rats in the control group, groups treated with 25mg/kg of cubiu extract, 50mg/kg of cubiu extract, 100mg/kg of cubiu extract and 150mg/kg of cubiu extract. Bar = 20 micrometers in the liver's photomicrography and 100 micrometers in the kidney's photomicrography.

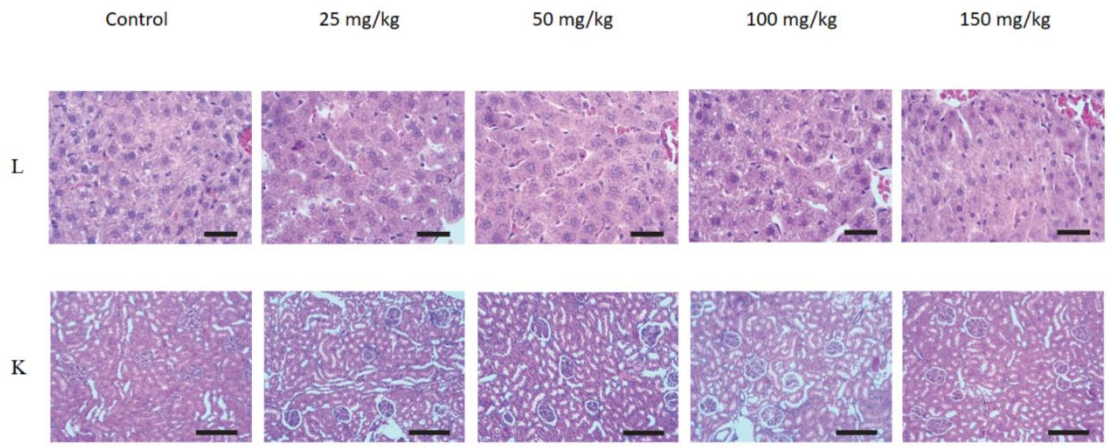
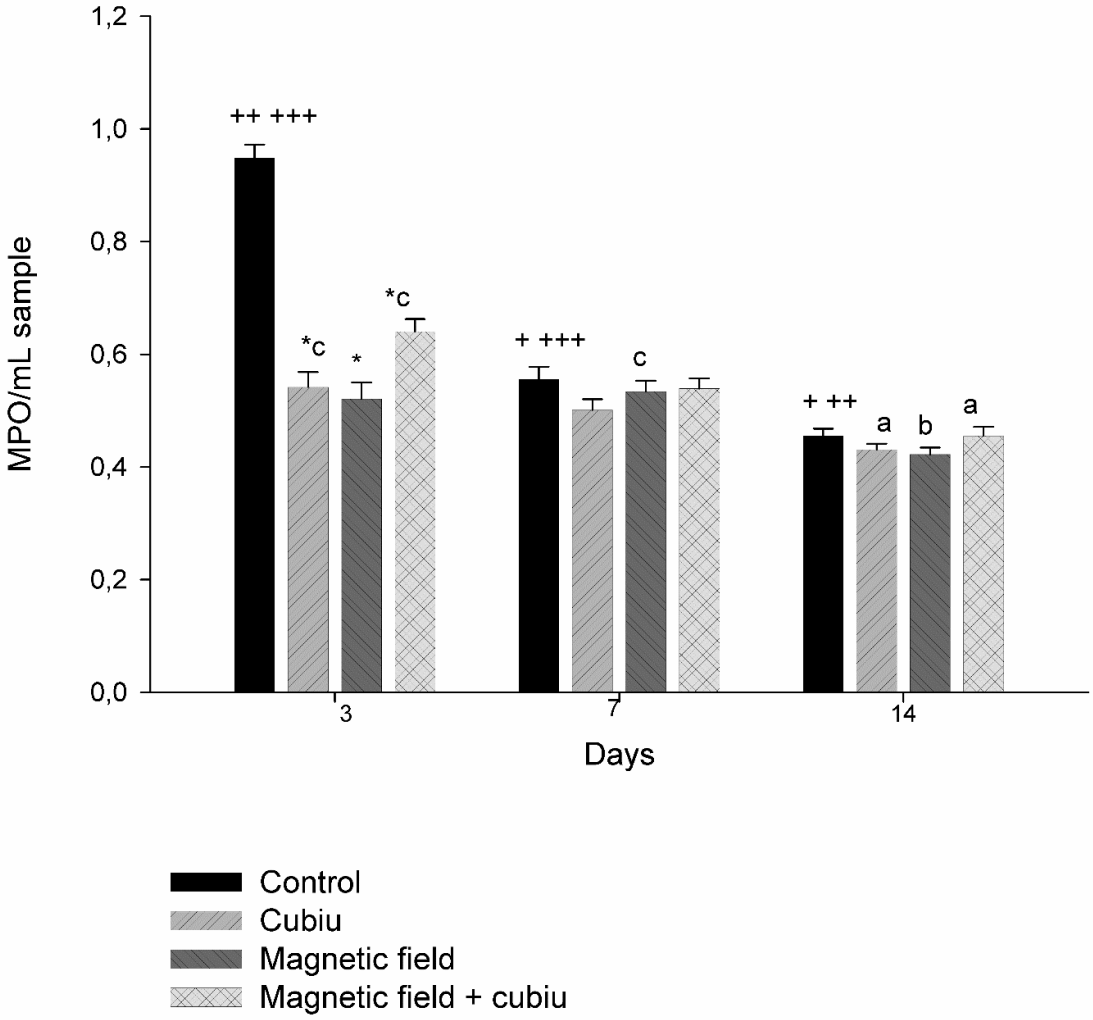


Figure 3- Graphical representation of myeloperoxidase activity. The results were expressed as optical density (OD)/mg of protein. * Indicates significant differences from the control group at the same time. Lowercase letters indicate significant differences between times at the same treatment. The letters “a” corresponds to the group cubiu, “b” corresponds to the group MF, “c” corresponds to the group MF + cubiu. To demonstrate significant difference between the control groups of different times are used + for the control 3rd day, ++ for the control 7th day, and +++ for the control 14th day.



4 DISCUSSÃO

O Cubiu é um fruto amazônico amplamente utilizado, tanto na alimentação quanto na medicina tradicional (ARGOTE; VARGAS; VILLADA, 2013; MONTAGNER et al., 2020). Em seu uso popular, são atribuídas diversas propriedades, dentre essas, seu uso para cicatrização de feridas. Nosso estudo é pioneiro em comprovar cientificamente que o cubiu realmente tem efeito cicatrizante, antimicrobiano e anti-inflamatório.

A cicatrização de pele se dá por meio de um processo complexo, o qual é dividido em 3 fases distintas e sobrepostas. A primeira fase é a fase inflamatória, marcada pela presença de células e mediadores inflamatórios, ocorrendo 0-3 dias após a lesão inicial. A próxima fase é conhecida como proliferação, caracterizada pela presença de fibroblastos e síntese da matriz extracelular. Esta fase ocorre do 3º ao 7º dia. A última fase é a remodelação tecidual, marcada pela reorganização da matriz extracelular e maturação das fibras colágenas. Didaticamente, a remodelação acontece do 7º ao 14º dia, mas pode persistir por meses (GONZALEZ et al., 2016; STUNOVA; VISTEJNOVA, 2018).

Sabe-se que os tratamentos de cicatrização de feridas atuam principalmente na primeira fase da cicatrização, a fase inflamatória, contribuindo para a reparação principalmente por meio de controlar o processo inflamatório e prevenir infecções. Dessa forma, os tratamentos que melhoram a cicatrização de feridas cutâneas devem promover atividades antimicrobianas e anti-inflamatórias, uma vez que infecções e processos inflamatórios prolongados retardam o processo de cicatrização (GONZALEZ et al., 2016; PAZYAR et al., 2014). Quanto ao extrato de cubiu, comprovamos que nele se encontram todas estas propriedades indispensáveis que promovem a cicatrização de feridas cutâneas, justificando assim o significativo potencial cicatricial desta fruta amazônica.

No primeiro estudo conduzido, foi realizada uma triagem dos efeitos farmacológicos do cubiu *in vitro*, evidenciando sua atividade antimicrobiana, anti-inflamatória e cicatrizante. Demonstrando também, que o mecanismo de atuação na cicatrização se dá por meio do controle dos mediadores inflamatórios.

Esses dados nos instigaram a darmos continuidade nas pesquisas desenvolvendo um estudo *in vivo*, onde analizamos a atuação do cubiu frente ao

processo de cicatrização de pele, e elencamos a terapêutica com campos magnéticos como escolha de tratamento padrão.

Embora os campos magnéticos sejam descritos na literatura como um tratamento para cicatrização de feridas cutâneas, sua contribuição para o perfil inflamatório e oxidante sistêmico ainda é controversa. Dependendo dos parâmetros de aplicação, como tempo e intensidade, os campos magnéticos podem ser pró-oxidantes ou antioxidantes, além de serem anti-inflamatórios ou promover inflamação em outros tecidos (ORTIZ et al., 2016; EMRE et al., 2011; GLINKA et al., 2018; CANSEVEN; COSKUN; SEYHAN, 2008). Diante disso, tem sido sugerido que o uso de substâncias antioxidantes juntamente com campos magnéticos deve ser investigado (CEYHAN et al., 2012; GHODBANE et al., 2015). Salientamos que o cubiu é amplamente utilizado por seus efeitos antioxidantes (COLODEL et al., 2017).

Isso despertou nosso interesse em elucidar a real atuação do campo magnético durante a cicatrização de pele nos parâmetros propostos nesse estudo, dessa forma, abrangemos duas terapêuticas distintas para cicatrização de pele, que se mostraram sinérgicas e com efeitos potencializados quando utilizadas em conjunto.

A atividade anti-inflamatória *in vivo* foi evidenciada a partir da redução dos níveis de citocinas pró-inflamatórias e aumento dos níveis de citocinas anti-inflamatórias (IL-10), que o cubiu e o campo magnético proporcionaram (potencializada quando utilizados em conjunto). Salientamos que, em concordância, no estudo prévio *in vitro*, o cubiu apresentou essa mesma modulação nas citocinas inflamatórias.

As citocinas inflamatórias são mediadores presentes durante o processo de cicatrização de feridas. Podem atuar aumentando a disseminação do processo inflamatório, como é o caso da IL-1, IL-6 e TNF-α; e também podem atuar reduzindo e controlando o processo inflamatório, como é o caso da IL-10 (STUNOVA; VISTEJNOVA, 2018). Assim, um aumento nos níveis de citocinas pró-inflamatórias durante a cicatrização acaba por retardar esse processo cicatricial, além de causar danos em nível sistêmico, como é o caso de lesões ou infecções graves, nas quais podem ocorrer alterações hemodinâmicas e metabólicas, danos nos órgãos e até mesmo levar a morte em casos de sepse (OLIVEIRA, 2011).

A ação do campo magnético na redução de citocinas pró-inflamatórias é justificada por seu mecanismo de ação que atua na modulação da adenosina e seus receptores A_{2A} e A_3ARs, aumentando a expressão destes, que são capazes de suprimir altos níveis de citocinas pró-inflamatórias (VARANI et al., 2017).

Quanto ao extrato de cubiu, atrubuimos sua atividade anti-inflamatória a sua composição fitoquímica. No estudo *in vitro*, evidenciamos que o composto bioativo majoritário presente no extrato de cubiu é o ácido 5-cafeiolquínico. Em concordância, Rodrigues e colaboradores (RODRIGUES; MARIUTTI; MERCADANTE, 2013) demonstraram por meio de HPLC-MS que o extrato de cubiu era constituído principalmente de compostos fenólicos, como ácido clorogênico, especialmente ácido 5-cafeoilquínico. Além disso, outras espécies de *Solanum*, como *Solanum lycocarpum* possui compostos fenólicos, incluem ácidos cafeoilquínicos em sua composição, aos quais foi atribuída a capacidade antioxidante e anti-inflamatória exercida pelas solanáceas (MORAES et al., 2020).

A atividade anti-inflamatória exercida pelos compostos fenólicos tem como mecanismo de ação a capacidade de inibir enzimas que estão relacionadas à inflamação, como a prostaglandina sintetase, a lipoxigenase e a ciclooxigenase (AMBRIZ-PÉREZ et al., 2016). Ainda, o ácido clorogênico interfere na resposta leucocitária às quimiocinas, impedindo sua interação com as moléculas de adesão envolvidas na migração celular, inibindo a adesão celular, controlando e reduzindo o processo inflamatório (CHANG et al., 2010).

Além disso, durante o processo de cicatrização de feridas, intimamente relacionado à inflamação, está a liberação de espécies reativas de oxigênio (ROS) e espécies reativas de nitrogênio (RNS), que desempenham um papel defensivo, principalmente contra potencial invasão microbiana, atuando também na sinalização celular no processo de cicatrização. Porém, quando há um aumento na produção dessas espécies reativas, em detrimento da capacidade antioxidante do organismo, é gerado o estresse oxidativo. Este, por sua vez, atua na propagação da resposta inflamatória, pois a produção de enzimas inflamatórias como a ciclooxigenase (COX) e a lipoxigenase é estimulada a partir da peroxidação lipídica, induzindo os leucócitos a liberarem citocinas pró-inflamatórias (BISWAS; DAS; BANERJEE, 2017). Assim, está bem descrito que o estresse oxidativo está intimamente relacionado à patogênese de feridas que

não cicatrizam. Portanto, o excesso de espécies reativas deve ser evitado, pois retarda o processo de cicatrização (SCHARFFETTER-KOCHANEK; WLASCHEK, 2005; LIM; PARK, 2011).

Perante isso, no estudo *in vivo,* demonstramos e elucidamos a atividade antioxidante do campo magnético e do cubiu (potencializada quando utilizados em conjunto), evidenciada ao reduzir os níveis dos biomarcadores pró-oxidantes e aumentar os antioxidantes. Sabe-se que os campos magnéticos promovem trocas com o potencial biomagnético do organismo, podendo assim ativar reações enzimáticas (WARNKE, 1980; GLINKA et al., 2013). A ação dos campos magnéticos no equilíbrio oxidativo pode ocorrer através do mecanismo de sinalização celular, como as vias de quinases reguladas por sinais extracelulares (MERT, 2017). Além disso, os composto fenólicos presentes no cubiu agem por meio de propriedades quelantes e antioxidantes.

Outro mecanismo de ação do cubiu na cicatrização de pele sugerido aqui nesta tese, se dá por sua atividade antimicrobiana, evidenciada no estudo *in vitro*. A infecção é um dos fatores que pode afetar a cicatrização de pele e contribuir para a patogênese de feridas crônicas. Dessa forma, a infecção de feridas cutâneas pode levar à alteração dos processos ao longo da via de cicatrização da pele. As bactérias produzem mais mediadores inflamatórios, que prolongam a fase inflamatória e inibem a fase de epitelização do processo de cicatrização da pele. Além disso, bactérias presentes em uma ferida infectada causam morte celular, o que levará a um aumento adicional na resposta inflamatória local e prolongamento da fase inflamatória aguda (GONZALEZ, et al., 2016).

O presente estudo evidenciou que o extrato de cubiu possui atividade bacteriostática e bactericida contra *A. caviae, S. paucimobilis e P. aeruginosa* (PA01), que são bactérias de relevância clínica que afetam a pele (e também outros tecidos) e causam infecções leves e graves (RYAN; ADLEY, 2010; WU et al., 2011; KRISHNA; MILLER, 2012). Além disso, sabendo que a PA01 é uma bactéria produtora de biofilme multirresistente (MAUNDERS; WELCH, 2017), também demonstramos a eficácia do extrato de cubiu em inibir a formação de biofilme e destruir esse biofilme quando previamente formado por PA01. O biofilme está associado principalmente a infecções prolongadas e é um importante mecanismo utilizado por microrganismos para sobreviver a

tratamentos com antibióticos. Além disso, o biofilme formado pelo PA01 está intimamente relacionado a infecções em feridas crônicas da pele (MAUNDERS; WELCH, 2017; DONLAN; COSTERTON, 2002).

Evidências sugerem que o possível mecanismo de ação de compostos antimicrobianos, está relacionado à cascata de reações envolvendo a célula bacteriana (DA ROSA et al., 2017; MARTÍNEZ-BUSI et al., 2019). Essas reações podem envolver a capacidade antioxidante de compostos fenólicos, o que pode ser importante no mecanismo de ação antimicrobiano, uma vez que esses compostos naturais podem reduzir a produção de metabólitos essenciais para a sobrevivência do microrganismo em condições de estresse. Portanto, uma vez que evidenciamos a presença de compostos fenólicos no extrato de cubiu, é plausível que este tenha se fixado e incorporado à estrutura do biofilme, prejudicando as vias de sinalização e induzindo a ruptura da membrana celular (DESBOIS; SMITH, 2010; DAMBOLENA; ZYGADLO; RUBINSTEIN, 2011).

Uma vez visto que o cubiu possui todas essas propriedades farmacológicas, e que ainda é benéfico em associação ao campo magnético, coube também elucidarmos se o mesmo é seguro para o uso. Investigamos a toxicidade do cubiu tanto *in vitro* quanto *in vivo*, e todos os biomarcadores averiguados revelaram que o cubiu não apresenta toxicidade nem a nível celular, nem a nível sistêmico. Em concordância, Hernandes e colaboradores (HERNANDES et al., 2014) relataram que o cubiu não apresenta efeitos citotóxicos ou genotóxicos, ecoando ainda mais que é seguro o seu consumo.

Em suma, a partir da triagem farmacológica realizada, este é o primeiro estudo a comprovar que o extrato de cubiu é um importante composto contra doenças de pele, podendo ser utilizado para tartar feridas, infecções e inflamações cutâneas.

5 CONCLUSÃO

A presente tese elucidou as propriedades farmacológicas do cubiu: demonstrando seu potencial para cicatrização de feridas de pele, isolado e em associação ao campo magnético; sua atividade antimicrobiana contra cepas de relevância clínica; atividade anti-inflamatória tanto sistêmica quanto na via

cicatricial; atividade antioxidante; e sua segurança para o uso, não apontando toxicidade celular ou orgânica.

O cubiu é popularmente utilizado para tratamentos de afecções cutâneas, incluindo a cicatrização de pele, porém carecia de confirmação científica do seu potencial para esses fins. Essa pesquisa é pioneira em demonstrar que o extrato de cubiu é um importante composto contra doenças de pele, promovendo a cicatrização de feridas cutâneas, atividade antimicrobiana e anti-inflamatória.

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ANEXO A – COMPROVANTE DE PUBLICAÇÃO (ARTIGO 2)

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 7562569, 12 pages
https://doi.org/10.1155/2022/7562569

Research Article

Toxicity, Anti-Inflammatory, and Antioxidant Activities of Cubiu (*Solanum sessiliflorum*) and Its Interaction with Magnetic Field in the Skin Wound Healing

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Received 15 August 2021; Revised 23 December 2021; Accepted 12 February 2022; Published 10 March 2022

Academic Editor: Rohit Sharma

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Cubiu, an Amazonian fruit, is widely used as food and popular treatment for pathologies that present an inflammatory pattern, such as skin wound healing. However, there is still no confirmation in the scientific literature about the safety profile, as well as the anti-inflammatory, antioxidant, and healing actions of cubiu. This study is divided into two experimental protocols using Wistar rats. Thus, the first objective (protocol 1) of this study was to evaluate the toxicity of an oral administration of cubiu extract at different doses for 28 days. The macroscopic and microscopic analyses of the liver and kidney were performed, and the following analysis was determined in plasma: glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, gamma-glutamyl transpeptidase, glucose, triglycerides, total cholesterol, urea, creatinine, and uric acid. After, we conducted the second protocol aimed to establish the potential antioxidant and anti-inflammatory capacity of cubiu and its interaction with magnetic field in skin wound healing. On days 3, 7, and 14 of treatment, skin and blood samples were collected and analyzed: the oxidative stress biomarkers (reactive substances to thiobarbituric acid, nonprotein thiols, superoxide dismutase, catalase, and glutathione S-transferase), myeloperoxidase enzymatic activity, and cytokines levels (interleukin 1, interleukin 6, interleukin 10, and tumor necrosis factor-alpha). The cubiu has shown to be safe and nontoxic. Both cubiu and magnetic field promoted decreased levels of proinflammatory and prooxidant biomarkers (interleukin 1, interleukin 6, tumor necrosis factor-alpha, and reactive substances to thiobarbituric acid), as well as increased levels of anti-inflammatory and antioxidant biomarkers (interleukin 10, nonprotein thiols, and superoxide dismutase), with greater potential when treatments are used in association. Thus, cubiu promotes antioxidant and anti-inflammatory action in skin wound healing, while also improving results of the conventional treatment for skin healing (magnetic field) when used in association.

1. Introduction

Solanum sessiliflorum (Dunal) is a Solanaceae native of the Amazon Basin, currently found throughout the Brazilian, Ecuadorian, Colombian, and Venezuelan Amazonian territory. Its fruit, called cubiu in Portuguese, also known as "apple/peach tomato, is widely used as food, and it can be used in numerous ways such as juices, sweets, jellies, and consumed in natura [1]. Furthermore, cubiu is very nutritious and rich in iron, niacin, citric acid, and pectin. Thus,

ANEXO B – COMPROVANTE DE SUBMISSÃO (ARTIGO 1)

Fitoterapia

Phytochemical characterization, pharmacological properties and toxicity of Amazonian fruit cubiu (Solanum sessiliflorum Dunal)

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Keywords:	antimicrobial; antioxidant; anti-inflammatory; skin wound healing; cocona
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Abstract:	The cubiu (Solanum sessiliflorum) is a tropical fruit native to the Amazon region and widely used in medicine and cosmetics, despite the lack of research regarding the actual safety and effectiveness of its use for these purposes. This study aimed to evaluated the phytochemical characterization, pharmacological properties (skin wound healing, antimicrobial and anti-inflammatory properties), and toxicity of cubiu extract. The cubiu antimicrobial capacity was determined against strains of Aeromonas caviae, Pseudomonas aeruginosa, and Sphingomonas paucimobilis. Additionally, cubiu toxicity (hemolysis, coagulation, cell viability, and genotoxicity tests), antioxidant activity (reactive oxygen species total levels), scratch assay (in vitro skin wound healing), and anti-inflammatory activity against phytohemagglutinin and in the scratch assay (interleukin 1, interleukin 6, interleukin 10, tumor necrosis factor-alpha, and interferon-gamma levels), were evaluated. Human fibroblast cells were used to evaluate skin wound healing, and human peripheral blood mononuclear cells were used for the other assessments. Our findings showed that the cubiu extract is rich in phenolic compounds, the major compound being 5-caffeoylquinic acid. In addition, was effective against the three bacterial strains tested and inhibited and destroyed the biofilm formed by Pseudomonas aeruginosa. The cubiu extract also was no toxicity, maintained the hemocompatibility parameters in the biological range, improved cell viability, decreased reactive oxygen species total levels and pro-inflammatory cytokine levels, increased anti-inflammatory cytokine levels, and accelerated the wound healing process. In conclusion, This is the first research to prove that cubiu is an important compound for use in the skin diseases, promoting skin wound healing, antimicrobial and anti-inflammatory activities.