

UNIVERSIDADE FEDERAL DE VIÇOSA

LYVIA LOPES MIRANDA

**EFEITO DE EXTRATOS VEGETAIS NO REPARO ÓSSEO E CUTÂNEO EM
MODELOS ANIMAIS**

**VIÇOSA
MINAS GERAIS – BRASIL
2018**

LYVIA LOPES MIRANDA

**EFEITO DE EXTRATOS VEGETAIS NO REPARO ÓSSEO E CUTÂNEO EM
MODELOS ANIMAIS**

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Biologia Celular e Estrutural, para obtenção do título de *Magister Scientiae*.

VIÇOSA
MINAS GERAIS – BRASIL
2018

**Ficha catalográfica preparada pela Biblioteca Central da Universidade
Federal de Viçosa - Câmpus Viçosa**

T

Miranda, Lyvia Lopes, 1993-
M672e Efeito de extratos vegetais no reparo ósseo e cutâneo em
2018 modelos animais / Lyvia Lopes Miranda. – Viçosa, MG, 2018.
vii, 84f. : il. (algumas color.) ; 29 cm.

Orientador: Reggiani Vilela Gonçalves.
Dissertação (mestrado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Essências e óleos essenciais. 2. Animais de laboratório.
3. Cicatrização de ferimentos. 4. Ossos - Doenças - Tratamento.
5. Queimaduras. I. Universidade Federal de Viçosa.
Departamento de Biologia Geral. Programa de Pós-Graduação
em Biologia Celular e Estrutural. II. Título.

CDD 22. ed. 573.76

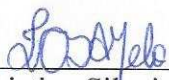
LYVIA LOPES MIRANDA

**EFEITO DE EXTRATOS VEGETAIS NO REPARO ÓSSEO E CUTÂNEO EM
MODELOS ANIMAIS**

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Biologia Celular e Estrutural, para obtenção do título de *Magister Scientiae*.

APROVADA: 31 de julho de 2018.


Rômulo Dias Novaes
(Coorientador)


Fabiana Cristina Silveira Alves de Melo


Reggiani Vilela Gonçalves
(Orientadora)

AGRADECIMENTOS

A Deus, o Rei da minha vida, responsável por tudo que sou e realizo, sem o qual eu nada seria.

Aos meus pais, Mariza e David, por me apoiar e incentivar, sempre me ensinando e inspirando, responsáveis, também, por essa conquista.

Aos meus irmãos, Alexander, Larissa e Thaís, pelo companheirismo, amizade em todos os momentos e por sempre torcerem por mim.

Ao meu namorado, Lucas, pelo amor, paciência, parceria e por sonhar junto comigo.

Aos meus amigos da CBPaz, por todos os momentos que me proporcionam, deixando a vida mais leve e alegre.

Aos meus familiares por todo encorajamento.

Aos amigos do Laboratório de Patologia Experimental, pela disposição em ajudar e pela convivência tão agradável, em especial à Luciana, parceira de todas as horas e ao Daniel Bastos, pela paciência e disposição em ajudar nas análises.

À professora Reggiani Vilela Gonçalves, pela orientação e confiança.

À Mariáurea pela coorientação e apoio em todos os momentos.

Aos professores Sérgio da Matta, Jorge Dergan, Mariella, Sirlene, Leandro Licursi, e ao NUPEB/UFOP, por cederem suas estruturas para o desenvolvimento da pesquisa.

À Beth, secretária da Biocel, pela sua paciência, simpatia e esforço, sempre ajudando no que precisamos.

À Universidade Federal de Viçosa, ao Departamento de Biologia Geral e ao Programa de Pós-graduação em Biologia Celular e Estrutural, pela oportunidade de desenvolvimento estudantil.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES, pelo apoio financeiro.

À FAPEMIG e ao CNPq.

Aos professores participantes da minha banca avaliadora pela disponibilidade em participar da avaliação desta pesquisa.

E a todos que, direta ou indiretamente, fazem parte dessa conquista.

“Aquele que é capaz de fazer infinitamente mais do que tudo o que pedimos ou pensamos. De acordo com o Seu poder que atua em nós. A Ele seja a glória.”

Efésios 3:20

SUMÁRIO

RESUMO	vi
ABSTRACT	vii
1. INTRODUÇÃO GERAL	1
1.1 Reparo tecidual	1
1.2 Fraturas ósseas	1
1.3 Queimaduras cutâneas	2
1.4 Extratos vegetais	3
2. OBJETIVOS	4
2.1 Objetivo geral	4
2.2 Objetivos específicos	4
REFERÊNCIAS	4
ARTIGO 1:	9
Preclinical relevance of the use of plant extracts in bone repair: a systematic review in animal studies	9
Abstract	10
1. Introduction	11
2. Methodology	12
3. Results	15
Screening	16
Included	16
Eligibility	16
Identification	16
4. Discussion	45
5. Conclusion	50
6. References	51
ARTIGO 2:	59
Pomada a base de extrato de <i>Brassica oleracea</i> var. <i>capitata</i> acelera o processo de cicatrização de queimaduras de terceiro grau em ratos Wistar	59
Resumo	59
1. Introdução	60
2. Material e métodos	61
2.1 Obtenção do material vegetal	61
2.2 Preparo da formulação	62
2.3 Animais	62
2.4 Realização das queimaduras de pele	62
2.5 Cálculo da área e taxa de contração da ferida	63

2.6	<i>Design experimental</i>	63
2.7	<i>Análises histológicas</i>	64
2.8	<i>Análises bioquímicas</i>	64
2.8.1	<i>Atividade de superóxido dismutase</i>	64
2.8.2	<i>Atividade de catalase</i>	65
2.8.3	<i>Atividade de glutathione S-transferase</i>	65
2.8.4	<i>Determinação de malondialdeído</i>	65
2.8.5	<i>Oxidação de proteínas</i>	65
2.8.6	<i>Produção de Óxido Nítrico (NO)</i>	65
2.8.7	<i>Produção de Peróxido de Hidrogênio (H₂O₂)</i>	66
2.9	<i>Análise estatística</i>	66
3.	<i>Resultados</i>	66
3.1	<i>Contração da ferida</i>	66
3.2	<i>Resultados histopatológicos</i>	67
3.3	<i>Resultados bioquímicos</i>	69
3.3.1	<i>Enzimas antioxidantes</i>	69
3.3.2	<i>Marcadores oxidativos</i>	70
3.3.3	<i>Óxido Nítrico e Peróxido de Hidrogênio (NO e H₂O₂)</i>	71
4.	<i>Discussão</i>	72
5.	<i>Conclusão</i>	75
	<i>Referências</i>	75
	<i>CONCLUSÕES GERAIS</i>	84

RESUMO

MIRANDA, Lyvia Lopes, M.Sc., Universidade Federal de Viçosa, julho de 2018. **Efeito de extratos vegetais no reparo ósseo e cutâneo em modelos animais**. Orientadora: Reggiani Vilela Gonçalves. Coorientadores: Mariáurea Matias Sarandy Souza e Rômulo Dias Novaes.

O reparo de lesões teciduais é um processo biológico natural essencial para a sobrevivência de todos os organismos superiores. A utilização de produtos naturais para tratamento de lesões vem crescendo em todo o mundo, pois representam uma alternativa eficaz e com baixos efeitos colaterais para os indivíduos. Esse estudo teve por objetivo investigar o efeito de extratos vegetais na cicatrização de fraturas ósseas e feridas cutâneas em animais. Esta dissertação é composta por dois artigos. No artigo 1, foi realizada uma revisão sistemática. Foram selecionados 27 estudos que avaliaram o efeito do extrato de plantas no reparo ósseo. A avaliação da qualidade dos estudos incluídos foi realizada utilizando os critérios descritos nas diretrizes ARRIVE para Relatórios de Pesquisa Animal. Apesar da grande variação entre alguns parâmetros analisados na revisão sistemática, os animais tratados com espécies vegetais, no geral, apresentaram resultados positivos quanto à estimulação da proliferação celular. O artigo 2, consistiu em uma investigação experimental, onde vinte e cinco ratos Wistar (± 349 g), com três meses de vida, foram obtidos do Biotério Central da Universidade Federal de Viçosa e individualizados em gaiolas com alimento e água *ad libitum*. Após anestesia, foram feitas duas feridas por queimadura (12 mm de diâmetro) no dorso de cada animal, os quais foram igualmente randomizados em cinco tratamentos: Sal: salina; VP: veículo da pomada (lanolina e vaselina); SS: controle positivo (sulfadiazina de prata 1%); PB1: pomada *B. oleracea* a 10%; e PB2: pomada *B. oleracea* a 20%. Os animais foram tratados com a pomada, diariamente, durante oito dias. A pomada à base do extrato de *Brassica oleracea* var. *capitata* nas concentrações 10 e 20% promoveu maior celularidade, vascularização, bem como aumento dos componentes fibrosos da matriz extracelular, aumentando a expressão das enzimas antioxidantes. Nossos achados demonstraram por meio da revisão sistemática que extratos vegetais estimulam o reparo ósseo e, o estudo experimental evidenciou a eficácia da pomada a base do extrato de *Brassica oleracea* na aceleração da cicatrização de feridas por queimadura em ratos Wistar.

ABSTRACT

MIRANDA, Lyvia Lopes, M.Sc., Universidade Federal de Viçosa, July, 2018. **Effect of plant extracts on bone and cutaneous repair in animal models.** Adviser: Reggiani Vilela Gonçalves. Co-Advisers: Mariáurea Matias Sarandy Souza and Rômulo Dias Novaes.

The repair of tissue lesions is a natural biological process essential for the survival of all higher organisms. The use of natural products for the treatment of injuries has been growing all over the world as they represent an effective alternative and with low side effects for individuals. This study aimed to investigate the effect of plant extracts on the healing of bone fractures and cutaneous wounds in animals. This dissertation is composed of two articles. In article 1, a systematic review was carried out. Twenty-seven studies evaluated the effect of plant extract on bone repair. The quality evaluation of the included studies was performed using the criteria described in the ARRIVE guidelines for Animal Research Reports. Despite the great variation among some parameters analyzed in the systematic review, the animals treated with plant species, in general, presented positive results regarding the stimulation of cell proliferation. Article 2 consisted of an experimental investigation, where twenty - five Wistar rats (± 349 g), with three months of life, were obtained from the Central Biotery of the Federal University of Viçosa and individualized in cages with food and water ad libitum. After anesthesia, two burn wounds (12 mm diameter) were made on the back of each animal, which were equally randomized in five treatments: Salt: saline; VP: ointment vehicle (lanolin and vaseline); SS: positive control (1% silver sulfadiazine); PB1: 10% *B. oleracea* ointment; and PB2: 20% *B. oleracea* ointment. The animals were treated with the ointment daily for eight days. The ointment based on the extract of *Brassica oleracea* var. *capitata* at concentrations 10 and 20% promoted greater cellularity, vascularization, as well as increase of the fibrous components of the extracellular matrix, increasing the expression of the antioxidant enzymes. Our findings demonstrated through systematic review that plant extracts stimulate bone repair and the experimental study evidenced the efficacy of *Brassica oleracea* extract ointment in accelerating the healing of burn wounds in Wistar rats.

1. INTRODUÇÃO GERAL

1.1 Reparo tecidual

Feridas provocadas por diferentes agentes lesivos surgem constantemente comprometendo a estrutura e função de tecidos (Basu et al., 2017) e expondo indivíduos ao risco de outras complicações de saúde como infecções (Ho et al., 2017).

O reparo de feridas causadas por diferentes agentes lesivos, é um processo biológico natural essencial para a sobrevivência de todos os organismos superiores (Takeo et al., 2015). Neste processo, células, matriz extracelular (MEC), proteínas derivadas de plasma e fatores de crescimento atuam juntos para restaurar a integridade do tecido danificado (Gibon et al., 2016; Eming et al., 2014; Basu et al., 2017).

O processo cicatricial ocorre em três fases: inflamatória, de proliferação e de remodelamento (Mulholland et al., 2017). Durante a primeira etapa, há liberação de mediadores e recrutamento de leucócitos, que são fundamentais para eliminar restos de tecido e infecções durante a cicatrização, ajudando assim na reparação do tecido lesado (Curtis et al., 2014). Na fase de proliferação ocorre a formação do tecido de granulação rico em colágeno tipo III (Hyldig et al., 2017; Werner & Antsiferova, 2016). Na fase de remodelamento, o colágeno tipo III é substituído pelo tipo I, que é mais espesso e resistente (Docheva et al., 2015) e, como resultado da interação entre células, matriz extracelular e citocinas, ocorre o fechamento da ferida (Bankoti et al., 2017). No caso de fraturas ósseas, na fase inflamatória, há o recrutamento de células-tronco mesenquimais específicas (CTMs), que irão proliferar e se diferenciar em células osteogênicas. Após isso ocorre a formação de um calo de fibrocartilagem, que sofre mineralização, reabsorção e é então substituído por osso (Marsell & Einhorn, 2011).

1.2 Fraturas ósseas

O osso é um tecido biológico dinâmico formado por células metabolicamente ativas que compõem uma estrutura rígida (Loi et al., 2016). Sua porção orgânica é formada por células (osteoblastos, osteócitos e osteoclastos), fibras colágenas e substância base (proteoglicanos e glicoproteínas) (Levengood & Zhang, 2014). A capacidade de regeneração do osso é influenciada por uma variedade de mecanismos bioquímicos, biomecânicos,

celulares, hormonais e patológicos (Agarwal & García, 2015). O remodelamento ósseo ocorre por meio da reabsorção e neoformação óssea, mediada por osteoclastos e osteoblastos, respectivamente (Saran et al., 2014).

Fraturas ósseas podem estar relacionadas a vários fatores como traumatismos (Rodriguez-Merchan et al., 2013) osteoporose (Cauley, 2013), má nutrição (Karpouzou et al., 2017), tabagismo (Prada et al., 2017), falta de exercícios físicos (Moreira-Marconi et al., 2016), envelhecimento e doenças (Zimmermann et al., 2015), além de poder afetar diferentes ossos (Palacios et al., 2014). Essas lesões associadas a variáveis como idade, sexo e comorbidades médicas podem aumentar os riscos de morbidade e mortalidade dos pacientes, sendo um importante problema de saúde pública (Belmont et al., 2014).

1.3 Queimaduras cutâneas

A pele, maior órgão do corpo humano, é composta pela epiderme e derme. Esse órgão é responsável pela manutenção da homeostase do organismo vivo, além de formar uma barreira de proteção contra agentes químicos, físicos e biológicos (Ho et al., 2017). Feridas provocadas por queimadura, trauma mecânico, cortes, e cirurgias surgem constantemente neste órgão comprometendo sua estrutura e função e expondo indivíduos ao risco de outras complicações de saúde como ulcerações e infecções (Basu et al., 2017).

Queimaduras são consideradas um problema de saúde pública, causando cerca de 265.000 mortes por ano e, quando não fatais, são uma das principais causas de morbidade, gerando altos gastos financeiros para o tratamento (World Health Organization, 2016).

As feridas por queimadura podem ser causadas por calor, produtos químicos, eletricidade, radiação, e são classificadas como primeiro grau (superficial - atingindo a epiderme), segundo grau (espessura parcial - atingindo epiderme e derme) e terceiro grau (espessura total - atingindo epiderme, derme, hipoderme e músculos) (Bahramsoltani et al., 2017). Há também queimaduras de quarto grau que podem atingir os ossos (Nasoori & Hoomand, 2017). A profundidade da queimadura é definida por temperatura, tempo de exposição, pressão de contato e é a principal determinante do prognóstico após as lesões (Singer et al., 2010).

Queimaduras cutâneas são um fator de risco para o desenvolvimento de infecções, já que destroem a primeira barreira de defesa contra agentes infecciosos, a pele, e quando ocorre em áreas extensas leva à imunossupressão, favorecendo o processo infeccioso (Ramirez-

Blanco et al., 2017). A maior compreensão dos mecanismos de aceleração do processo de reparo de feridas, bem como o desenvolvimento de novos fármacos, poderá beneficiar pacientes com queimaduras graves, levando a um processo de reparo mais rápido (Takeo et al., 2015; Rowan et al., 2015).

1.4 Extratos vegetais

Plantas medicinais e seus derivados possuem uma variedade de constituintes como alcaloides, óleos essenciais, flavonoides, taninos, terpenoides, saponinas, ácidos graxos e compostos fenólicos. Esses compostos potencializam os seus efeitos de melhora do processo cicatricial, além de ser uma forma de tratamento simples, barata e com menos efeitos colaterais (Rekik et al., 2016).

Para um processo de cicatrização eficiente, alguns cuidados são necessários. A escolha do fármaco ideal e a agilidade em iniciar o tratamento podem evitar infecções (Hashmi et al., 2017). Atualmente, a maioria das drogas disponíveis possuem atividade antimicrobiana em vez de efeito cicatrizante (Bahramsoltani et al., 2014). Porém, existe uma tendência nos estudos de fitoterápicos que possuam diferentes componentes ou princípios ativos com ação sinérgica, que tenham efeito antimicrobiano e cicatrizante (Agra et al., 2013).

As folhas de repolho (*Brassica oleraceae* var. *capitata*) são usadas na cura de feridas e para alívio da dor nas articulações na medicina tradicional (Samuelsen et al., 2007; Vale et al., 2014). Componentes fitoquímicos como glucosinolatos, taninos (ácidos fenólicos) e flavonoides têm sido relacionados à ação cicatrizante de plantas do gênero *Brassica* (Carvalho et al., 2008; Nugrahedhi et al., 2015; Hassini et al., 2017; Šamec et al., 2017). Estudos realizados pelo nosso grupo de pesquisa também têm mostrado a ação estimuladora dessa planta na cicatrização de feridas cutâneas de segunda intenção (Gonçalves et al., 2013; Sarandy et al., 2015), porém, a ação deste fitoterápico em queimaduras cutâneas de terceiro grau ainda não é conhecida.

2. OBJETIVOS

2.1 Objetivo geral

Avaliar o efeito de extratos vegetais no processo de cicatrização de fraturas ósseas e queimaduras cutâneas em animais.

2.2 Objetivos específicos

- Reunir as informações resultantes de um conjunto de estudos sobre o efeito de extratos vegetais e seus derivados no reparo ósseo em animais.
- Determinar, por meio da revisão sistemática, se existe um critério de seleção das espécies de plantas utilizadas para cicatrização óssea, a distribuição geográfica de cada espécie, bem como qualquer evidência de bioprospecção baseada em dados etnobotânicos.
- Avaliar a qualidade da escrita e/ou metodológica dos estudos incluídos na revisão sistemática.
- Avaliar os efeitos da pomada à base de *B. oleracea* (10 e 20%) sobre a velocidade de fechamento da área da queimadura.
- Analisar os efeitos da pomada à base de *B. oleracea* (10 e 20%) sobre a quantidade de fibras colágenas, elásticas, fibroblastos, mastócitos e vasos sanguíneos no tecido cicatricial de feridas por queimaduras de terceiro grau em ratos Wistar.
- Avaliar a ação da pomada à base de *B. oleracea* (10 e 20%) sobre enzimas antioxidantes (superóxido dismutase, catalase e glutathione S transferase), geração de marcadores de estresse oxidativo tecidual (malondialdeído e proteínas carboniladas) e espécies reativas de oxigênio (óxido nítrico e peróxido de hidrogênio).

REFERÊNCIAS

Agarwal R, García AJ. Biomaterial strategies for engineering implants for enhanced osseointegration and bone repair. *Advanced Drug Delivery Reviews* 2015;94:53-62.

Agra IKR, Pires LLS, Carvalho PSM, Silva-Filho EA, Smaniotto S, Barreto E. Evaluation of wound healing and antimicrobial properties of aqueous extract from *Bowdichia virgilioides* stem barks in mice. *Anais da Academia Brasileira de Ciências* 2013;85:945-954.

Bahramsoltani R, Farzaei MH, Rahimi R. Medicinal plants and their natural components as future drugs for the treatment of burn wounds: an integrative review. *Archives of Dermatological Research* 2014;306:601-617.

Bahramsoltani R, Farzaei H, Abdolghaffari AH, Rahimi R, Samadi N, Heidari M, Esfandyari M, Baeri M, Hassanzadeh G, Abdollahi M, Soltani S, Pourvaziri A, Amin G. Evaluation of phytochemicals, antioxidant and burn wound healing activities of *Cucurbita moschata* Duchesne fruit peel. *Iranian Journal of Basic Medical Sciences* 2017;20:798-805.

Bankoti K, Rameshbabu AP, Datta S, Maity PP, Goswami P, Datta P, Ghosh SK, Mitra A, Dhara S. Accelerated healing of full thickness dermal wounds by macroporous waterborne polyurethane-chitosan hydrogel scaffolds. *Materials Science & Engineering C-Materials for Biological Applications* 2017;81:133-143.

Basu P, Kumar UN, Manjubala I. Wound healing materials – a perspective for skin tissue engineering. *Current Science* 2017;112:2392-2404.

Belmont PJ Jr, Garcia EJ, Romano D, Bader JO, Nelson KJ, Schoenfeld AJ. Risk factors for complications and in-hospital mortality following hip fractures: a study using the National Trauma Data Bank. *Archives of Orthopaedic and Trauma Surgery* 2014;134:597-604.

Carvalho CA, Silva MB, Oliveira TG, Lima JM, Rosa MB. Spectrometric study at different phenologic stages of the cabbage (*Brassica oleraceae* var. *capitata*). *Brazilian Journal of Pharmacognosy* 2008;18:249-257.

Cauley JA. Public health impact of osteoporosis. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* 2013;68:1243-1251.

Curtis BJ, Hlavin S, Brubaker AL, Kovacs EJ, Radek KA. Episodic binge ethanol exposure impairs murine macrophage infiltration and delays wound closure by promoting defects in early innate immune responses. *Alcoholism: Clinical and Experimental Research* 2014;38:1347-1355.

Docheva D, Müller SA, Majewski M, Evans CH. Biologics for tendon repair. *Advanced Drug Delivery Reviews* 2015;84:222-239.

Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: mechanisms, signaling, and translation. *Science Translational Medicine* 2014;6:265sr6.

Gibon E, Lu L, Goodman SB. Aging, inflammation, stem cells, and bone healing. *Stem Cell Research & Therapy* 2016;7:44.

Gonçalves RV, Sarandy MM, da Matta SL, Novaes RD, Pinto MVM. Comparative study of the effects of laser photobiomodulation and extract of *Brassica oleracea* on skin wounds in wistar rats: A histomorphometric study. *Pathology – Research and Practice* 2013;209:648-659.

Hashmi MU, Khan F, Khalid N. Hydrogels incorporated with silver nanocolloids prepared from antioxidant rich *Aerva javanica* as disruptive agents against burn wound infections. *Colloids and Surfaces A* 2017;529:475-486.

Hassini I, Baenas N, Moreno DA, Carvajal M, Boughanmi N, Martinez Ballesta MDC. Effects of seed priming, salinity and methyl jasmonate treatment on bioactive composition of *Brassica oleracea* var. *capitata* (white and red varieties) sprouts. *Journal of the Science of Food and Agriculture* 2017;97:2291-2299.

Ho J, Walsh C, Yue D, Dardik A, Cheema U. Current advancements and strategies in tissue engineering for wound healing: a comprehensive review. *Advances in Wound Care* 2017;6:191-209.

Hyldig K, Riis S, Pennisi CP, Zachar V, Fink T. Implications of extracellular matrix production by adipose tissue-derived stem cells for development of wound healing therapies. *International Journal of Molecular Sciences* 2017;18:e1167.

Karpouzou A, Diamantis E, Farmaki P, Savvanis S, Troupis T. Nutritional aspects of bone health and fracture healing. *Journal of Osteoporosis* 2017;2017:4218472.

Levengood SL, Zhang M. Chitosan-based scaffolds for bone tissue engineering. *Journal of Materials Chemistry B* 2014;2:3161-3184.

Loi F, Córdova LA, Pajarinen J, Lin TH, Yao Z, Goodman SB. Inflammation, fracture and bone repair. *Bone* 2016;86:119-130.

Marsell R., Einhorn TA. The biology of fracture healing. *Injury* 2011;42:551-555.

Moreira-Marconi E, Dionello CF, Morel DS, Sá-Caputo DC, Souza-Gonçalves CR, Paineiras-Domingos LL, Guedes-Aguiar EO, Marin PJ, Cruz BP, Bernardo-Filho M. Could whole body vibration exercises influence the risk factors for fractures in women with osteoporosis? *Osteoporosis and Sarcopenia* 2016;2:214-220.

Mulholland EJ, Dunne N, McCarthy HO. MicroRNA as therapeutic targets for chronic wound healing. *Molecular Therapy: Nucleic Acids* 2017;8:46-55.

Nasoori A, Hoomand R. Maggot debridement therapy for an electrical burn injury with instructions for the use of *Lucilia sericata* larvae. *Journal of Wound Care* 2017;26:734-741.

Nugrahedhi PY, Verkerk R, Widianarko B, Dekker M. A mechanistic perspective on process-induced changes in glucosinolate content in *Brassica* vegetables: a review. *Critical Reviews in Food Science and Nutrition* 2015;55:823-838.

Palacios S, Neyro JL, Fernández de Cabo S, Chaves J, Rejas J. Impact of osteoporosis and bone fracture on health-related quality of life in postmenopausal women. *Climacteric* 2014;17:60-70.

Prada D, Zhong J, Colicino E, Zanobetti A, Schwartz J, Dagnacourt N, Fang SC, Kloog I, Zmuda JM, Holick M, Herrera LA, Hou L, Dominici F, Bartali B, Baccarelli AA. Association of air particulate pollution with bone loss over time and bone fracture risk: analysis of data from two independent studies. *The Lancet Planetary Health* 2017;1:e337-e347.

Ramirez-Blanco CE, Ramirez-Rivero CE, Diaz-Martinez LA, Sosa-Avila LM. Infection in burn patients in a referral center in Colombia. *Burns* 2017;43:642-653.

Rekik DM, Khedir SB, Moalla KK, Kammoun NG, Rebai T, Sahnoun Z. Evaluation of wound healing properties of grape seed, sesame, and fenugreek oils. *Evidence-Based Complementary and Alternative Medicine* 2016;2016:7965689.

Rodriguez-Merchan EC, Moraleta L, Gomez-Cardero P. Injuries associated with femoral shaft fractures with special emphasis on occult injuries. *The Archives of Bone and Joint Surgery* 2013;1:59-63.

Rowan MP, Cancio LC, Elster EA, Burmeister DM, Rose LF, Natesan S, Chan RK, Christy RJ, Chung KK. Burn wound healing and treatment: review and advancements. *Critical Care* 2015;19:243.

Šamec D, Pavlović I, Salopek-Sondi B. White cabbage (*Brassica oleracea* var. *capitata* f. *alba*): botanical, phytochemical and pharmacological overview. *Phytochemistry Reviews* 2017;16:117-135.

Samuelsen AB, Westereng B, Yousif O, Holtekjølen AK, Michaelsen TE, Knutsen SH. Structural features and complement-fixing activity of pectin from three *Brassica oleracea* varieties: White Cabbage, Kale, and Red Kale. *Biomacromolecules* 2007;8:644-649.

Saran U, Gemini Piperni S, Chatterjee S. Role of angiogenesis in bone repair. *Archives of Biochemistry and Biophysics* 2014;561:109-117.

Sarandy MM, Novaes RD, da Matta SLP, Mezencio JMS, da Silva MB, Zanuncio JC, Gonçalves RV. Ointment of *Brassica oleracea* var. *capitata* matures the extracellular matrix in skin wounds of wistar rats. *Evidence-Based Complementary and Alternative Medicine* 2015;2015:1-9.

Singer JA, Taira BR, Anderson R, McClain SA, Rosenberg L. Does pressure matter in creating burns in a porcine model? *Journal of Burn Care & Research* 2010;31:646-51.

Takeo M, Lee W, Ito M. Wound Healing and Skin Regeneration. Cold Spring Harbor Laboratory Press 2015;5:a023267.

Vale AP, Cidade H, Pinto M, Oliveira MB. Effect of sprouting and light cycle on antioxidant activity of *Brassica oleracea* varieties. *Food Chemistry* 2014;165:379-387.

Werner S, Antsiferova M. Wound healing: an orchestrated process of cell cycle, adhesion, and signaling. *Encyclopedia of Cell Biology* 2016;3:216-222.

World Health Organization. Burns: 2016 update; <http://www.who.int/mediacentre/factsheets/fs365/en/>.

Zimmermann EA, Busse B, Ritchie RO. The fracture mechanics of human bone: influence of disease and treatment. *BoneKEY Reports* 2015;4:743.

ARTIGO 1:

Preclinical relevance of the use of plant extracts in bone repair: a systematic review in animal studies

Lyvia Lopes Miranda¹, Vanessa de Paula Guimarães Lopes², Luciana Schulthais Altoé¹, Mariáurea Matias Sarandy², Fabiana Cristina Silveira Alves Melo², Rômulo Dias Novaes³, Reggiani Vilela Gonçalves^{2*}.

¹Departamento de Biologia Geral, Universidade Federal de Viçosa, 36570-900-Viçosa, Minas Gerais, Brasil.

²Departamento de Biologia Animal, Universidade Federal de Viçosa, 36570-900-Viçosa, Minas Gerais, Brasil.

³Departamento de Biologia Estrutural, Universidade Federal de Alfenas, Rua Gabriel Monteiro da Silva, 700, Campus Universitário, Centro, 37130-000, Alfenas, MG, Brasil.

*Autor de correspondência: Reggiani Vilela Gonçalves - reggiani.goncalves@ufv.br

Abstract

Bone tissue repair is a dynamic process coordinated by osteoprogenitor cells of the periosteum and endosteum, responsible for the formation of a new bone matrix. Studies using natural products for bone lesions treatment have been growing worldwide. We developed a systematic review to compile the results of works with animal models investigating the effect of plant extracts in the treatment of bone lesions and analyze the methodological quality of the studies on this subject. Studies were selected in the Pubmed/Medline and Scopus databases according to the PRISMA statement. The research filters were constructed using three parameters: animal model, bone repair, and plant extracts. The bias analysis of the 27 selected works was done using the ARRIVE guidelines. The animals treated with plant extracts presented positive results in the osteoblastic proliferation and, consequently, accelerated osteogenic differentiation and bone callus formation, as well as bone fracture repair. In addition, the bias analysis evidenced the absence of standardization of the experimental designs, showing that it is necessary to improve the methodology in further studies in order to achieve a better quality of the scientific evidence.

Keywords: bone healing, bone tissue, vegetal products, traditional medicine, animal studies.

1. Introduction

Bone remodeling is a mechanism of bone tissue replacement or reconstruction regulated by intracellular and hormonal factors [1]. This repair process is composed of a complex sequence of cellular events [2] that include phases of inflammation, cell proliferation and bone remodeling, which is controlled by osteogenesis and angiogenesis [3]. In most cases, bone fractures are caused by specific bone traumas or diseases. These diseases can cause bone tissue destruction or weakening, which can lead to fracture even in a trivial trauma [4].

The bone has high capacity of remodeling, being able to regenerate and maintain its structure and function. However, there are clinical situations in which the acceleration of bone formation is desirable [5]. Research has been carried out to better understand the mechanisms that regulate this repair process and identify new therapeutic targets for the treatment of bone fractures [6,7]. In this context, natural products, biomaterials and their derivatives have stood out as a promising alternative to minimize side effects, reduce costs and promote a fast and efficient repair process [8].

Drugs derived from medicinal plants are consumed by about 60% of the world's population and represent the main form of treatment of traditional medicine in some countries like Brazil, China and India [9]. Medications containing extracts, fractions and plant isolates can be distributed in capsules, pills, oils, gels, ointments, aqueous and hydroalcoholic solutions, infusions and poultices [10]. Studies have shown that some molecules extracted from natural compounds have high anti-inflammatory, antioxidant and regenerative effects, which justifies the successful use of these products in different diseases [11]. However, the mechanisms by which compounds of natural origin act in the process of bone healing are still poorly understood. Current evidences are sparse, fragmented and based on punctual researches, which makes inconsistent the results described in the literature.

Clinical and preclinical studies have attempted to demonstrate the positive effects of plant compounds on cell proliferation and bone matrix formation. However, this proposition is not always confirmed, mainly due to the great methodological variations involving the extract preparation, therapeutic schemes and mechanisms of action. Therefore, it is necessary to compile data from several studies in order to clarify the aforementioned discrepancies. In this context, the systematic review is a powerful tool that incorporates the variability between studies and allows the obtaining of an overall estimate of the use of plant extracts in the treatment of bone lesions. Thus, we systematically analyzed the preclinical evidence *in vivo*, to establish the relevance of the use of vegetal products in bone repair. In addition, we aimed to determine if there is a rational selection criterion of the plants species used, the geographic distribution of each species, as well as any evidence of bioprospection based on ethnobotanical data. We also performed a critical analysis of the studies, aiming to improve the quality of the reports, preventing the reproduction of methodological failures in new studies.

2. Methodology

2.1 Search strategy

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) strategy was applied to identify all studies included in this review [12]. A direct search was carried out from two comprehensive electronic databases PubMed/MEDLINE and SCOPUS. The secondary search was based on the screening of reference list of all relevant studies identified in the direct search.

Structured search filters were developed for each database. The search filters were initially constructed considering standardized descriptors extracted from PubMed thesaurus (MeSH - Medical Subject Headings). All descriptors were combined in a complete three-level

search strategy based on: (i) animal model, (ii) bone repair, and (iii) plant extracts. Standardized descriptors were defined by MeSH algorithm and non-MeSH descriptors were characterized by the [TIAB] algorithm, which was also used to recover recently published and studies in process of indexation. A previously published and optimized animal filter was applied in PubMed search interface [13]. The same search filters used for bone repair and intervention were adapted for Scopus. The own Scopus animal filter (Keyword – animals [limit to]) was used in this database. Only studies in English, Portuguese and Spanish were recovered and no chronological limits were applied in our search strategy. All relevant studies published until August 27, 2018 (updated search date) were recovered and included in the systematic review.

2.2 Records screening and eligibility

All research records recovered in database search were analyzed and duplicates were removed considering the authors, title, journal and year of publication. After title and abstract screening, all potentially relevant studies were evaluated in full-text for eligibility according specific inclusion and exclusion criteria. Only studies investigating the relevance of plant extracts on bone repair in preclinical studies with animal models. The exclusion criteria were based on: (i) it's not bone, (ii) it's not plant, (iii) laminectomy, (iv) absence of bone defect, (v) biomaterial, (vi) in vitro, (vii) review, (viii) secondary studies, (ix) marketed products, (x) associated treatment, (xi) other language, (xii) bone marrow. Literature reviews were admitted only when original data were additionally reported. Eligibility was independently analyzed by the researchers and disagreements were resolved by consensus. Considering enhance the research strategy comprehensiveness, the reference lists of all relevant papers identified from databases search were screened for additional studies.

2.3 Data extraction

An initial selection based on title and abstract (TIAB) was conducted by three independent reviewers (LLM, MMS, and FCSAM). In case of disagreements, a fourth reviewer (RVG) decided whether the study met the inclusion and exclusion criteria. In order to discard subjectivity in the data collection and selection strategy, the information was independently extracted by the four reviewers (LLM, MMS, FCSAM, and RVG) and analyzed separately.

Data were extracted and tabulated in a descriptive way (descriptors and results tables). The data extraction was categorized as follows: (I) characteristics of the publication: author, year and country; (II) characteristics of the animal model: species, sex, age and weight; (III) treatment characteristics: total number of animals, number of animals in each group, control group, treatment time, osteoporosis induction, bone type, bone defect model, lesion size, anesthetics and euthanasia procedure; (IV) plants: species, used part, popular indication, extraction and purification method, dose, administration, secondary metabolites and geographical distribution.

2.4 Methodological bias

Reporting bias was analyzed based on methodological requirements described in ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guideline [14]. This strategy requires the complete screening of all manuscript sessions (abstract to acknowledgement and funding) to evaluate the completeness of the scientific reports in animal studies. The screening strategy was based on short descriptions of essential characteristics such as baseline measurements, sample size, animal allocation, randomization, experimental concealment, statistical methods, ethical statement and generalizability power. A table summarizing all

relevant and applicable aspects was constructed considering the specificity and aims of the systematic review.

3. Results

3.1 PRISMA Guideline

From the PubMed/Medline and Scopus database, 274 articles were recovered. A total of 24 duplicated studies and 214 with thematic inadequacy were excluded after reading the title and abstract. Of the 36 remaining studies, 12 articles were excluded after reading the full text because they did not meet the eligibility criteria. Thus, 24 studies were included in the systematic review. The reference list of all included studies was analyzed to ensure the identification of additional relevant studies and 2 of them were recovered, totalizing 26 studies (Figure 1).

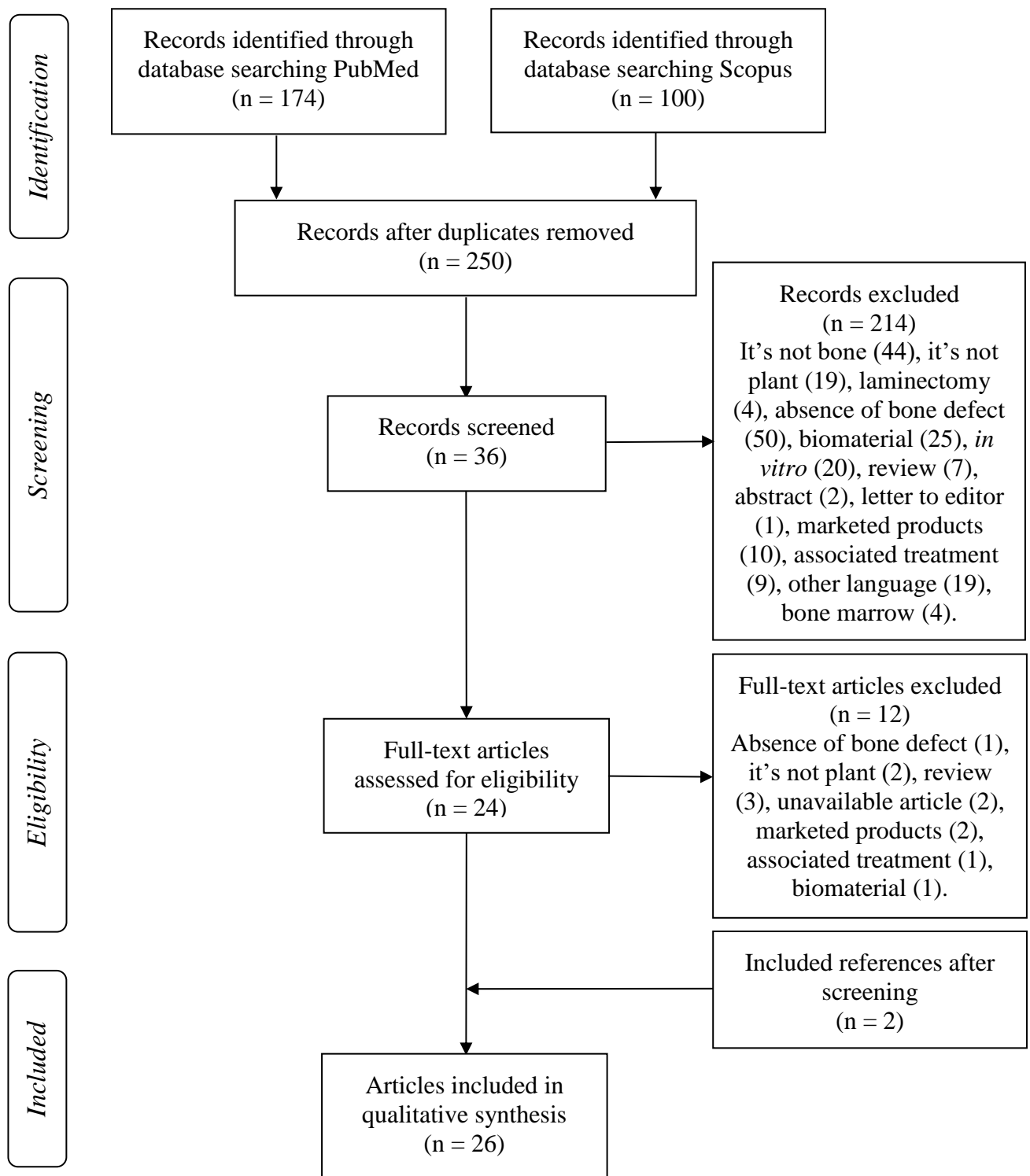


Figure 1. PRISMA diagram. Different phases of selection of studies for conducting qualitative and quantitative analyses. Flow diagram of the systematic review literature search results. Based on 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement'. <http://www.prisma-statement.org>. From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009).

3.2 Qualitative analysis

The analyzed studies were conducted in 10 different countries, especially China (26.9%, n=7), followed by India (23.1%, n=6), Cameroon (11.5%, n=3) and Brazil, Turkey and Germany (7.7%, n=2 each). The most commonly used animal models used were murine (88.5, n=23) and rabbits (11.5%, n=3). Considering the animal strain, 57.7% (n=15) were Sprague Dawley rats, 19.2% (n=5) Wistar rats, 11.5% (n=3) New Zealand white rabbits, and 11.5% (n=3) did not report the strain. From the experimental models, 61.5% (n=16) used female animals, 19.2% (n=5) used males, 3.8% (n=1) used both sexes, and 15.4% (n=4) of all studies did not report this information. The animal's age ranged from 7 weeks to 15 months for rats, 6 months for rabbits, and 38.5% (n=10) of the studies did not report this information. The weight of rats ranged from 150 to 310 g, 1.62 to 5 kg for rabbits, 28 to 33 g for mice, and 15.4% (n=4) of the studies did not report this data (Table 1).

Regarding the treatment time, there was great variation, from 12 days (15.4%, n=4) to 12 weeks (7.7%, n=2). Six studies (23.1%) reported that they had induced osteoporosis in the animals. The most evaluated bone was the femur (53.8%, n=14) followed by the tibia (23.1%, n=6). The methods used to perform the bone defects were described in 84.6% (n=22) of the studies. 34.6% (n=9) were performed by insertion of a drill bit. A lesion of 0.8 mm in diameter was created in 30.7% (n=8) of the studies. 53.8% (n=14) studies did not report this information. Regarding the anesthetic procedure, 38.5% (n=10) of the studies used ketamine and xylazine, 7.7% (n=2) used chloral hydrate, and 38.5% (n=10) did not report or did not use anesthesia. Most of the studies (84.6%, n=22) did not report or did not use pharmacology postoperatively. More than half of the studies (65.4%, n=17) did not report the euthanasia procedure of the animals. 11.5% (n=3) used decapitation under anesthesia. The data cited above can be analyzed in Table 2.

Table 1. Description of the main characteristics of the animal model in the studies demonstrating the action of plant extracts in the bone repair process.

Title	Study ID	Country	Animal model	Sex (M/F)*	Age (d,w,m)*	Weight
The effect of <i>Davallina orientalis</i> on bone healing-a preliminary report.	Chow et al., 1982 [15]	China	Mice	?	11-12w	28-33g
The use of Chinese herbal medicine on experimental fracture healing.	Huang and You, 1997 [16]	China	Rats	M	?	250-350g
The effects of <i>Lepidium sativum</i> seeds on fracture-induced healing in rabbits.	Juma Ab, 2007 [17]	Saudi Arabia	New Zealand white rabbits	?	6m	4-5kg
The effects of phytoestrogens on fracture healing: experimental research in New Zealand white rabbits.	Oztürk et al., 2008 [18]	Turkey	New Zealand white rabbits	?	?	1.62 ± 0.05kg
Formononetin promotes early fracture healing through stimulating angiogenesis by up-regulating VEGFR-2/Flk-1 in a rat fracture model.	Huh et al., 2009 [19]	Korea	Sprague-Dawley rats	M	2m	280-300g
Equol but not genistein improves early metaphyseal fracture healing in osteoporotic rats.	Kolios et al., 2009 [20]	Germany	Sprague-Dawley rats	F	3m	?
Absence of positive effect of black cohosh (<i>Cimicifuga racemosa</i>) on fracture healing in osteopenic rodent model.	Kolios et al., 2010 [21]	Germany	Sprague-Dawley rats	F	3m	?
Bone quality associated with daily intake of coffee: a biochemical, radiographic and histometric study.	Lacerda et al., 2010 [22]	Brazil	Wistar rats	?	?	250-300g
<i>In vitro</i> & <i>in vivo</i> assessment of a herbal formula used topically for bone fracture treatment.	Peng et al., 2010 [23]	China	New Zealand white rabbits	M	6m	?
<i>Piper sarmentosum</i> enhances fracture healing in ovariectomized osteoporotic rats: a radiological study.	Estai et al., 2011 [24]	Malaysia	Sprague-Dawley rats	F	?	200-250g
A novel quercetin analogue from a medicinal plant promotes peak bone mass achievement and bone healing after injury and exerts an anabolic effect on osteoporotic bone: the role of aryl hydrocarbon receptor as a mediator of osteogenic action.	Sharan et al., 2011 [25]	India	Sprague-Dawley rats	F	?	200±20g

Table 1. Continued.

Title	Study ID	Country	Animal model	Sex (M/F)*	Age (d,w,m)*	Weight
Evaluation of Cameroonian plants towards experimental bone regeneration.	Ngueguim et al., 2012 [26]	Cameroon	Sprague – Dawley rats	F	4m	220±20g
The bone fracture-healing potential of <i>Ormocarpum cochinchinense</i> , methanolic extract on albino wistar rats.	Kumar et al., 2013 [27]	India	Wistar albino rats	F	3m	150-200g
Ethanol extract of <i>Peperomia pellucida</i> (Piperaceae) promotes fracture healing by an anabolic effect on osteoblasts.	Ngueguim et al., 2013 [28]	Cameroon	Sprague-Dawley rats	F	4m	200±20g
Salvianolic acid B promotes bone formation by increasing activity of alkaline phosphatase in a rat tibia fracture model: a pilot study.	He and Shen, 2014 [29]	China	Sprague-Dawley rats	M	7w	225g
Evaluation of a topical herbal agent for the promotion of bone healing.	Siu et al., 2015 [30]	Hong Kong	Sprague-Dawley rats	F	15.2 ± 1.41m	?
Repair of critical calvarias defects with systemic <i>Epimedium sagittatum</i> extract.	Burim et al., 2016 [31]	Brazil	Wistar albino rats	M	?	200-250g
The effects of <i>Nigella sativa</i> seed extract on bone healing in an experimental model.	Ezirganli et al., 2016 [32]	Turkey	Wistar albino rats	F	3m	280-310g
Root bark of <i>Sambucus williamsii</i> Hance promotes rat femoral fracture healing by the BMP-2/Runx2 signaling pathway.	Yang et al., 2016 [33]	China	Sprague-Dawley rats	M/F	3m	220±20g
<i>Ulmus davidiana</i> extract improves lumbar vertebral parameters in ovariectomized osteopenic rats.	Zhuang et al., 2016 [34]	China	Rats	F	?	250-270g
Dried and free flowing granules of <i>Spinacia oleracea</i> accelerate bone regeneration and alleviate postmenopausal osteoporosis.	Adhikary et al., 2017 [35]	India	Sprague-Dawley rats	F	3m	180-200g
Tanshinol alleviates osteoporosis and myopathy in glucocorticoid-treated rats.	Chen et al., 2017 [36]	China	Sprague-Dawley rats	F	4-5m	250-275g

Table 1. Continued.

Title	Study ID	Country	Animal model	Sex (M/F)*	Age (d,w,m)*	Weight
Aqueous extract of <i>Peperomia pellucida</i> (L.) HBK accelerates fracture healing in Wistar rats.	Florence et al., 2017 [37]	Cameroon	Wistar rats	F	3m	150-200g
Heartwood extract from <i>Dalbergia sissoo</i> promotes fracture healing and its application in ovariectomy-induced osteoporotic rats.	Karvande et al., 2017 [38]	India	Sprague-Dawley rats	F	?	220±20g
Ethanollic extract of <i>Dalbergia sissoo</i> promotes rapid regeneration of cortical bone in drill-hole defect model of rat.	Khedgikar et al., 2017 [39]	India	Sprague-Dawley rats	F	?	180±20g
Guava fruit extract and its triterpene constituents have osteoanabolic effect: Stimulation of osteoblast differentiation by activation of mitochondrial respiration via the Wnt/ β -catenin signaling.	Porwal et al., 2017 [40]	India	Sprague-Dawley rats	F	?	220±20g

M: male; F: female; d: day; w: week; m: month; ?: not related.

Table 2. Description of the main characteristics of the treatment in the studies demonstrating the action of plant extracts in the bone repair process.

Study ID	Number of animals	Number of animals / group	Control group	Treatment time (d,w,m)*	Osteoporosis	Bone type	Induced defect	Lesion size	Anesthesia (pharmaco)	Post-operative drug	Euthanasia
Chow et al., 1982 [15]	30	10	Saline	?	No	Femur	Osteotomy	?	Ether inhalation	?	?
Huang and You, 1997 [16]	75	?	No treatment	12d	No	Tibia / fibula	?	?	?	?	?
Juma Ab, 2007 [17]	6	3	Routine diet	12w	No	Femur	Drill machine	?	Ketamine and xylazine	?	?
Oztürk et al., 2008 [18]	22	11	?	35d	No	Tibia	?	?	Alfazin and Propofol	?	?
Huh et al., 2009 [19]	?	144/12	Saline	21d	No	Femur	?	?	?	?	Paraformaldehyde / tiletamine-zolazepam
Kolios et al., 2009 [20]	48	12	Phytoestrogen-free	35d	Yes OVX	Tibia	Osteotomy	?	Ketamine and xylazine	?	Decapitated under deep CO ₂ anesthesia
Kolios et al., 2010 [21]	36	12	Phytoestrogen-free chow	35d	Yes OVX	Tibia	Osteotomy	?	Ketamine and xylazine	?	Decapitated while under deep CO ₂ anesthesia
Lacerda et al., 2010 [22]	42	?	Water	7, 21, 42d	No	Maxilla	Incisor teeth extraction	?	2,2,2 tribromoethanol	Veterinary pentabiotic	Anesthetic overdose
Peng et al., 2010 [23]	30	11/12	Without treatment with plants	8w	No	Tibia	Fracture device	?	?	?	?

Table 2. Continued.

Study ID	Number of animals	Number of animals / group	Control group	Treatment time (d,w,m)*	Osteoporosis	Bone type	Induced defect	Lesion size	Anesthesia (pharmaco)	Post-operative drug	Euthanasia
Estai et al., 2011 [24]	24	6	Saline	6w	Yes OVX	Femur	Blunt guillotine-like blade device	?	Xylazil and ketamine	Antibiotic Baytril and povidone-iodine solution	?
Sharan et al., 2011 [25]	60	10	Gum acacia in water	2w	Yes OVX	Skull	Insertion a drill bit with a diameter of 0.8 mm	0.8mm diameter	?	?	?
Ngueguim et al., 2012 [26]	42	6	Acacia gum in water	12d	No	Femur	Drill machine	0.8mm diameter	?	?	?
Kumar et al., 2013 [27]	?	3	Saline	0, 7, 14, 21d	No	Femur	Fracture device	?	Ketamine hydrochloride	?	?
Ngueguim et al., 2013 [28]	?	6	Acacia gum in distilled water	12d	No	Femur	Insertion a drill bit with a diameter of 0.8 mm	0.8mm diameter	?	?	?
He and Shen, 2014 [29]	20	10	Saline	12w	No	Tibia	Custom-made three-point bending device	?	?	?	Ketamine
Siu et al., 2015 [30]	20	10	Without any treatment	6w	No	Femur and tibia	Electric drill	2mm (femur), 2.4mm (tibia) diameter	Ketamine and xylazine	?	?

Table 2. Continued.

Study ID	Number of animals	Number of animals / group	Control group	Treatment time (d,w,m)*	Osteoporosis	Bone type	Induced defect	Lesion size	Anesthesia (pharmaco)	Post-operative drug	Euthanasia
Burim et al., 2016 [31]	40	20	Saline	7, 14, 21, 42d	No	Calvaria	Trephine drill with a 8 mm in diameter	8mm diameter	Ketamine and xylazine hydrochloride	Antibiotic prophylaxis (benzathine benzylpenicillin)	CO ₂ chamber
Ezirganli et al., 2016 [32]	32	16	?	2, 4w	Yes OVX	Calvaria	Trephine burr	5mm	Ketamine and xylazine	?	Barbiturate overdose
Yang et al., 2016 [33]	96	24	Distilled water	2, 4, 8w	No	Femur	Osteotomy with a wire saw	?	Chloral hydrate	Penicillin sodium	?
Zhuang et al., 2016 [34]	32	8	Methylcellulose	14d	No	Femur	Insertion a drill bit with a diameter of 0.8 mm	0.8mm diameter	Ketamine and xylazine	?	?
Adhikary et al., 2017 [35]	?	10	Vehicle / Parathyroid hormone (PTH)	14d	?	Femur	Insertion a drill bit with a diameter of 0.8 mm	0.8mm diameter	?	?	?
Chen et al., 2017 [36]	96	24 or 16	Distilled water / calcitriol	6w	Yes GIOP	Tibia	?	2mm	Chloral hydrate	?	Cardiac puncture
Florence et al., 2017 [37]	30	5	Distilled water	14d	No	Femur	Insertion a drill bit	?	?	?	Decapitated while under ketamine and valium anesthesia

Table 2. Continued.

Study ID	Number of animals	Number of animals / group	Control group	Treatment time (d,w,m)*	Osteoporosis	Bone type	Induced defect	Lesion size	Anesthesia (pharmaco)	Post-operative drug	Euthanasia
Karvande et al., 2017 [38]	40	10	Acacia gum in distilled water	2w	No	Femur	Insertion a drill bit with a diameter of 0.8 mm	0.8mm diameter	Ketamine and xylazine	?	?
Khedgikar et al., 2017 [39]	32	?	Acacia gum in distilled water	2w	No	Femur	Insertion a drill bit with a diameter of 0.8 mm	0.8mm diameter	?	?	?
Porwal et al., 2017 [40]	20	?	Water	12d	No	Femur	Insertion a drill bit with a diameter of 0.8 mm	0.8mm diameter	Ketamine and xylazine	?	?

d: day; w: week; m: month; OVX: ovariectomized; GIOP: glucocorticoid; ?: not related.

3.3 Treatment characteristics

From the 26 studies, 80.8% (n=21) reported the scientific name of the plant, and 19.2% (n=5) cited only the popular name. The most used plant structures were the leaves (19.2%, n=5), followed by whole plant and roots (11.5%, n=3 each), and 26.9% (n=7) did not record this information. Most of the authors (26.9%, n=7) did not report the solvent used to extract the components of the plant. Among the studies that presented such information, the most used methods were ethanol (38.5%, n=10) and a water/ethanol mixture (19.2%, n=5). The most used treatments in the control groups were saline solution (23.1%, n=6), followed by acacia gum in aqueous medium (19.2%, n=5), and 7.7% (n=2) reported no treatment. Most of the studies reported oral administration (46.2%, n=12); however, in 11.5% of the cases (n=3) this information was not reported.

India was the most cited country (11.5%, n=3) in relation to the geographical distribution of plant species, but many studies (65.4, n=17) did not record this information. In relation to the investigated plants, 38.5% (n=10) realized the phytochemical prospecting, 26.9% (n=7) of them quoted the phytochemical components already reported in the literature and 34.6% (n=9) not described this information. The most common secondary metabolites were flavonoids, cited in 30.8% (n=8) studies (Table 3).

Table 3. Treatment characteristics in the studies demonstrating the action of plant extracts in the bone repair process.

Study ID	Plant	Used parts	Indication	Solvent extraction	Obtaining plant material	Dose	Administration	Secondary metabolites	Geographic distribution
Chow et al., 1982 [15]	<i>Davallina orientalis</i>	?	Fractures (traditional uses)	Methanol	?	10mg/kg or 30mg/kg	Intraperitoneally	?	?
Huang and You, 1997 [16]	Ru-Yih-Jin-Huang Saan and Jie-Guu-Saan	?	Disorders of orthopedics (traditional uses)	?	China Medical College Hospital	0.8g	Topic/orally	?	?
Juma Ab, 2007 [17]	<i>Lepidium sativum</i>	Seeds	Diuresis, bile function, cough fracture healing (traditional uses)	?	From the local market of the type grown in the Al-Qaseem area	6g/day	Orally	?	?
Oztürk et al., 2008 [18]	<i>Vitex agnus-castus</i> L.	Fruits	Bone loss and resorption, heart disease (reports)	Ethanol	?	0.75mg	Injection	Flavonoids (reports)	Middle East and Southern Europe
Huh et al., 2009 [19]	<i>Astragalus membranaceus</i>	Root	Vascular diseases breast cancer, climateric bone diseases (reports)	Water/ethanol	From a herbal supplier in Seoul (Korea)	20µg/kg/day and 200µg/kg/day	Orally	Isoflavone Formononetin	?

Table 3. Continued.

Study ID	Plant	Used parts	Indication	Solvent extraction	Obtaining plant material	Dose	Administration	Secondary metabolites	Geographic distribution
Kolios et al., 2009 [20]	Soybeans	?	Osteoporosis (reports)	?	?	1g/kg	Orally	Isoflavone Genistein	?
Kolios et al., 2010 [21]	<i>Cimicifuga racemosa</i>	Rhizomes	Reduce climacteric complaints (proven)	Water/ethanol	Cultivated plants	24,9mg/day	Orally	?	?
Lacerda et al., 2010 [22]	Coffe	?	Protein expression of the vitamin D receptor, osteoblast activity (reports)	Water	Utam SA, Ribeirão Preto, SP, Brazil	1.2mL/day	?	Caffeine (reports)	?
Peng et al., 2010 [23]	Radix Dipsaci, Ramulus Sambucus Williamsii, Rhizoma Notoginseng, Flos Carthami, Rhizoma Rhei and Fructus Gardeniae	?	Anti-inflammatory and bone forming (traditional uses and reports)	Water/ethanol	From a herbal supplier in AnHui Province, China	?	Topic	?	?
Estai et al., 2011 [24]	<i>Piper sarmentosum</i>	Leaves	Diabetes, hypertension and joint aches (traditional uses)	?	From a supplier	125mg/kg/day	Orally	Alkaloids, amides, flavonoids, lignans, phenylpropanoids (reports)	?

Table 3. Continued.

Study ID	Plant	Used parts	Indication	Solvent extraction	Obtaining plant material	Dose	Administration	Secondary metabolites	Geographic distribution
Sharan et al., 2011 [25]	<i>Ulmus wallichiana</i>	Stem bark	Fractures (traditional uses)	Ethanol	?	1.0mg/kg/day and 5.0mg/kg/day	Orally	Flavonoid Quercetin	?
Ngueguim et al., 2012 [26]	<i>Elephantopus mollis</i> , <i>Spilanthes africana</i> , <i>Urena lobata</i> , <i>Momordica multiflora</i> , <i>Asystasia gangetica</i> and <i>Brillantaisia ovariensis</i>	Leaves, twigs or whole plant	Bone diseases and fracture repair (traditional uses)	Ethanol	Collected from Dschang region, Cameroon	250, 500 and 750mg/kg	Orally	?	?
Kumar et al., 2013 [27]	<i>Ormocarpum cochinchinense</i>	Leaves	Fractures (traditional uses)	Methanol	Collected from Kancheepuram district, Tamil Nadu	100 mg/kg ⁻¹	Topic/orally	?	Jungles of Coromandel coast and dry forest from Tamil Nadu, India
Ngueguim et al., 2013 [28]	<i>Peperomia pellucida</i> (L.) HBK	Whole plant	Fractures, abdominal pain, headache, hypertension (traditional uses)	Ethanol	Collected from Dschang region, Cameroon	100 and 200mg/kg	Orally	?	Damp areas from Cameroon
He and Shen, 2014 [29]	Radix <i>Salviae miltiorrhizae</i> (Danshen)	Root	Cardiovascular, hepatic, kidneys and lungs diseases, trauma wounds and fractures (used in clinical practice)	?	?	40mg/kg/day	Intraperitoneally	Salvianolic acid B	?
Siu et al., 2015 [30]	Carthami Flos (C), Dipsaci Radix (D), Notoginseng Rhizoma (N), Rhei Rhizoma (R)	?	Fractures (traditional uses)	Water/ethanol	Herbal supplier in Hong Kong	0.5mL	Topic	?	?

Table 3. Continued.

Study ID	Plant	Used parts	Indication	Solvent extraction	Obtaining plant material	Dose	Administration	Secondary metabolites	Geographic distribution
Burim et al., 2016 [31]	<i>Epimedium sagittatum</i>	Dried leaves	Bone-repair, osteoporosis (reports)	Water/ethanol	Imported from Shaanxi Jiahe Phytochem Co, Ltd, Shaanxi, China	0.3mL	Gavage	Flavonoid icariin	Asian countries
Ezirganli et al., 2016 [32]	<i>Nigella sativa</i>	Seed	Analgesic, antipyretic, anti-inflammatory, antimicrobial, antibacterial, antifungal, antiparasitic, antiasthmatic, antioxidant, antineoplastic	?	?	10mg/kg/day	Oro-gastric tube	Proteins, alkaloids, essential oils, saponin	?
Yang et al., 2016 [33]	<i>Sambucus williamsii</i> Hance (SWH)	Root bark	Fractures, osteoporosis (traditional uses)	Ethanol	Collected from Founder County of Harbin	340 and 680mg/kg	Orally	Lignans, Iridoids	China
Zhuang et al., 2016 [34]	<i>Ulmus davidiana</i> Planch	Stem-bark	Inflammation, edema, stomach cancer (traditional uses)	Ethanol	?	50, 100, 250 and 500mg/kg	Gavage	Flavonoids (catechin)	Korean peninsula
Adhikary et al., 2017 [35]	<i>Spinacia oleracea</i>	Whole plant	Increased satiety in females and lipid-lowering effects in postmenopausal women (previous reports)	Ethanol	Cultivated plants	125, 250, 500, and 750mg/kg ⁻¹ day ⁻¹	Orally	Ascorbate, carotenoids, tocopherols, phenolics, flavonoids, folate	?
Chen et al., 2017 [36]	<i>Salvia miltiorrhiza</i>	?	Osteoporosis, osteogenesis (reports)	?	From BIYU Bio-Tech, Inc.	25 and 50mg/kg	Intragastrically	Tanshinol	?

Table 3. Continued.

Study ID	Plant	Used parts	Indication	Solvent extraction	Obtaining plant material	Dose	Administration	Secondary metabolites	Geographic distribution
Florence et al., 2017 [37]	<i>Peperomia pellucida</i> (L.) HBK	Whole plant	Abdominal pain, abscesses, acne, boils, colic, fatigue, gout, rheumatic, joint pain, fracture management (traditional uses)	Water	Collected from Limbe, Cameroon	100, 200 and 400mg/kg	Orally	Flavonoids (reports)	America, Africa and Asia
Karvande et al., 2017 [38]	<i>Dalbergia sissoo</i>	Heartwood	Stimulation of new cell growth, tissue regeneration (reports) Fevers, inflammation (traditional uses)	Ethanol	Collected from Lucknow, India	250, 500 and 1000mg/kg/day	?	Neoflavanoides (reports)	Indian subcontinent
Khedgikar et al., 2017 [39]	<i>Dalbergia sissoo</i>	Leaves	Stimulation of new bone cells, tissue regeneration, eye ailments (reports)	Ethanol	Collected from Lucknow, India	250, 500 and 1000mg/kg/day	Orally	Phytoestrogens, Flavonoids (reports)	Indian subcontinent
Porwal et al., 2017 [40]	<i>Psidium guajava</i>	Fruits	Diabetes, obesity, osteoporosis (traditional uses)	Ethanol	Collected from Sitapur, Uttar Pradesh, India	250mg/kg	?	Polyphenols, carotenoids (reports)	?

?: not related.

3.4 Main parameters analyzed to evaluate the extract action in bone repair

The most analyzed parameters among the 26 papers found in this review were histopathological analyzes 84.6% (n=22) [16, 18, 19, 20, 21, 22, 25-40], followed by radiological analyzes 76.9% (n=20) [17-26, 28, 29, 31, 33, 35-40]. These analyzes demonstrated mainly osteoblastic proliferation, angiogenesis, and increased formation of bone matrix with fracture closure and bone callus formation. Only 23.1% (n=6) [22, 23, 27, 30, 33, 37] performed blood tests, and the most cited parameters were Ca⁺⁺ content and serum alkaline phosphatase. Only 38.5% (n=10) [16, 17, 23, 24, 26, 27, 31, 32, 36, 37] reported whether the fracture had complete, partial or absent closure. Other analyzes related to bone strength, tensile strength and expression of genes that stimulate bone formation and osteogenic differentiation were performed in 53.8% (n=14) [15, 18, 19, 21, 22, 28, 30, 31, 33-35, 37-39] of the studies (Table 4).

Table 4. Description of the main results of the studies demonstrating the action of plant extracts in the bone repair process.

Studies	Blood analyzes	Histopathology	Radiological analyzes	Total fracture closure	Other analyzes
Chow et al. 1982 [15]	?	?	?	?	<i>Davallina orientalis</i> extract increased bone strength at the fracture site and was the best result at the lowest concentration (10mg/0.5 mL saline)
Huang and You, 1997 [16]	?	Animals treated with Ru-Yih-Jin-Huang-Saan (RYJHS) and Jie-Guu-Saan (JGS) extracts showed ↑ hydroxyproline, collagen, ↑ DNA, RNA and protein synthesis	?	Treated animals presented complete closure of the fractures	?
Juma Ab, 2007 [17]	?	?	The group receiving supplementation with seeds of <i>L sativum</i> presented ↑ amount of bone callus	Fracture healing continued for up to 12 weeks and was nearly complete in all groups	?
Oztürk et al., 2008 [18]	?	Ossification, evaluation of spongy bone and bone marrow showed no significant difference between groups Intramembranous ossification was superior to endochondral ossification	The tissue repair was ↑ in the group treated with 0.75mg of <i>Vitex agnus-castus L.</i> (group 1)	?	Scintigraphy: The scintigraphy data of the fractured tibias were ↑ in group 1
Huh et al., 2009 [19]	?	The group treated with Formononetin presented ↑ amount of bone callus, ↑ angiogenesis, chondrogenesis in the bone callus adjacent to the newly formed trabecular bone, and endochondral ossification Immunohistochemistry: Formononetin ↑ VEGF and VEGFR-2 expression at the fracture site	Radiographs were taken at the time of sacrifice, and inappropriate callus fractures were excluded	?	RT-PCR: The expression of osteocalcin (OCN) and osteopontin (OPN) mRNA was further stimulated in Formononetin-treated group after 14 days

Table 4. Continued.

Studies	Blood analyzes	Histopathology	Radiological analyzes	Total fracture closure	Other analyzes
Kolios et al., 2009 [20]	?	In general, bone callus was more robust in 17 β -estradiol (E) -treated group, followed by Equol (EQ) -treated group. The Genistein (G) group was less effective in relation to callus formation	In general, EQ and E presented better number of nodes proximal to callus formation, \uparrow dorsal callus density, and EQ \uparrow density of endosteal bone callus Treatment with EQ followed by E induced the highest values of callus formation	?	?
Kolios et al., 2010 [21]	?	In CR group (<i>Cimicifuga racemosa</i>) the callus structure was slightly enhanced compared with control group (C), but the levels did not reach those of the 17- β -estradiol group (E)	The tibia osteotomies of all 36 rats healed adequately	?	Mechanical tests: The stiffness (S) and yield load (yL) were highest in the 17- β -estradiol group (E), followed by the <i>Cimicifuga racemosa</i> group (CR) Fluorochrome labeling: Most callus apposition occurred in the endosteal region in all three groups, followed by the dorsal region
Lacerda et al., 2010 [22]	Calcium levels (mg%) were statistically highest in animals treated with coffee when compared to controls	A possible delay in the osteogenic process was apparent in the alveoli of rats treated with coffee, which had lower volume bone density from the 7th to the 42nd day after surgery	Animals treated with coffee showed more radiotransparence when compared with controls	?	Colorimetry: calcium levels in the urine (mg/24 h) were statistically \uparrow in animals treated with coffee when compared to controls. In maxillary bone (mg Ca/mg bone), these levels were \downarrow in animals that ingested coffee throughout the experimental period
Peng et al., 2010 [23]	Group treated with extracts of the six herbs (FH) presented \uparrow levels of Serum Alkaline Phosphatase	?	Callus size \uparrow from 2 to 5 weeks in the group treated with FH. In addition, the callus compaction rate was \uparrow (1.9 times)	The application of FH promoted fracture consolidation	?
Estai et al., 2011 [24]	?	?	Ovarectomized control animals (OVXC) presented \uparrow number of callus with soft tissue. In Sham (SO), ovarectomized + estrogen (OVX + ERT) and ovarectomized + Piper sarmentosum (OVX + P.s.) groups it occurred callus reduction and remodeling	Fracture line slightly detectable	?

Table 4. Continued.

Studies	Blood analyzes	Histopathology	Radiological analyzes	Total fracture closure	Other analyzes
Sharan et al., 2011 [25]	?	In contrast to the controls, 6-C-b-D-glucopyranosyl- (2S, 3S) - (β) -3', 4', 5,7-tetrahydroxyflavanol (GTDF) at both doses ↑ bone mineral density and osteocytes The bone marrow did not present any cytomorphological differences	GTDF in both doses ↑ the mineral apposition rate, elastic modulus, maximum load and mineral deposition	?	?
Ngueguim et al., 2012 [26]	?	<i>Elephantopus mollis</i> (EM) and <i>Spilanthes africana</i> (SA) promoted ↑ expression of Runx2, and ↑ recruitment of osteoblasts at the fracture site	Computed tomography: The parameters at the dose of 750mg/kg indicated a more compact and structured assembly of the newly formed bone plate in SA and EM treatments	Fracture closure was better in SA-treated groups than in EM	?
Kumar et al., 2013 [27]	Ca levels: Lower levels in the treatment group than untreated/normal ↓ 7d, and ↑ 14, 21d. Except for the topical application group. Inorganic phosphorous levels: ↑ 7d, and ↓ 14, 21d of application in all the treated rats. Levels ↑ in the treated groups. (alkaline phosphatase levels were similar).	The bones in the treatment groups has fused and the tissues around the fracture area had grown, completely covering over the fused bones, which was not seen in the control group, which had incomplete and slow healing	?	The fusion of fractured bones is was not complete in untreated group	?
Ngueguim et al., 2013 [28]	?	The extract induced a significantly higher mineral (calcein label) deposition over the control. The increase was 9.45% at 100mg/kg dose and 84.85% at 200mg/kg dose	Compared to the control; the extract at both doses increased bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and decreased trabecular separation (Tb.Sp) and structure model index (SMI)	?	Culture: The <i>Peperomia pellucida</i> extract exhibited dose-dependent increased mineralized nodule formation in bone marrow stromal cells (BMC), in relation to control. PCR: <i>Peperomia pellucida</i> extract exhibited a dose dependant increase in the mRNA levels of genes col1, osteocalcin and BMP-2, over the control. The highest levels occurred at a dose of 200 mg/kg

Table 4. Continued.

Studies	Blood analyzes	Histopathology	Radiological analyzes	Total fracture closure	Other analyzes
He and Shen, 2014 [29]	?	The group treated with Salvianolic acid B 40mg/kg/d (Sal B) had ↑ cartilaginous cells, bone matrix, trabeculae formation, spongy and compact bone	Increased bone callus growth in Sal B group	?	?
Siu et al., 2015 [30]	There was a ↑ in the bone-specific alkaline phosphatase (BALP) level, and a ↓ trend of serum deoxypyridinoline (Dpd). However, there was no significant difference between the two groups at each time point throughout the study	Bone volume (BV) it changed from 15.43mm ³ to 17.11mm ³ in Chinese herbal paste (CDNR) while from 15.05mm ³ to 18.02mm ³ in control	?	?	Biomechanical tests: CDNR treated bone showed 16.5% higher normalized yield strength, and also 13.8% higher energy to reach the yield point, both of them in relation the control Transdermal transport: all the chemical markers of CDNR, except oleanolic acid (OA), were detected in the rat skin, and those 7 markers detected in the skin, except kaempferol (KAE) and OA, could be found in the muscle
Burim et al., 2016 [31]	?	The bone matrix deposition was more pronounced in the test group than in the control group in all of the experimental periods	The soft X-ray images showed a highest reduction in the bone defect area in the test group than that in the control	In both the test and control groups, the defect area was not completely refilled with bone at 42 days	HPLC: The mean concentration of icariin in the extract was 5.8 ± 0.1mg/mL
Ezirganli et al., 2016 [32]	?	The osteoblastic activity in the study groups at both 2 and 4 weeks was higher than that of the control groups. Osteoclastic activity was determined as higher in the control groups than in the study groups. Ossification was greater at 4 weeks	?	In control group, the defects contained mostly soft connective tissues and little ossification. In study group, the defects were filled with lamellar bone containing bone marrow. Both of them on the 4th week	?
Yang et al., 2016 [33]	Ethanol extract of root bark of <i>Sambucus williamsii</i> Hance (EE-rbSWH) ↑ serum alkaline phosphatase (ALP) level at weeks 2 and 4, and ↑ the serum osteocalcin (BGP) levels at weeks 4 and 8, compare with the Model group	Immunohistochemistry: At low dose, treatment ↑ BMP-2, BMPRII, BMPRII and Runx2 proteins at week 2, compared with the Model	The connective tissue at the fracture site had been replaced by the mature osteoblasts and fibrous callus in the EE-rbSWH groups, and the 340mg/kg group was better than 680mg/kg group, but it was still existing in the Model group	?	Column chromatography: Thirteen Iridoids and ten Lignans were found in EE-rbSWH. Culture: Compared with the Model group, EE-rbSWH it induced an 104% ↑ of mineralized nodules by 340mg/kg and 90% ↑ by 680mg/kg in BMC. PCR: At low dose, treatment ↑ expressions of BMP-2 and Runx2 mRNA genes at weeks 2 and 4, compared with the Model

Table 4. Continued.

Studies	Blood analyzes	Histopathology	Radiological analyzes	Total fracture closure	Other analyzes
Zhuang et al., 2016 [34]	?	In comparison to the control, <i>Ulmus davidiana</i> extract (UDE) ↑ BV/TV at 250mg/kg dose. At 100mg/kg dose had no significant effect on BV/TV. There was no difference in BV/TV between 250 and 500mg/kg dose. The maximum effective dose was 250mg/kg	?	?	HPLC: (-)-catechin was found to account for 7.46 ± 0.82% of the extract, and (+)-catechin for 3.74 ± 0.38% of the extract
Adhikary et al., 2017 [35]	?	In <i>Spinaceae oleracea</i> extract (SOE) treatment mineralized callus was observed at 750mg/kg-1/d-1 dose as compared to the vehicle-treated group and the defect region was bridged by the end of treatment. Dried granular form of <i>S. oleracea</i> extract (SOG) exhibited ↑ calcein intensity compared with control, was analogous to PTH-treated	SOE 750mg/kg-1/d-1: ↑ BV/TV in 25%, trabecular number by 24% (Tb.N); ↓ the trabecular separation (Tb.Sp) and structure model index (SMI) by 26% and 46%, respectively. SOG at the 750mg/kg-1/d-1 dose as compared to vehicle-treated control rats ↑ BV/TV in 39% and Tb.N in 58%. ↑ in bone volume was corroborated by a 45% ↓ in Tb.Sp and a 54% in SMI compared with the control group	?	PCR: SOE exhibited a dose-dependent ↑ in the expression of BMP2, COL1, BMP4 over the vehicle-treated control group. The highest ↑ occurred in SOE 750mg/kg-1/d-1. Intermittent PTH showed a greater ↑ in expression of bone formation genes as compared to the extract. SOG in 750mg/kg-1/d-1 increased the gene expression of BMP2, BMP4, COL1, and the difference in fold change was equivalent to that exhibited in PTH-treated animals. SOG at a lower dose did not show significant fold change in BMP2, BMP4, and COL1 expression
Chen et al., 2017 [36]	?	Tanshinol at a dosage of 25mg/kg reversed the glucocorticoid-induced (GC) decrease in the parameters % labeled perimeter (L.Pm), mineral apposition rate (MAR), bone formation rate (BFR) per unit of bone surface (BFR/BS), BFR/BV, and BFR/TV	GC-induced damage was attenuated by treatment with tanshinol (in both the 25 and 50mg/kg doses). BV/TV ↓, Tb.Sp and SMI were ↑ in the GC groups compared with the control, and were reversed by the administration of tanshinol or calcitriol	After six weeks, bone defects had healed completely in each of the experimental groups	?

Table 4. Continued.

Studies	Blood analyzes	Histopathology	Radiological analyzes	Total fracture closure	Other analyzes
Florence et al., 2017 [37]	100 mg/kg extract: ↑ white blood cells (WBCs) as compared to the normal control and fractured control. 200 mg/kg: ↑ WBCs with respect to the fractured control. 400 mg/kg for the fractured rats: ↓ granulocyte and a ↑ value of mean platelet volume (MPV) compared to the normal control	Callus deposition was dose dependent, with a slightly compact callus at the dose of 200 mg/kg while and a callus more compact and dense in 400 mg/ kg dose as compared to the fracture control	The treatment of fractured rats with the plant extract at the doses of 100, 200 and 400 mg/kg induced a dose dependent decrease in the drill hole diameter to 28.75, 71.43 and 100% decrease respectively in comparison to the fractured control	There was complete closure of the hole in the rats receiving the plant extract at 400 mg/kg	<p>Calcium concentrations: ↑ in bone with extract 100 and 200 mg/kg compared to the normal control. At dose 400 mg/kg did not modify in non-fractured rats as compared to the normal control</p> <p>Phosphorus concentrations: ↓ in serum with extract 100, 200 and 400 mg/kg and ↑ in bone with extract 100 mg/kg as compared to the normal control</p> <p>Alkaline phosphatase concentrations: ↓ in serum with extract 400 mg/kg fractured rats as compared to the fracture control. ↓ in bone with extract 400 mg/kg non-fractured rats with respect to the normal control</p>
Karvande et al., 2017 [38]	?	Compared to control, heartwood ethanolic extract (HEE) ↑ calcein fluorescence intensity by ~61% at 250mg/kg, ~73% at 500mg/kg and ~70% at 1000mg/ kg dose, respectively	HEE treatment to rats ↑ BV/TV, Tb.N and ↓ Tb.Sp, SMI	?	PCR: HEE dose dependently stimulated osteogenic and chondrogenic genes such as BMP-2 by ~50%, BMP-4 by ~45%, RUNX2 by ~60% and Col-1 by ~72% compared with control group
Khedgikar et al., 2017 [39]	?	The ethanolic extract (EE) induced a significantly higher mineral (calcein label) deposition over the control	Quantification of various mCT parameters showed that all the doses 250, 500 and 1000 mg/kg/d' of EE improved the bone microarchitecture compared to the control group	?	<p>PCR: EE at different doses ↑ the mRNA levels of the four genes over the control. However, 250 mg/kg dose of EE was most effective, ↑ the expression of BMP-2 (60%), BMP-4 (28%), RunX-2 (44%) and COL-1 (66%).</p> <p>Culture: Percentage change stained cells was approximately 35% at 250mg/kg and 28% at 500mg/kg dose compared to control, but 250mg/kg dose has better response</p>
Porwal et al., 2017 [40]	?	That compared to the control, guava triterpene-enriched extract (GE) increased mineral deposition (calcein label) by ~50%	Percentage bone volume at the tissue level (BV/TV%) was significantly higher at the fracture callus site in the GE group over the vehicle	?	?

↑: increase; ↓: decrease; d: days; ?: not related.

3.5 Bias analysis

Detailed results of the bias analysis are depicted in Figure 2 and Table 5. An average of $57,50 \pm 8,73$ ARRIVE items were met by the original included studies. In general, the works published more recently have better met the methodological quality criteria analyzed. Primary and secondary objectives were clearly stated by 73.1% (n=19) of the studies, while 80.8% (n=21) reported ethics committee permission for performing the research. The number of animals per group was reported in 88.5% (n=23) of the studies, and only 19.2% (n=5) reported a blind controlled study. Most studies (92.3%, n=24) provided information about the treatment description, the therapeutic dose administered and treatment duration (96.2%, n=25 each). However, only 7.7% (n=2) reported the period when the treatment was administered. All studies (100%, n=26) reported the animal species and 88.5% (n=23) described the animal strain. The sex and weight were reported in 84.6% (n=22) and 88.5% (n=23) of the works, respectively, and 65.4% (n=17) of the studies provided information about the age of the animals. No study reported description of genetic modification status, and 46.2% (n=12) presented information regarding previous procedures performed on the animals. Among the articles, 30.8% (n=8) reported the housing of experimental animals (facility type, cage or housing type, material and number of cage companions), and 53.8% (n=14) provided information about the experimental conditions (temperature, humidity, light cycles, feed and water). Only 26.9% (n=7) of the studies performed assessments and interventions related to animal welfare. Regarding the sample size, 76.9% (n=20) of the studies reported the total number of animals used and the number of animals in each experimental group, but only 3.8% (n=1) explained the reason for choosing such numbers. The details of how the animals were allocated to experimental groups (randomization or matching) were reported by 34.6% (n=9) of the studies, and no study described the order in which animals in different groups were treated and assessed. The experimental outcomes were clear in 84.6% (n=22) of the studies.

Statistical analyzes were performed by 88.5% (n=23) of the studies, 84.6% (n=22) of them specified the unit of analysis for each dataset, and 88.5% (n=23) specified the methods used to assess whether the data met the assumptions of the statistical approach. Information regarding mortality was described in 7.7% (n=2) of the studies, and no study described modifications to the experimental protocols made to reduce adverse events. A coherent interpretation of the results and the direct relationship between objectives and hypothesis were described in all included studies (100%, n=26), and 26.9% (n=7) commented about the limitations of the studies. Comments on the importance of applying the results to human biology were found in 53.8% (n=14) of the studies, and 57.7% (n=15) mentioned sources of funding and the role of the funder in the study (Table 5).

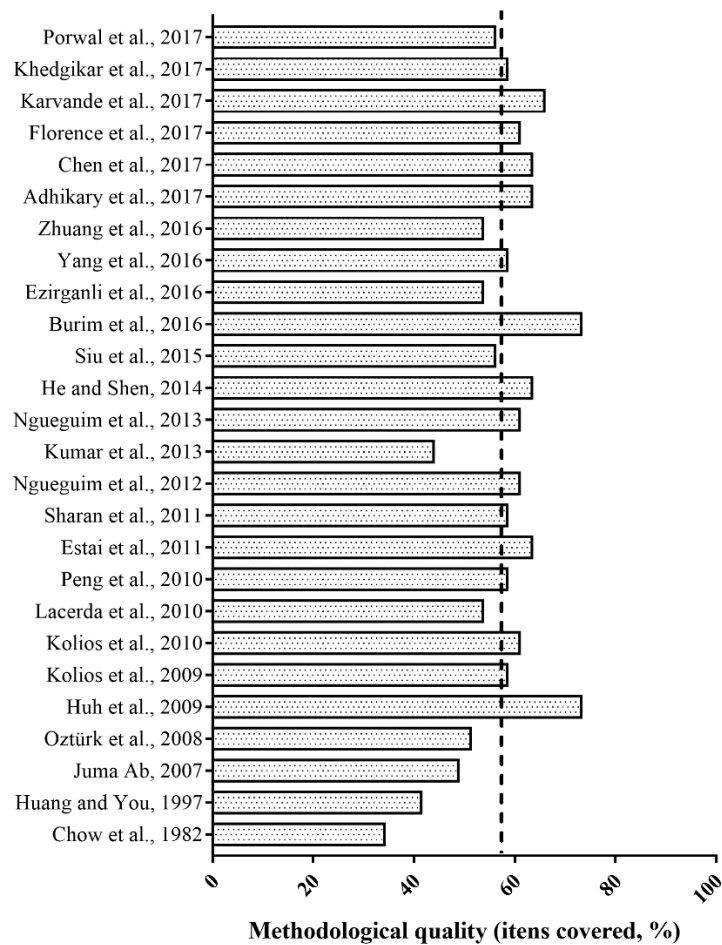


Figure 2. Analysis of methodological bias (reporting quality) for each study included in the review. Based on Animal Research: Reporting of *In Vivo* Experiments - ARRIVE guidelines (www.nc3rs.org.uk/arrive-guidelines). The dotted line indicated the mean quality score (%). Detailed bias analysis stratified by domains and items evaluated is presented in Table 4.

Table 5. Continued.

	Chow et al., 1982 [15]	Huang and You, 1997 [16]	Juma Ab, 2007 [17]	Oztürk et al., 2008 [18]	Huh et al., 2009 [19]	Kolios et al., 2009 [20]	Kolios et al., 2010 [21]	Lacerda et al., 2010 [22]	Peng et al., 2010 [23]	Estai et al., 2011 [24]	Sharan et al., 2011 [25]	Ngueguim et al., 2012 [26]	Kumar et al., 2013 [27]	Ngueguim et al., 2013 [28]	He and Shen, 2014 [29]	Siu et al., 2015 [30]	Burim et al., 2016 [31]	Ezirganli et al., 2016 [32]	Yang et al., 2016 [33]	Zhuang et al., 2016 [34]	Adhikary et al., 2017 [35]	Chen et al., 2017 [36]	Florence et al., 2017 [37]	Karvande et al., 2017 [38]	Khedgikar et al., 2017 [39]	Porwal et al., 2017 [40]		
<i>Experimental procedures</i>																												
Treatment Description	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	24	92,30%
Treatment dosage	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	25	96,15%
Treatment Duration		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	25	96,15%
Time of day of treatment administration																	X					X				2	7,69%	
<i>Experimental animals</i>																												
Information regarding animal species	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	26	100%
Animals' strain			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	23	88,46%
Animals' sex		X			X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	22	84,61%
Animals' Weight range	X	X	X	X	X	X		X		X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	23	88,46%
Animals' Age	X		X		X	X	X		X		X	X	X	X	X	X		X	X		X	X	X			17	65,38%	
Description of genetic modification status (Knock-out, transgenic, SPF)																											0	0,00%
Information related to previous procedures performed on the animals							X	X		X			X			X		X	X		X	X	X	X		X	12	46,15%
<i>Housing and husbandry</i>																												
Housing of experimental animals (facility type, cage or housing type, material, number of cage companions)		X	X		X		X	X					X				X								X		8	30,77%
Husbandry conditions (breeding program, light/dark cycle, temperature, water)							X	X		X			X			X	X	X		X	X	X	X	X	X	X	14	53,84%

Table 5. Continued.

	Chow et al., 1982 [15]	Huang and You, 1997 [16]	Juma Ab, 2007 [17]	Oztürk et al., 2008 [18]	Huh et al., 2009 [19]	Kolios et al., 2009 [20]	Kolios et al., 2010 [21]	Lacerda et al., 2010 [22]	Peng et al., 2010 [23]	Estai et al., 2011 [24]	Sharan et al., 2011 [25]	Ngueguim et al., 2012 [26]	Kumar et al., 2013 [27]	Ngueguim et al., 2013 [28]	He and Shen, 2014 [29]	Siu et al., 2015 [30]	Burim et al., 2016 [31]	Ezirganli et al., 2016 [32]	Yang et al., 2016 [33]	Zhuang et al., 2016 [34]	Adhikary et al., 2017 [35]	Chen et al., 2017 [36]	Florence et al., 2017 [37]	Karvande et al., 2017 [38]	Khedgikar et al., 2017 [39]	Porwal et al., 2017 [40]		
<i>Housing and husbandry</i>																												
Welfare-related assessments and interventions that were carried out before, during, or after the experiment										X			X			X	X	X	X		X						7	26,92%
<i>Sample size</i>																												
Total number of animals used in each experimental and the number of animals in each experimental group	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X		X	X	X			20	76,92%
Explanation regarding the decision of the number of animals and details of sample size calculation					X																						1	3,84%
<i>Allocating animals to experimental groups</i>																												
Full details of how animals were allocated to experimental groups (including randomization or matching)		X		X					X	X					X		X				X	X			X		9	34,61%
Order in which the animals in the different experimental groups were treated and assessed																											0	0,00%
<i>Experimental outcomes</i>																												
Clear experimental outcomes assessed	X		X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	22	84,61%
<i>Statistical methods</i>																												
Statistical methods used for each analysis		X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	23	88,46%

Table 5. Continued.

	Chow et al., 1982 [15]	Huang and You, 1997 [16]	Juma Ab, 2007 [17]	Oztürk et al., 2008 [18]	Huh et al., 2009 [19]	Kolios et al., 2009 [20]	Kolios et al., 2010 [21]	Lacerda et al., 2010 [22]	Peng et al., 2010 [23]	Estai et al., 2011 [24]	Sharan et al., 2011 [25]	Ngueguim et al., 2012 [26]	Kumar et al., 2013 [27]	Ngueguim et al., 2013 [28]	He and Shen, 2014 [29]	Siu et al., 2015 [30]	Burim et al., 2016 [31]	Ezirganli et al., 2016 [32]	Yang et al., 2016 [33]	Zhuang et al., 2016 [34]	Adhikary et al., 2017 [35]	Chen et al., 2017 [36]	Florence et al., 2017 [37]	Karvande et al., 2017 [38]	Khedgikar et al., 2017 [39]	Porwal et al., 2017 [40]			
Statistical methods																													
Unit of analysis specifications for each dataset	X		X	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	22	84,61%
Methods used to assess whether the data met the assumptions of the statistical approach	X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	23	88,46%
Results																													
Baseline data																													
Description of animals health status, for each experimental group, before treatment																												0	0,00%
Number analyzed																													
Number or animals in each group included in each analysis (absolute numbers)	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	25	96,15%
Animals or data not included in the analysis (and explanation for the exclusion)					X		X																					2	7,69%
Outcomes and estimation																													
Information (Mean= Standard Deviation)	X		X	X	X	X	X	X	X	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X	22	84,61%
Adverse events																													
Information regarding mortality of experimental animals (Mean=Standard Deviation)				X											X													2	7,69%

Table 5. Continued.

	Chow et al., 1982 [15]	Huang and You, 1997 [16]	Juma Ab, 2007 [17]	Oztürk et al., 2008 [18]	Huh et al., 2009 [19]	Kolios et al., 2009 [20]	Kolios et al., 2010 [21]	Lacerda et al., 2010 [22]	Peng et al., 2010 [23]	Estai et al., 2011 [24]	Sharan et al., 2011 [25]	Ngueguim et al., 2012 [26]	Kumar et al., 2013 [27]	Ngueguim et al., 2013 [28]	He and Shen, 2014 [29]	Siu et al., 2015 [30]	Burim et al., 2016 [31]	Ezirganli et al., 2016 [32]	Yang et al., 2016 [33]	Zhuang et al., 2016 [34]	Adhikary et al., 2017 [35]	Chen et al., 2017 [36]	Florence et al., 2017 [37]	Karvande et al., 2017 [38]	Khedgikar et al., 2017 [39]	Porwal et al., 2017 [40]																												
Adverse events																																																						
Modifications to the experimental protocols made to reduce adverse events																											0	0,00%																										
Discussion																																																						
Interpretation /scientific implications																																																						
Interpretation of the results, taking into account the study objectives and hypotheses, current theory and relevant studies																											X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	26	100%
Comments on the study limitations (sources of bias, limitations of the animal model, imprecision associated with the results)																															X	X	X		X					X		X											7	26,92%
Generalisability /translation																																																						
Comments on how the findings are likely to translate to other species or systems, including relevance to human biology																													X		X	X		X	X		X	X				X	X			X	X		X	X	X	X	14	53,84%
Funding																																																						
List of funding sources and the role of the funder(s) in the studt																														X	X	X		X	X	X	X		X	X					X		X	X	X	X	X	X	15	57,69%
Results of total																											14	17	20	21	30	24	25	22	24	26	24	25	18	25	26	23	30	22	24	22	26	26	25	27	24	23		

X: related; unmarked: not related.

4. Discussion

4.1 General aspects

In this study, we conducted a systematic review to analyze the effect of plant extracts and its derivatives on bone repair in animal models. Despite the great heterogeneity of the studies, in general, the use of plant extracts was effective for treating bone lesions. Histopathological and radiological analyzes demonstrating bone remodelling (new bone formation, bone callus, cell proliferation and osteogenesis) were the main findings of this study, suggesting that some components of the extracts may favour the synthesis of certain cell types, probably due to the interactions of these cells with the components of the extracts. Taking into consideration that the biological activity of a natural product is generally due to the synergism between its constituents, which potentiate its therapeutic properties, the study of plants for the treatment of many different diseases has been increasing gradually [41, 42, 43]. We believe that the development of therapeutic strategies based on the use of plants is opening a new perspective and represents a promising therapy as an alternative to conventional medicines and synthetic products [44, 45, 46].

The use of natural products for the treatment of injuries is an old practice [47] and represents an important source of bioactive compounds that contribute directly to the development of new drugs [48]. Our findings showed that most of the studies were conducted in China and India, countries known for having a millenary practice in traditional medicine [49]. This interest is probably due to the extensive and diverse flora found in these countries and to the vast traditional ethnomedicinal knowledge that serve as a basis for the researches [50, 51]. It is already known the great ethnopharmacological potential of different phytotherapies whose popular knowledge has favors and potentiate research in different health areas, thus directing the rational choice of medicinal plant [52, 53]. In addition, it is noteworthy that in China, 40% of all health care provision is based on medicinal plants [54,

55]. However, a limiting factor found here was the language, which hinders access to information and reduces the dissemination of the data obtained to the scientific community [56].

As one of the objectives was to research experimental models closer to the human model, our studies focused on *in vivo* experiments. Initially all animal species were considered. However, after selection by inclusion criteria, only studies with rats, mice and rabbits were admitted. It is noteworthy that there was a predominance of studies performed in murine models. Although these models do not allow the direct extrapolation of the results to human models [57], they can provide important insights into the biology and pathophysiology of the lesions and are indispensable for researchers [58]. The advantage of working with such animals is mainly due to reduced costs, as more animals can be housed in a limited space, and the reproductive cycle is shorter. These characteristics allow, in a shorter time, a sufficient number of animals for large study groups, enabling a relevant statistical analysis [59].

4.2 Main parameters analyzed and therapeutic findings

The studies presented different methodologies, and this could be justified by the difference of objectives and parameters analysed. However, important information such as sex, age, weight of the animals and description of the methods for performing the bone defect were neglected in some studies. The absence of this information compromises the comprehension of the studies, since biological and methodological variables directly affect the response to the treatments [60]. It was also observed that, in addition to bone fracture, some studies have induced osteoporosis in animals, mainly evaluating the action of phytotherapies as estrogen stimulants [20, 21, 24, 25, 32, 36]. Warriner et al. (2010) [61] published a systematic review investigating different works involving the association between bone fractures and osteoporosis and reported that the main fracture sites related to this disease

were vertebrae, femoral neck, radius and ulna. In our review, we found that the most evaluated bone was the femur followed by the tibia, probably due to the greater resistance and size of these bones.

Two other parameters that varied widely were the size of the lesion or bone fracture, and the time of treatment, which can also compromise the reproduction of the work as well as the comparison between the different groups treated with extracts. Image analysis, such as radiological findings and tomography, are fundamental for studies with fractures [62]. However, histopathological analyses play a complementary role in helping to interpret and confirm the cellular action of phytotherapeutic compounds in tissue repair [63]. The action of osteogenic cells on bone callus formation, cell organization and osteoconductive targeting are factors that can be confirmed histologically [64, 65, 66], thus leading the experiment to a greater reliability of its results. The trabecular area, regions of spongy bone and compact bone, indicated in radiological and histopathological analyses, were higher in the phytotherapeutic treatment groups when compared to the control groups, demonstrating an efficiency of extracts in the bone repair. This observation suggests that some of the components of the extract, or the synergism between them may favour the synthesis of certain cell types and, consequently, accelerate the synthesis of bone matrix, accelerating the formation of bone callus [67]. In this context, radiological and histopathological analyses also confirm whether the fracture was totally, partially or not closed. Similar results were found by Neto et al., who evaluating the effect of a poultice prepared from the leaves of *Chenopodium ambrosioides L.* on bone repair in rabbits. Phytochemical analysis of the aqueous extract of this plant revealed the strong presence of saponins, flavonoids, tannins and alkaloids, which may contribute to its effect on bone formation [68].

The use of phytotherapies has been increasing considerably in the last years, indicating that phytotherapy currently represents an effective way to treat the most varied tissue

dysfunctions [69, 70]. This curative effect is probably related to the extensive source of bioactive compounds that are found in extracts obtained from natural products [71, 72]. However, the positive effects may vary according to the species of the plant and its part used. It is common to find studies that demonstrate, through different chromatographic analyses, different concentrations of flavonoids, tannins and triterpenes in bark and leaves of the same plant species [73, 74, 75], and that the protective effect of an extract should take into account the part of the plant [76]. In this review, we observed that approximately a quarter of the studies do not provide the scientific name of the species or the part of the plant used, which reduces the accuracy and reproducibility of the studies. The diversity of the compounds directly interferes on their performance in the organism, and their metabolites differ according to the parts of the plant, as well as the region and climate where they were collected [77]. As for the secondary metabolites found in the phytochemical description of the studies, we observed that 30.8% were flavonoids, indicating the positive action of this compound on the bone repair. The role of flavonoids in skin repair is already known, but recent studies have demonstrated the action of this compound also on bone repair [18, 24, 25, 31, 34, 35, 37, 39]. Another important fact to be informed in works with phytotherapics is the knowledge about the extraction techniques as well as solvents used, since they can determine the isolation of a biologically active compound, directing the research [78]. This bias was found frequently in our study, since 26.9% of the studies did not report the solvent used for extraction, which makes it difficult to obtain the extract again.

For the analysis of the quality of the works, we used an approach based on the ARRIVE Guide, describing minimum information that can compromise the quality of the writing as well as the reproduction of the study [14]. Aspects related to the organization and writing of the evaluated articles showed that more than half of the studies presented introduction with relevant scientific base as well as clearly written objectives. Through the

bias analysis, flaws were detected in the reporting of the methodological procedures of the experiments, and it was found that many papers neglected information about the Ethics Committee approval, double-blind studies and experimental conditions such as light cycles and randomization. These results point to the need to improve experimental designs and current guidelines in animal experiments reporting as a means to ensure an adequate level of scientific evidence [79].

4.3 Limitations of the study

A great contribution of this work is based on the global estimation of the use of plant extracts for the treatment of bone lesions. However, the results presented here should be interpreted with caution, since it can be argued that the selection process of the studies may be biased due to different factors, such as the initial exclusion based only on the reading of the titles and abstracts or the inclusion of more than one study of the same group of researchers. However, the work selection process carried out in our review was based on widely recommended and accepted practices for performing systematic reviews [12, 80].

Another relevant issue highlighted in our work is related to the bias of the publication. For this, we used the ARRIVE Guidelines, which allowed us to test the bias of the studies individually and, later, collectively. After this analysis, we realized that aspects related to the organization of the experiments were neglected, including lack of randomization. These factors highlight the need to enhance experimental designs and current guidelines in reporting animal experiments as a means to ensure an adequate level of scientific evidence. Finally, it was observed that the methodologies used and evaluation parameters are extremely heterogeneous, with different measures being reported in all the studies, such as analysis of the size of the lesion or fracture, as well as histopathological analyses. Despite the improvement in the methodological quality of individual studies from 2009, much still needs

to be done to allow the reproducibility of the studies. Interestingly, most papers did not report whether the results of their studies could be translated into other species and systems, including some relevance to human biology. Considering the experimental model used in most studies and the social relevance of bone lesions for the world population, the translation of the results and its applicability for the treatment of the disease in humans is fundamental to allow the continuity of studies with medicinal plants, since the goal is to develop a drug that improves the quality of life of humans.

5. Conclusion

The results of this study demonstrated that the use of plant extracts stimulates bone repair, increasing osteogenesis, rate of calcification, and the formation and mineralization of bone callus, accelerating the process of new bone formation in the fracture region. However, the methodological flaws found in some studies make it difficult to understand and use data in studies for the human condition. Therefore, more complete methodological descriptions are needed to better compare the studies and to allow the reproducibility of future trials.

Conflicts of Interest

The Author(s) declare(s) that there is no conflict of interest.

Acknowledgments

Thanks are due to: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

6. References

- [1] Amadei SU, Silveira VAS, Pereira AC, Carvalho YR, Rocha RF. A influência da deficiência estrogênica no processo de remodelação e reparação óssea. *Jornal Brasileiro de Patologia e Medicina Laboratorial*. 2006; 42:5-12. <http://dx.doi.org/10.1590/S1676-24442006000100003>.
- [2] Beil FT, Barvencik F, Gebauer M, Beil B, Pogoda P, Rueger JM, Ignatius A, Schinke T, Amling M. Effects of increased bone formation on fracture healing in mice. *The Journal of Trauma*. 2011; 70:857-862. <http://dx.doi.org/10.1097/TA.0b013e3181de3dd9>. PMID: 20664377.
- [3] Levy S, Feduska JM, Sawant A, Gilbert SR, Hensel JA, Ponnazhagan S. Immature myeloid cells are critical for enhancing bone fracture healing through angiogenic cascade. *Bone*. 2016; 93:113-124. <https://doi.org/10.1016/j.bone.2016.09.018>. PMID: 27664567.
- [4] Oryan A, Monazzah S, Bigham-Sadegh A. Bone injury and fracture healing biology. *Biomedical and Environmental Sciences*. 2015; 28:57-71. <https://doi.org/10.3967/bes2015.006>. PMID: 25566863.
- [5] Hankenson KD, Gagne K, Shaughnessy M. Extracellular signaling molecules to promote fracture healing and bone regeneration. *Advanced Drug Delivery Reviews*. 2015; 94:3-12. <https://doi.org/10.1016/j.addr.2015.09.008>. PMID: 26428617.
- [6] Dimitriou R, Jones E, McGonagle D, Giannoudis PV. Bone regeneration: current concepts and future directions. *BMC Medicine*. 2011, 9:66. <https://doi.org/10.1186/1741-7015-9-66>. PMID: 21627784.
- [7] Bukata SV. Systemic administration of pharmacological agents and bone repair: What can we expect. *Injury-International Journal of the Care of the Injured*. 2011; 42:605-608. <https://doi.org/10.1016/j.injury.2011.03.061>. PMID: 21507402.
- [8] Patel C, Ayaz RM, Parikh P. Studies on the osteoprotective and antidiabetic activities of *Moringa Oleifera* plant extract. *IOSR Journal of Pharmacy and Biological Sciences*. 2015; 5:19-22. <https://doi.org/10.9790/3008-0521017>.
- [9] Potu BK, K. Bhat MR, Rao MS, Nampurath GK, Chamallamudi MR, Nayak SR, Muttigi MS. Petroleum ether extract of *Cissus quadrangularis* (Linn.) enhances bone marrow mesenchymal stem cell proliferation and facilitates osteoblastogenesis. *Clinics*. 2009; 64:993-998. <https://doi.org/10.1590/S1807-59322009001000010>. PMID: 19841707.
- [10] Jalil MAA, Shuid AN, Muhammad N. Role of medicinal plants and natural products on osteoporotic fracture healing. *Evidence-Based Complementary and Alternative Medicine*. 2012; 2012:ID 714512. <https://doi.org/10.1155/2012/714512>. PMID: 22973405.
- [11] Udalamaththa VL, Jayasinghe CD, Udagama PV. Potential role of herbal remedies in stem cell therapy: proliferation and differentiation of human mesenchymal stromal cells. *Stem Cell Research & Therapy*. 2016; 7:110. <https://doi.org/10.1186/s13287-016-0366-4>. PMID: 27515026.

- [12] Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA Statement. *PLoS Medicine*. 2009; 6:e1000097. <https://doi.org/10.1371/journal.pmed.1000097>. PMID: 19621072.
- [13] Hooijmans CR, Tillema A, Leenaars M, Ritskes-Hoitinga M. Enhancing search efficiency by means of a search filter for finding all studies on animal experimentation in PubMed. *Laboratory Animals*. 2010; 44:170-175. <https://doi.org/10.1258/la.2010.009117>. PMID: 20551243.
- [14] Kilkeny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biology*. 2010; 8:e1000412. <https://doi.org/10.1371/journal.pbio.1000412>. PMID: 20613859.
- [15] Chow SP, Yeung HW, Law LK, Chan TM, Lau C. The effect of *Davallina Orientalis* on bone healing – a preliminary report. *The American Journal of Chinese Medicine*. 1982; 10:101-106. <https://doi.org/10.1142/S0192415X82000166>. PMID: 7183201.
- [16] Huang HF, You JS. The use of Chinese herbal medicine on experimental fracture healing. *The American Journal of Chinese Medicine*. 1997; 25:351-356. <https://doi.org/10.1142/S0192415X97000391>. PMID: 9358909.
- [17] Juma AbHbA. The Effects of *Lepidium sativum* seeds on fracture-induced healing in rabbits. *MedGenMed: Medscape General Medicine*. 2007; 9:23. PMID: 17955079.
- [18] Oztürk A, Ilman AA, Sağlam H, Yalçinkaya U, Aykut S, Akgöz S, et al. The effects of phytoestrogens on fracture healing: experimental research in New Zealand white rabbits. *Ulusal Travma Ve Acil Cerrahi Dergisi-Turkish Journal of Trauma & Emergency Surgery*. 2008; 14:21-27. PMID: 18306063.
- [19] Huh JE, Kwon NH, Baek YH, Lee JD, Choi DY, Jingushi S, et al. Formononetin promotes early fracture healing through stimulating angiogenesis by up-regulating VEGFR-2/Flk-1 in a rat fracture model. *International Immunopharmacology*. 2009; 9:1357-1365. <https://doi.org/10.1016/j.intimp.2009.08.003>. PMID: 19695348.
- [20] Kolios L, Sehmisch S, Daub F, Rack T, Tezval M, Stuermer KM, Stuermer EK. Equol but not genistein improves early metaphyseal fracture healing in osteoporotic rats. *Planta Medica*. 2009; 75:459-465. <https://doi.org/10.1055/s-0029-1185380>. PMID: 19241330.
- [21] Kolios L, Schumann J, Sehmisch S, Rack T, Tezval M, Seidlova-Wuttke D, et al. Effects of black cohosh (*Cimicifuga racemosa*) and estrogen on metaphyseal fracture healing in the early stage of osteoporosis in ovariectomized rats. *Planta Medica*. 2010; 76:850-857. <https://doi.org/10.1055/s-0029-1240798>. PMID: 20104444.
- [22] Lacerda SA, Matuoka RI, Macedo RM, Petenusci SO, Campos AA, Brentegani LG. Bone quality associated with daily intake of coffee: a biochemical, radiographic and histometric study. *Brazilian Dental Journal*. 2010; 21:199-204. <http://dx.doi.org/10.1590/S0103-64402010000300004>. PMID: 21203700.
- [23] Peng LH, Ko CH, Siu SW, Koon CM, Yue GL, Cheng WH, et al. *In vitro* & *in vivo* assessment of a herbal formula used topically for bone fracture treatment. *Journal of*

Ethnopharmacology. 2010; 131:282-289. <https://doi.org/10.1016/j.jep.2010.06.039>. PMID: 20600749.

[24] Estai MA, Suhaimi FH, Das S, Fadzilah FM, Alhabshi SMI, Shuid NA, Soelaiman IN. *Piper sarmentosum* enhances fracture healing in ovariectomized osteoporotic rats: a radiological study. Clinics. 2011; 66:865-872. <http://dx.doi.org/10.1590/S1807-59322011000500025>. PMID: 21789393.

[25] Sharan K, Mishra JS, Swarnkar G, Siddiqui JA, Khan K, Kumari R, et al. A novel quercetin analogue from a medicinal plant promotes peak bone mass achievement and bone healing after injury and exerts an anabolic effect on osteoporotic bone: The role of aryl hydrocarbon receptor as a mediator of osteogenic action. Journal of Bone and Mineral Research. 2011; 26:2096-2111. <https://doi.org/10.1002/jbmr.434>. PMID: 21638315.

[26] Ngueguim FT, Khan MP, Donfack JH, Siddiqui JA, Tewari D, Nagar GK, et al. Evaluation of Cameroonian plants towards experimental bone regeneration. Journal of Ethnopharmacology. 2012; 141:331-337. <https://doi.org/10.1016/j.jep.2012.02.041>. PMID: 22414477.

[27] Kumar MD, John KMM, Karthik S. The bone fracture–healing potential of *Ormocarpum cochinchinense*, methanolic extract on albino Wistar rats. Journal of Herbs, Spices & Medicinal Plants. 2013; 19:1-10. <https://doi.org/10.1080/10496475.2012.726954>.

[28] Ngueguim FT, Khan MP, Donfack JH, Tewari D, Dimo T, Kamtchouing P, et al. Ethanol extract of *Peperomia pellucida* (Piperaceae) promotes fracture healing by an anabolic effect on osteoblasts. Journal of Ethnopharmacology. 2013; 148:62-68. <https://doi.org/10.1016/j.jep.2013.03.063>. PMID: 23578859.

[29] He X, Shen Q. Salvianolic acid B promotes bone formation by increasing activity of alkaline phosphatase in a rat tibia fracture model: a pilot study. BMC Complementary and Alternative Medicine. 2014; 14:493. <https://doi.org/10.1186/1472-6882-14-493>. PMID: 25510675.

[30] Siu WS, Ko CH, Lam KW, Wat E, Shum WT, Lau CBS, et al. Evaluation of a topical herbal agent for the promotion of bone healing. Evidence-Based Complementary and Alternative Medicine. 2015; 2015:10. <https://doi.org/10.1155/2015/905270>.

[31] Burim RA, Sendyk DI, Hernandez LS, de Souza DF, Correa L, Deboni MC. Repair of critical calvarias defects with systemic *Epimedium sagittatum* extract. Journal of Craniofacial Surgery. 2016; 27:799-804. <https://doi.org/10.1097/SCS.0000000000002451>. PMID: 26982112.

[32] Ezirganli S, Kazancioglu HO, Ozdemir H, Inan DS, Tek M. The effects of *Nigella sativa* seed extract on bone healing in an experimental model. Journal of Craniofacial Surgery. 2016; 27:1905-1909. <https://doi.org/10.1097/SCS.0000000000002986>. PMID: 27513784.

[33] Yang B, Lin X, Tan J, She X, Liu Y, Kuang H. Root bark of *Sambucus williamsii* Hance promotes rat femoral fracture healing by the BMP-2/Runx2 signaling pathway. Journal of Ethnopharmacology. 2016; 191:107-114. <https://doi.org/10.1016/j.jep.2016.05.017>. PMID: 27178636.

- [34] Zhuang X, Fu C, Liu W, Wang Y, Xu F, Zhang Q, et al. *Ulmus davidiana* extract improves lumbar vertebral parameters in ovariectomized osteopenic rats. *American Journal of Translational Research*. 2016; 8:298-313. PMID: 27158327.
- [35] Adhikary S, Choudhary D, Ahmad N, Kumar S, Dev K, Mittapelly N, et al. Dried and free flowing granules of *Spinacia oleracea* accelerate bone regeneration and alleviate postmenopausal osteoporosis. *Menopause*. 2017; 24:686-698. doi: 10.1097/GME.0000000000000809. PMID: 28118295.
- [36] Chen G, Zhang X, Lin H, Huang G, Chen Y, Cui L. Tanshinol alleviates osteoporosis and myopathy in glucocorticoid-treated rats. *Planta Medica* 2017; 83:1264-1273. <https://doi.org/10.1055/s-0043-108761>. PMID: 28427101.
- [37] Florence NT, Huguette ST, Hubert DJ, Raceline GK, Desire DD, Pierre K, Theophile D. Aqueous extract of *Peperomia pellucida* (L.) HBK accelerates fracture healing in Wistar rats. *BMC Complementary and Alternative Medicine*. 2017; 17:188. <https://doi.org/10.1186/s12906-017-1686-3>. PMID: 28372562.
- [38] Karvande A, Khedgikar V, Kushwaha P, Ahmad N, Kothari P, Verma A, et al. Heartwood extract from *Dalbergia sissoo* promotes fracture healing and its application in ovariectomy-induced osteoporotic rats. *Journal of Pharmacy and Pharmacology*. 2017; 69:1381-1397. <https://doi.org/10.1111/jphp.12764>. PMID: 28664619.
- [39] Khedgikar V, Kushwaha P, Ahmad N, Gautam J, Kumar P, Maurya R, Trivedi R. Ethanolic extract of *Dalbergia sissoo* promotes rapid regeneration of cortical bone in drill-hole defect model of rat. *Biomedicine & Pharmacotherapy*. 2017; 86:16-22. doi: 10.1016/j.biopha.2016.11.140. PMID: 27936389.
- [40] Porwal K, Pal S, Dev K, China SP, Kumar Y, Singh C, et al. Guava fruit extract and its triterpene constituents have osteoanabolic effect: Stimulation of osteoblast differentiation by activation of mitochondrial respiration via the Wnt/ β -catenin signaling. *The Journal of Nutritional Biochemistry*. 2017; 44:22-34. doi: 10.1016/j.jnutbio.2017.02.011. PMID: 28343085.
- [41] Dubey NK, Kumar R, Tripathi P. Global promotion of herbal medicine: India's opportunity. *Current Science*. 2004; 86:37-41. ISSN: 0011-3891.
- [42] Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal and Aromatic Plants*. 2015; 4:196. <https://doi.org/10.4172/2167-0412.1000196>.
- [43] Perviz S, Khan H, Pervaiz A. Plant alkaloids as an emerging therapeutic alternative for the treatment of depression. *Frontiers in Pharmacology*. 2016; 7:1663-9812. <https://doi.org/10.3389/fphar.2016.00028>. PMID: 26913004.
- [44] Mishra G, Srivastava S, Nagori BP. Pharmacological and therapeutic activity of *Cissus quadrangularis*: An overview. *International Journal of PharmTech Research*. 2010; 2:1298–1310. ISSN: 0974-4304.

- [45] Staren ED, Omer S. Hormone replacement therapy in postmenopausal women. *The American Journal of Surgery*. 2004; 188:136-149. <https://doi.org/10.1016/j.amjsurg.2003.12.063>. PMID: 15249239.
- [46] Abdel-Naim AB, Alghamdi AA, Alghandaby MM, Al-Abbasi FA, Al-Abd AM, Eid BG, et al. Rutin isolated from *Chrozophora tinctoria* enhances bone cell proliferation and ossification markers. *Oxidative Medicine and Cellular Longevity*. 2018; 2018:5106469. <https://doi.org/10.1155/2018/5106469>. PMID: 29636845.
- [47] Thomford NE, Senthebane DA, Rowe A, Munro D, Seele P, Maroyi A, Dzobo K. Natural products for drug discovery in the 21st century: innovations for novel drug discovery. *International Journal of Molecular Sciences*. 2018; 19. <https://doi.org/10.3390/ijms19061578>. PMID: 29799486.
- [48] Suvarna V, Sarkar M, Chaubey P, Khan T, Sherje A, Patel K, Dravyakar B. Bone health and natural products- An insight. *Frontiers in Pharmacology*. 2018; 9:981. <https://doi.org/10.3389/fphar.2018.00981>. PMID: 30283334.
- [49] Nooreen Z, Rai VK, Yadav NP. Phytopharmaceuticals: A new drug class regulated in India. *Annals of Phytomedicine*. 2018; 7:27-37. <https://doi.org/10.21276/ap.2018.7.1.4>.
- [50] Sangma AJT, Sahoo UK. Utilization pattern of medicinal plants by different tribes of garo hills of meghalaya, North-East India. *American Journal of Ethnomedicine*. 2017; 4:1. <https://doi.org/10.21767/2348-9502.100001>.
- [51] Singh G, Passsari AK, Leo VV, Mishra VK, Subbarayan S, Singh BP, et al. Evaluation of phenolic content variability along with antioxidant, antimicrobial, and cytotoxic potential of selected traditional medicinal plants from India. *Frontiers in Plant Science*. 2016; 7:407. <https://doi.org/10.3389/fpls.2016.00407>. PMID: 27066046.
- [52] Zhang L, Zhuang H, Zhang Y, Wang L, Zhang Y, Geng Y, et al. Plants for health: An ethnobotanical 25-year repeat survey of traditional medicine sold in a major marketplace in North-west Yunnan, China. *Journal of Ethnopharmacology*. 2018; 224:119-125. <https://doi.org/10.1016/j.jep.2018.05.029>. PMID: 29800743.
- [53] Shi Y, Mon AM, Fu Y, Zhang Y, Wang C, Yang X, Wang Y. The genus *Ficus* (Moraceae) used in diet: Its plant diversity, distribution, traditional uses and ethnopharmacological importance. *Journal of Ethnopharmacology*. 2018; 226:185-196. doi: 10.1016/j.jep.2018.07.027. PMID: 30055253.
- [54] Shengji P, Hamilton AC, Lixin Y, Huyin H, Zhiwei Y, Fu G, Quangxin Z. Conservation and development through medicinal plants: a case study from Ludian (Northwest Yunnan, China) and presentation of a general model. *Biodiversity and Conservation*. 2010; 19:2619-2636. <https://doi.org/10.1007/s10531-010-9862-5>.
- [55] Lao L, Xu L, Xu S. Traditional Chinese Medicine. In: Längler A., Mansky P., Seifert G. (eds) *Integrative Pediatric Oncology*. *Pediatric Oncology*. Springer, Berlin, Heidelberg. 2012; 125-135. https://doi.org/10.1007/978-3-642-04201-0_9.

- [56] Amano T, González-Varo JP, Sutherland WJ. Languages are still a major barrier to global science. *PLoS Biology*. 2016; 14:e2000933. <https://doi.org/10.1371/journal.pbio.2000933>. PMID: 28033326.
- [57] Perlman RL. Mouse models of human disease: An evolutionary perspective. *Evolution, Medicine, and Public Health*. 2016; 2016:170-176. <https://doi.org/10.1093/emph/eow014>. PMID: 27121451.
- [58] Wong VW, Sorkin M, Glotzbach JP, Longaker MT, Gurtner GC. Surgical approaches to create murine models of human wound healing. *Journal of Biomedicine and Biotechnology*. 2011; 2011:8. ID 969618. <https://doi.org/10.1155/2011/969618>.
- [59] Histing T, Garcia P, Holstein JH, Klein M, Matthys R, Nuetzi R, et al. Small animal bone healing models: standards, tips, and pitfalls results of a consensus meeting. *Bone*. 2011; 49: 591-599. <https://doi.org/10.1016/j.bone.2011.07.007>. PMID: 21782988.
- [60] Xiong Y, Mahmood A, Chopp M. Animal models of traumatic brain injury. *Nature Reviews Neuroscience*. 2013; 14:128-142. <https://doi.org/10.1038/nrn3407>. PMID: 23329160.
- [61] Warriner AH, Patkar NM, Curtis JR, Delzell E, Gary L, Kilgore M. et al, Which fractures are most attributable to osteoporosis?. *Journal of Clinical Epidemiology*. 2010; 64:46-53. <https://doi.org/10.1016/j.jclinepi.2010.07.007>. PMID: 21130353.
- [62] Hastrup SG, Chen X, Bechtold JE, Kyle RF, Rahbek O, Keyler DE, et al. Effect of nicotine and tobacco administration method on the mechanical properties of healing bone following closed fracture. *Journal of Orthopaedic Research*. 2010; 28:1235-1239. <https://doi.org/10.1002/jor.21106>. PMID: 20225318.
- [63] Pereira-Júnior OC, Rahal SC, Iamaguti P, Felisbino SL, Pavan PT, Vulcano LC. Comparison between polyurethanes containing castor oil (soft segment) and cancellous bone autograft in the treatment of segmental bone defect induced in rabbits. *Journal of Biomaterials Applications*. 2007; 21:283-297. <https://doi.org/10.1177/0885328206063526>. PMID: 16543284.
- [64] Aspenberg P, Sandberg O. Distal radial fractures heal by direct woven bone formation. *Acta Orthopaedica*. 2013; 84:297-300. doi: 10.3109/17453674.2013.792769. PMID: PMC3715812.
- [65] Hobbenaghi R, Mahboob P, Saifzadeh S, et al. Retraction note: Histopathological features of bone regeneration in a canine segmental ulnar defect model. *Diagnostic Pathology*. 2016;11:124. doi:10.1186/s13000-016-0573-4. PMID: 27802815.
- [66] Padovan LEM, Garcia e Sousa D, Padovan SHM, Lopes de Oliveira GJP, Silva RD, Claudino M. Histological analysis of the use of biphasic ceramics based on hydroxyapatite and β -tricalcium phosphate in maxillary sinus floor elevation surgery: pilot study. *Rev. odontol. UNESP [online]*. 2018; 47:63-68. <http://dx.doi.org/10.1590/1807-2577.10917>.
- [67] Verma S. Role of *Cissus quadrangularis* in the treatment of osteoporosis: A review. *Acute Medicine Research*. 2018; 1:1.

- [68] Pinheiro Neto VF, Ribeiro RM, Morais CS, Campos MB, Vieira DA, Guerra PC, et al. *Chenopodium ambrosioides* as a bone graft substitute in rabbits radius fracture. BMC Complementary and Alternative Medicine. 2017; 17:350. <https://doi.org/10.1186/s12906-017-1862-5>. PMID: 28676049.
- [69] Almeida SA, Salome GM, Dutra RAA, Ferreira LM. Feelings of powerlessness in individuals with either venous or diabetic foot ulcers. Journal of Tissue Viability. 2014; 23:109-114. <https://doi.org/10.1016/j.jtv.2014.04.005>. PMID: 24882082.
- [70] Kant V, Gopal A, Kumar D, Pathak NN, Ram M, Jangir BL. Curcumin-induced angiogenesis hastens wound healing in diabetic rats. Journal of Surgical Research. 2015; 193:978-988. <https://doi.org/10.1016/j.jss.2014.10.019>. PMID: 25454972.
- [71] Subramanian R, Asmawi MZ, Sadikun A. A bitter plant with a sweet future? A comprehensive review of an oriental medicinal plant: *Andrographis paniculata*. Phytochemistry Reviews. 2012; 11:39-75. <https://doi.org/10.1007/s11101-011-9219-z>.
- [72] Bais S, Gill NS, Rana N, Shandil S. A Phytopharmacological review on a medicinal plant: *Juniperus communis*. International Scholarly Research Notices. 2014; 2014:1-6. <http://dx.doi.org/10.1155/2014/634723>.
- [73] Bensaid I, Atik Bekkara F, El Hacı IA, Belarbi K, Beddou F, Bekhechi C. Phytochemical analysis and antioxidant properties of organic extracts obtained from *Cynoglossum cheirifolium* L. Oriental Pharmacy and Experimental Medicine. 2017; 17:381-387. <https://doi.org/10.1007/s13596-017-0291-9>.
- [74] Russo M, Fanali C, Tripodo G, Dugo P, Muleo R, Dugo L, et al. Analysis of phenolic compounds in different parts of pomegranate (*Punica granatum*) fruit by HPLC-PDA-ESI/MS and evaluation of their antioxidant activity: application to different Italian varieties. Analytical and Bioanalytical Chemistry. 2018; 410:3507-3520. <https://doi.org/10.1007/s00216-018-0854-8>. PMID: 29350256.
- [75] Lavola A, Maukonen M, Julkunen-Tiitto R. Variability in the composition of phenolic compounds in winter-dormant *Salix pyrolifolia* in relation to plant part and age. Phytochemistry. 2018; 153:102-110. <https://doi.org/10.1016/j.phytochem.2018.05.021>. PMID: 29906656.
- [76] Bristy NJ, Nazmul Hasan AHM, Alam MN, Wahed TB, Roy P, Khairul Alam KM. Characterization of antioxidant and cytotoxic potential of methanolic extracts of different parts of *Aegle marmelos* (L.). IJPSR. 2017; 8:1476-1484. [https://doi.org/10.13040/IJPSR.0975-8232.8\(3\).1476-84](https://doi.org/10.13040/IJPSR.0975-8232.8(3).1476-84).
- [77] Bennett BC, Balick MJ. Does the name really matter? The importance of botanical nomenclature and plant taxonomy in biomedical research. Journal of Ethnopharmacology. 2014; 152:387-392. <https://doi.org/10.1016/j.jep.2013.11.042>. PMID: 24321863.
- [78] Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African Journal of Traditional, Complementary and Alternative Medicines. 2011; 8:1-10. PMID: 22238476.

[79] Sampaio R. F. and Mancini M. C. Estudos de revisão sistemática: um guia para síntese criteriosa da evidência científica. *Revista Brasileira de Fisioterapia*. 2007; 11:83-89. <http://dx.doi.org/10.1590/S1413-35552007000100013>.

[80] Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009; 339:b2700. <https://doi.org/10.1136/bmj.b2700>.

ARTIGO 2:

Pomada a base de extrato de *Brassica oleracea* var. *capitata* acelera o processo de cicatrização de queimaduras de terceiro grau em ratos Wistar

Resumo

Queimaduras são responsáveis por mais de 265 mil mortes por ano no mundo. As infecções são a principal causa de morte em pacientes queimados, por isso quanto menor o tempo de cicatrização, melhor é o prognóstico. Baseado nisso, este estudo teve por objetivo investigar o efeito da pomada a base de *Brassica oleracea* var. *capitata* na aceleração da cicatrização de feridas cutâneas produzidas por queimadura. Vinte e cinco ratos Wistar (± 349 g), com três meses de vida, foram obtidos do Biotério Central da Universidade Federal de Viçosa e individualizados randomicamente em gaiolas com alimento e água *ad libitum*. Após anestesia, foram feitas duas feridas por queimadura (12 mm de diâmetro) no dorso de cada animal, os quais foram aleatoriamente divididos em cinco tratamentos: Sal: salina; VP: veículo da pomada (lanolina e vaselina); SS: controle positivo (sulfadiazina de prata 1%); PB1: pomada *B. oleracea* a 10%; e PB2: pomada *B. oleracea* a 20%. Os animais receberam a pomada nas feridas, diariamente, durante oito dias. A área da ferida foi mensurada a cada quatro dias e, simultaneamente, foram retirados fragmentos para análises histopatológicas (celularidade, vasos sanguíneos e componentes da matriz extracelular) e análises do perfil oxidativo (enzimas antioxidantes, marcadores de oxidação lipídica e proteica, além de óxido nítrico (NO) e água oxigenada (H₂O₂)). A pomada a base do extrato de *Brassica oleracea* var. *capitata* nas concentrações 10 e 20% promoveu aumento de células e vasos sanguíneos, bem como aumento dos componentes fibrosos da matriz extracelular, aumentando também a atividade das enzimas antioxidantes e acelerando o processo de recuperação do tecido cutâneo após queimaduras de terceiro grau.

Palavras-chave: Repolho; queimaduras; cicatrização de feridas; estereologia; estresse oxidativo; atividade antioxidante.

1. Introdução

A pele, maior órgão do corpo, é composta pela epiderme e derme, além de ser responsável pela manutenção da homeostase do corpo, e servir como barreira de proteção química, física e bacteriana [1]. Feridas provocadas por queimadura, trauma mecânico, cortes, tumores, má circulação sanguínea e cirurgias podem comprometer a estrutura e função da pele [2], expondo indivíduos ao risco de outras complicações de saúde como ulcerações e infecções [3].

Feridas por queimadura afetam mais de 11 milhões de pessoas anualmente em todo o mundo [4] e podem ser causadas por calor, produtos químicos, eletricidade, radiação, entre outras, e são classificadas como primeiro grau (superficial - atingindo a epiderme), segundo grau (espessura parcial - atingindo epiderme e parte da derme) e terceiro grau (espessura total - atingindo epiderme, derme, hipoderme e músculos) [5, 6]. A profundidade da queimadura está relacionada ao tempo de exposição ao agente agressor, temperatura e pressão sob o tecido e estes fatores determinam o prognóstico após as lesões [7]. Análises histológicas do tecido podem ser usadas para confirmar o grau da lesão, entretanto, após a queimadura os elementos celulares e parenquimatosos da pele são comprometidos, dificultando a avaliação da profundidade da queimadura [8].

O processo cicatricial é essencial para a sobrevivência de todos os organismos superiores [9] e ocorre em três fases: inflamatória, de proliferação e de remodelamento [10]. Durante a primeira etapa, quando a barreira epidérmica é violada, ocorre a liberação de mediadores derivados de plaquetas, subprodutos bacterianos e quimioatrativos que promovem a hemostasia do tecido [11]. Além disto, leucócitos são recrutados, eliminando restos de tecido lesado e infecções, permitindo que a próxima etapa ocorra de forma harmoniosa e eficaz [12]. Na fase seguinte, ocorre a proliferação de células e vasos, levando a formação do tecido de granulação, muito vascularizado e rico em colágeno tipo III [13, 14]. O tecido de granulação, apesar de frágil, é essencial para a maturação tecidual e deve estar presente para que ocorra a deposição do próximo colágeno na ferida [15]. Na fase de remodelamento, o colágeno tipo III é substituído pelo tipo I, que é mais espesso e se organiza em feixes de fibras, conferindo à cicatriz força e resistência [16]. O resultado final deste processo que envolve a interação entre células, matriz e citocinas é o fechamento rápido e eficaz da ferida [17].

A indução da fase inflamatória e o recrutamento de células imunes em lesões por queimaduras, levam à produção de Espécies reativas de Oxigênio (ERO's) [18]. Na tentativa de neutralizar o estresse oxidativo, pode haver um aumento no consumo das defesas

antioxidantes no tecido, ocasionando redução da capacidade antioxidante. Assim, resultando em um aumento exagerado das ERO's, excedendo a capacidade do sistema para neutralizá-las e eliminá-las, causando excesso na produção de radicais livres no tecido [19]. O organismo defende-se contra ERO's envolvendo vários sistemas antioxidantes endógenos enzimáticos e não enzimáticos, entre eles destacamos as enzimas antioxidantes, superóxido dismutase (SOD), catalase (CAT) e glutathione S-transferase (GST) [20–22], que atuam neutralizando ou impedindo a formação de radicais livres, que podem ser prejudiciais às biomoléculas vitais e, conseqüentemente, aos tecidos do corpo [23]. A diminuição das enzimas de defesa antioxidante e o processo de degradação dos constituintes celulares comprometem o processo cicatricial, levando a um reparo tecidual ineficaz [24].

Feridas por queimaduras graves precisam de tratamento rápido para evitar infecções, que são a causa mais comum de mortalidade em pacientes queimados [25]. Por isto, existe uma necessidade crescente de fármacos adequados com este objetivo no mercado [26], já que a maioria das drogas disponíveis atualmente possui atividade antimicrobiana em detrimento ao efeito cicatrizante [27]. Neste contexto, o uso de plantas medicinais e seus derivados são importantes, uma vez que vários compostos fitoquímicos podem atuar de forma sinérgica e ser eficazes para inibir a proliferação de microorganismos e estimular o fechamento das feridas [28, 29], além de ser uma forma de tratamento simples, barata e provavelmente apresentar menos efeitos colaterais [30].

O repolho (*Brassica oleraceae* var. *capitata*) pertence ao gênero *Brassica* e à família Brassicaceae (Cruciferae) [31], é uma planta com grande importância econômica, cultivada e usada na alimentação em todo o mundo [32]. As folhas de repolho são usadas para a cura de feridas e alívio da dor nas articulações na medicina tradicional [33, 34]. Componentes fitoquímicos de plantas do gênero *Brassica* como glucosinolatos, sulforafanos, taninos (ácidos fenólicos) e flavonoides, já foram relacionadas à ação cicatrizante [31, 35–38] e antimicrobiana [39–41]. Baseado nisto, nosso objetivo foi desenvolver um estudo único para avaliar o efeito da pomada a base de *Brassica oleracea* var. *capitata* (10 e 20%) no processo de cicatrização de queimaduras cutâneas de terceiro grau em ratos Wistar.

2. Material e métodos

2.1 Obtenção do material vegetal

Folhas de *Brassica oleracea* var. *capitata* foram coletadas em uma área preservada de Mata Atlântica no estado de Minas Gerais, Brasil (20°43'00"S e 42°29'10"W, a 1,200

metros acima do nível do mar). As amostras foram selecionadas, higienizadas por imersão em hipoclorito de sódio a 2% em água destilada por 2 minutos, e lavadas em água potável autoclavada por 2 minutos. A extração foi feita por maceração empregando-se veículo álcool/propilenoglicol como solvente extrator. Em seguida, a suspensão foi submetida à ultrassonicação (Unique, MaxiClean 1400, São Paulo, SP) e filtração a vácuo e o extrato concentrado foi aquecido a 55°C em estufa por 24 h para retirada do solvente. Após o processo da extração e filtração, o extrato concentrado foi incorporado ao veículo da pomada formada por lanolina anidra a 30% e vaselina sólida 70%, até completa homogeneização do extrato.

2.2 Preparo da formulação

A formulação da pomada foi preparada como segue: 1) extrato glicólico de *B. oleracea* var. *capitata* (10% ou 20% em peso); 2) Veículo da pomada: álcool cetostearílico (5% em peso), monooleato de sorbitano etoxilado (10% em peso), vaselina (10% em peso), ácido graxo com éster polioliol (5% em peso), lanolina anidra (15% em peso), carbamida (5% em peso), conservantes (metilparabeno, propilparabeno e hidroxitolueno butilado [1:1, 10% em peso]), óleo de rícino hidrogenado (5% em peso) e água desmineralizada (15% em peso). Finalmente, o extrato foi incorporado no veículo da pomada até a homogeneização completa [42].

2.3 Animais

Ratos Wistar (*Rattus norvegicus*) ($n=25$), machos, com peso de $335,4\pm 16g$, três meses de vida, provenientes do Biotério Central da Universidade Federal de Viçosa (UFV) foram transportados em veículo particular, em microisoladores com filtro de proteção, até o Biotério da Biologia Geral (UFV), onde foram alocados randomicamente em gaiolas individuais, diariamente higienizadas e mantidas em ambiente com temperatura controlada ($\pm 22^{\circ}C$). O fotoperíodo foi estabelecido em ciclos de 12 horas de claridade e 12 horas de escuridão. Água e ração foram fornecidos aos animais *ad libitum*. O estudo foi aprovado pela Comissão de Ética no Uso de Animais (CEUA) da UFV (n° 90/2017).

2.4 Realização das queimaduras de pele

Os animais foram anestesiados com Pentobarbital (70 mg/ kg) para a realização das queimaduras e não foi administrado analgésico após o procedimento cirúrgico, uma vez que a aplicação de medicamentos pode alterar a migração e proliferação celular, comprometendo o processo de reparo cutâneo. A região dorso-lateral dos animais foi raspada e duas feridas

circulares por queimadura de terceiro grau foram criadas por meio de um cilindro de ferro de 12 mm de diâmetro, que foi aquecido em água fervente (100 °C) e pressionado sobre o dorso dos animais durante 30 segundos [adaptado de [43]]. A área das feridas por queimaduras foi mensurada com a utilização de paquímetro analógico (Mitutoyo Sul Americana Ltda®, São Paulo, Brasil) [44].

2.5 Cálculo da área e taxa de contração da ferida

A área e a taxa de contração da ferida foram avaliadas a cada quatro dias, utilizando imagens digitalizadas com 320 × 240 pixels (24 bits / pixel) obtidas por câmera de smartphone Asus Zenfone 2 ZE551ML (ASUS, Brasil). A área da ferida foi calculada pela fórmula $A = \pi \times \text{raio} (r)^2$. A taxa de contração da ferida (TCF) foi calculado pela razão: área inicial da área da ferida (A_0) - área medida em um determinado dia (A_i) / área da ferida inicial (A_0) × 100 [45].

O processo de cicatrização foi analisado na amostra coletada no último dia do experimento.

2.6 Design experimental

Os animais foram divididos aleatoriamente em cinco grupos (n= 5/grupo): Grupo SAL (feridas tratadas com solução salina a 0,9%); Grupo VP (feridas tratadas com o veículo da pomada); Grupo SS (feridas controle tratadas com Sulfadiazina de prata 1%); Grupo PB1 (feridas tratadas com pomada à base do extrato de *Brassica oleracea* na concentração de 10%); Grupo PB2 (feridas tratadas com pomada à base do extrato de *Brassica oleracea* na concentração de 20%). A quantidade de pomada aplicada foi de 0,1g em cada ferida, diariamente.

As feridas foram limpas diariamente com solução salina a 0,9% antes da aplicação da pomada. Todos os tratamentos foram iniciados 24 horas após a realização das feridas e repetidos uma vez ao dia ao longo de oito dias. Fragmentos do tecido em regeneração foram coletados nos dias zero (Ferida 0= F0), quatro (Ferida 1=F1) e oito (Ferida 2=F2), repetindo o processo anestésico relatado anteriormente para retirada das amostras (Pentobarbital - 70 mg/kg). Os fragmentos continham tecido do centro da ferida e parte do tecido ileso, presente na região adjacente à borda da lesão. Ao final do tratamento, os animais foram eutanasiados por meio de exsanguinação por punção cardíaca após procedimento anestésico.

2.7 Análises histológicas

Os fragmentos retirados das feridas nos dias quatro e oito foram fixados em solução de formaldeído 10% tamponado em fosfato de sódio 0,1 M (pH 7,2), desidratados em álcool etílico, diafanizados em xilol e incluídos em parafina. Foram obtidos cortes histológicos de 4 μm de espessura em micrótomo rotativo Leica Multicut® 2045 (Reichert-Jung Products, Germany) os quais foram corados com Hematoxilina e Eosina para análise de fibroblastos e vasos sanguíneos [46]. Pela técnica modificada de Sirius Red foi realizada a análise das fibras colágenas [47] e para a diferenciação de fibras elásticas foi utilizada a coloração pelo método de Verhoeff [48]. A coloração com azul de toluidina foi utilizada para identificar mastócitos [49]. Secções semi-seriadas foram realizadas aproveitando um a cada dez cortes, para evitar a análise repetida do tecido. Imagens histológicas foram obtidas em microscópio de luz BX-60® (Olympus, São Paulo, Brasil) acoplado a uma câmera fotográfica digital modelo QColor-3® (Olympus, São Paulo, Brasil). Para cada corte foram obtidas, de forma randomizada, cinco imagens com resolução de 2592×1944 pixels para contagem de fibroblastos e vasos sanguíneos e de 1024×768 pixels para fibras elásticas e colágenas. Células, vasos sanguíneos e fibras foram analisados em aumento de 200×. Foi utilizada uma grade de 300 intersecções para contagem de fibroblastos e vasos sanguíneos e de 256 intersecções para fibras elásticas, colágenas, associada ao software de análise de imagens Image Pro-plus 4.5 (Media Cybernetics®, Silver Spring, USA). Mastócitos foram analisados em aumento de 400×, 10 campos histológicos foram analisados randomicamente em cada secção histológica para obtenção de uma área total (AT) de $3.11 \times 10^6 \mu\text{m}^2$. O número de mastócitos por unidade de área histológica foi calculado de acordo com a equação $QA = \Sigma \text{ mast cells} / AT$ [50].

2.8 Análises bioquímicas

Amostras de tecido retiradas de cada ferida foram imediatamente congeladas em nitrogênio líquido (-196 °C) e armazenadas em freezer a -80 °C. Os fragmentos de pele dessas amostras foram homogeneizados em tampão fosfato e centrifugados a 4 °C [51].

2.8.1 Atividade de superóxido dismutase

A atividade da SOD foi determinada pelo método baseado na redução do superóxido (O^{-2}) e do peróxido de hidrogênio, diminuindo assim a auto-oxidação do pirogalol [52]. A atividade da SOD foi calculada como unidades por miligrama de proteína, com U de SOD definido como a quantidade que inibiu a taxa de auto-oxidação do pirogalol em 50%.

2.8.2 Atividade de catalase

A atividade da catalase (CAT) foi determinada pelo método de Aebi, 1984, usando H_2O_2 como substrato. Uma unidade de atividade CAT foi definida como a quantidade de enzima que decompõe 1 mmol de H_2O_2 por 1 minuto. A atividade da catalase foi calculada como U por miligrama de proteína [53].

2.8.3 Atividade de glutathione S-transferase

A atividade da glutathione S-transferase (GST) foi medida [54] de acordo com a formação de 2,4-dinitroclorobenzeno conjugado com glutathione (CDNB). Uma unidade de atividade de GST foi definida como a quantidade de enzima que catalisou a formação de um μmol de produto/min/ml. A atividade da GST foi expressa em U por miligrama de proteína.

2.8.4 Determinação de malondialdeído

A extensão da peroxidação lipídica (LPO) foi medida por meio dos níveis de malondialdeído total (MDA) [55]. A concentração de MDA foi determinada usando a curva padrão de concentrações conhecidas de 1,1,3,3-tetrametoxipropano (TMPO). Os resultados foram expressos em $\mu\text{mol/L}$ por mg de proteína.

2.8.5 Oxidação de proteínas

O teor de proteínas carboniladas foi medido utilizando o procedimento 2,4-dinitrofenilhidrazina (DNPH) [56] com base nos grupos carbonil que reagem com DNPH. Os pellets resultantes dos homogenatos obtidos dos fragmentos de pele foram usados para quantificação. Os resultados foram expressos em nmol por mg de proteína.

Todos os dados bioquímicos descritos acima foram normalizados em relação aos níveis de proteínas totais no sobrenadante de acordo com o protocolo de Bradford (1976) e os resultados foram expressos em $\mu\text{mol/L}$ por mg de proteína [57].

2.8.6 Produção de Óxido Nítrico (NO)

A produção de NO foi estimada pela produção de NO_2/NO_3 pela reação padrão de Griess [58]. Foram incubados 50 μL de sobrenadantes de cada grupo de tratamento com um volume igual de reagente Griess (1% sulfanilamida, 0,1% N-(1-Naftil) etilenodiamina e 2,5% H_3PO_4) à temperatura ambiente, por 10 minutos. A conversão da absorbância em concentrações micromolares de NO foi obtida a partir de uma curva padrão de nitrito de sódio (0–125 μM) e expressa em concentrações de NO ($\mu\text{mol/L}$).

2.8.7 Produção de Peróxido de Hidrogênio (H₂O₂)

A produção de H₂O₂ foi medida em sobrenadantes de tecidos homogeneizados, como descrito acima. Foram incubados 50 µL de sobrenadante com 50 µL de dicloridrato de α-fenilenodiamina (OPD) e um volume igual de peroxidase tipo II 15 mmol / L. A conversão da absorbância em concentrações micromolares de H₂O₂ foi calculada a partir de uma curva padrão utilizando uma concentração conhecida de H₂O₂. Os resultados foram expressos em µmol/L [59].

2.9 Análise estatística

A análise estatística foi realizada por meio do software GraphPad Prism 7 (GraphPad Software Inc., San Diego, Calif., USA). Os dados foram relatados como média e desvio padrão (média ± DP) e foi feita uma análise de variância One-way ANOVA seguido pelo teste paramétrico de Student-Newman-Keuls. Uma probabilidade de p <0,05 foi considerada estatisticamente significativa.

3. Resultados

3.1 Contração da ferida

Nos dias quatro (F1) e oito (F2), a área da ferida foi significativamente reduzida nos grupos tratados com *B. oleracea* a 10% e 20% quando comparado aos demais grupos. A taxa de contração da ferida foi maior no grupo tratado com a pomada de *B. oleracea* var. *capitata* a 20% (PB2) do que nos outros grupos no dia oito (F2). (Tabela 1).

Tabela 1. Área (mm²) e taxa de contração da ferida (TCF) (%) em animais tratados com extrato de *Brassica oleracea* var. *capitata* em diferentes concentrações.

		SAL	VP	SS	PB1	PB2
F0	Area	156,6±20,1	161,6±29,4	141,3±15,7	126,9±13,3	134,9±13,5
	TCF	0,0±0,0	0,0±0,0	0,0±0,0	0,0±0,0	0,0±0,0
F1	Area	153,1±33,4	178,4±18,8	138,4±32,3	87,4±19,7*	97,7±22,4*
	TCF	10,3±6,9	3,3±20,6	2,4±18,4	31,4±10,8	27,8±13,3
F2	Area	142,2±30,0	155,4±34,7	133,9±50,4	67,6±20,9*	66,4±21,4*
	TCF	15,9±14,3	10,4±19,8	28,1±18,0	47,2±12,2	51,4±12,5*

SAL: solução salina a 0,9%; VP: veículo da pomada; SS: sulfadiazina de prata (1%), PB1: pomada *B. oleracea* a 10%; e PB2: pomada *B. oleracea* a 20%. F0 = tecido intacto; F1, F2 =

tecido cicatricial após quatro e oito dias, respectivamente. Dados representados como média \pm DP. * Diferença estatística entre os tratamentos: SAL, VP e SS.

3.2 Resultados histopatológicos

O número de células e vasos sanguíneos no grupo tratado com a pomada *B. oleracea* 10% (PB1) aumentou em relação aos grupos controle SAL, VP e SS no dia quatro. Ainda no dia quatro, o grupo PB2 apresentou número maior de células quando comparado a PB1 e SAL. No dia oito, a celularidade foi maior nos dois grupos tratados com a pomada *B. oleracea* 10% (PB1) e 20% (PB2) em relação ao grupo SAL e SS, e o número de vasos sanguíneos foi maior no grupo PB2 comparado à SAL e SS (Figura 1-A, B).

O número de mastócitos foi maior no grupo tratado com a pomada *B. oleracea* 20% (PB2) em comparação aos grupos SAL, VP e SS no dia quatro. No dia oito, foi maior em ambos os tratamentos com a pomada (10% e 20%, PB1 e PB2 respectivamente) comparada aos grupos SAL e VP (Figura 1-C). A Figura 2-D mostra um corte histológico do grupo tratado com a pomada *B. oleracea* 20% (PB2) no dia quatro.

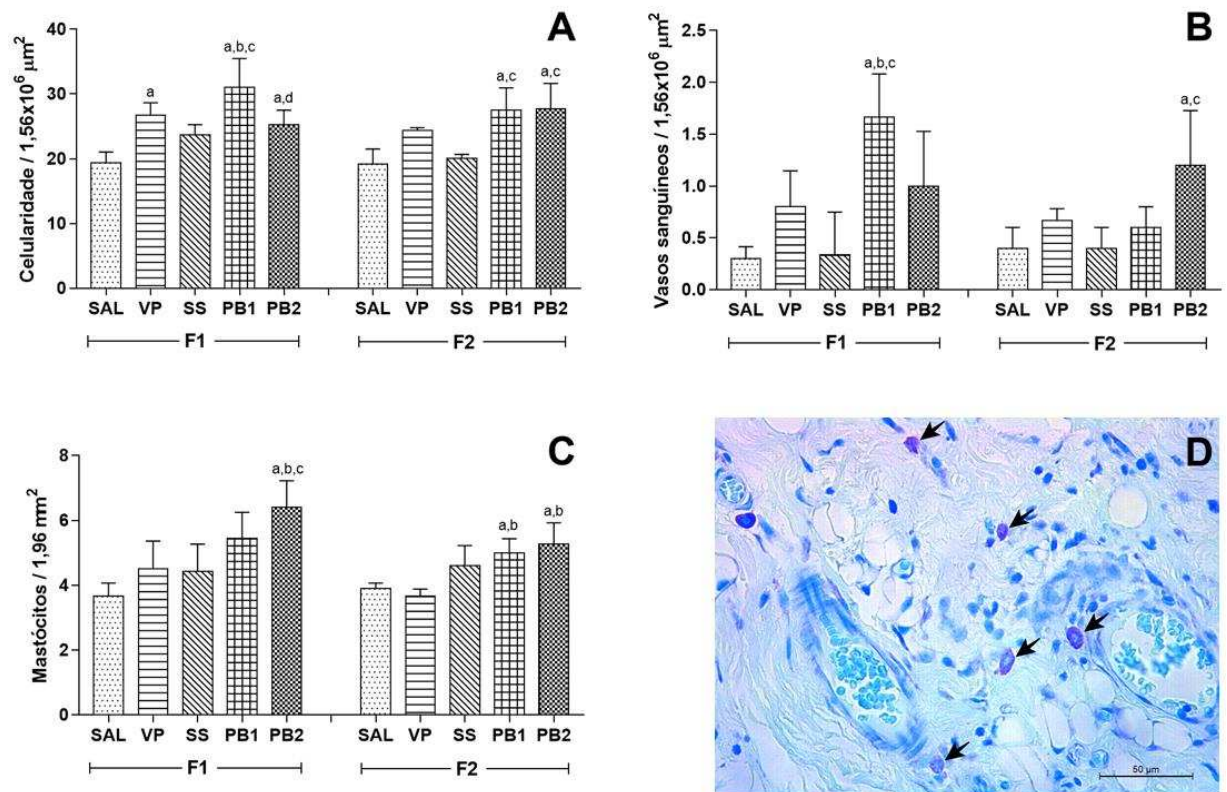


Figura 1. Proporção de: A - Células, B - Vasos sanguíneos, C - Mastócitos no tecido cicatricial de ratos em diferentes tratamentos. F1: tecido tratado após quatro dias, F2: tecido tratado após oito dias. SAL: solução salina a 0,9%; VP: veículo da pomada; SS: sulfadiazina

de prata (1%), PB1: pomada *B. oleracea* a 10%; e PB2: pomada *B. oleracea* a 20%. Os dados estão representados como média \pm DP; ^{a, b, c, d}, representam diferenças estatísticas em relação aos grupos: SAL, VP, SS e PB1, respectivamente. ($p < 0,05$) (teste de Student-Newman-Keuls). D - Fotomicrografia representativa de mastócitos (setas) no tecido cicatricial de queimadura tratada com a pomada a base do extrato de *B. oleracea*, coloração azul de toluidina (barra = 50 μ m).

A proporção de fibras colágenas do tipo III não foi diferente estatisticamente entre os grupos no dia quatro. No dia oito, foi menor nos grupos tratados com *B. oleracea* 10% (PB1) e 20% (PB2) quando comparado aos demais. O grupo tratado com sulfadiazina de prata (SS) teve menor proporção quando comparado a SAL e VP (Figura 2-A).

A proporção de fibras colágenas do tipo I foi maior no grupo com concentração da pomada em 20% (PB2) em relação a todos os outros grupos no dia quatro. No dia oito, foi maior nos grupos tratados com *B. oleracea* (10% e 20%) e sulfadiazina de prata (SS) em relação a SAL e VP (Figura 2-B). Além disso, a proporção do grupo tratado com a maior dose da pomada (PB2) foi maior do que o grupo que recebeu a menor dose (PB1).

Em relação à proporção de fibras elásticas, no dia quatro, não houve diferença estatística entre os grupos. No dia oito, a proporção foi maior no grupo tratado com a pomada *B. oleracea* 20% em relação aos grupos SAL e VP (Figura 2-C). Os grupos PB1 e SS apresentaram valores maiores quando comparados a VP. A Figura 3-D mostra um corte histológico do grupo tratado com *B. oleracea* 20% (PB2) no dia oito.

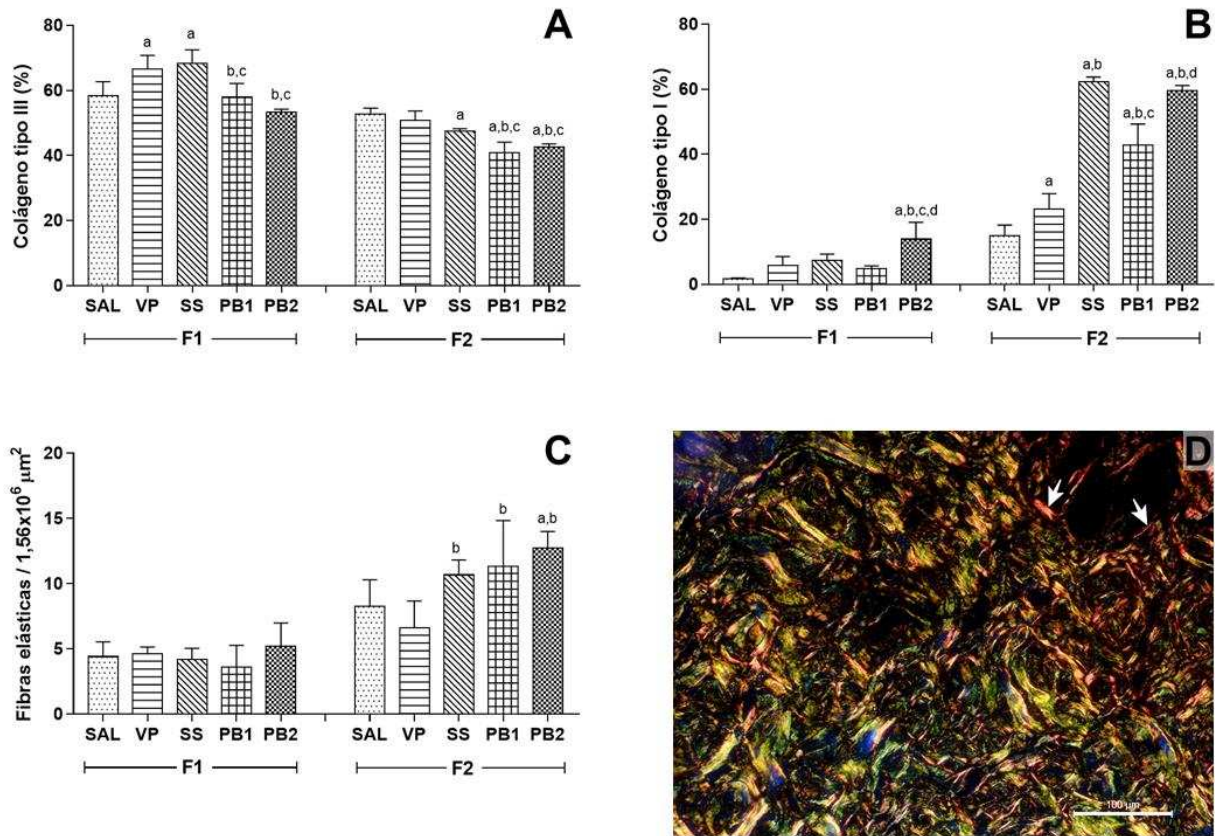


Figura 2. Proporção de: A - Fibras colágenas Tipo I, B - Fibras colágenas Tipo III, C - Fibras elásticas no tecido cicatricial de ratos em diferentes tratamentos. F1: tecido tratado após quatro dias, F2: tecido tratado após oito dias. SAL: solução salina a 0,9%; VP: veículo da pomada; SS: sulfadiazina de prata (1%), PB1: pomada *B. oleracea* a 10%; e PB2: pomada *B. oleracea* a 20%. Os dados estão representados como médias \pm DP; ^{a, b, c, d}, representam diferenças estatísticas em relação aos grupos: SAL, VP, SS e PB1, respectivamente ($p < 0,05$) (teste de Student-Newman-Keuls). D - Fotomicrografia representativa de corte histológico de pele mostrando o efeito do tratamento com *B. oleracea* 20% (PB2) no dia oito em ratos Wistar com lesão por queimadura, evidenciando a presença de colágeno Tipo I (vermelho - setas) e Tipo III (verde) coloração sirius red (barra = 100μm).

3.3 Resultados bioquímicos

3.3.1 Enzimas antioxidantes

A atividade de SOD foi significativamente maior no grupo tratado com a pomada *B. oleracea* a 20% (PB2) quando comparado aos outros grupos nos dias quatro e oito (Figura 3-A). A atividade de CAT também foi maior no grupo tratado com *B. oleracea* 20% (PB2) em comparação a todos os outros grupos no dia quatro. No dia oito, CAT foi maior em ambos os grupos tratados com a pomada *B. oleracea* (PB1 e PB2) em relação a SAL, VP e SS (Figura

3-B). Os valores de GST foram maiores no grupo tratado com a pomada *B. oleracea* 10% (PB1) no dia quatro em relação a SS, e em ambos os grupos tratados com *B. oleracea* (PB1 e PB2), em relação a SAL e VP, no dia oito (Figura 3-C).

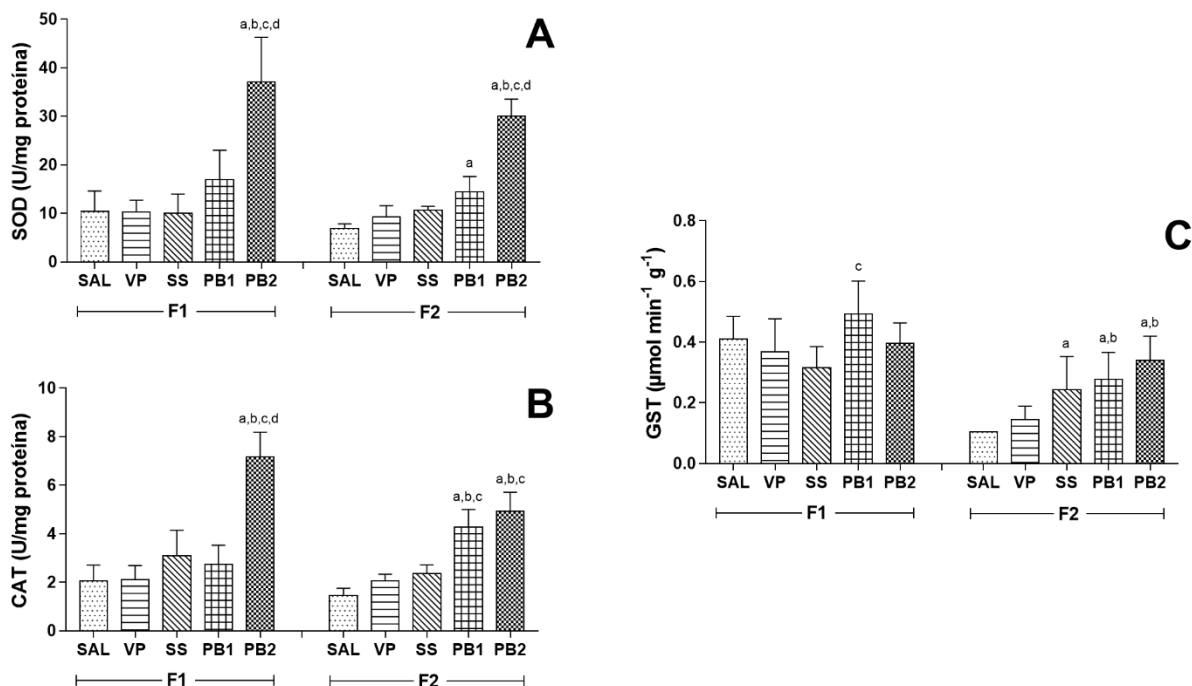


Figura 3. Atividade de: A - superóxido dismutase (SOD), B - catalase (CAT), C - glutiona S-transferase (GST) no tecido cicatricial de ratos em diferentes tratamentos. F1: tecido tratado após quatro dias, F2: tecido tratado após oito dias. SAL: solução salina a 0,9%; VP: veículo da pomada; SS: sulfadiazina de prata (1%), PB1: pomada *B. oleracea* a 10%; e PB2: pomada *B. oleracea* a 20%. Os dados estão representados como médias \pm DP; a, b, c, d, representam diferenças estatísticas em relação aos grupos: SAL, VP, SS e PB1, respectivamente ($p < 0,05$) (teste de Student-Newman-Keuls).

3.3.2 Marcadores oxidativos

Os níveis de MDA foram significativamente menores no grupo tratado com a pomada *B. oleracea* 10 e 20% e no grupo SS em relação ao grupo VP no dia quatro. No oitavo dia não houve diferença estatística entre os grupos (Figura 4-A).

Os valores de PCN foram menores nos grupos tratados com a pomada *B. oleracea* 10 e 20% quando comparado ao grupo SAL no dia quatro. No dia oito, não houve diferença estatística entre os grupos (Figura 4-B).

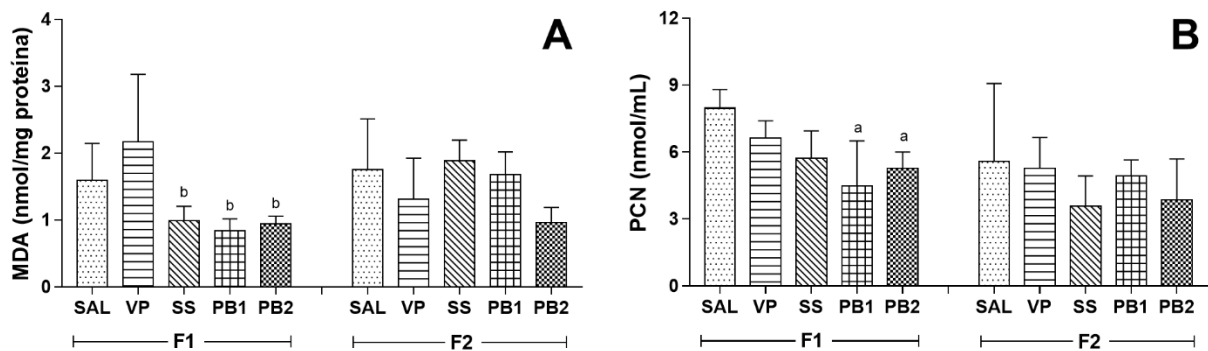


Figura 4. Níveis de: A - malondialdeído (MDA), B - proteínas carboniladas (PCN) no tecido cicatricial de ratos em diferentes tratamentos. F1: tecido tratado após quatro dias, F2: tecido tratado após oito dias. SAL: solução salina a 0,9%; VP: veículo da pomada; SS: sulfadiazina de prata (1%), PB1: pomada *B. oleracea* a 10%; e PB2: pomada *B. oleracea* a 20%. Os dados estão representados como médias \pm DP; ^{a, b}, representam diferenças estatísticas em relação aos grupos: SAL, VP, respectivamente. ($p < 0,05$) (teste de Student-Newman-Keuls).

3.3.3 Óxido Nítrico e Peróxido de Hidrogênio (NO e H₂O₂)

A produção de nitrito e nitrato (NO₂/NO₃) não foi diferente estatisticamente entre os grupos no dia quatro. No dia oito, foi maior nos grupos tratados com a pomada à base de *B. oleracea* a 10% e 20% (PB1 e PB2) em relação ao grupo controle SAL (Figura 5-A).

Os níveis de H₂O₂ não apresentaram diferenças estatísticas no dia quatro. No dia oito, o conteúdo de H₂O₂ foi maior nos grupos tratados com a pomada *B. oleracea* 10% e 20% (PB1 e PB2) e no grupo SS em relação aos grupos SAL e VP (Figura 5-B).

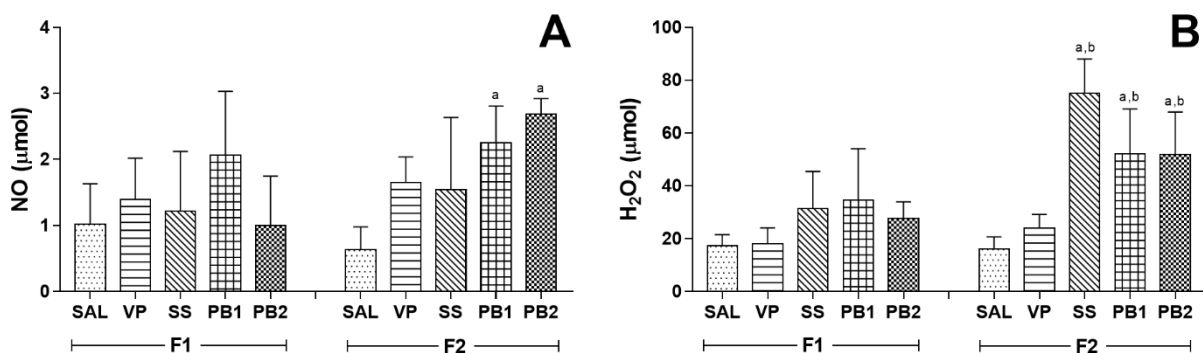


Figura 5. Níveis de: A - Óxido Nítrico (NO), B - Peróxido de hidrogênio (H₂O₂) no tecido cicatricial de ratos em diferentes tratamentos. F1: tecido tratado após quatro dias, F2: tecido tratado após oito dias. SAL: solução salina a 0,9%; VP: veículo da pomada; SS: sulfadiazina de prata (1%), PB1: pomada *B. oleracea* a 10%; e PB2: pomada *B. oleracea* a 20%. Os dados

estão representados como médias \pm DP; ^{a, b}, representam diferenças estatísticas em relação aos grupos: SAL e VP, respectivamente. ($p < 0,05$) (teste de Student-Newman-Keuls).

4. Discussão

Queimaduras ocorrem devido à exposição da pele a agentes químicos, físicos ou biológicos, ocasionando destruição tecidual, infecção, dor e até a morte [60]. Por essa razão, pesquisas recentes se concentraram em encontrar compostos que melhorem o processo de reparo [61]. No presente estudo, utilizamos o extrato de *Brassica oleracea* var. *capitata*, uma planta muito utilizada na culinária e popularmente conhecida como repolho [62], para avaliar seu efeito na cicatrização de feridas por queimadura na pele. Nossos resultados mostram que a pomada à base de repolho promove a proliferação celular e vascular, bem como síntese da matriz extracelular, acelerando o processo de cicatrização de feridas de terceiro grau.

Em nosso estudo, a redução significativa da área das queimaduras de pele nos grupos tratados com *B. oleracea* a 10% e 20% evidencia o aumento na proliferação e diferenciação celular e na síntese de proteínas. Resultados semelhantes foram obtidos por Sarandy et al., [63], que demonstrou que o extrato de *Brassica oleracea* var. *capitata* promoveu um reparo tecidual mais rápido em feridas cutâneas de segunda intenção. Essa aceleração do fechamento das feridas se deve, provavelmente, à elevada presença de flavonoides e antocianinas no extrato de *B. oleracea* [64, 65]. Esses compostos têm elevado poder terapêutico, pois, além de possuírem alta atividade antioxidante, estimulam a formação de novos vasos e garantem ao tecido maior nutrição, promovendo a recuperação rápida do tecido lesado [66–68].

O aumento de células e vasos nos grupos tratados com o extrato de *Brassica*, principalmente no quarto dia, demonstra uma fase inflamatória acelerada e intensa [69]. Os mediadores liberados na fase inflamatória estimulam a síntese de colágeno e, conseqüentemente, a formação do tecido de granulação, rico em vasos e células [70, 71]. A alta celularidade e vascularização podem estar relacionadas ao aumento do metabolismo celular e, possivelmente, da nutrição tecidual, importantes para o fechamento do tecido [72]. A potencialização do processo inflamatório no início do reparo pode levar a uma recuperação mais rápida do tecido [73], principalmente associando esses resultados aos de contração da ferida, onde foi demonstrada a maior redução da área da queimadura nos grupos tratados com a pomada a base do extrato de *B. oleracea*.

A diminuição do colágeno Tipo III e o aumento do colágeno Tipo I e de fibras elásticas nos grupos tratados com a pomada observado no oitavo dia, demonstram elevados índices de maturação do tecido. O colágeno III, frágil e pouco resistente, deve predominar no início do

processo, durante as fases inflamatórias e de proliferação, pois serve de arcabouço e suporte para a deposição do colágeno do tipo I, que confere maior força e resistência ao tecido [46, 61]. Ambos os tipos de colágeno são fundamentais para um processo de cicatrização saudável [74]. Possivelmente, a maior deposição de colágeno I está relacionada ao aumento no número de células e vasos neste tecido, indicando a alta atividade metabólica do mesmo após o tratamento com *B. oleracea*. Quanto maior concentração destas proteínas no tecido, maior é a atividade dos fibroblastos durante o reparo e mais rápido será o fechamento da ferida [75]. Estes achados podem estar relacionados também ao predomínio de flavonoides, já que este composto influencia a síntese de colágeno e está ligado ao aumento da tradução de proteínas importantes para o processo de recuperação de feridas cutâneas [76–78]. Estas moléculas são bioativas para o tratamento de várias desordens com efeito antioxidante e regenerativo, e possuem elevada capacidade de estimular o fechamento rápido e eficaz de feridas [79, 80].

A diminuição nos níveis de MDA e PCN nos grupos que receberam a pomada de *B. oleracea* indica o elevado poder antioxidante deste extrato, reduzindo o dano tecidual causado pela oxidação de lipídeos e proteínas. O MDA é um subproduto da peroxidação lipídica derivado da quebra de ácidos graxos poli-insaturados [81]. No processo de queimaduras ocorre intensa lesão tecidual e, por isso, é comum encontrar excesso de MDA no tecido, uma vez que o aumento deste marcador indica lesão das membranas celulares, frequentemente associada ao aumento local do estresse oxidativo [82]. Outro bom marcador de injúria tecidual é o conteúdo de proteínas carboniladas (PCN), já que mostra o nível de oxidação e desnaturação das proteínas [83]. Sarandy et al. [51], relataram que houve redução dos níveis de MDA e PCN analisadas em feridas cutâneas de segunda intenção em ratos Wistar tratados com *S. pseudoquina*. Ainda nesse estudo, os autores relatam a ação dos flavonoides como antioxidante efetivo, reiterando nossos achados com o tratamento com extrato de *B. oleracea*. Provavelmente este potencial antioxidante pode ser justificado pelo elevado número de compostos com efeito protetor presentes no extrato de *Brassica*, como compostos fenólicos, antocianinas e sulforafanos.

Os resultados demonstrados acima para marcadores de estresse oxidativo corroboraram com os nossos resultados para enzimas antioxidantes, já que os grupos que receberam *B. oleracea* apresentaram aumento das principais enzimas responsáveis pela proteção do tecido, como SOD, CAT e GST. A partir daí podemos sugerir que o potencial antioxidante do extrato de *B. oleracea* se deve à sua capacidade de inibir a oxidação lipídica e proteica, por estimular fortemente a ação das enzimas antioxidantes, contribuindo para um reparo tecidual mais rápido e eficaz das feridas por queimadura. As enzimas superóxido dismutase (SOD), catalase

(CAT) e glutathione S-transferase (GST) são responsáveis pela proteção do tecido contra danos oxidativos causados por espécies reativas de oxigênio em excesso (ERO's) [84, 85]. A importância destas enzimas para o processo de reparo cutâneo já é conhecida [86–88]. Resultados semelhantes foram demonstrados por Al-Roujayee [89], onde após o tratamento com o composto fenólico Naringenin em ratos Wistar queimados, houve aumento das enzimas antioxidantes SOD, CAT e GST e consequente redução da peroxidação lipídica das células e aumento da síntese de matriz, tornando a cicatriz mais resistente.

Quando um tecido está sofrendo injúria ocorre aumento de espécies reativas de oxigênio, como ânion superóxido, que eliminam o óxido nítrico (NO) tecidual, reduzindo assim suas concentrações efetivas e ações de sinalização nas células [90]. Normalmente os níveis de NO em um tecido lesado por queimadura é baixo, devido às elevadas concentrações de ERO's e baixos níveis de enzimas antioxidantes [91]. Em nosso estudo houve um aumento de NO nos grupos tratados com *B. oleracea*, o que pode indicar diminuição de ERO's no tecido, demonstrando novamente o efeito antioxidante deste extrato. O óxido nítrico (NO) é uma molécula importante que regula diversas funções fisiológicas vitais, como a resposta imune, e, em alguns casos, níveis elevados deste marcador podem sinalizar para homeostase do tecido [92]. Resultados semelhantes foram encontrados por Brisbois [93], que demonstrou que o NO melhora a cicatrização em camundongos feridos por queimaduras, reduzindo a infecção e o crescimento bacteriano, acelerando o processo de cura das feridas.

O peróxido de hidrogênio (H_2O_2) é gerado *in vivo* pela dismutação do ânion-radical superóxido ($O_2^{\cdot-}$) por enzimas como superóxido dismutase (SOD) [94]. Em lesões, é formado rapidamente e é um promotor essencial da resposta inflamatória da cicatrização de feridas [95]. Quando os níveis de H_2O_2 estão elevados no tecido há um aumento de enzimas antioxidantes como CAT e peroxidases ligadas à tioredoxina [96]. Aumentos nos níveis de H_2O_2 sob condições de estresse têm sido relatados, como em situações de feridas por queimadura [97]. Em geral, níveis elevados desta molécula no tecido indicam estresse tecidual, no entanto, podem mostrar também uma via ativa e eficaz de desintoxicação das células pelas enzimas antioxidantes. Levando em consideração os resultados encontrados em nosso estudo acreditamos que a segunda hipótese é a mais aceita, já que todos os sistemas de defesa estão super-estimulados e que os marcadores de estresse estão diminuídos no tratamento com Brassica. Estes achados poderiam justificar também o aumento de CAT tecidual, uma vez que esta enzima utiliza H_2O_2 como substrato, portanto, o aumento de CAT pode indicar a necessidade de eliminar o excesso de peróxido, para manter a homeostase tecidual [98].

5. Conclusão

Nossos resultados mostram que a pomada à base do extrato de *Brassica oleracea* var. *capitata*, quando aplicada nas doses de 10 e 20%, acelera o fechamento da área da ferida, bem como a proliferação de células e vasos sanguíneos, modulando a produção dos componentes da matriz, estimulando a formação de uma cicatriz forte e resistente em queimaduras de terceiro grau. Além disso, o repolho é amplamente usado na alimentação humana e possui baixo potencial de toxicidade, sendo uma alternativa segura de tratamento. Este estudo deve ampliar o conhecimento científico em relação a eficácia deste produto fitoterápico na cicatrização, podendo ser útil para atenuar o risco de complicações como infecções, beneficiando os pacientes que sofreram lesão por queimadura.

Conflitos de interesses

Os autores declaram que não há conflitos de interesses.

Declaração de financiamento

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); e Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG PPM)

Agradecimentos

Os agradecimentos são para: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); e Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

Referências

- [1] J. Ho, C. Walsh, D. Yue, A. Dardik, and U. Cheema, “Current advancements and strategies in tissue engineering for wound healing: a comprehensive review,” *Adv. Wound Care*, vol. 6, pp. 191–209, 2017.
- [2] P. Basu, U. Narendra Kumar, and I. Manjubala, “Wound healing materials - a perspective for skin tissue engineering,” *Curr. Sci.*, vol. 112, p. 2392, 2017.
- [3] S. Guo and L. A. Dipietro, “Factors affecting wound healing,” *J. Dent. Res.*, vol. 89, pp. 219–229, 2010.

- [4] T. J. Hamilton, J. Patterson, R. Y. Williams, et al., “Management of head and neck burns—A 15-year review,” *J. Oral Maxillofac. Surg.*, vol. 76, pp. 375–379, 2018.
- [5] R. Bahramsoltani, M. H. Farzaei, A. H. Abdolghaffari, et al., “Evaluation of phytochemicals, antioxidant and burn wound healing activities of *Cucurbita moschata* Duchesne fruit peel,” *Iran. J. Basic Med. Sci.*, vol. 20, pp. 798–805, 2017.
- [6] R. Fekrazad, A. Nikkerdar, K. Joharchi, and K. A. M. Kalhori, “Effect of laser photostimulation on the healing of third-degree burn wounds in rats,” *Acta Cir. Bras.*, vol. 2, pp. 0–5, 2014.
- [7] A. J. Singer, B. R. Taira, R. Anderson, S. A. McClain, and L. Rosenberg, “Does pressure matter in creating burns in a porcine model?,” *J. Burn Care Res.*, vol. 31, pp. 646–651, 2010.
- [8] H. Ye and S. De, “Thermal injury of skin and subcutaneous tissues: A review of experimental approaches and numerical models,” *Burns*, vol. 43, pp. 909–932, 2017.
- [9] M. Takeo, W. Lee, and M. Ito, “Wound healing and skin regeneration,” *Cold Spring Harb. Perspect. Med.*, vol. 5, p. a023267, 2015.
- [10] E. J. Mulholland, N. Dunne, and H. O. McCarthy, “MicroRNA as therapeutic targets for chronic wound healing,” *Mol. Ther. Nucleic Acids*, vol. 8, pp. 46–55, 2017.
- [11] B. K. Sun, Z. Siplashvili, and P. A. Khavari, “Advances in skin grafting and treatment of cutaneous wounds,” *Science (80-.)*, vol. 346, pp. 941–944, 2014.
- [12] B. J. Curtis, S. Hlavin, A. L. Brubaker, E. J. Kovacs, and K. A. Radek, “Episodic binge ethanol exposure impairs murine macrophage infiltration and delays wound closure by promoting defects in early innate immune responses,” *Alcohol. Clin. Exp. Res.*, vol. 38, no. 5, pp. 1347–1355, 2014.
- [13] K. Hyldig, S. Riis, C. P. Pennisi, V. Zachar, and T. Fink, “Implications of extracellular matrix production by adipose tissue-derived stem cells for development of wound healing therapies,” *Int. J. Mol. Sci.*, vol. 18, p. 1167, 2017.
- [14] S. Werner and M. Antsiferova, “Wound healing: an orchestrated process of cell cycle, adhesion, and signaling,” *Encycl. Cell Biol.*, vol. 3, pp. 216–222, 2016.
- [15] D. Chouhan, B. Chakraborty, S. K. Nandi, and B. B. Mandal, “Role of non-mulberry silk fibroin in deposition and regulation of extracellular matrix towards accelerated wound healing,” *Acta Biomater.*, vol. 48, pp. 157–174, 2017.
- [16] D. Docheva, S. A. Müller, M. Majewski, and C. H. Evans, “Biologics for tendon repair,” *Adv. Drug Deliv. Rev.*, vol. 84, pp. 222–239, 2015.
- [17] K. Bankoti, A. P. Rameshbabu, S. Datta, et al., “Accelerated healing of full thickness

- dermal wounds by macroporous waterborne polyurethane-chitosan hydrogel scaffolds,” *Mater. Sci. Eng. C*, vol. 81, pp. 133–143, 2017.
- [18] F. A. D. T. G. Wagener, C. E. Carels, and D. M. S. Lundvig, “Targeting the redox balance in inflammatory skin conditions,” *Int. J. Mol. Sci.*, vol. 14, pp. 9126–9167, 2013.
- [19] M. Schieber and N. S. Chandel, “ROS function in redox signaling and oxidative stress,” *Curr. Biol.*, vol. 24, pp. R453–R462, 2014.
- [20] M. Kirici, C. Turk, C. Caglayan, and M. Kirici, “Toxic effects of copper sulphate pentahydrate on antioxidant enzyme activities and lipid peroxidation of freshwater fish capoeta Umbla (Heckel, 1843) tissues,” *Appl. Ecol. Environ. Res.*, vol. 15, pp. 1685–1696, 2017.
- [21] Y. Chtourou, B. Aouey, M. Kebieche, and H. Fetoui, “Protective role of naringin against cisplatin induced oxidative stress, inflammatory response and apoptosis in rat striatum via suppressing ROS-mediated NF- κ B and P53 signaling pathways,” *Chem. Biol. Interact.*, vol. 239, pp. 76–86, 2015.
- [22] K. M. Holmström and T. Finkel, “Cellular mechanisms and physiological consequences of redox-dependent signalling,” *Nat. Rev. Mol. Cell Biol.*, vol. 15, pp. 411–421, 2014.
- [23] O. M. Ighodaro and O. A. Akinloye, “First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid,” *Alexandria J. Med.*, pp. 1–7, 2017.
- [24] R. V. Gonçalves, R. D. Novaes, J. P. V. Leite, et al., “Hepatoprotective effect of *Bathysa cuspidata* in a murine model of severe toxic liver injury,” *Int. J. Exp. Pathol.*, vol. 93, pp. 370–376, 2012.
- [25] Y. Wang, J. Beekman, J. Hew, et al., “Burn injury: Challenges and advances in burn wound healing, infection, pain and scarring,” *Adv. Drug Deliv. Rev.*, vol. 123, pp. 3–17, 2018.
- [26] M. U. Hashmi, F. Khan, N. Khalid, et al., “Hydrogels incorporated with silver nanocolloids prepared from antioxidant rich *Aerva javanica* as disruptive agents against burn wound infections,” *Colloids Surfaces A Physicochem. Eng. Asp.*, vol. 529, pp. 475–486, 2017.
- [27] R. Bahramsoltani, M. H. Farzaei, and R. Rahimi, “Medicinal plants and their natural components as future drugs for the treatment of burn wounds: an integrative review,”

- Arch. Dermatol. Res.*, vol. 306, pp. 601–617, 2014.
- [28] T. Efferth and E. Koch, “Complex interactions between phytochemicals. The multi-target therapeutic concept of phytotherapy,” *Curr. Drug Targets*, vol. 12, pp. 122–132, 2011.
- [29] K. Akter, E. C. Barnes, J. J. Brophy, et al., “Phytochemical profile and antibacterial and antioxidant activities of medicinal plants used by aboriginal people of New South Wales, Australia,” *Evidence-Based Complement. Altern. Med.*, vol. 2016, p. 14, 2016.
- [30] D. Moalla Rekik, S. Ben Khedir, K. Ksouda Moalla, et al., “Evaluation of wound healing properties of grape seed, sesame, and fenugreek oils,” *Evidence-Based Complement. Altern. Med.*, vol. 2016, pp. 1–12, 2016.
- [31] D. Šamec, I. Pavlović, and B. Salopek-Sondi, “White cabbage (*Brassica oleracea* var. *capitata* f. *Alba*): botanical, phytochemical and pharmacological overview,” *Phytochem. Rev.*, vol. 16, pp. 117–135, Feb. 2017.
- [32] E. Hallmann, R. Kazimierzczak, K. Marszałek, et al., “The nutritive value of organic and conventional white cabbage (*Brassica oleracea* L. var. *capitata*) and anti-Apoptotic activity in gastric adenocarcinoma cells of sauerkraut juice produced Therof,” *J. Agric. Food Chem.*, vol. 65, pp. 8171–8183, 2017.
- [33] A. B. Samuelsen, B. Westereng, O. Yousif, et al., “Structural features and complement-fixing activity of pectin from three *Brassica oleracea* varieties: white cabbage, kale, and red kale,” *Biomacromolecules*, vol. 8, pp. 644–649, 2007.
- [34] A. P. Vale, H. Cidade, M. Pinto, and M. B. P. P. Oliveira, “Effect of sprouting and light cycle on antioxidant activity of *Brassica oleracea* varieties,” *Food Chem.*, vol. 165, pp. 379–387, 2014.
- [35] C. A. de Carvalho, M. B. da Silva, T. G. de Oliveira, J. de M. Lima, and M. B. da Rosa, “Estudo espectrométrico de diferentes estágios fenológicos da *Brassica oleracea* var. *capitata*,” *Rev. Bras. Farmacogn.*, vol. 18, pp. 249–257, 2008.
- [36] V. Dal Prá, C. B. Dolwitsch, G. D. da Silveira, et al., “Supercritical CO₂ extraction, chemical characterisation and antioxidant potential of *Brassica oleracea* var *capitata* against HO·, O₂(·-) and ROO·,” *Food Chem.*, vol. 141, pp. 3954–3959, 2013.
- [37] P. Y. Nugraedi, R. Verkerk, B. Widianarko, and M. Dekker, “A mechanistic perspective on process-induced changes in glucosinolate content in Brassica vegetables: a review,” *Crit. Rev. Food Sci. Nutr.*, vol. 55, pp. 823–838, 2015.
- [38] I. Hassini, N. Baenas, D. A. Moreno, et al., “Effects of seed priming, salinity and methyl jasmonate treatment on bioactive composition of *Brassica oleracea* var.

- capitata* (white and red varieties) sprouts,” *J. Sci. Food Agric.*, vol. 97, pp. 2291–2299, 2017.
- [39] S. H. Hu, J. C. Wang, H. F. Kung, et al., “Antimicrobial effect of extracts of cruciferous vegetables,” *Kaohsiung J. Med. Sci.*, vol. 20, pp. 591–599, 2004.
- [40] T. Sotelo, M. Lema, P. Soengas, M. E. Cartea, and P. Velasco, “*In vitro* activity of glucosinolates and their degradation products against Brassica-Pathogenic bacteria and fungi,” *Appl. Environ. Microbiol.*, vol. 81, pp. 432–440, 2014.
- [41] V. Dal Prá, C. B. Dolwitsch, F. O. Lima, et al., “Ultrasound-assisted extraction and biological activities of extracts of *Brassica oleracea* var. *capitata*,” *Food Technol. Biotechnol.*, vol. 53, pp. 102–109, 2015.
- [42] R. V. Gonçalves, R. D. Novaes, M. C. Cupertino, et al., “*Bathysa cuspidata* extract modulates the morphological reorganization of the scar tissue and accelerates skin wound healing in rats: a time-dependent study,” *Cells Tissues Organs*, vol. 199, pp. 266–277, 2014.
- [43] A. Partoazar, N. Kianvash, M. Darvishi, et al., “Ethosomal curcumin promoted wound healing and reduced bacterial flora in second degree burn in rat,” *Drug Res. (Stuttg.)*, vol. 66, pp. 660–665, 2016.
- [44] R. V. Gonçalves, M. M. Sarandy, S. L. P. da Matta, R. D. Novaes, and M. V. de M. Pinto, “Comparative study of the effects of laser photobiomodulation and extract of *Brassica oleracea* on skin wounds in wistar rats: A histomorphometric study,” *Pathol. - Res. Pract.*, vol. 209, pp. 648–653, 2013.
- [45] M. S. Agren, P. M. Mertz, and L. Franzén, “A comparative study of three occlusive dressings in the treatment of full-thickness wounds in pigs,” *J. Am. Acad. Dermatol.*, vol. 36, pp. 53–58, 1997.
- [46] R. Gonçalves, J. Mezêncio, G. Benevides, et al., “Effect of gallium-arsenide laser, gallium-aluminum-arsenide laser and healing ointment on cutaneous wound healing in Wistar rats,” *Braz. J. Med. Biol. Res.*, vol. 43, pp. 350–355, 2010.
- [47] P. C. Dolber and M. S. Spach, “Conventional and confocal fluorescence microscopy of collagen fibers in the heart,” *J. Histochem. Cytochem.*, vol. 41, pp. 465–469, 1993.
- [48] L. C. Junqueira, José; Carneiro, *Biologia celular e molecular*, 8 ed. Rio de Janeiro, 2005.
- [49] C. J. Churukian and E. A. Schenk, “A toluidine blue method for demonstrating mast cells,” *J. Histotechnol.*, vol. 4, pp. 85–86, 1981.
- [50] C. A. Mandarin-de-Lacerda, “Stereological tools in biomedical research,” *An. Acad.*

- Bras. Cienc.*, vol. 75, pp. 469–486, 2003.
- [51] M. M. Sarandy, L. L. Miranda, L. S. Altoé, et al., “*Strychnos pseudoquina* modulates the morphological reorganization of the scar tissue of second intention cutaneous wounds in rats,” *PLoS One*, vol. 13, p. e0195786, 2018.
- [52] S. Dieterich, U. Bieligk, K. Beulich, G. Hasenfuss, and J. Prestle, “Gene expression of antioxidative enzymes in the human heart: increased expression of catalase in the end-stage failing heart,” *Circulation*, vol. 101, pp. 33–39, 2000.
- [53] H. Aebi, “Catalase in vitro,” *Methods Enzymol.*, vol. 105, pp. 121–126, 1984.
- [54] W. H. Habig, M. J. Pabst, and W. B. Jakoby, “Glutathione S-transferases. The first enzymatic step in mercapturic acid formation,” *J. Biol. Chem.*, vol. 249, pp. 7130–7139, 1974.
- [55] B. Wallin, B. Rosengren, H. G. Shertzer, and G. Camejo, “Lipoprotein oxidation and measurement of thiobarbituric acid reacting substances formation in a single microtiter plate: its use for evaluation of antioxidants,” *Anal. Biochem.*, vol. 208, pp. 10–15, 1993.
- [56] R. L. Levine, D. Garland, C. N. Oliver, et al., “Determination of carbonyl content in oxidatively modified proteins,” *Methods Enzymol.*, vol. 186, pp. 464–478, 1990.
- [57] M. M. Bradford, “A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding,” *Anal. Biochem.*, vol. 72, pp. 248–254, 1976.
- [58] D. Tsikas, “Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: Appraisal of the Griess reaction in the l-arginine/nitric oxide area of research,” *J. Chromatogr. B*, vol. 851, pp. 51–70, 2007.
- [59] J. M. Oliveira, N. F. Losano, S. S. Condessa, et al., “Exposure to deltamethrin induces oxidative stress and decreases of energy reserve in tissues of the Neotropical fruit-eating bat *Artibeus lituratus*,” *Ecotoxicol. Environ. Saf.*, vol. 148, pp. 684–692, 2018.
- [60] P. Brassolatti, A. L. M. de Andrade, P. S. Bossini, A. N. Otterço, and N. A. Parizotto, “Evaluation of the low-level laser therapy application parameters for skin burn treatment in experimental model: a systematic review,” *Lasers Med. Sci.*, vol. 33, pp. 1159–1169, 2018.
- [61] M. Li, L. Qiu, W. Hu, et al., “Genetically-modified bone mesenchymal stem cells with TGF- β 3 improve wound healing and reduce scar tissue formation in a rabbit model,” *Exp. Cell Res.*, vol. 367, pp. 24–29, 2018.
- [62] K. S. Silva, E. da C. dos Santos, C. G. Benett, et al., “Produtividade e desenvolvimento

- de cultivares de repolho em função de doses de boro,” *Hortic. Bras.*, vol. 30, pp. 520–525, 2012.
- [63] M. M. Sarandy, R. D. Novaes, S. L. P. da Matta, et al., “Ointment of *Brassica oleracea* var. *capitata* matures the extracellular matrix in skin wounds of Wistar rats,” *Evidence-Based Complement. Altern. Med.*, vol. 2015, p. 919342, 2015.
- [64] F. Xu, Y. Zheng, Z. Yang, et al., “Domestic cooking methods affect the nutritional quality of red cabbage,” *Food Chem.*, vol. 161, pp. 162–167, 2014.
- [65] S. Park, M. V. Arasu, N. Jiang, et al., “Metabolite profiling of phenolics, anthocyanins and flavonols in cabbage (*Brassica oleracea* var. *capitata*),” *Ind. Crops Prod.*, vol. 60, pp. 8–14, 2014.
- [66] A. Senthilkumar, N. Karuvantevida, L. Rastrelli, S. S. Kurup, and A. J. Cheruth, “Traditional uses, pharmacological efficacy, and phytochemistry of *Moringa peregrina* (Forssk.) Fiori. —a review,” *Front. Pharmacol.*, vol. 9, p. 465, May 2018.
- [67] M. Wei, Y. Ma, Y. Liu, et al., “Urinary metabolomics study on the anti-inflammation effects of flavonoids obtained from Glycyrrhiza,” *J. Chromatogr. B*, vol. 1086, pp. 1–10, 2018.
- [68] A. Valenza, C. Bonfanti, M. E. Pasini, and P. Bellosta, “Anthocyanins function as anti-inflammatory agents in a *Drosophila* model for adipose tissue macrophage infiltration,” *Biomed Res. Int.*, vol. 2018, pp. 1–9, 2018.
- [69] D. Mehrabani, M. Farjam, B. Geramizadeh, et al., “The healing effect of curcumin on burn wounds in rat,” *World J. Plast. Surg.*, vol. 4, pp. 29–35, 2015.
- [70] U. Das, S. S. Behera, and K. Pramanik, “Ethno-herbal-medico in wound repair: an incisive review,” *Phyther. Res.*, vol. 31, pp. 579–590, 2017.
- [71] T. Kurahashi and J. Fujii, “Roles of antioxidative enzymes in wound healing,” *J. Dev. Biol.*, vol. 3, pp. 57–70, 2015.
- [72] J. C.-Y. Lai, H.-Y. Lai, N. K. Rao, and S.-F. Ng, “Treatment for diabetic ulcer wounds using a fern tannin optimized hydrogel formulation with antibacterial and antioxidative properties,” *J. Ethnopharmacol.*, vol. 189, pp. 277–289, 2016.
- [73] N. Pringle, T. Koekemoer, A. Holzer, et al., “Potential therapeutic benefits of green and fermented rooibos (*Aspalathus linearis*) in dermal wound healing,” *Planta Med.*, vol. 84, pp. 645–652, 2018.
- [74] E. Wulandari, S. W. A. Jusman, Y. Moenadjat, A. A. Jusuf, and M. Sadikin, “Expressions of collagen I and III in hypoxic keloid tissue,” *Kobe J. Med. Sci.*, vol. 62, pp. E58-69, 2016.

- [75] L. E. Tracy, R. A. Minasian, and E. J. Caterson, "Extracellular matrix and dermal fibroblast function in the healing wound," *Adv. Wound Care*, vol. 5, pp. 119–136, 2016.
- [76] T. Stipcevic, J. Piljac, and D. Vanden Berghe, "Effect of different flavonoids on collagen synthesis in human fibroblasts," *Plant Foods Hum. Nutr.*, vol. 61, pp. 29–34, 2006.
- [77] C. Faggio, A. Sureda, S. Morabito, et al., "Flavonoids and platelet aggregation: a brief review," *Eur. J. Pharmacol.*, vol. 807, pp. 91–101, 2017.
- [78] F. R. S. Corrêa, F. S. Schanuel, N. Moura-Nunes, A. Monte-Alto-Costa, and J. B. Daleprane, "Brazilian red propolis improves cutaneous wound healing suppressing inflammation-associated transcription factor NFκB," *Biomed. Pharmacother.*, vol. 86, pp. 162–171, 2017.
- [79] J. Nazaruk and A. Galicka, "The influence of selected flavonoids from the leaves of *Cirsium palustre* (L.) Scop. on collagen expression in human skin fibroblasts," *Phyther. Res.*, vol. 28, pp. 1399–1405, 2014.
- [80] R. H. Tuhin, M. M. Begum, M. S. Rahman, et al., "Wound healing effect of *Euphorbia hirta* linn. (Euphorbiaceae) in alloxan induced diabetic rats," *BMC Complement. Altern. Med.*, vol. 17, p. 423, 2017.
- [81] A. Ayala, M. F. Muñoz, and S. Argüelles, "Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal," *Oxid. Med. Cell. Longev.*, vol. 2014, pp. 1–31, 2014.
- [82] A. Pisoschi and A. Pop, "The role of antioxidants in the chemistry of oxidative stress: A review," *Eur. J. Med. Chem.*, vol. 97, pp. 55–74, 2015.
- [83] R. C. Bollineni, M. Fedorova, M. Blüher, and R. Hoffmann, "Carbonylated plasma proteins as potential biomarkers of obesity induced type 2 diabetes mellitus," *J. Proteome Res.*, vol. 13, pp. 5081–5093, 2014.
- [84] S. L. Manoto, M. J. Maepa, and S. K. Motaung, "Medical ozone therapy as a potential treatment modality for regeneration of damaged articular cartilage in osteoarthritis," *Saudi J. Biol. Sci.*, vol. 25, pp. 672–679, 2018.
- [85] S. Türedi, E. Yuluğ, A. Alver, A. Bodur, and İ. İnce, "A morphological and biochemical evaluation of the effects of quercetin on experimental sciatic nerve damage in rats," *Exp. Ther. Med.*, vol. 15, pp. 3215–3224, 2018.
- [86] Y. Iuchi, D. Roy, F. Okada, et al., "Spontaneous skin damage and delayed wound healing in SOD1-deficient mice," *Mol. Cell. Biochem.*, vol. 341, pp. 181–194, 2010.

- [87] R. V. Gonçalves, R. D. Novaes, M. M. Sarandy, et al., “5 α -Dihydrotestosterone enhances wound healing in diabetic rats,” *Life Sci.*, vol. 152, pp. 67–75, 2016.
- [88] M. M. Sarandy, R. D. Novaes, A. A. Xavier, et al., “Hydroethanolic extract of *Strychnos pseudoquina* accelerates skin wound healing by modulating the oxidative status and microstructural reorganization of scar tissue in experimental type I diabetes,” *Biomed Res. Int.*, vol. 2017, pp. 1–11, 2017.
- [89] A. S. Al-Roujaye, “Naringenin improves the healing process of thermally-induced skin damage in rats,” *J. Int. Med. Res.*, vol. 45, pp. 570–582, 2017.
- [90] N. S. Bryan, “Functional nitric oxide nutrition to combat cardiovascular disease,” *Curr. Atheroscler. Rep.*, vol. 20, p. 21, 2018.
- [91] A. Magenta, S. Greco, M. C. Capogrossi, C. Gaetano, and F. Martelli, “Nitric oxide, oxidative stress, and p66Shc interplay in diabetic endothelial dysfunction,” *Biomed Res. Int.*, vol. 2014, p. 193095, 2014.
- [92] D. Pierini and N. S. Bryan, “Nitric oxide availability as a marker of oxidative stress,” *Methods Mol. Biol.*, vol. 1208, pp. 63–71, 2015.
- [93] E. J. Brisbois, J. Bayliss, J. Wu, et al., “Optimized polymeric film-based nitric oxide delivery inhibits bacterial growth in a mouse burn wound model,” *Acta Biomater.*, vol. 10, pp. 4136–4142, 2014.
- [94] M. Mittal, M. R. Siddiqui, K. Tran, S. P. Reddy, and A. B. Malik, “Reactive Oxygen Species in inflammation and tissue injury,” *Antioxid. Redox Signal.*, vol. 20, pp. 1126–1167, 2014.
- [95] J. V. Cordeiro and A. Jacinto, “The role of transcription-independent damage signals in the initiation of epithelial wound healing,” *Nat. Rev. Mol. Cell Biol.*, vol. 14, pp. 249–262, 2013.
- [96] A. Sofo, A. Scopa, M. Nuzzaci, and A. Vitti, “Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses,” *Int. J. Mol. Sci.*, vol. 16, pp. 13561–13578, 2015.
- [97] P. Sharma, A. B. Jha, R. S. Dubey, and M. Pessarakli, “Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions,” *J. Bot.*, vol. 2012, pp. 1–26, 2012.
- [98] H. Sies, “Role of metabolic H₂O₂ generation,” *J. Biol. Chem.*, vol. 289, pp. 8735–8741, 2014.

CONCLUSÕES GERAIS

Os resultados dos estudos demonstraram que o uso de extratos vegetais estimula o processo rápido e eficaz de reparo ósseo e de cicatrização de queimaduras cutâneas.