Luis Henrique Almeida Castro
(Organizador)

CIÊNCIAS DA SAÚDE:

PLURALIDADE DOS ASPECTOS QUE INTERFEREM NA SAÚDE HUMANA

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Ciências da saúde: pluralidade dos aspectos que interferem na saúde humana 6

Diagramação: Maria Alice Pinheiro
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Dados Internacionais de Catalogação na Publicação (CIP)

C569 Ciências da saúde: pluralidade dos aspectos que interferem na saúde humana 6 / Organizador Luis Henrique Almeida Castro. – Ponta Grossa - PR: Atena, 2021.

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui bibliografia
ISBN 978-65-5983-677-2
DOI: https://doi.org/10.22533/at.ed.772210911

1. Ciências da saúde. I. Castro, Luis Henrique Almeida (Organizador). II. Título.

CDD 613

Elaborado por Bibliotecária Janaina Ramos – CRB-8/9166

Atena Editora
Ponta Grossa – Paraná – Brasil
Telefone: +55 (42) 3323-5493
www.atenaeditora.com.br
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Atena Editora
Ano 2021

Ciências Biológicas e da Saúde

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APRESENTAÇÃO

A obra “Ciências da saúde: pluralidade dos aspectos que interferem na saúde humana 6” traz ao leitor 65 artigos de ordem técnica e científica elaborados por pesquisadores de todo o Brasil; são produções que em sua maioria englobam revisões sistemáticas, revisões de escopo, relatos de casos clínicos, investigações epidemiológicas, e estudos de caracterização de amostra.

Seguindo a primícia que o próprio título deste e-book sugere, os textos foram organizados em três volumes – cada qual representando um pilar da tríade da nova estrutura da educação em saúde: o modelo biopsicossocial. Segundo Mario Alfredo De Marco em seu artigo “Do modelo biomédico ao modelo biopsicossocial: um projeto de educação permanente” (2006), esta abordagem “proporciona uma visão integral do ser e do adoecer que compreende as dimensões física, psicológica e social” e que “quando incorporada ao modelo de formação do médico coloca a necessidade de que o profissional, além do aprendizado e evolução das habilidades técnico-instrumentais, evolua também as capacidades relacionais que permitem o estabelecimento de um vínculo adequado e uma comunicação efetiva”.

Desta forma o primeiro volume, com 27 textos, é dedicado aos trabalhos que abordam os aspectos que interferem na saúde humana na esfera biológica; o segundo contêm 17 artigos e traz investigações acerca dos aspectos psíquicos da saúde; e, em seu último volume a obra contempla 21 estudos focados na dinâmica social da saúde coletiva, especialmente no Brasil.

Boa leitura!

Luis Henrique Almeida Castro

SUMÁRIO

CAPÍTULO 7

ANTIBACTERIAL ACTIVITY AND HEALING PERFORMANCE OF *Ruellia angustiflora* EXTRACTS

Data de aceite: 01/11/2021
Data de submissão 30/08/2021

Fernanda Brum Pires
Federal University of Santa Maria, UFSM; Post-Graduate Program in Pharmaceutical Sciences
Santa Maria – RS
http://lattes.cnpq.br/4905052653071160

Carolina Bolsoni Dolwitsch
Federal University of Santa Maria, UFSM; Post-Graduate Program in Pharmaceutical Sciences
Santa Maria – RS
http://lattes.cnpq.br/3808382659019370

Camilla Filippi dos Santos Alves
Federal University of Santa Maria, UFSM; Post-Graduate Program in Pharmaceutical Sciences
Santa Maria – RS
http://lattes.cnpq.br/3094550372129682

Bryan Brummelhaus de Menezes
Department of Chemistry - Federal University of Santa Maria, UFSM
Santa Maria – RS
http://lattes.cnpq.br/4005723344102461

Lucas Mironuk Frescura
Department of Chemistry - Federal University of Santa Maria, UFSM
Santa Maria – RS
http://lattes.cnpq.br/0907539763536975

Marina Zadra
Federal University of Santa Maria, UFSM; Post-Graduate Program in Pharmaceutical Sciences
Santa Maria – RS
http://lattes.cnpq.br/5637430699482543

Liliana Essi
Federal University of Santa Maria, UFSM; Department of Biology
Santa Maria – RS
http://lattes.cnpq.br/5776430118696894

Camilo Amaro de Carvalho
Federal University of Viçosa- UFV; Departament of Medicine and Nursing
Visçosa – MG
http://lattes.cnpq.br/2890862161339721

Marcelo Barcellos da Rosa
Federal University of Santa Maria, UFSM; Department of Chemistry
Santa Maria – RS
http://lattes.cnpq.br/0308293154958870

ABSTRACT: Leaves of *Ruellia angustiflora* (Acanthaceae), which is known as flower-of-fire, have been popularly used in wound healing, but this traditionally claimed application is not yet scientifically proven. Thus, this study aimed to investigate the antibacterial and healing activities of extracts of *R. angustiflora* obtained by supercritical fluid extraction with carbon dioxide (SFE-CO_2) and by ultrasound probe with ethanol (UAE-EtOH). Antibacterial activity was evaluated using the agar well diffusion method. Wound healing assessment included analysis of wound area, rate of wound contraction, cellularity, vascularization and proportion of types I and III collagen fibers in scar tissue of mice. The UAE-EtOH extract exhibited antibacterial activity against *Proteus vulgaris*, *Citrobacter freudii*, *Staphylococcus aureus* and *Staphylococcus*

eppidermidis at concentrations up to 17.5 mg/ml, and against *Escherichia coli* up to 35 mg/ml. The SFE-CO_2 extract showed no ability to inhibit bacterial growth at the tested concentrations (up to 70 mg/ml). Evaluation of the healing process in an animal model revealed the greater efficacy of the UAE-EtOH extract over silver sulfadiazine. The antibacterial and wound healing activities of *R. angustiflora* UAE-EtOH extract may be explained by the fact that it contains phenolic compounds, which are known to possess the observed properties. The present findings support the medicinal use of *R. angustiflora*; its UAE-EtOH extract has the potential to be explored as an active ingredient in the development of formulations intended for clinical use in future studies.
KEYWORDS: Biological activity; Healing wound; Medicinal plant; Phenolic

ATIVIDADE ANTIBACTERIANA E AÇÂO CICATRIZANTE DE EXTRATOS DE *RUELLIA ANGUSTIFLORA*

RESUMO: Folhas de *Ruellia angustiflora* (Acanthaceae), conhecidas como flor de fogo, têm sido popularmente utilizadas na cicatrização de feridas, mas essa aplicação tradicionalmente reivindicada ainda não foi comprovada cientificamente. Assim, o presente estudo teve como objetivo investigar as atividades antibacteriana e cicatrizante de extratos de *R. angustiflora* obtidos por extração de fluido supercrítico com dióxido de carbono (SFE-CO_2) e por sonda de ultrassom com etanol (UAE-EtOH). A atividade antibacteriana foi avaliada usando o método de difusão em ágar bem. A avaliação da cicatrização da ferida incluiu a análise da área da ferida, taxa de contração da ferida, celularidade, vascularização e proporção das fibras de colágeno dos tipos I e III no tecido cicatricial de camundongos. O extrato UAE-EtOH exibiu atividade antibacteriana contra *Proteus vulgaris*, *Citrobacter freudii*, *Staphylococcus aureus* e *Staphylococcus eppidermidis* em concentrações de até 17,5 mg/ml e contra *Escherichia coli* de até 35 mg/ml. O extrato SFE-CO_2 não mostrou capacidade de inibir o crescimento bacteriano nas concentrações testadas (até 70 mg/ml). A avaliação do processo de cicatrização em modelo animal revelou a maior eficácia do extrato UAE-EtOH sobre a sulfadiazina de prata. As atividades antibacteriana e cicatrizante do extrato de *R. angustiflora* UAE-EtOH podem ser explicadas pelo fato de conter compostos fenólicos, que são conhecidos por possuírem as propriedades observadas. Os presentes achados apoiam o uso medicinal de *R. angustiflora*; seu extrato UAE-EtOH tem potencial para ser explorado como ingrediente ativo no desenvolvimento de formulações destinadas ao uso clínico em estudos futuros.
PALAVRAS-CHAVE: Atividade biológica; Cicatrização de feridas; Planta Medicinal; Fenólico.

1 | INTRODUCTION

The growing interest in using medicinal plants and their extracts in clinical practice constitutes advances in primary health care and therapeutic supplementation. In this regard, efficacy of such products must be ensured via biological assays (Firmo et al., 2011). *Ruellia angustiflora* Lindau ex Rambo has stimulated interest in the scientific community due to its great medicinal potential and lack of biological studies (Alice et al., 1995; Fuhro, Vargas, & Larocca, 2005).

This plant species belongs to family Acanthaceae, order Lamiales (Afzal et al., 2015,

Samy et al., 2015). It is a shrub that displays red flowers, hence its common name flower-of-fire (Alice et al., 1995; Fuhro, Vargas, & Larocca, 2005), and its leaves are used in folk medicine for showing wound healing activity (Alice et al., 1995).

Earlier investigations into the potential of *R. angustiflora* have identified intermediate polarity compounds such as rutin, caffeic acid, vanillic acid, p-coumaric acid, ferulic acid, kaempferol, apigenin, quercitrin, luteolin, 6-hydroxycoumarin and resveratrol, and non-polar constituents as linolenic (methyl ester), lycopersene, γ-tocopherol, α-tocopherol, vitamin E (acetate), campesterol, β-stigmasterol, β-sitosterol, methyl commate A and methyl commate D. Moreover, analysis of the antioxidant potential and cyto-genotoxicity of *R. angustiflora* extracts revealed a great antioxidant capacity by the antiradical activity against the 1-diphenyl-2-picrylhydrazylil radical (DPPH), while no cyto-genotoxic effects were triggered in Peripheral Blood Mononuclear Cells (PBMCs) (Pires et al., 2020). Such promising findings have motivated further research on the species properties.

Based on the increasing concern about bacterial resistance to conventional antibiotics (Borges et al., 2013, Carvalho et al., 2014) and on the popular use of *R. angustiflora* in wound healing (Alice et al., 1995), this study was aimed at improving scientific knowledge on this species by evaluating its *in vitro* antimicrobial activity as well as its *in vivo* healing effect in order to support its medicinal application.

2 | EXPERIMENTAL

2.1 Plant material

Leaves of *R. angustiflora* were harvested in Santo Antão District, in the Municipality of Santa Maria, RS, Brazil (S 29º 37´ 18.6´´ W 053º 51´ 35.6) in March 2017. A voucher specimen was deposited in the Herbarium of the Botanical Garden of the Federal University of Santa Maria (SMDB) under registration number 17547. Samples were oven dried at 40 °C until constant mass was attained. Next, grinding was performed in a knife mill (Marconi, SP, Brazil), then the samples were stored at -12 ºC until extractions.

2.2 Preparation, extraction and characterization of *R. angustiflora*

R. angustiflora extracts were obtained by ultrasonic probe with ethanol (UAE-EtOH) and supercritical fluid extraction with carbon dioxide (SFE-CO_2). Obtainment of the extracts as well as chemical characterization have been detailed in Pires et al. (2020).

2.3 Antibacterial activity

2.3.1 Sample preparation for antibacterial assay

Solvents were evaporated, then the residue was resuspended in sterile distilled

water for the UAE-EtOH extract, and sterile distilled water and Tween 80 for the SFE-CO_2 extract. Both extracts were initially evaluated at 70 mg/mL. Subsequently, serial dilutions of 1:2, 1:4, 1:6 and 1:8 were made to obtain the remaining concentrations.

2.3.2 Microorganisms and culture conditions

In vitro antibacterial testing used the following strains: *Staphylococcus aureus* ATCC 29213; *Staphylococcus aureus* ATCC 33591; *Staphylococcus eppidermidis* ATCC 35984; *Salmonella tiphimurium* ATCC 14028; *Proteus vulgaris* ATCC 13315; *Listeria monocytogenes* ATCC 7644; *Bacillus cereus* ATCC 14579; *Citrobacter freudii* ATCC 8090; *Listeria innocua* ATCC 33090; *Shigella flexinerii* ATCC 12022; *Moroxell*a *catarrhalis* ATCC 25238; and *Escherichia coli* ATCC 29214. Bacteria were inoculated into test tubes filled with 3-4 mL of Luria-Bertani medium liquid (LB) and placed in an oven for overnight incubation at 37 °C. Then, a sample size of 1 mL of each bacterial inoculum as well as 1 mL of blank were added to a different tube. Microbial growth was measured by OD600 in spectrophotometer. Blank control was used to calibrate the instrument. Samples that did not show absorbance of 0.1 were diluted to obtain the aforementioned reading.

2.3.3 Agar well diffusion assay

The agar well diffusion method was used for evaluating *in vitro* antibacterial activity in Mueller-Hinton medium (Himedia®) (Bauer et al., 1966). After growing for 24 h at 37 °C, the bacterial suspension was diluted to the final concentration of 10^8 CFU/ml and added to 20 mL of Mueller-Hinton agar. After solidification, wells of about 6 mm in diameter and 3 mm in height were made in the medium. Next, 15 µL of each extract at the initial concentration of 70 mg/ml and dilutions of 1:2, 1:4, 1:6, 1:8 for both extracts, DMSO (negative control) and ampicillin 50 mg/mL were inoculated into each well (positive control), and chloramphenicol 30 µg/mL for the *E. coli* test. Plates were then transferred to an oven to grow overnight at 37 °C. Subsequently, the zones of inhibition were measured in millimeters. The test was performed in triplicate.

2.4 Wound-healing activity of the extracts

Tests used 14 45-day-old male mice (Balb C) obtained from the Animal Resource Center of the Federal University of Viçosa (UFV), Minas Gerais state, Brazil. Statistical analysis justified the total number of animals proposed for use in this research. The mice were house in individual polypropylene cages, which were daily sanitized, in an environment with controlled temperature (22-24 °C) and 12/12 h light/dark cycle. Animals were provided with free access to food and water throughout the experimental period; acclimation to the experimental area lasted 15 days. At the end of the experimental period, animals were euthanized in accordance with the recommendations of the Animal Experimentation Ethics

Committee of UFV, which approved the present project (CEUA/UFV, protocol number 597/2017); carcasses were collected by the biosafety service of the institution.

2.4.1 Surgical incision and animal treatment

Based on previously published literature, the protocol applied in this research lasted 8 days. On day 0, the mice were anesthetized intraperitoneally with a combination of 8.0 mg/kg xylazine hydrochloride and 140 mg/kg ketamine hydrochloride. Next, the dorsal region was trichotomized and chlorhexidine gluconate 2% was used for skin antisepsis. Then, an incision was made by excising the skin with a round scalpel blade (6 mm) as described in Carvalho et al. (2013); the skin fragment was considered the control (day 0). The wounds were left open and solely manipulated for treatment application once a day from day 1 to day 8, and for sampling on days 4 and 8. Treatments were divided into three groups: SFE-CO_2 treatment (n=6; a mouse from this group died during the acclimation period), UAE-EtOH treatment (n=7), and positive control treatment with silver sulphadiazine (Sulf) (n=10). The extracts were tested at 70 mg/ml, and 1% silver sulfadiazine was used in the control mice, with the same amount of each substance being given to the respective group.

2.4.2 Wound evaluation

Lesions were assessed by visual examination once daily; possible quantitative and qualitative clinical alterations, such as signs of inflammation and erythematous halo, as well as time course of wound healing and epithelialization were observed. There were no macroscopic signs of infection in any of the test groups throughout the trial. A manual caliper was used for measuring the wound area in two directions: the largest length and the largest width. Measurements were made on the 1st, 4th and 8th days after injury.

2.4.3 Material Collection and Histological Processing

Three animals in each group were euthanized at days 4 and 8 post-injury. A surgical incision was made with a round scalpel blade (6 mm) to collect a tissue fragment for histopathological analysis. These analyses were carried out at the Pathology Laboratory of the Department of Veterinary Medicine at UFV.

The skin fragments were fixed in 4% paraformaldehyde for 24 h and then placed in 70% alcohol until preparation of the histological slides. For paraffin inclusion, samples were dehydrated in graded ethanol concentrations (70% to 100%) and transferred to xylol for diaphanization using the conventional processing. Sections of 5 μm were obtained on a rotating microtome and stained with Picro Sirius and Hematoxylin and Eosin (H&E) for evaluation of fibroblast, vascularization and inflammatory cells. Picro Sirius red staining is intended to differentiate between types I and III collagen fibers under polarized light.

2.4.4 Histopathological and histomorphometric evaluation of the healing process

H&E staining was performed to count the cells which participate in the inflammatory process as well as fibroblasts. Images of the histological slides were captured by a digital camera coupled to the optical microscope. Ten fields were randomly photographed per slide with the 20x objective, resulting in a total tissue area of 7.2 x 106 μm^2, which was subjected to stereological analysis. For this analysis, a 300-point test grid consisting of a reference area of 1.2 × 105 μm^2 was placed over each image to collect the measurements (Vieira et al., 2015). Examination of collagen fibers was done using Picro Sirius red staining to obtain a qualitative analysis of types I and III in the connective tissue. In polarized light observation, thick type I collagen fibers appear as bright yellow to red, while thin, more delicate, fragile and immature type III collagen fibers are visualized in bright green colors (Vieira et al., 2015). Stereological analysis was performed using the specialized software Image-Pro Plus® (Media Cybernetics).

2.4.5 Statistical analysis

Data were assessed by One-Way ANOVA followed by post-hoc Tukey's test using GraphPad Prism 5.01 statistical software (GraphPad Software, Inc, CA, USA). Differences were considered significant at $p<0.05$. Data are expressed as the Mean±SEM.

3 I RESULTS AND DISCUSSION

3.1 Antibacterial activity

The UAE-EtOH extract was inhibitory to six of the tested bacterial strains; the zones of inhibition obtained for its different concentrations are shown in Table 1. The extract demonstrated antibacterial activity at concentrations up to 17.5 mg/ml against *P. vulgaris* (ATCC 13315), *C. freudii* (ATCC 8090), *S. aureus* (ATCC29213), *S. aureus* (ATCC33591) and *S. epidermidis* (ATCC35984), and up to 35 mg/ml against *E. coli* (ATCC 29214). No inhibitory capacity was observed against the remaining bacteria. The largest zone of inhibition was observed against *E. coli* (ATCC 29214) (16 mm), and the smallest against *C. freudii* (ATCC 8090) (10 mm), both at 70 mg/ml UAE-EtOH extract. Several compounds of phenolic origin, as phenolic acids and flavonoids, were identified in the extract of *R. angustiflora* (Pires et al., 2020). These substances are known to be bioactive antibacterial components (Borges et al., 2013; Stojković et al., 2013), thus explaining the results reported herein.

Samples	*P. vulgaris* ATCC 13315	*E. coli* ATCC 2921 4	*C. freudii* ATCC 8090	*S. aureus* ATCC33591	*S. aureus* ATCC2921 3	*S. epidermidis* ATCC3598 4
R. angustiflora 70 mg/ml	12 mm	16 mm	10 mm	12 mm	11 mm	12 mm
R. angustiflora 35 mg/ml	11 mm	12 mm	9 mm	10 mm	10 mm	11 mm
R. angustiflora 17.5 mg/ml	8 mm	Absent	7 mm	09 mm	9 mm	10 mm
R. angustiflora 11.6 mg/ml	Absent	Absent	Absent	Absent	Absent	Absent
R. angustiflora 8.75 mg/ml	Absent	Absent	Absent	Absent	Absent	Absent
Negative control	Absent	Absent	Absent	Absent	Absent	Absent
Ampicillin 50 mg/ml	22 mm	-	22 mm	21 mm	17 mm	19 mm
Chloramphenicol 30 µg/ml	-	18 mm	-	-	-	-

TABLE 1Inhibition Halo obtained for the UAE-EtOH extract of *R. angustiflora.*

The SFE-CO_2 extract showed no ability to inhibit the growth of the evaluated bacteria. According to Silveira et al. (2009), the methodology applied in the current research is more suitable to test the diffusion of substances in ethanolic extracts of plants, hence the lack of activity of the SFE-CO_2 extract.

In keeping with the present results, some studies have observed antimicrobial activity of other *Ruellia* species by the agar well diffusion method. Ramadevi et al. (2016) assessed different extracts of *R. patula* leaves against the bacteria *Bacillus subtilis* and *E. coli* and the fungus *Aspergillus niger*. Senthilkumar et al. (2013) evaluated the methanolic extract of *R. tuberosa* leaves at 100 mg mL^{-1} and found significant activity against the bacteria *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *B. subtilis* and *Proteus mirablis* and the fungi *Aspergillus sp*, *Mucor sp*, *Penicillium sp* and *Fusarium sp*.

3.2 Wound evaluation: histopathology and clinical analysis

Macroscopic evaluation of the healing process allowed to verify that both extracts of *R. angustiflora* as well as silver sulfadiazine (positive control) promoted wound contraction (Fig. S1, supplementary material).

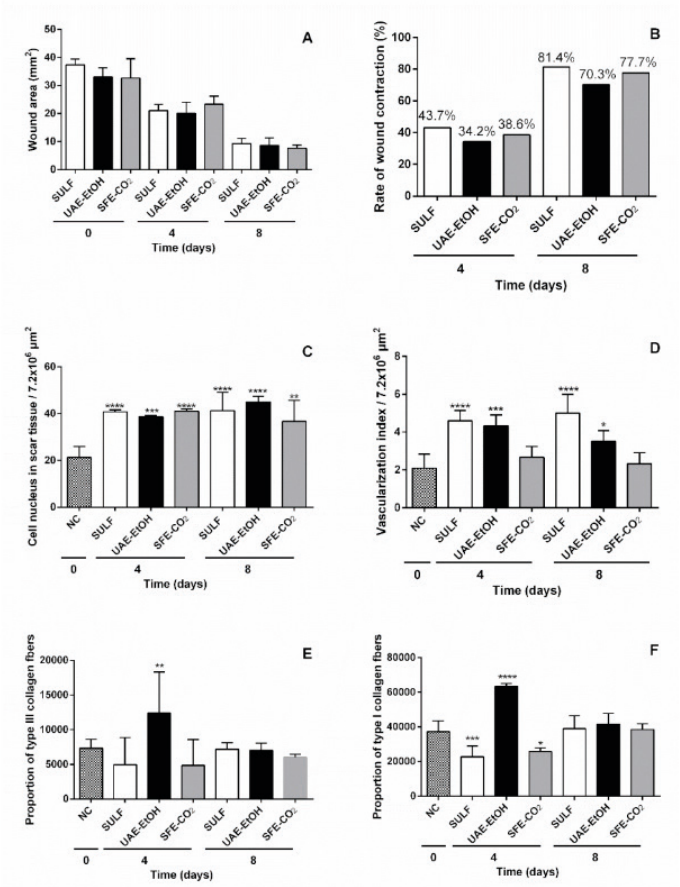

Fig. 1Effect of wound treatment with SFE-CO2and UAE-EtOH extracts, negative control (NC) and positive control -silver sulfadiazine (SULF) in mice. Wound area (A), rate of wound contraction (B), cellularity (C), vascularization (D) and proportion of types III (E) and I (F) collagen fibers in scar tissue of mice evaluated at days 4 and 8 of treatment. *=p<0.05, **=p<0.01, ***=p<0.001 and p<0.0001.

Wound healing evaluation (Fig. 1) consisted of analysis of wound area (A), rate of wound contraction (B), cellularity (C), vascularization (D) and proportion of types III (E) and I (F) collagen fibers in scar tissue of mice. There was no significant difference between SFE-CO_2 and UAE-EtOH extracts and sulfadiazine treatment groups when evaluated separately at days 0, 4 and 8 (Fig. 1A). Treatment efficacy is demonstrated by the reduction in wound area over time.

Nevertheless, absolute numbers indicate that Sulf-treated mice had a higher rate of wound contraction (43.7%) at day 4, followed by SFE-CO_2 (38.6%) and UAE-EtOH (34.2%) treatments. At day 8 after injury, Sulf-treated group showed the highest percentage of wound contraction (81.4%), followed by SFE-CO_2 (77.7%) and UAE-EtOH (70.3%) treatments (Fig. 1B). Macroscopic evaluation of the healing process at the various time points after wounding indicated that the mice which received Sulf presented greater rates of wound contraction in

comparison to those subjected to UAE-EtOH and SFE-CO_2 extracts.

Regarding total cell count, significantly higher numbers ($p<0.01$) were obtained for all treatment groups compared to control with intact skin (Fig. 1C and 2, supplementary material). However, there was no significant difference between them ($p<0.05$) from day 4 to day 8.

The vascularization index in control group (NC) was significantly lower than that in Sulf ($p<0.0001$ at days 4 and 8) and UAE-EtOH ($p<0.001$ at day 4 and $p<0.05$ at day 8) groups (Fig. 1D). No difference was observed between Sulf and UAE-EtOH treatments on the 4th and 8th days post-injury. The vascularization index in Sulf group was greater than the one found in SFE-CO_2 group at days 4 and 8 ($p<0.05$ and $p<0.01$, respectively). Nonetheless, no significant difference was observed between the treatments with the extracts on days 4 and 8. On the 4th experimental day, no statistically significant difference was observed between the group which received SFE-CO_2 treatment and control.

As for collagen synthesis, mice treated with UAE-EtOH exhibited a greater production of type III collagen fibers on day 4 when compared to the remaining treatments on both assessment days, with a significant difference from control group ($p <0.01$). The group treated with UAE-EtOH showed a decline in the amount of type III collagen fibers from day 4 to day 8 ($p<0.05$); this suggests a greater efficacy of such treatment over the other two, since production of new collagen in a wound starts with a rapid synthesis chiefly of type III and later of type I collagen (Fig. 1E).

Analysis of type I collagen synthesis, which is an essential process in skin wound healing, shows a greater proportion in the group treated with UAE-EtOH at day 4, with significant differences being observed from control ($p<0.0001$), Sulf ($p<0.001$) and SFE-CO_2 ($p<0.05$) groups (Fig. 1F and 3, supplementary material). It may be concluded that, in a short period of time, the UAE-EtOH treatment triggered the production of a greater quantity of fibers than that present in the intact tissue of control mice. The amount of type I collagen fibers seen throughout the groups at day 8 did not differ and was similar to that occurring in the intact skin (control-day 0).

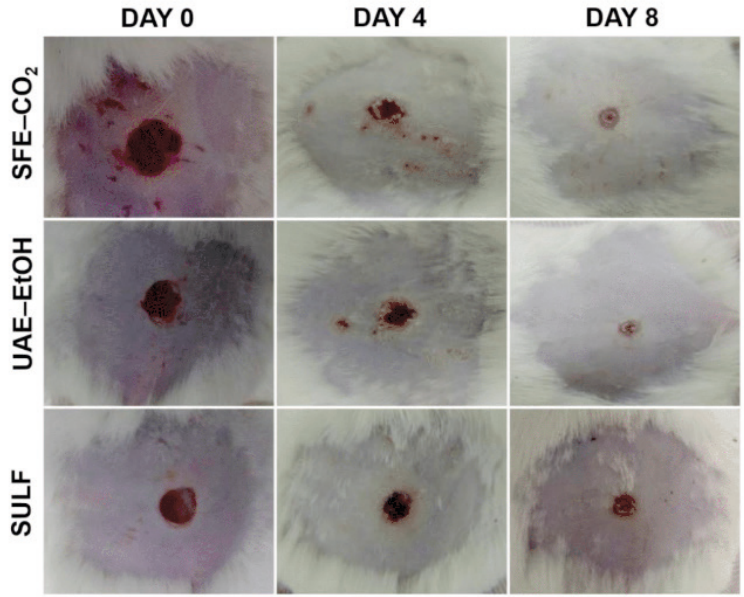

Fig. S1 Macroscopic evaluation of excisional wound healing and treatment with SFE-CO2and UAE-EtOH extracts and positive control -silver sulfadiazine (SULF) in mice on days 0, 4 and 8.

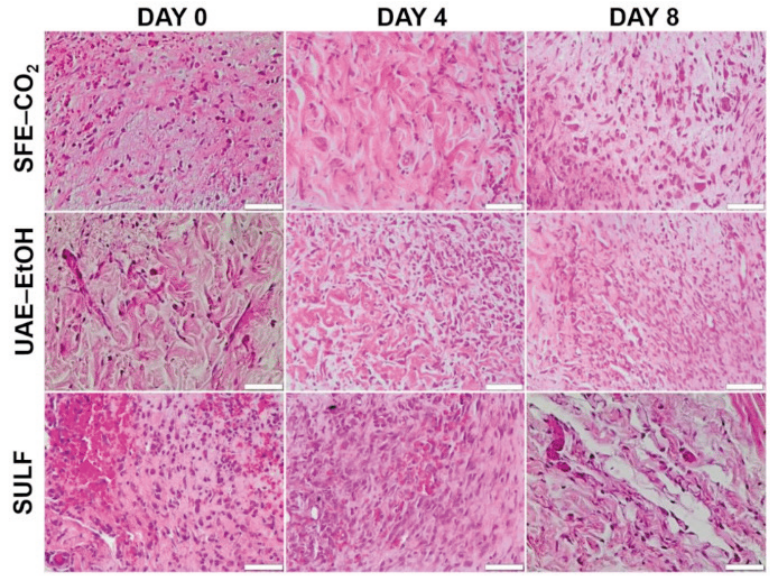

Fig. S2Representative photomicrographs showing cellularity in HematoxylinandEosinstained sections from rat skin observed under light microscopy. Tissue fragments were collected on days 0, 4 and 8 of treatment with SFE-CO2, UAE-EtOH and silver sulfadiazine (Sulf-Positive control). Scale bar: 200 µm.

Even though macroscopic evaluation of the wound area indicated sulfadiazine as the most satisfactory treatment, the UAE-EtOH extract showed the most promising results in

the histomorphometric analysis. There are numerous events in re-epithelialization playing a key role in wound healing, and visual examination permits only a superficial analysis of the entire process.

Wound healing is a complex process which begins after impairment of skin integrity. It involves several physiological processes and can be divided into three phases: inflammatory, proliferative and remodeling. The inflammatory phase is characterized by a vascular phenome with the occurrence of hemostasis and coagulation. Cellular debris are removed and the tissue is protected against colonization and invasion of microorganisms. It usually starts 24 to 48 h after injury and lasts up to 2 weeks (Laureano & Rodrigues, 2011; Blanck & Giannini, 2014). The proliferative phase occurs approximately on the 4th day after the onset of the lesion and involves angiogenesis, production of extracellular matrix, re-epithelization and wound contraction (fibroplasia) to reconstruct dermis integrity. Lastly, in the remodeling phase, excess collagen produced in the previous phase is remodeled in the extracellular matrix as the fibrin clot is replaced by granulation tissue (Laureano & Rodrigues, 2011; Blanck & Giannini, 2014).

It should be noted that treatment with the UAE-EtOH extract promoted a decrease in vascularization on day 8 comparing to sulfadiazine, thus indicating a reduction in the inflammatory response and the beginning of the proliferative phase in the scar tissue. Analysis of collagen synthesis supports these findings: the UAE-EtOH treatment induced greater stimulation of fibroblasts and synthesis of type I collagen fibers. Tests demonstrated a higher efficacy of the UAE-EtOH extract over silver sulfadiazine.

The results attained for the UAE-EtOH extract may be explained by the fact that it contains bioactive compounds, e.g. phenolic and flavonoid acids, as reported in Pires et al. (2020). Flavonoids are plant constituents which are known to stimulate wound healing (Vieira et al., 2008). Rutin, a flavonoid that is the main component of the UAE-EtOH extract (Pires et al., 2020), has been tested in dermatological formulations and improvements in skin wound healing were observed (Almeida et al., 2012). The healing activity exhibited by this extract in the present assessment may be attributed to rutin and to the synergistic interactions between this and other compounds; as stated by Brglez Mojzer et al. (2016), synergism of polyphenolic mixtures in herbal therapy aids to produce a faster and more efficient healing.

Data on the antibacterial property as well as on the collagen-stimulating activity disclosed in this study suggest that the UAE-EtOH extract may be used in the development of dermocosmetics with potential clinical application.

Literature reports indicate wound healing activity of *R. patula* and *R. tuberosa* (Prakash, et al., 2005; Ranjani & Manjula, 2012). Prakash et al. (2005) used an ethanolic extract of *R. patula* in the formulation of an ointment to verify the healing activity of the plant; the preparation showed better activity than the standard drug (Framycetin Sulphate Cream 1% w/w). Ranjani & Manjula (2012) also demonstrated the healing effect of *R. tuberosa*

leaves. Moreover, Nejjari et al. (2019) assessed the wound healing activity of a topical application containing the hydroalcoholic extract of *Telephium imperati* (L.); evaluation consisted of three groups, ointment formulated with 5% extract, standard medication (Madecassol®) and control (vaseline), and reduction in wound area at the end of treatment (55 days) was 95.5%, 97.5% and 75.75%, respectively. Regarding tissue evaluation, Nejjari et al. (2019) found that the ointment-treated group showed a greater effect on the healing process with good proliferation of fibroblasts, few inflammatory cells and well-organized collagen.

4 | CONCLUSION

The UAE-EtOH extract of *R. angustiflora* was able to inhibit bacterial growth of *P. vulgaris*, *C. freudii*, *S. aureus*, *S. epidermidis*, *E. coli* and *C. freudii*, thus proving to be a promising alternative for the development of new antibacterial and wound healing agents, either as part of a combination therapy with conventional drugs or as the sole treatment. With regard to collagen fibers production, which is fundamental for a more rigid, firm and effective healing, satisfactory results were also achieved with the UAE-EtOH extract. Such findings indicate a greater efficacy of the UAE-EtOH extract over the SFE-CO_2 extract, thus supporting the medicinal use of the former.

REFERENCES

AFZAL, Khurram et al. **Genus Ruellia: Pharmacological and phytochemical importance in ethnopharmacology.** Acta Poloniae Pharmaceutica-Drug Research, v. 72, n. 5, p. 821, 2015.

ALICE, Cecilia Ballvé. **Plantas medicinais de uso popular: atlas farmacognóstico**. Editora da ULBRA, 1995.

ALMEIDA, J. S. et al. **Hydrogels containing rutin intended for cutaneous administration: efficacy in wound healing in rats.** Drug development and industrial pharmacy, v. 38, n. 7, p. 792-799, 2012.

BAUER, A. W. **Antibiotic susceptibility testing by a standardized single disc method.** Am J clin pathol, v. 45, p. 149-158, 1966.

BLANCK, Mara; GIANNINI, Tereza. Úlcera e feridas, as feridas têm alma. Uma abordagem interdisciplinar do plano de cuidados e da reconstrução estética. Rio de Janeiro: Dilivros, 2014.

BORGES, Anabela et al. **Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria**. Microbial Drug Resistance, v. 19, n. 4, p. 256-265, 2013.

DE CARVALHO, Camilo Amaro et al. **Aspectos químicos e atividade antibacteriana de Piptadeniagonoacantha (FABACEAE)**. Ciência e Natura, v. 36, n. 2, p. 732-744, 2014.

CARVALHO, Gabriel D.; VARGAS VILÓRIA, Marlene I.; VALENTE, Fabrício L. **REPARAÇÃO DE FERIDAS EXPERIMENTAIS EM COELHOS TRATADOS ORALMENTE COM ÁGUA-DE-COCO E ÁGUA MAGNETIZADA**. Archives of Veterinary Science, v. 18, n. 3, 2013.

FIRMO, Wellyson da Cunha Araújo et al. **Contexto histórico, uso popular e concepção científica sobre plantas medicinais**. Cadernos de pesquisa, 2011.

FUHRO, Daniela; VARGAS, D.; LAROCCA, João. **Levantamento florístico das espécies herbáceas, arbustivas e lianas da floresta de encosta da Ponta do Cego, Reserva Biológica do Lami (RBL), Porto Alegre, Rio Grande do Sul, Brasil**. Pesquisas, ser. Botânica, v. 56, p. 239-256, 2005.

LAUREANO, André; RODRIGUES, Ana Maria. **Cicatrização de feridas**. Journal of the Portuguese Society of Dermatology and Venereology, v. 69, n. 3, p. 355-355, 2011.

BRGLEZ MOJZER, Eva et al. **Polyphenols: Extraction methods, antioxidative action, bioavailability and anticarcinogenic effects.** Molecules, v. 21, n. 7, p. 901, 2016.

NEJJARI, R. et al. **Phytochemical screening and wound healing activity of** *Telephium imperati* (L.) **in rats**. South African Journal of Botany, v. 123, p. 147-151, 2019.

PIRES, Fernanda B. et al. **Chemical study, antioxidant activity, and genotoxicity and cytotoxicity evaluation of** *Ruellia angustiflora*. Natural product research, p. 1-6, 2020.

PRAKASH, TNK Suriya et al. **Wound healing activity of** *Ruellia patula*. Hamdard Medicus (Pakistan), 2005.

RAMADEVI, S.; KALEESWARAN, B.; NATARAJAN, P. **Phytochemicals analysis and antimicrobial activity of** *Ruellia patula* L. **against pathogenic microorganisms**. South Indian J Biol Sci, v. 2, n. 2, p. 306-313, 2016.

RANJANI, P.; MANJULA, P. *Ruellia Tuberosa* **and Wound Healing**. International Journal of Nursing Education, v. 4, n. 2, 2012.

SAMY, **Mamdouh Nabil et al. Chemical constituents and biological activities of genus** Ruellia. International Journal of Pharmacognosy, v. 2, n. 6, p. 270-279, 2015.

SENTHILKUMAR, P.; SAMBATH, R.; VASANTHARAJ, S. **Antimicrobial potential and screening of antimicrobial compounds of** *Ruellia tuberose* **using GC-MS.** Int. J. Pharm. Sci. Rev. Res, v. 20, n. 1, p. 184-189, 2013.

SILVEIRA, Luiz Mário da Silva et al. **Metodologias de atividade antimicrobiana aplicadas a extratos de plantas: comparação entre duas técnicas de ágar difusão.** Revista Brasileira de Farmácia, v. 90, n. 2, p. 124-128, 2009.

STOJKOVIĆ, Dejan et al. **In situ antioxidant and antimicrobial activities of naturally occurring caffeic acid, p-coumaric acid and rutin, using food systems**. Journal of the Science of Food and Agriculture, v. 93, n. 13, p. 3205-3208, 2013.

VIEIRA, Alno Poiares et al. **Ação dos flavonóides na cicatrização por segunda intenção em feridas limpas induzidas cirurgicamente em ratos Wistar**. Semina: Ciências Biológicas e da Saúde, v. 29, n. 1, p. 65-74, 2008.

VIEIRA, Geisla et al. **Efeito cicatrizante do extrato da casca de Pseudopiptadenia contorta (DC.) GP Lewis & MP Lima feridas cutâneas de segunda intenção**. Revista Cubana de Plantas Medicinales, v. 20, n. 4, 2015.

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