American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AJEONP

American Journal of Essential Oils and Natural Products

AkiNik

ISSN: 2321-9114
AJEONP 2021; 9(3): 01-06

Received: 04-05-2021
Accepted: 06-06-2021

Márcia Nunes da Silva Warken
Laboratory of Applied Genetics and Toxicology, Lutheran University of Brazil, Canoas, Rio Grande do Sul, Brazil

Flavia Tasmin Techera Antunes
Postgraduate Program in Cellular and Molecular Biology for the Health Sciences, Lutheran University of Brazil, Canoas, Rio Grande do Sul, Brazil

Eliane Sempé Obach
Laboratory of Applied Genetics and Toxicology, Lutheran University of Brazil, Canoas, Rio Grande do Sul, Brazil

Elenir de Fátima Wiilland
Postgraduate Program in Cellular and Molecular Biology for the Health Sciences, Lutheran University of Brazil, Canoas, Rio Grande do Sul, Brazil

Alessandra Hubner de Souza
Postgraduate Program in Cellular and Molecular Biology for the Health Sciences, Lutheran University of Brazil, Canoas, Rio Grande do Sul, Brazil

Alexandre de Barros Falcão Ferraz
Postgraduate Program in Cellular and Molecular Biology for the Health Sciences, Lutheran University of Brazil, Canoas, Rio Grande do Sul, Brazil

Corresponding Author:
Flavia Tasmin Techera Antunes
Postgraduate Program in Cellular and Molecular Biology for the Health Sciences, Lutheran University of Brazil, Canoas, Rio Grande do Sul, Brazil

Healing properties of an emulsion containing essential oil of *Rosmarinus officinalis*

Márcia Nunes da Silva Warken, Flavia Tasmin Techera Antunes, Eliane Sempé Obach, Elenir de Fátima Wiilland, Alessandra Hubner de Souza and Alexandre de Barros Falcão Ferraz

Abstract
Rosmarinus officinalis commonly known as rosemary, alecrim, or romero is a worldwide plant with antioxidant and antimicrobial potential. The present study aimed to evaluate an emulsion containing the essential oil of *R. officinalis* (EORO) as an open wound healer in rats. The emulsion containing 20% of EORO was produced by the technique of hot emulsification. After the creation of a full-thickness wound in each of the 30 male Wistar rats used, they were divided into three groups treated once a day with the EORO emulsion, emulsion base, or an irrigated saline solution. Cutaneous wound contraction measurements, exudate scores, and histological analysis were performed on the 0, 3rd, 6th, 9th, 12th, and 15th. The saline group presented a great contraction on days 3, 6 ($p<0.05$), and 9 ($p<0.001$) when became similar to the EORO emulsion until the next days of evaluation. After that, there were no differences between the groups. The group treated with EORO emulsion reduced the exudate scores from the 9th day ($p<0.01$) different from saline. On the 6th day, the EORO emulsion group showed a little granulation tissue which did increase on the 9th day and became a thin epithelium on the 15th day. Also, the EORO emulsion enhanced an accented collagenesis in the 15th day seen by the histological analysis. The study reinforces the healing properties from EORO formulated as a topical emulsion.

Keywords antimicrobial activity; collagenesis; open wound; topical treatment

1. Introduction
Rosmarinus officinalis also isnamedas *Salvia rosmarinus* Schleid. and *Rosmarinus angustifolius* Mill, but commonly known as rosemary, alecrim, or romero [1]. That is a worldwide plant with pharmacological properties due to its antioxidant and antimicrobial potential, besides antinociceptive effects [1-6].
Sincethe topical application is an important administration route for drugs requiring local action on the skin, the extract from *R. officinalis* leaves demonstrated potential applications in cosmetic formulations in the treatment of cellulite, alopecia, ultraviolet damage, and aging [7-8]. Its essential oil also showed the biological activities reported both *in vitro* and *in vivo* assays [9-10]. It can be attributed to several molecules present in the essential oil such as β-pinene, 1, 8-cineole, borneol, camphor, limonene, and verbenone [11].
Related to the topical healing properties from *R. officinalis*, although widely studied, the researchers have shown the use of the emulsion with associations of other plant extracts [12, 13], or topically only the oil [14-16]. Therefore, the present study aimed to evaluate a feasible and inexpensive emulsion containing the essential oil of *R. officinalis* (EORO) as an open wound healer in rats.

2. Material and Methods
2.1. Materials
The EORO used in this work was produced and donated by the company Tekton® Óleos Essenciais Ltda (Brazil). To obtain this oil, steam extraction of *R. officinalis* leaves was used and analyzed by gas chromatography coupled to mass spectrum (GC/MS). The chemical analysis of the volatile oil provided by Tekton® is detailed in Supplementary Table 1.
For the emulsion, it was used Cetearyl Alcohol (and) Diacetyl Phosphate (and) Ceteth-10 Phosphate (Crodafos CES®, UK), Butylated hydroxytoluene (BHT) (Sigma Aldrich, US), Propylparaben (Sigma Aldrich, US), Methylparaben (Sigma Aldrich, US), Glycerin(Sigma Aldrich, US), Disodium EDTA (ethylenediaminetetraacetic acid) (Sigma Aldrich, US) and Hydroxypropyl Starch Phosphate (STRUCTURE® XL, Underwriters Laboratories, US).

2.2. Emulsionpreparation

The formulation consisted of an emulsion produced by the technique of hot emulsification, with separation of the aqueous and oily phases and mixing of both with constant agitation until cooling below 40ºC. EORO was incorporated into the oil phase in order to obtain a more stable emulsion. All components were selected based on criteria such as low toxicity and high skin tolerance (Table 1). The concentration of EORO in the final product was determined previously after evaluating the inhibition zones developed with the serial dilutions of the EORO through the Agar disk-diffusion testing [17] using *Staphylococcus aureus* ATCC 25.923 e *Staphylococcus epidermidis* ATCC 1228 as test microorganisms. Therefore, EORO 20% was used considering that the bacterial growth inhibition was similar to the positive control (chlorhexidine 0.05%) in the antimicrobial assessment (data not shown). A second product containing the same constituents and established concentrations, substituting only EORO for mineral oil was also obtained for use as a negative control in skin healing tests.

Table 1: Emulsion composition containing essential oil of *Rosmarinus officinalis* (EORO)

Components	Concentration (%)	Basic Function
Cetearyl Alcohol/ Diacetyl Phosphate/ Ceteth-10 Phosphate	5.00	Surfactant stabilizer and thickening agent
EORO	20.0	Emollient
Butylated hydroxytoluene (BHT)	0.01	Antioxidant
Propylparaben	0.05	Preservative
Methylparaben	0.15	Preservative
Glycerin	5.00	Humectant
Disodium EDTA	0.05	Chelating agent
Hydroxypropyl Starch Phosphate	0.50	Thickening agent and sensorial corrective
Distilled water	69.24	Vehicle

2.3. Animals

The experiments were conducted using a total of 30 male Wistar rats (160-250 g) that were obtained from the Vivarium of the Lutheran University of Brazil (ULBRA), Canoas, the Rio Grande do Sul, Brazil. All experiments were approved according to the guidelines established by the Ethics Committee of Animals (ECA) from the ULBRA, Canoas, RS, Brazil, nº 2013/19P. The animals were maintained alone in standard cages under a 12h light/dark cycle and in a temperature-controlled room (25 ± 2ºC). Food and water were available ad libitum. This study followed the Guidelines for Animal Research, as set out by the Brazilian College of Animal Experimentation (COBEA) and conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in for example the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

2.4. Wound creation and treatments

The procedure was carried out under anesthesia by an intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). The rat's dorsum was shaved and disinfected with 70% ethylic alcohol. A single lesion in each rat was created up to the muscle fascia, 2x2 cm in size, using a sterile scalpel. After the operation and according to the treatment to be applied on their lesions, the animals were equally divided into three groups (n=10). They were treated once a day with a thin layer (± 2 g) of the emulsion containing EORO, emulsion base, or an irrigated saline solution (± 1 mL), for 15 days.

2.5. Morphological and exudate evaluation

The evaluation of tissue healing of each animal was evaluated for measurement by a digital caliper. They were photographed on the 0, 3rd, 6th, 9th, 12th, and 15th days using a Cyber Shot® digital camera (Sony®, Tokyo, Japan),with a pre-set height (30 cm) and zoom, and in a standard animal body position. The images were evaluated with ImageJ image-processing software (NIH, Bethesda, MD, USA), to determine the area of the wound from the formula: WCR = [(A0-At)/A0]*100%, where A0 and at refers to the initial area and the wound's area at a time t, respectively.

The analysis of the exudate was transformed into scores from zero to four where the amount of exudate (evaluated before any treatment was applied) could be: absent = 0, small = 1, moderate = 2, and large = 3.

2.6. Histologicalevaluation

A fragment from each wound was collected and immersed in 10% buffered formalin for 24 hours. After the conventional ethanol gradient dehydration, the samples were sectioned at 7µm in paraffin and stained with hematoxylin and eosin (HE). The animals were anesthetized from this procedure on the 9th and 15th days. The parameters evaluated in the epidermis were the presence of collagenesis, fibrin, bleeding, hyperemia, and inflammatory infiltrate.

2.7. Statistical analysis

The analysis results were studied by Two-Way Analysis of Variance (ANOVA), followed by Bonferroni's test when using Graph Pad 5 Software (USA). All data were expressed as mean ± standard error and it was considered a significant value if $p<0.05$.

3. Results

3.1. Morphological and exudate evaluation

The saline group presented a great contraction on days 3, 6, and 9 when became similar to the EORO emulsion until the next days of evaluation (Figure 1a). The base emulsion showed the worst result from the beginning of the experiment (day 3: 13.8 ±5.01%, $p<0.05$) until the 9th day (54.6 ± 8.26%, $p<0.001$), being significantly different from the saline group (day 3: 28.04 ± 4.17%; day 9: 81.7 ± 3.50%) and EORO emulsion (day 9: 75.28 ± 3.55%, $p<0$, 01). After that, there were no differences between the groups, wherein the 15th-day EORO emulsion group presented 97.22 ± 0.71 %, the saline group presented 93.65 ±1.05%, and the base emulsion 86.44 ± 2.74% of wound contraction.

Related to the exudate score, the group base emulsion presented the higher scores from the first's treatment days (Figure 1b). The EORO emulsion reduced the exudate scores from the 9th day (1.8 ± 0.03) until the 15th day (0.2 ± 0.13), being significantly different from saline until the end of the experiment (day 9: 2.9 ± 0.28, $p<0.05$; day 12: 2.6 ± 0.37, $p<0.01$; day 15:2.0 ± 0.30, $p<0.001$).

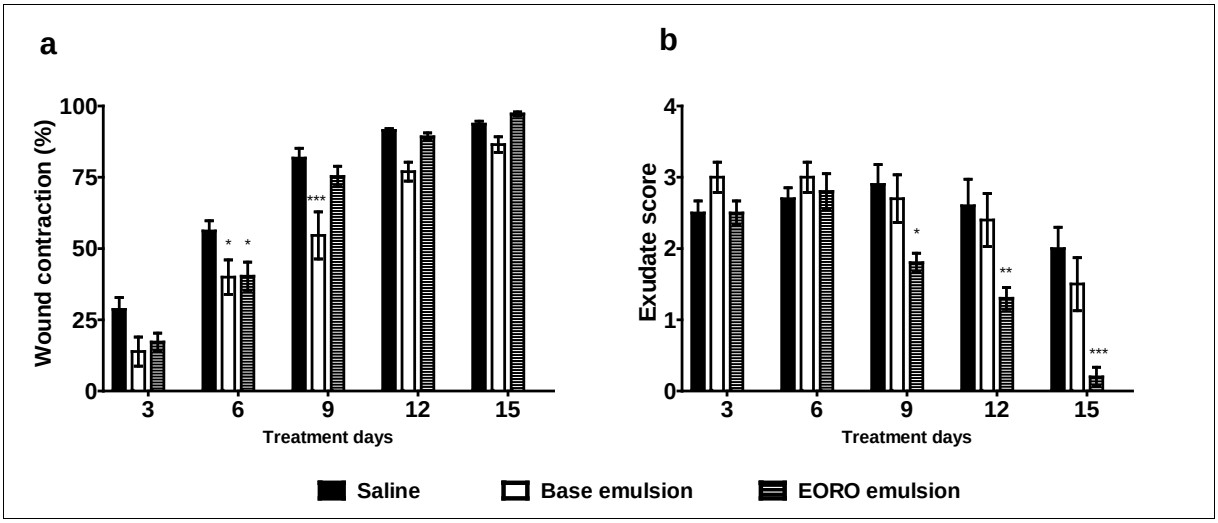


Fig 1: Cutaneous wound contraction measurements (a) and the exudate scores of the cutaneous wounds in rats treated with saline, base emulsion, or emulsion containing essential oil of *Rosmarinus officinalis*(EORO) on the 3rd, 6th, 9th, 12th, and 15th days

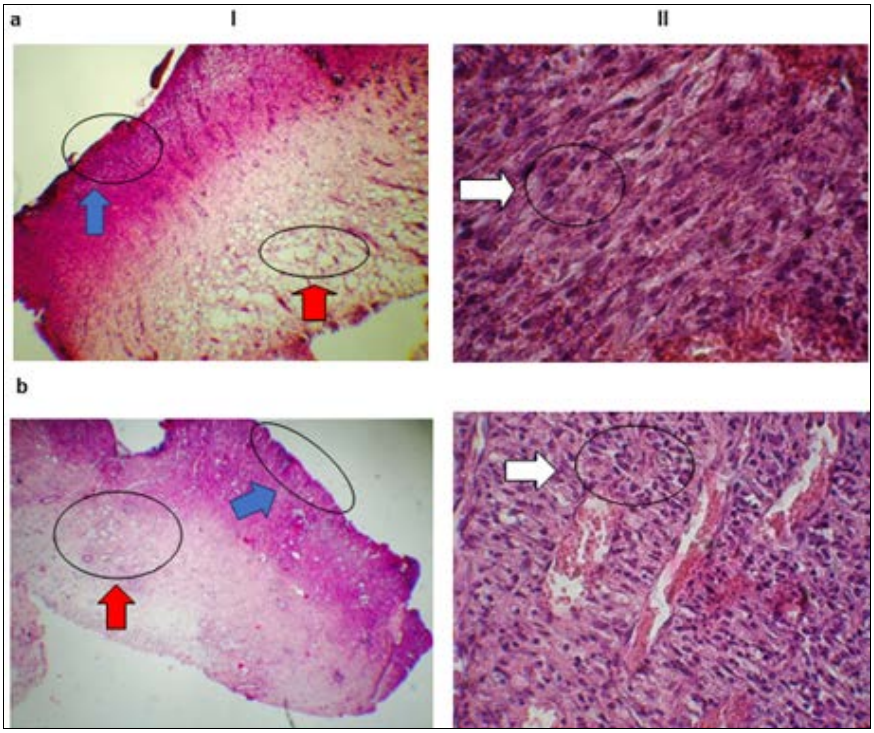
Each column represents the mean ± standard error of 10 animals. **p*<0.05 and ****p*<0.001 represents the level of significance when compared to the saline group and ##*p*<0.01 represents the level of significance when compared to the EORO emulsion group (Two-Way ANOVA, followed by Bonferroni's test).

3.2. Histological evaluation

Table 2 shows the studied parameters on the 9th and 15th days of treatment. Besides, all groups showed an absence of epithelium on the 9th day (Figure 2a, b, and c). On the 15th day, the group EORO emulsion showed a higher epithelium than the other groups (Figure 2d, e, and f).

Table 2: Histological parameters were evaluated after 9 and 15 days after treatment of open wound with saline, base emulsion, or emulsion containing essential oil of *Rosmarinus officinalis* (EORO)

Histological parameters	After 9 days			After 15 days		
	Saline	Base emulsion	EORO emulsion	Saline	Base emulsion	EORO emulsion
Collagenesis	Absent	Absent	Absent	Moderate	Absent	Accented
Fibrin	Absent	Absent	Accented	Absent	Absent	Absent
Bleeding	Accented	Absent	Accented	Discreet	Absent	Absent
Hyperemia	Absent	Accented	Absent	Absent	Absent	Absent
Inflammatory Infiltrate	Accented	Accented	Discreet	Absent	Discreet	Absent



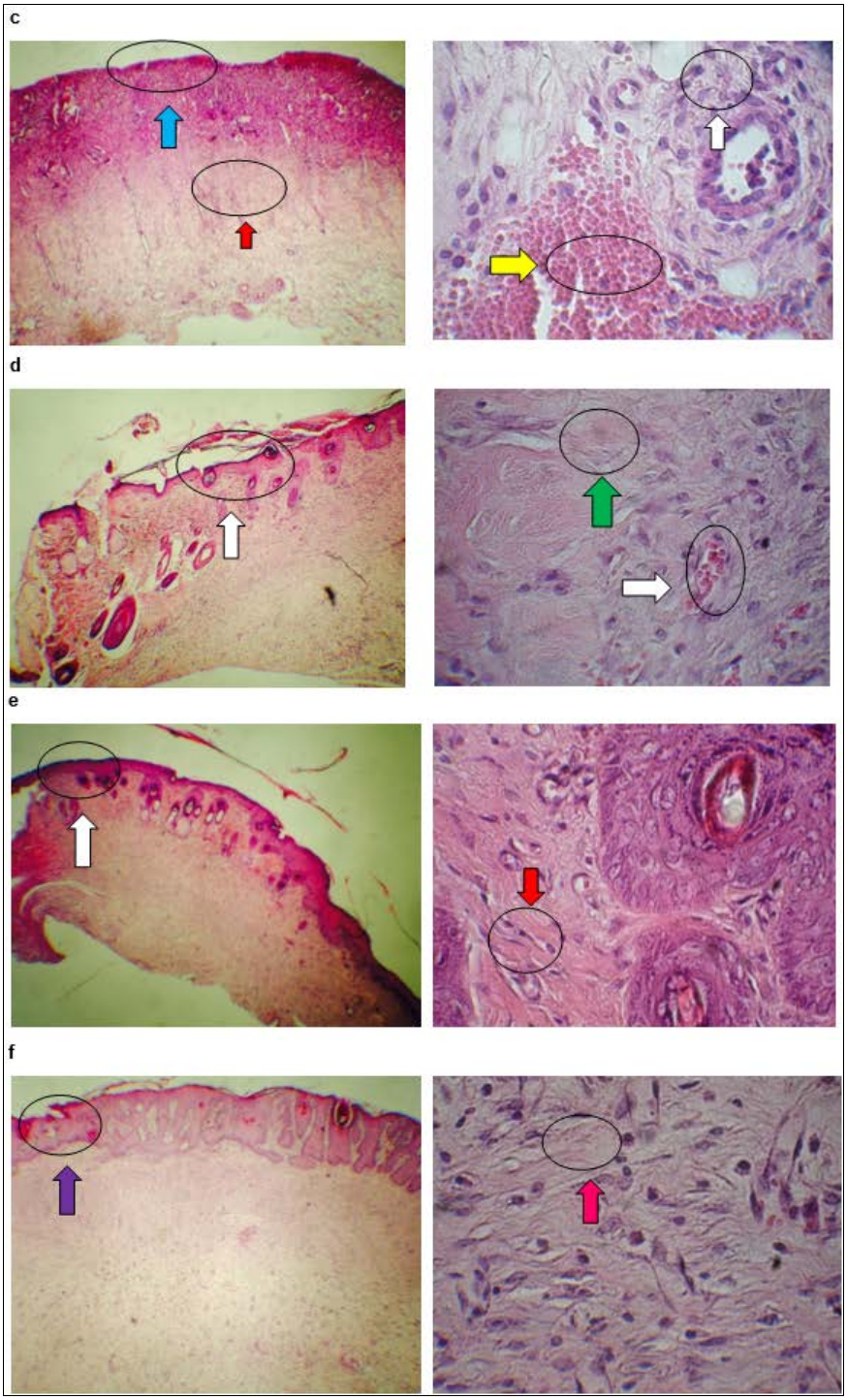


Fig 2: Histology of the fragment of a wound from animals on days 9 (a, b and c), and 15 (d, e, and f), after the wonder lesion treatments with saline (a and d); base emulsion (b and e), and emulsion containing essential oil of *Rosmarinus officinalis* (EORO) (c and f). Staining H&E, 40x(I),and 400x(II) magnification. Blue arrows: absence of epithelium; red arrows: inflammatory infiltrate; white arrows: bleeding; yellow arrows: fibrine; purple arrow: thin epithelium; pink arrow: accented Collagenesis; green arrow: moderate collagenesis

4. Discussion

According to Cizauskaite *et al.* [18], EORO offers advantages as a stabilizer in the formation of dermopharmaceutical and cosmetic preparations, besides, containing active ingredients that work as functional cosmetic agents due to its biological activities. Moreover, its dermal toxicity is low in rabbits, and dermal irritation could occur only with 24 hours of direct contact, being skin sensitization reported as negative [19]. In addition, The Cosmetic Ingredient Review Expert Panel concluded that the *R. officinalis*-derived ingredients are safe as used in cosmetics when formulated to be non-sensitizing [20].

The main compounds found in the EORO used in the present work were α-pinene, 1.8-cineole, and camphene, which is in line with the previous studies showed by Borges *et al.* [9], in their review. These chemical components are responsible for antimicrobial [21-24] and anti-inflammatory activities [5, 9, 25]. In this sense, based on these useful features, the work aimed to evaluate the healing properties from an emulsion containing EORO in the open wound in rats.

A wound leads to a cascade of events that ends with the complete and organized closure of the wound aiming at the reconstruction of the structural and functional barrier of the skin, preventing an invasion of microorganisms into the body [26]. Pérez-Recalde *et al.* [27], reviewed that EORO has shown positive results in rodent wounds like faster closure rate,

better collagen deposition, and/or enhanced fibroblasts proliferation. In the present results, the researchers showed that the emulsion containing EORO can reduce the wound area within the fifteen-day evaluation, alike the treatment with saline. Also, knowing that conditions of excessive and extreme exudate adversely affect healing [28], the product decreased the exudate score significantly on days 12 and 15 after the injury. Still macroscopically, on the 6th day, the EORO emulsion group showed a little granulation tissue which did increase on the 9th day and became a thin epithelium on the 15th day. Finally, the EORO emulsion enhanced an accented collagenesis in the 15th day seen by the histological analysis, differently from the other groups studied. These findings suggest that through the formation of collagen and re-epithelialization, the migration of fibroblasts to the extracellular matrixis confirmed [29]. In this sense, similar results were found in animals treated topically only with EORO when the wound was evaluated until the 21st day postoperative [16].

A promising resource for the discovery of new antiseptic and healing agents with lesser side effects has been found in oils and plant extracts used in folk medicine [30]. The interest in the use of herbal medicines does not aim to replace drugs already registered and marketed, but to provide a variety of products with the same effectiveness, low cost, legitimizing the empiricism of popular medicine [31].

Although there are many works studying ways to formulate topical emulsion with EORO, the topical healing properties of the essential oil are not well known yet, mainly in control animals. The present findings indicate a potential healer to be used in the cosmetic field since the increased of aesthetic procedures evolving skincare like cleaning, peels, laser treatments, micro blading, needling, lip-blushing, need a post-care being recommended the association of antimicrobial and healing properties. Based on this, the EORO emulsion would have some interesting features to fit in the cosmeceutical market. Nevertheless, it is needed more studies evaluating the emulsion stability, viable bacterial count, and cytotoxicity, as well as acute and chronic toxicity tests in order to evaluate the safety of the emulsion.

6. Conclusion

The study reinforces the healing properties from EORO formulated as a topical emulsion, which could be useful in the aesthetic cosmeceutical market due also to its antimicrobial activity.

5. References

1. Muñoz-Centeno L. Plantas medicinalesespañolas: *Rosmarinus officinalis* L. (Lamiaceae) (romero). Ediciones Univ. Salamanca, 2012.
2. Borrás-Linares I, Stojanovic Z, Quirantes-Pin R, Arráez-Román D, Svarc-Gajié J, Fernández-Gutiérrez A, *et al*. *Rosmarinus officinalis* leaves as a natural sourceofbioactivecompounds. Int. J Mol. Sci. 2014;15:20585-20606. Doi: 10.3390/ijms151120585
3. Akbari J, Saeedi M, Farzin D, Morteza-Semnani K, Esmaili Z. Transdermal absorption enhancing effect of the essential oil of *Rosmarinus officinalis* on percutaneous absorption of Na diclofenac from topical gel. Pharm Biol2015;53(10):1442-7. Doi: 10.3109/13880209.2014.984855.
4. Andrade JM, Faustino C, Garcia C, Ladeiras D, Reis CP, Rijo P. *Rosmarinus officinalis* L. An update review of its phytochemistry and biological activity. Future Sci OA. 2018;4(4):FSO283. Doi:10.4155/fsoa-2017-0124.
5. Borges RS, Keita H, Ortiz BLS, Dos Santos Sampaio TI, Ferreira IM, Lima ES, *et al*. Anti-inflammatory activity of nanoemulsions of essential oil from *Rosmarinus officinalis* L. *in vitro* and in zebra fish studies. Inflammopharmacology. 2018a;26(4):1057-1080. Doi:10.1007/s10787-017-0438-9.
6. Borges RS, Lima ES, Keita H, Ferreira IM, Fernandes CP, Cruz RAS, *et al*. Anti-inflammatory and antialgic actions of a nanoemulsion of *Rosmarinus officinalis* L. essential oil and a molecular docking study of its major chemical constituents. Inflammopharmacology. 2018b;26(1):183-195. Doi:10.1007/s10787-017-0374-8.
7. Borges RS, Ortiz BLS, Pereira ACM, Keita H, Carvalho JCT. *Rosmarinus officinalis* essential oil: A review of its phytochemistry, anti-inflammatory activity, and mechanisms of action involved. J Ethnopharmacol. 2019;229:29-45. Doi: 10.1016/j.jep.2018.09.038.
8. De Macedo LM, Santos ÉMD, Militão L, Tundisi LL, Ataide JA, Souto EB, *et al*. Rosemary (*Rosmarinus officinalis* L., syn *Salvia rosmarinus* Spenn.) and Its Topical Applications: A Review. Plants (Basel). 2020;9(5):651. Doi:10.3390/plants9050651.
9. Takaki I, Bersani-Amado LEE, Vendruscolo A, Sartoretto SMM, Diniz SPP, Bersani-Amado CAA, *et al*. Anti-Inflammatory and Antinociceptive Effects of *Rosmarinus officinalis* L. Essential Oil in Experimental Animal Models. J Med. Food. 2008;11:741-746. Doi:10.1089/jmf.2007.0524.
10. De Melo GAN, Grespan R, Fonseca JP, Farinha TO, Silva EL, Romero AL, *et al*. *Rosmarinus officinalis* L. Essential Oil Inhibits *in vivo* and *in vitro* Leukocyte Migration. J Med. Food. 2011;14:944-946. Doi:10.1089/jmf.2010.0159
11. Pintore G, Usai M, Bradesi P, Juliano C, Boatto G, Tomi F, *et al*. Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica. Flavour Fragr J. 2001;17:15-19. Doi:10.1002/ffj.1022.
12. Zlabiene U, Baranauskaite J, Kopustinskiene DM, Bernatoniene J. *In vitro* and clinical safety assessment of the multiple W/O/W Emulsion Based on the Active Ingredients from *Rosmarinus officinalis* L., *Avena sativa* L. and *Linum usitatissimum* L. Pharmaceutics. 2021;13(5):732. Doi:10.3390/pharmaceutics13050732.
13. Farhan A, Alsuwayt B, Alanazi F, Yaseen A, Ashour MA. Evaluation and HPLC characterisation of a new herbal ointment for the treatment of full-thickness burns in rats. J Taibah Univ Med Sci. 2020 Dec 13;16(2):152-161. Doi:10.1016/j.jtumed.2020.10.023.
14. Abu-Al-Basal MA. Healing potential of *Rosmarinus officinalis* L. on full-thickness excision cutaneous wounds in alloxan-induced-diabetic BALB/c mice. J Ethnopharmacol. 2010;131(2):443-50. Doi:10.1016/j.jep.2010.07.007.
15. Umasankar K, Nambikkairaj B, Backyavathy DM. Effect of topical treatment of *Rosmarinus officinalis* essential oil on wound healing in streptozotocin induced diabetic rats. Nat. Environ. Pollut. Technol. 2012;11:607-611.
16. De Araújo JTC, Pantoja FO, Sá PS, Távora NPL, Pinheiro AV, Pinto VHF, *et al*. Effect of Essential Oil of *Rosmarinus officinalis* L. (Rosemary) on the Healing of Cutaneous Lesions in Mice. J Chem Pharm Res. 2017;9(5):381-386.
17. Valgas C, De Souza SM, Smânia EFA. Screening

methods to determine antibacterial activity of natural products. Braz. J Microbiol. 2007;38:369-380.
18. Cizauskaite U, Ivanauskas L, Jakštas V, Marksiene R, Jonaitiene L, Bernatoniene J. *Rosmarinus officinalis* L. extract and some of its active ingredients as potential emulsion stabilizers: a new approach to the formation of multiple (W/O/W) emulsion. Pharm Dev Technol. 2016;21(6):716-24. Doi:10.3109/10837450.2015.1048554.
19. Baker BP, Grant JA. Rosemary & Rosemary Oil Profile. Cornell University Library. New York State Integrated Pest Management Program. 2018. Available: https://ecommons.cornell.edu/handle/1813/56138
20. Fiume MM, Bergfield WF, Belsito DV, Hill RA, Klaassen CD, Liebler DC, *et al* Safety Assessment of *Rosmarinus officinalis* (Rosemary)-Derived Ingredients as Used in Cosmetics. Int. J Toxicol. 2018;37(3):12S-50S. Doi:10.1177/1091581818800020.
21. Mekonnen A, Yitayew B, Tesema A, Taddese S. *In vitro* Antimicrobial Activity of Essential Oil of *Thymus schimperi, Matricaria chamomilla, Eucalyptus globulus, and Rosmarinus officinalis.* Int. J Microbiol. 2016, 9545693. Doi:10.1155/2016/9545693
22. Bajalan I, Rouzbahani R, Pirbalouti AG, Maggi F. Antioxidant and antibacterial activities of the essential oils obtained from seven Iranian populations of *Rosmarinus officinalis*. Ind. Crops Prod. 2017;107:305-311. Doi:10.1016/j.indcrop.2017.05.063.
23. Jardak M, Elloumi-Mseddi J, Aifa S, Mnif S. Chemical composition, anti-biofilm activity and potential cytotoxic effect on cancer cells of *Rosmarinus officinalis* L. essential oil from Tunisia. Lipids Health Dis. 2017 Oct 2;16(1):190. Doi:10.1186/s12944-017-0580-9.
24. Khezri K, Farahpour MR, Rad SM. Accelerated infected wound healing by topical application of encapsulated Rosemary essential oil into nanostructured lipid carriers, Artif. Cells Nanomed. Biotechnol. 2019;47:1:980-988. Doi: 10.1080/21691401.2019.1582539.
25. Nogueira de Melo GA, Grespan R, Fonseca JP, Farinha TO, Silva EL, Romero AL, *et al*. *Rosmarinus officinalis* L. essential oil inhibits *in vivo* and in vitro leukocyte migration. J Med Food. 2011;14(9):944-6. Doi:10.1089/jmf.2010.0159.
26. Eggleston RB. Wound Management: Wounds with Special Challenges. Vet Clin North Am Equine Pract. 2018;34(3):511-538. Doi:10.1016/j.cveq.2018.07.003.
27. Pérez-Recalde M, Ruiz Arias IE, Hermida ÉB. Could essential oils enhance biopolymers performance for wound healing? A systematic review. Phytomedicine. 2018;38:57-65. Doi:10.1016/j.phymed.2017.09.024.
28. Spear M. Wound exudate - the good, the bad, and the ugly. Plast Surg Nurs. 2012;32(2):77-9. Doi:10.1097/PSN.0b013e318256d638.
29. Gonzalez ACO, Costa TF, Andrade ZA, Medrado ARAP. Wound healing – A literature review. An. Bras. Dermatol. 2016;91(5):614-20. Doi:10.1590/abd1806-4841.20164741
30. Oliveira ST, Leme MC, Pippi NL, Raiser AG, Manfron MP. Formulações de confrei (Symphytumofficinale L.) na cicatrização de feridas cutâneas de ratos. Rev Fac Zootec Vet Agro. 2000;7:61-5.
31. Cancelieri NM. Plantas medicinais: uma abordagem multidisciplinar. UNESC em Revista. 2003;6(14):181-91.