DEVELOPMENT OF NANOEMULSIONS CONTAINING *Copaifera officinalis* OIL AND *Syagrus coronata* OIL FOR THE TREATMENT OF SKIN INJURIES: FORMULATION, CHARACTERIZATION AND CYTOTOXICITY

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ABSTRACT

Despite its efficient protective function, the skin can present wounds that require complex treatment, which drives the search for new bioactives that present, above all, antioxidant, healing and anti-inflammatory activities. Thus, *Copaifera officinalis* oil and *Syagrus coronata* oil have been shown to be efficient in treating skin lesions due to their composition. Therefore, the development of nanoemulsions that incorporate these two oils will enhance the stability and delivery of active ingredients for the treatment of skin lesions. In this context, the objective of this work was to develop and characterize nanoemulsions containing *Copaifera officinalis* oil and *Syagrus coronata* oil for potential application in the treatment of skin lesions. The nanoemulsions (E7, BL, ECP1), developed by the low energy method, presented average size, PdI and Zeta Potential between 206.9±2.1 nm, 211.3±3.9 nm and 255.7±1, 5 nm, 0.274, 0.227 and 0.276 and -12.0 mV, -11.7 mV and -15.3 mV, respectively. Furthermore, they presented a pH, within the established range, of 4.55±0.04, conductivity characteristic of an O/W system and density between 1.031±0.049 and 1.160±0.016. The formulations did not present cytotoxicity, which reinforces the potential topical application, in addition to being stable in terms of the physical-chemical parameters analyzed over the 30 days.

Keywords: Nanoemulsion. Copaiba. Syagrus coronata. Skin injury.

1 INTRODUCTION

The skin is the largest and most visible organ of the human body, being the protective barrier with the ability to keep the body's internal organism constant and in balance, regardless of changes that may occur in the external environment[1]. Despite its efficient protective function, this organ can suffer injuries or wounds. Wound can be defined as damage or interruption of the normal anatomical structure of the skin, following physical or thermal trauma or as a consequence of underlying medical or pathophysiological conditions[2]. And skin repair occurs through the healing process, which is dynamic, complex and leads to the reestablishment of tissue integrity and homeostasis and involves inflammation, re-epithelialization, formation of granulated tissue, neovascularization, wound contraction and remodeling of the extracellular matrix[3-5]. Wounds that exhibit impaired healing, including acute wounds and chronic wounds, generally do not follow normal physiological healing processes. These wounds often enter a state of pathological inflammation due to a delayed, incomplete, or uncoordinated healing process[3].

Thus, it is noted that the treatment of these injuries still represents a great challenge and there is an increasing search for new treatments with bioactives that present, above all, antioxidant, healing and anti-inflammatory activities. *Copaifera officinalis* (Cop) oil stands out for its healing action, once that composition contains sesquiterpenes (80% of the total) and diterpenes. Those comopounds have astringent, anti-inflammatory and antioxidant action, which promote wound contraction and increase wound healing. epithelialization rate[6]. According to the literature, *Copaifera officinalis* oil demonstrated a predominance of sesquiterpenes such as β-caryophyllene, α-copaene and α-humulene, which were associated with biological activities[7]. Another biocomplex highlighted is *Syagrus coronata* (SC) oil, which has a high content of medium-chain fatty acids, the main one being lauric acid, giving it several biological activities such as antimicrobial, anti-inflammatory and healing[8].

However, as Cop oil has high lipophilicity and volatile characterisitc (sesquiterpenes compounds), it is unsuitable for topical applications due to physicochemical and biopharmaceutical properties. Nanoemulsion systems can be an alternative to modify the bioavailability of natural oils by different mechanisms, including (i) protecting the oils from degradation; (ii) enhancing cellular uptake and apparent solubility on body fluids; and (iii) promoting an effective drug release on different tissues[9]. Thus, SC oil was chosen as carrier (oily phase) and adjuvant in the wound healing process. As a result, it is expected that nanosystem will be able to protect, stabilize and enhance the healing, antioxidant and anti-inflammatory activity of *Copaifera officinalis* and *Syagrus coronata* oils. So, this work aims to develop and characterize nanoemulsions containing Cop oil and SC oil for potential application in the treatment of skin lesions.

2 MATERIAL & METHODS

S. coronata oil, produced by the Piedmont Region Production Cooperative of Diamantina (Coopes), was used. The polysorbate 80 (Tween 80) and Span 80 used are from the company Croda pharma. *C. officinalis* oil was collected by extraction in the city of

Cuiabá - MT, being removed from the stem and in a polypropylene container. This oil was donated to the Medical Material Research Laboratory (LAPEMM) and shared with the Polymers and Bioprocesses Laboratory, both at the Federal University of Bahia (UFBA).

The nanoemulsions produced were of the oil-in-water type, using the low-energy method of spontaneous emulsification[10]. The formulations ECS 8% (Cop 8% w/w+SC 8% w/w), EC 5% (Cop 5% w/w) and SC 8% w/w were proposed. Nanoemulsion containing only mineral oil was used as blank. The oily phase, coomposed of SC oil, Cop oil, Tween 80 (6% w/w) and Span 80 (1.5% w/w), was stirred at 1000 rpm for twenty minutes, After that, oily phase was dripped to the aqueous phase (qsp 100%) with a flow rate of 1mL/min under stirring for twenty minutes.

Those formulations were subjected to physicochemical characterizations, regarding average size, polydispersity index (PdI), zeta potential (ZP) through the dynamic light scattering technique (Zetasizer Nano, from the Malvern brand) pH, density, conductivity) and to the cell viability assay with human fibroblasts.

3 RESULTS & DISCUSSION

The formulations presented mean diameter and size distribuitions compatible with topical application (Table 1).

Table 1 Mean diameter, PDI (polidispersity index) and Zeta Potential (ZP) of the nanoemulsions of Cop+SC, Cop and SC.

Sample	Mean diameter fresh	PDI fresh	ZP fresh	Mean diameter after 30 days	PDI after 30 days	ZP after 30 days
ECS 8%	206.9±2.1 nm	0.274	-12.0 mV	224.7±5.55 nm	0.395	-19.2 mV
EC 5%	211.3±3.9 nm	0.227	-11.7 mV	201.6±6.7 nm	0.226	-16.2 mV
SC 8%	255. 7±1.5 nm	0.276	-15.3 mV	198.3±11.4 nm	0.247	-16.9 mV

According to the literature, nanoemulsions need to have a mean diameter between 100 and 300 nm for topical application[11]. All formulations presented PDI below 0.3, indicanting monomodal distribution. In Figure 1, a monomodal profile is observed for all nanoemulsions during 30 of storage at 4˚C. According to literature, polydispersity values between 0.1 and 0.25nm indicate a small size distribution, whereas PDI values greater than 0.5 indicate wide size distribution[12].

A relatively high ZP value, ±30vmV, is important to have good physical stability of the dispersions, once that large repulsive forces tend to prevent aggregation. However, this is not the only important point to verify stability[13-14]. So, in this research, nanoemsulsion showed physical stability over the 30 days without phase separation or creaming along the time (30 days evaluated).

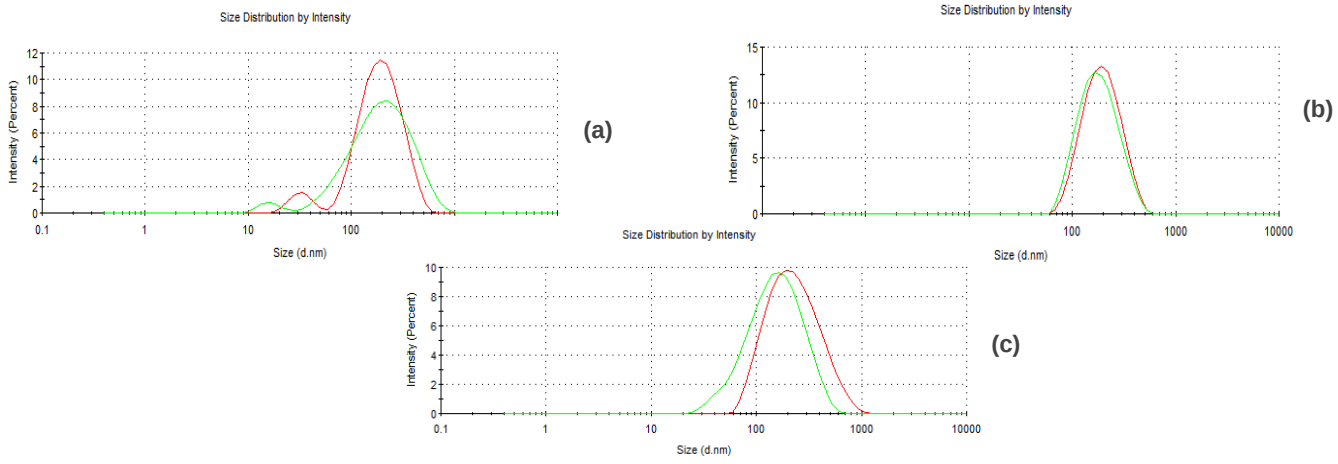

Figura 1- (a) Size distribution of the ECS 8% after 24 h (red line) and 30 days (green line). **(b)** Size distribution of the EC 5% after 24 h (red line) and 30 days (green line). **(c)** Size distribution of the SC 8% after 24 h (red line) and after 30 days (green line).

The pH values for all nanoemulsions were between 4.11 and 4.93. According to literature, skin has a pH between 4 and 6.5[15]. So, all formulations are suitable for topical application. The conductivity values were 157.8±0.2mV to ECS 8%, 181.8±0.2 mV to ECP 5% and 121.3±0.2 mV to SC 8%. Electrical conductivity values are proportional to the amount of oil in the formulation. Those values did not change over 30 days, showing that nanoemulsions kept their stability in that period.

Citotoxicity evaluation showed that free *C. officinalis* oil did not show cytotoxic effects on fibroblastos in concentrations of up 50 µg/mL. Free *S. coronata* oil did not show cytotoxic effects on fibroblasts at any concentration evaluated. (200, 100, 50, 25, 12.5, 6.25 and 3.1 µg/mL). However, nanoemulsion EC 5% did not show significant reduction ($p > 0.05$) in the fibroblast cellular viability at concentrations of 100, 50, 25, 12.5, 6.25 and 3.1 µg/mL. Cop nanoemulsion was cytotoxic only at a concentration of 200 µg/mL. Nanoemulsion ECS 8% did not show significant reduction in the fibroblasts up to 200 µg/mL. For the nanoemulsions containing mineral oil 8% (control) did not induce cytotoxic effects on fibroblastos.

4 CONCLUSION

In the presente study, the nanoemulsions was successfully produced and showed particle size distribution of approximately 200 nm and a negative zeta potential, parameters that remained stable for 30 days. The nanomemulsion containing *C. officinalis* and *S. coronata* oil 8% did not alter cells' integrity at concentrations up to 200 $\mu g \cdot mL^{-1}$. These results show the prospective use of *C. officinalis* oil in future for the treatment of skin injuries.

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