Antimicrobial Activity of Copaiba (*Copaifera officinalis*) and Pracaxi (*Pentaclethra macroloba*) Oils against *Staphylococcus Aureus*: Importance in Compounding for Wound Care

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ABSTRACT

The Amazon rainforest is the largest reserve of natural products in the world. Its rich biodiversity of medicinal plants has been utilized by local populations for hundreds of years for the prevention and treatment of various diseases and ailments. Oil extracts from plant species such as *Copaifera officinalis* and *Pentaclethra macroloba* are used in compounded formulations for their anti-inflammatory, antimicrobial, emollient, moisturizing, and wound-healing activities. The objective of this study was to investigate the *in vitro* bacteriostatic effect of two Amazonian oils, Copaiba and Pracaxi, against *Staphylococcus aureus*, a clinically important microorganism responsible for wound infection, to support the use of these oils as novel natural products for compounded wound-treatment modalities. The antibacterial activity of Copaiba and Pracaxi oils against a standard strain of *Staphylococcus aureus* was assessed using broth microdilution to determine the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the oil extracts. Copaiba oil demonstrated antibacterial activity against *Staphylococcus aureus*, with a Minimum Inhibitory Concentration of 0.3125 mg/mL and a Minimum Bactericidal Concentration of 0.3125 mg/mL. Conversely, Pracaxi oil failed to inhibit *Staphylococcus aureus* growth. While additional studies are required to further evaluate the antimicrobial activity of Pracaxi oil, even low concentrations of Copaiba oil effectively inhibited *Staphylococcus aureus* growth, supporting its potential use as a promising adjuvant in compounded topical formulations for wound and scar healing.

INTRODUCTION

The use of natural products for the prevention and treatment of diseases is one of the oldest medical practices. Knowledge of the therapeutic potential of plants has provoked scientific interest in the search for new ways to control and treat various ailments.[1]

Herbal remedies are used in many areas of modern medicine due to their excellent properties, such as availability, simple preparation and application, good efficiency, and minimal side effects.[2] In pharmacy, particularly in pharmaceutical compounding, natural products have been used to efficiently treat a range of ailments and may be formulated into various dosage forms, including ointments, creams, gels, shampoos, and capsules.[3,4]

The antimicrobial activity of substances used in wound healing is critical, as the wound provides an attractive environment for bacterial growth and, if left untreated, may result in the formation of biofilm and potentially cause significant damage.[5]

Wound infections are typically polymicrobial, and most of these bacteria are aerobic and gram-positive cocci (mostly *Staphylococcus aureus [S. aureus]*). Infected wounds, particularly those associated with *S. aureus,* require the most attention from health professionals due to their tendency to acquire antimicrobial resistance.[6-8]

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Recent studies have demonstrated the use of certain plants from the Amazon rainforest in treating and healing wounds. The antimicrobial, anti-inflammatory, antioxidant, and emollient properties, as well as the ability to stimulate cellular proliferation, of plant species such as *Copaifera officinalis* (Copaiba) and *Pentaclethra macroloba* (Pracaxi) may be associated with improved wound healing[9,10] and can be used in compounding preparations.

COPAIBA OIL

Copaiba is the most widely used medicinal plant among the populations of the Brazilian Amazon region. Copaiba oil is extensively marketed and used in folk medicine for its antimicrobial, anti-inflammatory, antifungal, anesthetic, emollient, and moisturizing properties and is commonly used for treating wounds, applied topically in concentrations of 2% to 10%. This oil is comprised of both volatile substances (sesquiterpenes), corresponding to approximately 90% of the resin oil mass, and non-volatile substances (diterpenes). It is believed that terpenes are the main chemicals responsible for the therapeutic properties of Copaiba oil.[10-13]

PRACAXI OIL

The Pracaxi plant is distributed throughout northern Brazil, Guyana, and some parts of Central America. Pracaxi oil presents various medicinal applications including wound healing, due to its antihemorrhagic, moisturizing, and antimicrobial properties. The usual concentration of this Amazonian oil in compounded preparations is between 1% and 5%. Pracaxi oil is composed mainly of fatty acids, with oleic (approximately 50%) and linoleic acids constituting the major components[9,13-17]

The objective of this study was to investigate the *in vitro* antimicrobial activity of Copaiba and Pracaxi oils against a standard strain of *S. aureus*, a clinically important microorganism responsible for wound infection, to support the use of these oils as novel natural products for effective compounded wound-treatment modalities.

MATERIALS AND METHODS

MATERIALS

Copaiba (Lot 07133310R) and Pracaxi (Lot 09135160R) oils were acquired from a manufacturer of Amazonian oils (Pará, Brazil).

MICROBIAL STRAIN

S. aureus (ATCC 25923) was obtained from American Type Culture Collection (Manassas, Virginia). The strain was inoculated in Brain Heart Infusion (BHI) broth (RenyLab, Brazil) and incubated at 37°C for 24 hours. The inoculum was then spread onto Mueller-Hinton agar plates (RenyLab, Brazil) and incubated at 37°C for 24 hours, to assess for possible contaminants.[18]

Three isolated *S. aureus* colonies were selected from the agar plates and transferred to test tubes containing 2 mL of sterile 0.85% NaCl solution. The suspensions were then adjusted to achieve a turbidity corresponding to a 0.5 MacFarland standard, which is equivalent to approximately 1.5×10^8 CFU/mL. The bacterial suspension was then further diluted to 1.5×10^3 CFU/mL, and this concentration was used in the following experiments.[19]

TABLE 1. Concentration of Copaiba and Pracaxi Oils in Minimum Inhibitory Concentration Assay.

WELLS	CONCENTRATION (MG/ML)
Row A	20
Row B	10
Row C	5
Row D	2.5
Row E	1.25
Row F	0.625
Row G	0.3125
Row H	0.16525

The antimicrobial activity of the Copaiba and Pracaxi oils was assessed *in vitro* by determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the oils that inhibits the visible growth of *S. aureus,* using the microdilution method.[20]

MINIMUM INHIBITORY CONCENTRATION

Determination of the MIC was performed in 96-well microdilution plates.[20,21] Copaiba and Pracaxi oils were first weighed and combined with 1% Tween 80 (Lot 142234) to form emulsions with a final concentration of 4% w/v (40 mg/ mL) for each oil. Tween 80 was added to improve the incorporation of the oils into the aqueous medium and subsequent incorporation of these samples with the culture medium.[22]

To each well of the microdilution plate, 100 ×L of double concentrated BHI medium was added. Then, in the first row of the plate, 100 µL of the Copaiba (columns 1–3) and Pracaxi (columns 4–6) oil emulsions was added, resulting in a final concentration of 2% w/v (20.0 mg/mL). Subsequent concentrations of the tested oils were obtained through two-fold serial dilutions on the plate, starting from the initial concentration of 2% (20 mg/mL – Row A) to 0.015625% (0.15625 mg/mL – Row H), by transferring 100 µL of the contents of a well to the subsequent well. Finally, 100 µL of the contents of the wells in Row H was removed and discarded in order for the total volume of each well to be the same. The concentrations of the oil emulsions in each row are listed in Table 1. To each well in columns 7–9, 100 µL of BHI broth with 1% Tween 80 was added as a positive bacterial growth control. In columns 10 and 11, 100 µL of 20% chlorhexidine solution (Lot SMAART/CHG/2012/028 3) was added as a positive antimicrobial control, as this was expected to inhibit bacterial growth in these wells. To assess sterility, BHI broth alone was added to the wells in column 12, as a negative-growth control.

Following this, 10 µL of the bacterial suspension (1.5×10^3 CFU/mL) was added to each well, except those in the negative-control column. The plates were then incubated at 37°C for 48 hours. Finally, 10 µL of tetrazolium bromide was added to each well, the plate was further

incubated for 1 hour, and cell viability was assessed qualitatively. The MIC corresponded to the lowest concentration of the oils at which neither a change in the medium color (yellow to purple) nor the presence of precipitate was observed.

MICs were determined as the lowest concentration of Copaiba or Pracaxi oil able to inhibit bacterial growth, and 100% MIC was defined as the lowest concentration able to inhibit 100% of the isolates.[23]

MINIMUM BACTERICIDAL CONCENTRATION

The MBC was determined by plating, on Mueller-Hinton agar, 10 µL of the dilutions corresponding to the MIC and the two immediately preceding concentrations (2MIC and 4MIC). These concentrations above the MIC are sufficient to show the bactericidal activity of the tested oils, since the bacteriostatic effect was determined by the absence of growth in the tested samples. The plates were incubated at 37°C for 24 hours, after which the amount of growth was noted.

The MBC was defined as the lowest concentration of the oil emulsions that prevented visible growth of bacteria or that allowed the formation of up to three colony forming units (CFU). Thus, concentrations at which the formation of more than three CFU were found were not considered inhibitory for bacterial growth, while the concentrations in which no growth or less than 3 CFU was observed, were considered bactericidal.[23,24] All experiments were performed in triplicate.

RESULTS

Results from the *in vitro* antimicrobial activity assays indicate that Copaiba oil effectively inhibited *S. aureus* growth, as there was neither formation of precipitate nor a color change in the medium with given concentrations of the oil. The MIC of the Copaiba oil was found to be 0.3125 mg/mL (Figure 1). Based on the MIC, the MBC of the Copaiba oil was also determined to be 0.3125 mg/mL, as evidenced by the absence of bacterial growth on Mueller-Hinton agar plates with this concentration of the oil (Figure 2a).

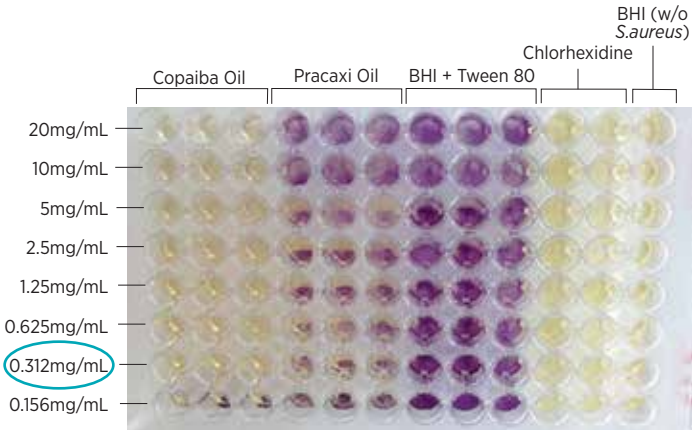
Conversely, the Pracaxi oil demonstrated no *in vitro* antibacterial activity against the *S. aureus* strain. In the MIC assay, all samples containing Pracaxi oil, regardless of concentration, resulted in bacterial growth (Figure 1). Results from the MBC assay indicate that even at the highest concentration tested, the Pracaxi oil failed to inhibit bacterial growth (Figure 2b).

As expected, no bacterial growth was observed in the chlorhexidine samples or in the negative-control (BHI broth alone) samples. On the other hand, a color change (purple) was indeed observed in the all positive control wells (BHI + Tween 80), indicating bacterial growth in these samples (Figure 1).

DISCUSSION

The use of plant oils for wound healing is widespread throughout populations in the Amazon region and has been described in several studies.[25,26] In particular, Copaiba oil has been reported as an effective antimicrobial agent.[27-29] The antimicrobial activity of Copaiba oil against *S. aureus* found in the current study (Table 2) supports these findings and may serve to explain the frequent use of this oil in treating wounds.[26] Reduction of the microbial load, and particularly *S. aureus,* is a significant factor in reducing both the healing time and the inflammatory process.[6,7,30.]

FIGURE 1. Determination of Minimum Inhibitory Concentration. The susceptibility of *S. aureus* to Copaiba oil (columns 1–3) and Pracaxi oil (columns 4–6) at the indicated concentrations was assessed *in vitro* by the microdilution method. Samples in columns 7–9 (BHI medium + Tween 80) served as a positive control for bacterial growth. Chlorhexidine (columns 10–11) was used as a positive control for antimicrobial activity. Samples in column 12 (BHI medium) were incubated without *S. aureus* as a negative growth control.



While numerous species of the *Copaifera* genus have been shown to exhibit antimicrobial activity, the *Copaifera officinalis* species is the most noted.[11] In a study by Santos *et al,* Copaiba oil from eight different species was assessed for antimicrobial activity. Oil from the *Copaifera officinalis* species was among those with the lowest MIC (62.5 µg/mL) and MBC (62.5 µg/mL). Moreover, the oil from this species presented moderate antimicrobial activity (MIC of 125 µg/mL and MBC of 250 µg/mL) against the methicillin-resistant strain of *S. aureus*.

The anti-inflammatory, antimicrobial, and healing properties are the most commonly studied attributes of Copaiba oil cited in ethnopharmacological studies. Researchers attribute these properties to active phytochemical components, such as sesquiterpenes (β-caryophyllene, α- and β-humulene, α-bergamotene, germacrene D, and β-bisabolene) and diterpenes (copalic and kaurenoic acid).[11,27,31,32]

The terpene activity on the bacterial membrane may be related to the lipophilic properties, the potency of the functional groups, and the solubility of these substances in water. The antimicrobial activity is attributed to the interaction of the terpenes with structural

FIGURE 2. The Minimum Bactericidal Concentration of the Copaiba oil (a) and Pracaxi oil (b) was assessed after being subcultured on Mueller-Hinton agar, based on the Minimum Inhibitory Concentration values determined previously.

FIGURE 2A

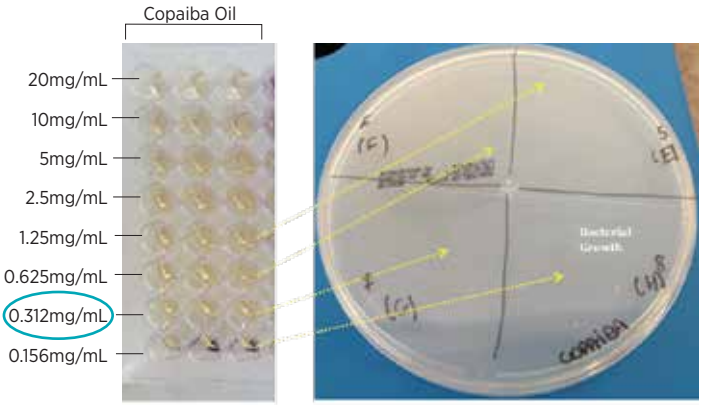
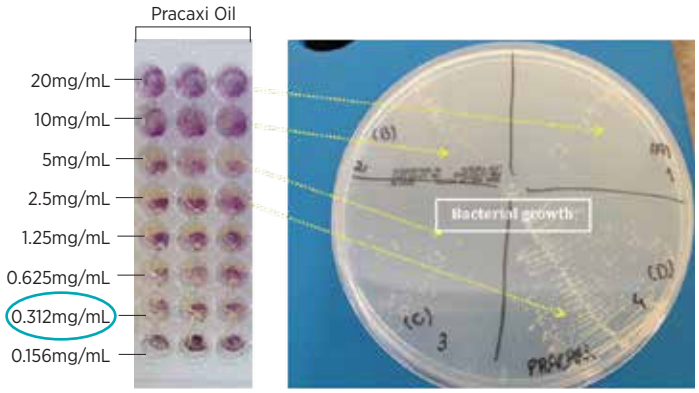


FIGURE 2B



components of bacterial cells, such as the phospholipid bilayer of the cell membrane, increasing permeability and binding to vital constituents of the bacteria.[33] Damage to the bacterial cell membrane as well as inhibition of the cellular proliferation of *S. aureus* by the terpenes in essential oils have been described previously.[20]

When selecting therapeutic options for wound and scar healing, pharmacists should consider the mechanism of action of the agents available as well as the condition of the wound to be treated. The use of Copaiba oil combined with other compounds traditionally used in wound and scar healing, such as aloe vera, pentoxifylline, hyaluronic acid, tranilast, antibiotics, corticosteroids, calcium channel blockers, and retinoids could potentially be considered to enhance the desired therapeutic effect (Table 3).[34,35]

The antimicrobial activity of Pracaxi oil (*Pentaclethra macroloba*) has yet to be reported. However, in a recent study by Oliveira et al, the antibacterial activity of the aqueous extract of Pracaxi was assessed against gram-negative and gram-positive bacteria. The results demonstrated that this extract showed antibacterial activity against gram-negative bacteria (*Klebsiella ozaenae* and *Acinetobacter baumannii*) but was ineffective against gram-positive bacteria (*S. aureus*).[14]

TABLE 2. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Copaiba and Pracaxi Oils (n = 3).

MICROORGANISM	COPAIBA OIL		PRACAXI OIL	
	MIC (mg / mL)	MBC (mg / mL)	MIC (mg / mL)	MBC (mg / mL)
Staphylococcus aureus	0.3125	0.3125	*	*

*No antimicrobial activity was observed
MBC = Minimum Bactericidal Concentration; MIC = Minimum Inhibitory Concentration

TABLE 3. Suggested Compounded Formulations for Wound and Scar Healing Using Topical Gel with Copaiba Oil.

Formula 01	Aloe Vera		0.2%
	Hyaluronic acid		0.2%
	Mupirocin		1%
	Pentoxifyllin		5%
	Topical Gel with Copaiba oil	qs	20 g
Formula 02	Fluticasone propionate		1%
	Pentoxifylline		5%
	Tranilast		1%
	Topical Gel with Copaiba oil	qs	20 g
Formula 03	Tamoxifen citrate		0.1%
	Pentoxifylline		3%
	Retinoic acid		0.05%
	Topical Gel with Copaiba oil	qs	20 g

While some studies support the antimicrobial activity of Pracaxi and its use in wound care, in the current study, Pracaxi oil showed no antimicrobial activity against the *S. aureus* strain, suggesting that it is not the most effective natural oil for reducing the microbial load, particularly that of *S. aureus*, in the treatment of wounds. Perhaps its high content of fatty acids, such as oleic and linoleic acid, may better explain its healing capability, as these acids provide emolliency and moisturization.[17]

CONCLUSION

Additional studies are required to further assess the antibacterial properties of Pracaxi oil against gram-positive bacteria such as S. aureus, particularly since these bacteria are the first to colonize an affected area of the skin. However, the antimicrobial activity of Copaiba oil against S. aureus observed in the current study presents a promising opportunity to develop new commercial and compounded products for wound and scar healing. Compounding topical medica-

tions can help tailor wound care treatments to individual patient needs and may offer alternative or enhanced wound healing effects.

REFERENCES

1. Ferreira SB, Dantas IC, Catão RMR. Avaliação da atividade antimicrobiana do óleo essencial de sucupira (*Pterodon emarginatus Vogel*). *Rev Bras Pl Med* 2014; 16(2): 225–230.
2. Carvalho CT. *Formulário Médico-Farmacêutico de Fitoterapia*. 3rd ed. São Paulo, Brazil: Pharmabooks; 2012: 5–15.
3. Zdoryk OA, Khokhlova KO, Georgiyants VA et al. Investigation of physical and chemical stability of ointment with herbals. *IJPC* 2014; 18(3): 248–252.
4. Kovác I, Durkác J, Holly M et al. *Plantago lanceolata L.* water extract induce transition of fibroblasts into myofibroblast and increases tensile strength of healing skin wounds. *J Pharm Pharmacol* 2015; 67(1): 117–125.
5. AWMA – Australian Wound Management Association Inc. Bacterial impact on wound healing: From contamination to infection 2011; Version 1.5.
6. Li M, Du X, Villaruz AE et al. MRSA epidemic linked to a quickly spreading colonization and virulence determinant. *Nat Med* 2012; 18(5): 816–819.
7. Dawson AL, Dellavalle RP, Elston DM. Infections skin diseases: A review and needs assessment. *Dermatol Clin* 2012; 30(1): 141–151.
8. Davis SC, Perez R. Cosmeceuticals and natural products: Wound healing. *Clin Dermatol* 2009; 27(5): 502–506.
9. Leal IC, Júnior II, Pereira EM et at. *Pentaclethra macroloba* tannins fractions active against methicillin-resistant staphylococcal and Gram-negative strains showing selective toxicity. *Brazilian J Pharmacog* 2011; 21(6): 991–999.
10. Masson-Meyers DS, Enwemeka CS, Bumah VV et al. Antimicrobial effects of *Copaifera langsdorffii* oleoresin in infected rat wounds. *International J Applied Microbiol Sci* 2013; 2(3): 9–20.
11. Leandro LM, Vargas FS, Barbosa PC et al. Chemistry and biological activities of terpenoids from copaiba (*copaifera spp.*) oleoresins. *Molecules* 2012; 17(4): 3866–3889.
12. Deus RJ, Alves CN, Arruda MS. Avaliação do efeito antifúngico do óleo resina e do óleo essencial de copaíba (*Copaifera multijuga Hayne*). Revista Brasileira de Plantas Medicinais 2011; 13(1): 1–7.
13. Souza VM, Junior DA. *Ativos Dermatológicos: Dermocosméticos e Nutracêuticos*. San Paulo: Pharmabooks; 2013.
14. Oliveira AA, Segovia JF, Sousa VY et al. Antimicrobial activity of Amazonian medicinal plants. *Springerplus* 2013; 2: 371.
15. Teixeira RS, Rocha PF, Polonini HC et al. Mushroom tyrosinase inhibitory activity and major fatty acid constituents of Amazonian native flora oils. *Brazilian J Pharmaceut Sci* 2012; 48(3): 399–404.
16. Silva JO, Coppede JS, Fernandes VC et al. Antihemorrhagic, antinucleolytic and other antiophidian properties of the aqueous extract from *Pentaclethra macroloba. J Ethnopharmacol* 2005; 100(2005): 145–152.
17. Santos Costa M, Muniz M, Negrão C et al. Characterization of *Pentaclethra macroloba oil. J Therm Anal Calorim* 2014; 115: 2269–2275.
18. Clinical Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.* Approved Standard —Ninth Edition. 2012; 32(2).
19. Amatiste S, Sagrafoli D, Giacinti G et al. Antimicrobial activity of essential oils against *Staphylococcus aureus* in fresh sheep cheese. *Italian J Food Safety* 2014; 3(1696): 148–150.
20. Ziech RE, Farias LD, Balzan C et al. Atividade antimicrobiana do oleorresina de Copaíba (*Copaifera reticulata*) frente a *Staphylococcus* coagulase positiva isolados de casos de otite em cães. *Pesq Vet Bras* 2013; 33(7): 909–913.
21. Pacheco TA, Barata LE, Duarte MC. Antimicrobial activity of copaíba (*Copaifera spp*) balsams. *Rev Bras Pl Med* 2006; 8: 123–124.
22. Silva MT, Ushimaru PI, Barbosa LN et al. Atividade antibacteriana de óleos essenciais de plantas frente a linhagens de *Staphylococcus aureus e Escherichia coli* isoladas de casos clínicos humanos. *Rev Bras Pl Med* 11(3): 257–262.
23. Freire IC, Pérez AL, Cardoso AM. Atividade antibacteriana de Óleos Essenciais sobre *Streptococcus mutans e Staphylococcus aureus. Rev Bras Pl Med* 2014; 16(2): 372–377.
24. Pozzatti P, Loreto ES, Lopes PG et al. Comparison of the susceptibilities of clinical isolates of *Candida albicans* and *Candida dubliniensis* to essential oils. *Mycoses* 2010; 53(1): 12–15.
25. Batista JS, Olinda RG, Medeiros VB et al. Atividade antibacteriana e cicatrizante do óleo de buriti *Mauritia flexuosa* L. *Ciência Rural* 2012; 42(1): 136–141.
26. Souza MH, Monteiro CA, Figueredo PM et al. Ethnopharmacological use of Babassu (*Orbignya phalerata Mart*) in communities of Babassu nut breakers in Maranhão, Brazil. *J Ethnopharmacol* 2011; 133: 1–5.
27. Heck MC, Viana LA, Vicentini VE. Importância do óleo de *Copaifera sp.* (Copaíba). SaBios: *Revista de Saúde e Biologia* 2012; 7(1): 82–90.
28. Yamaguchi MH, Garcia RF. Óleo de Copaíba e suas propriedades medicinais: Revisão Bibliográfica. *Revista Saúde e Pesquisa* 2012; 5(1): 137–146.
29. Santos AO, Ueda-Nakamura T, Filho BPD et al. Antimicrobial activity of Brazilian Copaíba oils obtained from different species of the *Copaifera* genus. *Mem Inst Oswaldo Cruz* 2008; 103(3): 277–281.
30. Kincaid MR. Options in Wound Care. *IJPC* 2002; 6(2): 92–95.
31. Veiga-Junior VF, Pinto AC. O gênero *Copaifera* L. *Química Nova* 2002; 25(2): 273–286.
32. Pieri FA, Mussi MC, Moreira MA. Óleo de copaíba (Copaifera sp.): Histórico, extração, aplicações industriais e propriedades medicinais. *Revista Brasileira de Plantas Medicinais* 2009; 11(4): 465–472.
33. Dorman HJ, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *J Applied Microbiol* 2000; 88: 308–316.
34. Helmke CD. Current Topical Treatments in Wound Healing, Part 1. *IJPC* 2014; 8(4): 269–274.
35. Helmke CD. Current Topical Treatment in Wound Healing, Part 2. *IJPC* 2014; 8(5): 354–357.

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