Research, Society and Development, v. 9, n. 10, e1869108593, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.8593

Chemical composition and antibacterial activity of commercial copaiba (*Copaifera* spp.) oils against bacterial pathogens isolated from postoperative mammoplasty surgery

Composição química e atividade antibacteriana de óleos comerciais de copaíba (*Copaifera* spp.) contra patógenos bacterianos isolados de cirurgia de mamoplastia pós-operatória

Composición química y actividad antibacteriana de los aceites comerciales de copaiba (*Copaifera* spp.) contra patógenos bacterianos aislados de mamoplastia posoperatoria

Received: 09/18/2020 | Reviewed: 09/20/2020 | Accept: 09/21/2020 | Published: 09/23/2020

Raquel Costa Machado
ORCID: https://orcid.org/0000-0002-1489-9024
Paranaense University, Brazil
E-mail: raquel@prof.unipar.br
Ana Karina Vargas Soares
ORCID: https://orcid.org/0000-0001-6076-166X
Paranaense University, Brazil
E-mail: ana.vargas@edu.unipar.br
Isabela Carvalho dos Santos
ORCID: https://orcid.org/0000-0002-7971-5126
Paranaense University, Brazil
E-mail: isabela_carvalhoxd@hotmail.com
Wanessa de Campos Bortolucci
ORCID: https://orcid.org/0000-0002-7233-8313
Paranaense University, Brazil
E-mail: wanessa.bortolucci@edu.unipar.br
Luis Fernando Espinoza Luizar
ORCID: https://orcid.org/0000-0003-4952-3286
Paranaense University, Brazil
E-mail: luis.f_espinoza@hotmail.com

Caio Franco de Araújo Almeida Campos
ORCID: https://orcid.org/0000-0001-5689-4785
UniCesumar University, Brazil
E-mail: caiofaac@hotmail.com
José Eduardo Gonçalves
ORCID: https://orcid.org/0000-0002-2505-0536
UniCesumar University, Brazil
E-mail: jose.goncalves@unicesumar.edu.br
Lidiane Nunes Barbosa
ORCID: https://orcid.org/0000-0001-5762-8091
Paranaense University, Brazil
E-mail: lidianebarbosa@prof.unipar.br
Samantha Wietzikoski
ORCID: https://orcid.org/0000-0003-4611-6642
Paranaense University, Brazil
E-mail: swietzikoski@prof.unipar.br
Lisiane de Almeida Martins
ORCID: https://orcid.org/0000-0003-0700-2634
Paranaense University, Brazil
E-mail: lisiane.almeida.martins@gmail.com
Zilda Cristiani Gazim
ORCID: https://orcid.org/0000-0003-0392-5976
Paranaense University, Brazil
E-mail: cristianigazim@prof.unipar.br
Francislaine Aparecida dos Reis Lívero
ORCID: https://orcid.org/0000-0001-6533-0850
Paranaense University, Brazil
E-mail: francislaine@prof.unipar.br
Evellyn Claudia Wietzikoski Lovato
ORCID: https://orcid.org/0000-0002-8511-0086
Paranaense University, Brazil
E-mail: evellyn@prof.unipar.br

Abstract

Plastic surgeries are considered clean or potentially contaminated procedures. The incidence of infection in reduction mammoplasty is 1.1 to 22% and the main etiological agents are bacteria found on the skin and mucous membranes such as *Staphylococcus aureus*. Due to the increase in bacterial resistance with the widespread use of antibiotics, identify natural compounds with antibacterial action on postoperative surgery wounds are fundamental. Thus, the objective of this research was the identification of compounds and assessment of the antibacterial action of *Copaifera* spp. (copaiba) oil against standard strains and bacterial pathogens isolated from postoperative mammoplasty surgery. For this, four commercial copaiba oils (1, 2, 3 and 4) were submitted to a gas chromatography/mass spectrometry analysis. The *in-vitro* antimicrobial activity and the minimum inhibitory concentration (MIC) of oils on standard strains and clinical samples, as well as the disk antibiotics sensitivity and the synergistic effect of the oils and antibiotics, were assessed. A total of 72 compounds were identified, accounting for ~99% of the volatile constituents in the oils. Sesquiterpenes comprised 67.24– 90.11% of the components, with *β*-caryophyllene being the most common. Oils 1 and 2 were the most active on the *S. aureus* strain, with MIC similarto Oil 3, while Oil 4presented no activity. The same pattern was observed in the clinical samples. In addition, Oil 2 presented synergism when associated with amoxicillin. The synergistic effects of Copaiba oils may represent a source of therapeutic compounds against bacterial infections in surgical wound.

Keywords: Microbial viability; Gram-negative bacteria; Gram-positive bacteria; Mammoplasty; *Staphylococcus aureus*.

Resumo

As cirurgias plásticas são consideradas procedimentos limpos ou potencialmente contaminados. A incidência de infecção na mamoplastia redutora é de 1,1 a 22% e os principais agentes etiológicos são bactérias presentes na pele e nas mucosas, como o *Staphylococcus aureus*. Devido ao aumento da resistência bacteriana com o uso generalizado de antibióticos, identificar compostos naturais com ação antibacteriana em feridas pós-operatórias é fundamental. Assim, o objetivo desta pesquisa foi a identificação de compostos e avaliação da ação antibacteriana do óleo de *Copaifera* spp. (copaíba) contra cepas padrão e patógenos bacterianos isolados do pós-operatório de cirurgia de mamoplastia. Para tanto, quatro óleos comerciais de copaíba (1, 2, 3 e 4) foram submetidos à análise por cromatografia gasosa / espectrometria de massa. A atividade antimicrobiana *in vitro* e a concentração inibitória mínima (CIM) de óleos em cepas padrão e amostras clínicas, bem como a sensibilidade a antibióticos em disco e o efeito sinérgico

dos óleos e antibióticos, foram avaliados. Um total de 72 compostos foram identificados, representando ~ 99% dos constituintes voláteis dos óleos. Os sesquiterpenos representaram 67,24–90,11% dos componentes, sendo o *β*-cariofileno o mais comum. Os óleos 1 e 2 foram os mais ativos na cepa de *S. aureus*, com CIM semelhante ao óleo 3, enquanto o óleo 4 não apresentou atividade. O mesmo padrão foi observado nas amostras clínicas. Além disso, o óleo 2 apresentou sinergismo quando associado à amoxicilina. Os efeitos sinérgicos dos óleos de copaíba podem representar uma fonte de compostos terapêuticos contra infecções bacterianas em feridas cirúrgicas.

Palavras-chave: Viabilidade microbiana; Bactérias gram-negativas; Bactérias gram-positivas; Mamoplastia; *Staphylococcus aureus.*

Resumen

Las cirugías plásticas se consideran procedimientos limpios o potencialmente contaminados. La incidencia de infección en la mamoplastia de reducción es del 1,1 al 22% y los principales agentes etiológicos son las bacterias presentes en la piel y mucosas como *Staphylococcus aureus*. Debido al aumento de la resistencia bacteriana con el uso generalizado de antibióticos, identificar compuestos naturales con acción antibacteriana sobre las heridas de la cirugía postoperatoria es fundamental. Así, el objetivo de esta investigación fue la identificación de compuestos y la evaluación de la acción antibacteriana de *Copaifera* spp. (copaiba) contra cepas estándar y patógenos bacterianos aislados de la cirugía de mamoplastia postoperatoria. Para ello, cuatro aceites de copaiba comerciales (1, 2, 3 y 4) se sometieron a un análisis de cromatografía de gases / espectrometría de masas. Se evaluó la actividad antimicrobiana in vitro y la concentración mínima inhibitoria (CMI) de los aceites en cepas estándar y muestras clínicas, así como la sensibilidad a los antibióticos de disco y el efecto sinérgico de los aceites y antibióticos. Se identificaron un total de 72 compuestos, que representan ~ 99% de los constituyentes volátiles de los aceites. Los sesquiterpenos comprendieron entre el 67,24 y el 90,11% de los componentes, siendo el *β*-cariofileno el más común. Los aceites 1 y 2 fueron los más activos en la cepa de *S. aureus*, con CMI similar al aceite 3, mientras que el aceite 4 no presentó actividad. El mismo patrón se observó en las muestras clínicas. Además, Oil 2 presentó sinergismo cuando se asoció con amoxicilina. Los efectos sinérgicos de los aceites de Copaiba pueden representar una fuente de compuestos terapéuticos contra infecciones bacterianas en heridas quirúrgicas.

Palabras clave: Viabilidad microbiana; Bacterias gramnegativas; Bacterias grampositivas; Mamoplastia; *Staphylococcus aureus*.

1. Introduction

The large trees belonging to the *Copaifera* spp. genus, commonly known as copaiba, are native of Latin America and West Africaand can be found in the north of Brazil (Arruda et al., 2009). Copaiba oil is extracted from the inner part of the trunk of those trees through schizolizigous channels formed by the dilation of intercellular (meat) spaces (Balouiri, Sadiki & Ibnsouda, 2016) and is characterized by a transparent liquid ranging from yellow to light brown in color.

The chemical composition of copaiba oil extracted from the species studied in Brazil is the result of some secondary metabolites, which may be influenced by factors such as environment, attacks caused by insects and fungi, plant development period and genetic factors (Barbosa et al., 2012). Its main compounds are β and *trans*-caryophyllene, α-humulene, α-cadinol, α-cubebene, β-elemene, α-copaene, *trans*-α-bergamotene and β-bisabolene, which may vary among species due to injury caused to the plant. In addition, β-caryophyllene is one of the main chemical markers of the copaiba oil (Balouiri, Sadiki & Ibnsouda, 2016), with anti-inflammatory, anti-edema, antibacterial and antifungal actions (Arruda et al., 2009; Balouiri, Sadiki & Ibnsouda, 2016; Barbosa et al., 2012; Belleti et al., 2004).

The antibacterial action is justified by the different substances present in the copaiba oil and other natural products, acting on different cellular targets in a synergistic manner in the various structures in the bacterial cell. Therefore, it may be an alternative to hinder or prevent the emergence of resistant microorganism (Chiavari-Frederico et al., 2020; CLSI, 2018; Wietzikoski Lovato et al., 2017; Cascon & Gilbert, 2000). Resistant bacteria are responsible for post-surgical infections (Okdakowska-Jedynak et al., 2003) with *Staphylococcus aureus* being the most common microorganism among infections in hospital environments, mainly causing cutaneous infections, presenting multi-resistant fronts to the antimicrobials agents (Fung, Kirschenbaum & Ojofeitimi, 2001; Zetola, Francis, Nuermberger & Bishai, 2005).

Plastic surgeries, according to the general classification, are considered clean or potentially contaminated procedures. Due to the rupture of the physical barrier of the skin, through the surgical incision, it becomes a "gateway" to microorganisms, allowing access to the surgical site. The risk of surgical infection is established by the relationship between the microbial load of the contamination, its virulence and degree of injury to the wound tissues against the resistance capacity of the host influenced by its local and systemic immune response. Another factor to be considered is the presence of foreign body in the wound, which may compromise the local defense of the tissues, reducing the inoculum necessary for the

development of infection even by microorganisms that are not very virulent (Franco, Cardoso & Franco, 2006; Gutwein, Panigrahi, Schultz & Mast, 2012).

Mammoplasty infection is a complication that can have serious consequences and often a conservative approach may not be enough. Thus, another surgical intervention with capsulectomy, debridement, abundant irrigation and removal of implants is required (Gutwein, Panigrahi, Schultz & Mast, 2012). The incidence of infection in reduction mammoplasty is 1.1 to 22% and its risk factors are obesity, duration of surgery, hospital environment, surgical material, among others. The main etiological agents are bacteria found on the skin and mucous membranes such as *Staphylococcus aureus*, different species of coagulase-negative *Staphylococcus*, and, to a lesser extent, gram-negative bacteria (Gravante, Caruso, Araco & Cervelli, 2008; Kaye et al., 2009; Spear & Seruya, 2010). In addition, it has been increasing the frequency of these bacteria due to the increasing number of implanted surgeries of immunocompromised patients who undergo surgical interventions (Kaye et al., 2009; Spear & Seruya, 2010), including the isolation of strains of *S. aureus* resistant to methicillin (Feldman et al., 2010), which generates a great concern in public health.

Due to the increase in bacterial resistance with the widespread use of antibiotics, the purpose of this paper is to quantitatively identify the compounds and assess the antibacterial action of four commercial copaiba oils against standard and clinical strain samples isolated from postoperative mammoplasty surgery wounds, following the good standards of scientific research methodology (Pereira et al., 2018).

2. Methodology

2.1 Chemical composition of copaiba oils

2.1.1 Copaiba oils

Four commercial copaiba oil samples were purchased for the experiments, being two of extractive origin made by traditional communities and two industrially sold commercially: Oil 1 (Comflona, Pará, BR), Oil 2 (Oriximina,Pará, BR), Oil 3 (Anil, Paraná, BR, production lot L0002), and Oil 4 (By Samia, São Paulo, BR, production lot L1016077B5).

The resinous oils were extracted directly from the tree trunk, puncturing the trunk and depleting the oil inside. The orifice is sealed so that the plant produces oil again. The oil is used in a fresh way, without it being processed. The *Copaifera officinallis* oil samples were previously submitted to a chromatographic analysis in order to define their chemical

compositions.

2.1.2 Gas chromatography/mass spectrometry (GC-MS) analysis

The copaiba oil samples were analyzed in a gas chromatograph (Agilent 7890 B) coupled to mass spectrum (Agilent 5977 A) equipped (GC-MS) with an Agilent HP-5MS UI capillary column (30 m X 0.250 mm X 0.25 µm), using the following conditions: 270ºC injector temperature, 1 µL injection volume at a 1:20 ratio (split mode), 2 mL/mincarrier gas (helium) flow, with initial column temperature at 110ºC, gradually heated to 130ºC at a 3ºC/min rate, before increasing to 290ºC at 8.5ºC/min, reaching 300ºC at 10ºC/min rate and being held for 1 min at 300ºC.

The transfer line, ion source, and quadrupole temperatures were 290 °C, 230 °C and 150ºC, respectively. Mass spectra were obtained in a 40-500 (*m/z*) range provided through scan mode with 3-min solvent delay time. The compounds were identified based on comparison with their retention indexes (RI) obtained using several *n*-alkanes (C_8-C_{27}). In addition, their EI-mass spectra were compared with the Wiley 275 spectra and according to Adams (2012).

2.1.3 Principal components analysis (PCA)

The contents of the components identified in the copaiba oil samples constitute a multivariate data set that was interpreted using Principal Component Analysis (PCA). The data was pre-processed using log normalization in order to reduce the influence of undesirable variations in the data set.

A data matrix was created using the values obtained in order to assess the classes of identified compounds and major components. The PCA presented the sample distribution and the importance of the variables. All the mathematical and statistical operations were carried out using the Statistic Software, version 13.3.

2.1.4 Collection and identification of clinical samples

Before the collection of the mammoplasty isolates, the study was submitted to the approval of the Ethics Committee in research involving human beings of the University of Paraná (CAAE 66185417.0.0000.0109). Participants provided their free and informed consent in writing about participation in the research.

Twelve healthy patients who underwent breast reduction and augmentation surgery were used. Postoperative samples were collected with the help of swab two days after the surgical procedure during dressing change. To date the patient had already received two doses of ceftriaxone (1g) as the surgeon's standard protocol. Each sample was cultured in blood agar and incubated for 24 hours at 37ºC.

Subsequently, it was sown in Baird Parker medium 37 °C for 48 hours for the isolation of *Staphylococcus* spp. The macroscopic and microscopic characteristics of the colonies were evaluated through gram staining and biochemical tests. After identification, each sample was frozen at -20°C in Brain Heart Infusion (BHI) with glycerol for future susceptibility testing of copaiba oils and conventional antimicrobials.

2.2 Antibacterial activity

2.2.1 Antibacterial activity in standard strains and growth conditions

Standard strains of NEWPROV (NEWP) and American Type Culture Collection (ATCC) were used. Gram-positive bacteria *Streptococcus pyogenes* (NEWP 0015), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) and gram-negative bacteria *Proteus mirabilis* (NEWP 0133), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). The standard strains of bacteria were kept at -20ºC and were reactivated in BHI medium for 24 hours at 37ºC.

After the turbidity of the BHI broth the inoculum was seeded in blood agar plates plus 5-8% defibrillated ovine blood for gram-positive bacteria and MacConkey gram-negative agar and again incubated at 37ºC for 24 hours. The bacteria standard strains were reactivated by being placed in BHI for 24 hours at 37 ºC. After the BHI broth presented turbidity, 5% sheep blood agar platesfor *S. pyogenes*, Mueller Hinton Agar (MHA) plates for *S. aureus*, and MacConkeyagar plates for gram-negative bacteria and *E. faecalis* were set up again. The bacterial strains were cultured overnight at 37 ºC.

2.2.2 Determination of minimum inhibitory concentration (MIC)

The MIC of copaiba oils was determined according to the procedure established by the Clinical and Laboratory Standards Institute (CLSI, 2018). A standardized bacterial suspension was prepared for each sample according to the McFarland 0.5 scale. Each microorganism was

tested on a microplate consisting of 96 wells in triplicate. Copaiba oils were tested at an initial concentration of 500 mg/mL at serial dilutions (500.00, 250.00, 125.00, 62.50, 31.25, 15.625, 7.81, 3.90, 1.95, 0.97 mg/mL), plus positive and negative controls.

The oils were diluted in an aqueous solution with 2% Tween 80. All assays were placed in wells containing 100 µL BHI medium, 100 µL dilutions of copaiba oil and 5 µL of the bacterial suspension. The plates were homogenized and incubated at 37 ° C for 24h or 48h, depending on the microorganism tested. The reading was performed after the addition of 5 µL of 10% solution of 2,3,5-triphenyltetrazolium chloride and incubated for 30 minutes. Bacterial growth was considered if the wells had any noticeable pink tones after incubation (Gazim et al., 2010).

2.2.3 Determination of antibacterial activity by disc diffusion method

The *in-vitro* antimicrobial activity of copaiba oils was determined using the agar disk diffusion method on the nine clinical samples presenting bacterial growth. Samples were thawed and spiked in mannitol medium (selective for *Staphylococcus* spp.), and their integrity and purity were checked.

The samples were then submitted to the disk sensitivity test according to CLSI (2018). Sterile filter-paper discs (6.5 mm in diameter) were impregnated with 10 µL of the oil (250 mg/mL concentration) and placed on the inoculated plates. Amoxicillin (10 µg), ampicillin (10 µg), azithromycin (15 µg), cefotaxime (30 µg), erythromycin (15 µg), gentamycin (10 µg), linezolid (10 µg), meropenem (10 µg), oxacillin (1 µg), penicillin (10 µg), and vancomycin (30 µg) discs were used as positive references for the bacteria in order to verify the sensitivity of the clinical sample isolates. Discs without samples were used as a negative control.

The plates were incubated at 37 °C for 24 h for the bacterial strains. Tests were also performed where the antibiotics were associated with the oils (synergism) by the addition of 10 µL of the copaiba oil at (concentration of 250 mg/mL) applied on each antibiotic disc. Discs containing only oil were also used to check their isolated actions. Antibacterial activity was evaluated by measuring the diameter of the growth inhibition zones in millimeters (including the disc diameter of 6.5 mm) for the tested organisms and for comparison with the controls. The inhibition zones were measured in three sample replicates, and therefore, the values presented herein are the means of three replicates.

2.2.4 Multidrug resistance index (MRI)

In order to evaluate the multiresistance index of each sample, the formula described by Krumperman (1983), a/b, was used, where ais the number of antibiotics against which the isolate was resistant, and b the number of antibiotics tested. The index chosen to delimit if the samples are high or low risk was 0.200, as in the work cited, where below 0.199 is considered low risk, and above 0.200 considered high risk.

2.3 Statistical analysis

The tests were performed in triplicates and the results were expressed as means followed by their corresponding standard error media (S.E.M.). The data were processed and submitted to Analysis of Variance (ANOVA). Differences between means were determined by Tukey's test with 5% significance level.

3. Results

3.1 Chemical composition

The chemical composition of the copaiba oils as identified using by GC-MS is presented in Table 1 and Figure 1 (A and B).

Table 1. Chemical composition of copaiba (*Copaifera* sp.) oils from four commercial varieties.

Peak	[a]RI	[b]Compounds	Relative area %				Identification Methods
			Oil 1	Oil 2	Oil 3	Oil 4	
1	1322	*δ*-eIemene	1.13	2.86	2.04	1.35	a, b, c
2	1332	*α*-cubebene	0.20	0.97	0.83	0.96	a, b, c
3	1355	cyclosativene	0.24	-	-	-	a, b, c
4	1358	*α*-copaene	0.55	-	7.28	7.36	a, b, c
5	1370	*β*-elemene	-	3.21	1.24	2.99	a, b, c
6	1398	cyperene	0.79	-	0.98	0.73	a, b, c
7	1421	*β*- caryophyllene	24.25	46.91	41.59	32.12	a, b, c
8	1434	*trans*-*α*-bergamotene	8.77	-	3.67	6.90	a, b, c
9	1436	aromadendrene	-	-	0.75	-	a, b, c
10	1439	*epi*-*β*-santalene	0.40	-	0.67	0.71	a, b, c
11	1445	*α*-himachalene	0.26	-	-	-	a, b, c

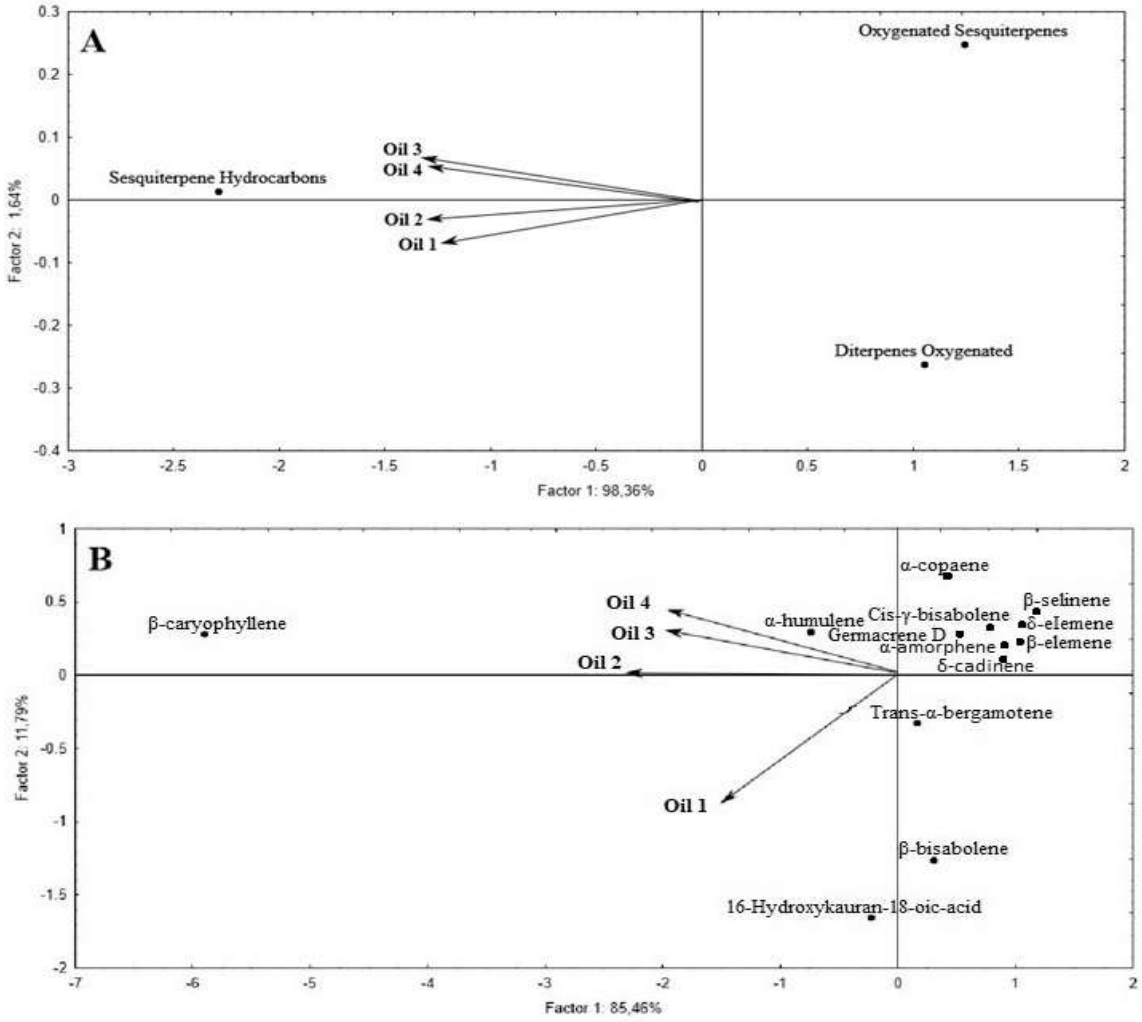
12	1446	*α*-humulene	6.06	13.75	12.04	8.70	a, b, c
13	1448	*α*-patchoulene	0.17	-	-	-	a, b, c
14	1452	*allo*-aromadendrene	0.15	-	-	-	a, b, c
15	1455	*γ* -gurjunene	-	-	0.50	1.77	a, b, c
16	1459	*α*-amorphene	0.87	-	3.74	0.19	a, b, c
17	1461	germacrene D	1.75	5.20	4.36	3.05	a, b, c
18	1462	*β*-selinene	1.42	-	0.65	5.57	a, b, c
19	1463	*δ*-selinene	-	-	0.29	-	a, b, c
20	1464	zingiberene	-	-	-	0.81	a, b, c
21	1465	valencene	-	0.13	-	0.52	a, b, c
22	1466	*α*-selinene	1.26	0.38	-	-	a, b, c
23	1467	bicyclogermacrene	-	-	1.51	-	a, b, c
24	1471	*α*-muurolene	-	-	0.82	1.16	a, b, c
25	1473	*β*-bisabolene	14.30	-	1.49	1.12	a, b, c
26	1474	*γ*-cadinene	0.46	-	0.95	-	a, b, c
27	1478	*cis*-*γ*-bisabolene	-	-	-	3.27	a, b, c
28	1482	*δ*-cadinene	1.86	-	3.34	1.28	a, b, c
29	1569	*trans*-*γ*-bisabolene	0.18	-	0.23	3.93	a, b, c
30	1571	*trans*-cadina-1,4-diene	-	-	0.15	0.25	a, b, c
31	1572	*α*-cadinene	2.06	-	0.16	-	a, b, c
32	1578	*α*-calacorene	-	-	0.10	0.44	a, b, c
33	1581	selina-3,7(11)-diene	-	-	-	0.50	a, b, c
34	1583	germacrene B	0.11	1.94	0.73	0.21	a, b, c
35	1588	7-*epi*-*α*-cadinene	-	-	-	0.11	a, b, c
36	1595	selin-7(11) -en-4-ol	-	-	-	0.09	a, b, c
37	1598	palustrol	-	-	0.27	0.99	a, b, c
38	1603	spathulenol	0.18		0.05	1.85	a, b, c
39	1604	caryophyllene oxide	0.29	0.19	1.21	0.30	a, b, c
40	1608	globulol	0.19	-	0.05	0.20	a, b, c
41	1611	viridiflorol	-	1.89	0.80	0.12	a, b, c
42	1614	cedrol	-	0.95	-	0.25	a, b, c
43	1617	guaiol	-	0.09	0.25	0.11	a, b, c
44	1619	ledol	-	0.16	0.20	0.80	a, b, c
45	1621	γ-eudesmol	0.17	0.10	-	0.36	a, b, c
46	1627	*α*-acorenol	0.10	0.18	0.16	0.40	a, b, c
47	1628	*epi*-*α*-muurolol	-	0.14	0.10	1.01	a, b, c
48	1630	*α*-muurolol	-	0.06	0.06	0.88	a, b, c
49	1632	cubenol	0.13	0.15	0.56	0.22	a, b, c
50	1636	*α*-cadinol	-	0.20	0.27	0.76	a, b, c
51	1642	*cis*-*α*-santalol	1.78	0.24	0.75	0.34	a, b, c
52	1643	2,6-*cis*-farnesal	-	-	1.34	0.05	a, b, c
53	1649	8-cedren-13-ol	0.13	-	-	-	a, b, c

54	1656	shyobunol	2.32	0.30	-	0.32	a, b, c
55	1666	eudesm-7(11)-en-4-ol	0.24	0.18	0.68	0.36	a, b, c
56	1667	2,6-*trans,cis*-farnesol	0.32	0.60	0.16	-	a, b, c
57	1694	*cis*-*β*-santalol	0.41	1.45	0.75	-	a, b, c
58	1708	methyl eudesmate	0.66	-	0.06	-	a, b, c
59	1712	*trans*-*β*-santalol	0.16	-	0.15	-	a, b, c
60	1750	*β*-costol	0.35	-	0.47	-	a, b, c
61	1791	ni	0.44	-	-	-	a, b, c
62	1794	ni	-	-	-	0.03	a, b, c
63	1913	2,6-*trans*-farnesyl acetate	0.10	-	-	0.04	a, b, c
64	1925	sclareoloxide	0.56	-	-	-	a, b, c
65	1930	costunolide	-	-	-	0.15	a, b, c
66	1931	ni	-	-	-	0.04	a, b, c
67	1932	ni	-	-	-	0.23	a, b, c
68	1994	kaur-15-ene	1.40	-	-	0.78	a, b, c
69	2039	kaur-16-ene	0.26	-	-	0.03	a, b, c
70	2040	Ni	0.25	-	-	-	a, b, c
71	2043	ni	0.19	-	-	-	a, b, c
72	2061	*epi*-13-manool	1.36	1.93	0.44	0.41	a, b, c
73	2199	sclareol	-	0.74	0.09	0.97	a, b, c
74	2300	(+)-copaiferic acid	0.17	-	-	0.22	a, b, c
75	2307	verticillol	-	-	0.14	0.34	a, b, c
76	2311	ni	-	-	-	0.28	a, b, c
77	2318	ni	-	-	-	0.41	a, b, c
78	2324	labd-7-en-15-oic acid	1.32	1.64	-	0.26	a, b, c
79	2334	2,6,10,14-hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E,E,E)-	1.86	-	-	0.08	a, b, c
80	2401	n.i	0.21	-	-	0.29	a, b, c
81	2483	16-hydroxykauran-18-oic acid	16.89	13.18	-	-	a, b, c
Total Identified			**98.59**	**99.72**	**99.12**	**98.69**	
Sesquiterpene Hydrocarbons			67.24	75.35	90.11	86.00	
Oxygenated Sesquiterpenes			6.77	6.88	8.28	9.56	
Diterpenes Hydrocarbons			1.66	-	-	0.81	
Diterpenes Oxygenated			19.74	17.49	0.67	2.20	
Other Compounds			3.18	0.00	0.06	0.12	
Unidentified Compound			1.09	0.00	0.00	1.28	

[a]RI = identification based on the calculated retention index (RI) utilizing a standard homologous series of *n*-alkanes C_7-C_{25} in Agilent HP-5MS UI column; [b]Compounds = compounds listed in elution order in HP-5MS UI column; [c]Identification based on comparison with mass spectra from Wiley 275 spectra and according to Adams[17]; Relative area (%): percentage of the area occupied by the compound within the chromatogram; ni = unidentified compound.

A total of 72 compounds were identified, accounting for 99.12 - 99.72% of the volatile constituents in the copaiba oil samples (Table 1).

Figure 1. Principal components analysis (PCA) score and loading biplot for the gas chromatograph coupled to mass spectrum (GC-MS) volatile profile of copaiba oil (Oil 1, Oil 2, Oil 3 and Oil 4) samples. A: PCA biplot evaluating the classes of the identified compounds. B: PCA biplot evaluating the major components.



Source: Authors.

Figure 1 presents the difference in chemical composition of the four samples of commercial copaiba oils. Sesquiterpene hydrocarbons were the dominant compounds, comprising 67.24– 90.11% of the copaiba oil samples, as presented in Figure 1A. The PCA analysis (Figure 1B) presented the formation of four groups, with different information: first the separation of Oil 1 from the other oils (2, 3 and 4).

This separation occurred due to the presence of *β*-caryophyllene and 16-hydroxykauran-18-oic acid. The *β*-caryophyllene is the major compound and found in the highest concentration in Oil 2 (46.91%); Oil 3 (41.59%) and Oil 4 (32.12%). It was present in Oil 1 in a smaller amount (24.25%). 16-hydroxykauran-18-oic acid was found in both Oil 1 (16.89%) and Oil 2 (13.18%). Humulene was also found in high concentrations in the four oil samples Oil 2 (13.75%), Oil 3 (12.04%), Oil 4 (8.70%) and Oil 1(6.06%).The following compounds were present in high concentrations only in this sample: *β*-bisabolene (14.30%); *Trans-α*-bergamotene (8.77%) in Oil 1; *α*-copaene (7.28 and 7.36%) in Oil 3 and 4, respectively.

After determining the composition of the copaiba oils, the purpose of this researchwas to evaluate their bactericidal effectiveness on standard and clinical strain samples.

3.2 Antibacterial activity

Table 2 presents the results obtained for the antibacterial activity of commercial copaiba oils against standard bacterial strains using the broth microdilution method.

Table 2. Minimum Inhibitory Concentration (mg/mL) of four commercial copaiba (*Copaifera*sp.) oils on standard bacterial strains.

Microorganisms	Source	Oil 1	Oil 2	Oil 3	Oil 4
ProteusMirabilis	NEWP0133	500 ± 0.0	>500.00	>500.00	500 ± 0.0
Staphylococcus aureus	ATCC25923	52.08 ± 10.41*	104.16 ± 72.91*	333.33 ± 83.33	>500.00
Streptococcuspyogenes	NEWP0015	333.33 ± 83.33	104.16 ± 20.83#	333.33 ± 83.33	125.0 ± 72.16
Enterococcusfaecalis	ATCC29212	>500.00	>500.00	>500.00	500 ± 0.0
Escherichia coli	ATCC25922	>500.00	>500.00	>500.00	500 ± 0.0
Pseudomonasaeruginosa	ATCC27853	416.66 ± 83.33	>500.00	>500.00	>500.00

Values expressed as median ± S.E.M. for triplicate experiments. Statistical comparison performed using one-way ANOVA followed by Tukey's test.*$p \leq 0.05$ when compared with Oil 3 group. #$p \leq 0.05$ when compared with Oil 1 and Oil 3 groups. Source: Authors.

The one-way ANOVA indicated a difference among the groups treated with different copaiba commercial oils against the standard strains ($F(23.48) = 25.37$, $p < 0.001$). Tukey's *post-hoc* showed that Oil 1 (52.08 mg/mL) and Oil 2 (104.16 mg/mL) were the most active on the *S. aureus* strain with MIC ($p<0.05$) when compared to Oil 3. No activity effect was observed in Oil 4 against *S. aureus*. It was also observed that Oil 2 (104.16 mg/mL) was more effective when compared to Oil 1 and Oil 3 against *S. pyogenes* ($p<0.05$). However, no significant effect was found when comparingOil 4to Oil 1 and Oil 3 against *S. pyogenes* ($p=0.08$).

Table 2 also showed that Oil 1 and Oil 4were active against *P. mirabilis* strains (500 mg/mL). *E. faecalis* and *E. coli* were sensitive to Oil 4 (500 mg/mL), and *P. aeruginosa* was only inhibited by Oil 1 (416.66 mg/mL).After the *in-vitro* screening for antibacterial activity in the four copaiba commercial oils against the bacterial strains, bactericidal action against the bacteria isolated from postoperative mammoplasty wounds was assessed and the results are presented in Table 3.

Table 3. Minimum Inhibitory Concentration (mg/mL) of four commercial copaiba(*Copaifera*sp.) oils on postoperative mammoplasty field samples.

Field Sample	Coagulase	Oil 1	Oil 2	Oil 3	Oil 4
*Staphylococcus*sp.	Positive	7.81 ± 0.0*	7.81 ± 0.0*	166.66 ± 41.66*	416.66 ± 83.33
*Staphylococcus*sp.	Negative	21.35 ± 8.81*	9.63 ± 0.89*	15.10 ± 3.13*	141.66 ± 29.94

Values expressed as median ± S.E.M. for triplicate experiments. Statistical comparison performed using two-way ANOVA followed by Tukey's test. *p ≤ 0.001 when compared with Oil 4 group. Source: Authors.

Staphylococcus coagulase positive and negative coagulase samples were identified, and of the 12 samples collected, only 9 presented bacterial growth.

The two-way ANOVA showed a significant difference for the following factors: groups (Oil 1, 2, 3, and 4), $F(3,124) = 23.70$, $p<0.001$; coagulase (positive, negative), $F(1.124) = 15.72$, $p<0.001$; interaction between groups X Coagulase $F(3,124) = 7.00$, $p<0.001$. Tukey's *post hoc* showed that, except for Oil 4, all others presented bactericidal activity against coagulase positive (MIC: 7.81 – 166.66 mg/mL) and negative *Staphylococcus* sp. (MIC: 9.63 – 21.35 mg/mL) ($p<0.001$) (Table 3).

Table 4 shows the results determined by the agar disk diffusion method for the susceptibility variation to *Staphylococcus* sp. isolated from postoperative mammoplasty wounds for amoxicillin, ampicillin, azithromycin, cefotaxime, erythromycin, gentamycin, linezolid, meropenem, oxacillin, penicillin, and vancomycin associated with the four groups of copaiba oils.

Table 4. Antimicrobial activity of four commercial copaiba (*Copaifera* ssp.) oils on postoperative mammoplasty field samples determined by agar disk diffusion method.

Group	Oil 1		Oil 2		Oil 3		Oil 4	
	Halo (mm)	INT	Halo (mm)	INT	Halo (mm)	INT	Halo (mm)	INT
Control	1.89 ± 1.27	-	3.44 ± 1.39	-	1.44 ± 0.98	-	0 ±0	-
AMO	23.11 ± 1.75*	R	23.11 ± 1.75*	R	23.11 ± 1.75*	R	23.11 ± 1.75*	R
AMO + Oil	25.78 ± 2.86*	R	29.0 ± 3.58*	S	28.0 ± 4.37*	R	25.66 ± 2.66*	R
AMP	24.0 ± 2.65*	S	24.0 ± 2.65*	S	24.0 ± 2.65*	S	24.0 ± 2.65*	S
AMP + Oil	26.33 ± 1.84*	S	29.55 ± 3.51*	S	28.78 ± 4.45*	S	27.55 ± 2.66*	S
AZI	8.44 ± 4.99	R	8.44 ± 4.99	R	8.44 ± 4.99	R	8.44 ± 4.99	R
AZI + Oil	5.22 ± 3.08	R	5.33 ± 3.53	R	4.0 ± 2.89	R	2.78 ± 2.77	R
CEF	28.44 ± 1.91*	S	28.44 ± 1.91*	S	28.44 ± 1.91*	S	28.44 ± 1.91*	S
CEF + Oil	31.00 ± 1.90*	S	33.77 ± 2.41*	S	33.22 ± 2.35*	S	31.55 ± 2.42*	S
ERI	19.22 ± 5.50	I	19.22 ± 5.51	I	19.22 ± 5.51	I	19.22 ± 5.50*	I
ERI + Oil	16.00 ± 3.56	I	17.89 ± 4.14	I	21.44 ± 4.22*	I	15.89 ± 5.77	S
GEN	22.89 ± 1.77*	S	22.89 ± 1.77*	S	22.89 ± 1.77*	S	22.89 ± 1.77*	S
GEN + Oil	24.22 ± 1.92*	S	24.55 ± 1.71*	S	24.89 ± 1.60*	S	25.0 ± 2.42*	S
LIN	37.0 ± 1.81*	S	37.0 ± 1.81*	S	37.0 ± 1.81*	S	37.0 ± 1.81*	S
LIN + Oil	39.44 ± 1.66*	S	34.44 ± 4.57*	S	35.0 ± 4.80*	S	33.55 ± 4.74*	S
MEN	32.89 ± 2.89*	S	32.89 ± 2.89*	S	32.89 ± 2.89*	S	32.89 ± 2.89*	S
MEN + Oil	35.67 ± 2.75*	S	40.78 ± 2.90*	S	39.66 ± 3.08*	S	34.55 ± 3.40*	S
OXA	15.55 ± 3.47	R	15.55 ± 3.47	R	15.55 ± 3.47	R	15.55 ± 3.47	R
OXA + Oil	19.11 ± 2.31	R	19.66 ± 3.45	R	17.55 ± 3.20	R	11.86 ± 3.26	R
PEN	17.00 ± 2.10	R	17.0 ± 2.10	R	17.0 ± 2.10	R	17.0 ± 2.10	R
PEN + Oil	20.31 ± 2.80*	R	23.0 ± 3.86*	R	23.55 ± 4.29*	R	19.66 ± 3.08*	R
VAN	24.11 ± 1.13*	S	24.11 ± 1.13*	S	24.11 ± 1.13*	S	24.11 ± 1.13*	S
VAN + Oil	24.66 ± 1.10*	S	21.89 ± 2.84*	S	21.66 ± 2.97*	S	20.22 ± 3.12*	S

Values expressed as median ± S.E.M. of inhibition halos (mm), (n = 9). Statistical comparison performed using one-way ANOVA followed by Tukey's test. INT: Interpretation, AMO: amoxicillin, AMP: ampicillin, AZI: azithromycin, CEF: cefotaxime, ERI: erythromycin, GEN: gentamycin, LIN: linezolid, MEN: meropenem, OXA: oxacillin, PEN: penicillin, VAN: vancomycin. R: resistant, I: intermediary, S: sensitivity. Interpretation according to CLSI, 2015. * $p \leq 0.001$ when compared with control group. Source: Authors.

It is observed that oil 2 presented synergism when associated with amoxicillin, where *Staphylococcus* sp. was sensitive to the resistance antibiotic.

The MRI of *Staphylococcus* sp. isolated from mammoplasty are in Table 5, which ranged from 0.091 to 0.636 and eight of the nine bacteria had MAR higher than 0.200.

Table 5. Multiple antibiotic resistance (MAR) index of bacteria isolated from postoperative mammoplasty.

Samples	Absolute number of resistances	MAR index
1	3	0.273
2	3	0.273
3	3	0.273
4	5	0.455
5	5	0.455
6	6	0.545
7	4	0.364
8	1	0.091
9	7	0.636

Note: MAR was calculated according to the method described by Krumperman (1983). Source: Authors.

4. Discussion

This study assessed the antibacterial activity of four copaiba oils commercially available. Initially, the four oils were analyzed in order to obtain their chemical composition in order to know their major components using GC-MS. The results showed that the samples had mainly sesquiterpenes, the most common being *β*-caryophyllene, 16-hydroxykauran-18-oic acid, Humulene, *Trans*-*α*-bergamotene and *α*-copaene. The presence of sesquiterpenes in approximately 80% of the oils was also found by Veiga Junior and Pinto (2002), with the most common being *α*-copaene, *β*-caryophyllene, *β*-bisabolene, *α* and *β*-selinene, *α*-humulene, and *δ* and *γ*-cadinene. It is known that there are several species of copaiba with an immense variety of chemical compounds already identified; however, the presence of sesquiterpenes suggests a relationship between the structure and the activity produced by the compounds (Santos et al., 2008).

After the compounds were identified, *in-vitro* bactericidal activity was assessed on samples obtained from standard strains and isolated from postoperative mammoplasty wounds. Growth inhibition was observed in standard strains of *S. aureus*. It can be concluded that copaiba oil has chemical compounds capable of inhibiting microorganisms, thus justifying its use as an antimicrobial agent in the treatment of human, animal and conservation infections (Pieri et al., 2012). In fact, sesquiterpenes are herbal secondary metabolites with bactericidal and bacteriostatic activities against gram-positive bacteria (Teng et al., 2010). These compounds are widely present in the oils tested in this study, corroborating with Goren et al. (2011), who observed *β*-caryophyllene antibacterial activity against *E. coli*, *S. aureus*, *P.*

aeruginosa, *C. albicans*. However, according to Leandro et al. (2012), the functionality of the copaiba oil compounds should not be attributed in isolation, but synergistically.

In fact, it is possible that different sesquiterpenes present in the copaiba oils present a synergistic interaction, thus contributing to the antibacterial effects observed in this investigation. It should also be clarified that there is a common chemical variation and biological activity between the different species of copaiba and even in similar species with collections carried out in different places (Cascon & Gilbert, 2000). According to Arruda et al. (2009) these differences are due to factors such as climate, soil, genetics, complex chemical composition in relation to oleoresin and other parts of the plant; which may influence the presence and quantity of each compound, in addition to frequent cases of adulteration.

The antimicrobial activity of gurjunene (Njume, Afolayan, Green & Ndip, 2011), *β*-elemene (Hashim, Sirat & Yen, 2014), viridiflorol (Monzote, Scull, Cos & Setzer, 2017), germacrene B (El-Kalamouni et al., 2017), and *cis*-*β*-santalol (Matsuo, Sakagami &Mimaki, 2014) is well described in the literature. In the same way, the antimicrobial effects of 16-hydroxykauran-18-oic acid, widely present in Oils 1 and 2, were also described (Saepou et al., 2010; Sebisubi et al., 2010).

It can also justify that antimicrobial synergism can occur in several situations, among them that the drug can affect the cell membrane, facilitating the penetration of the second drug or substance or two drugs can block the microbial metabolic pathway, thus, the combined action is significantly superior to the effect of both (Sebisubi et al., 2010), but it should be noted that even with the absence of inhibition halo in the other samples, the presence of antimicrobial substances cannot be ruled out. According to Valgas et al (2007), the free hydroxyl groups present in each glucose residue makes the surface of the disk hydrophilic, thus, if the natural products were cationic, they would attach to the surface of the disk and would not diffuse into the medium, interfering with the susceptibility of the antibiogram.

The observed differences in antibacterial activity through broth and disk diffusion in agar shows that the latter may not be the best alternative for the evaluation of copaiba oil. The results are directly affected by the intrinsic limitations of each technique and reinforce the importance of using more than one approach in the evaluation of natural products (Balouiri, Sadiki & Ibnsouda, 2016; Nascimento et al., 2007).

The MRI above 0.2 in 88.89% of the samples demonstrates the importance of the results of the antibacterial activity of copaiba oil. This index reflects these samples were frequently exposed to antibiotics and which present high public health risk. The major problem is that the increasing number of resistant strains is not accompanied by the development of new

antimicrobial drugs (Fair & Tor, 2014) and that natural products should be exploited as a source of new biologically active molecules targeting alternatives to existing treatments (Negi, 2012).

This study shows that the copaiba oils haveantibacterial activity action on standard strains and onclinical samples, suggesting that copaiba oil may be a potential source forassistance in the treatment of surgical infections and to help accelerate the healing process. It should also be noted that there are several reasons justifying the urgent need for new antibiotic agents, such as: infectious diseases are the second greatest cause of mortalityworldwide; high rates of microbial resistance, especially in hospital settings; and the need for agents that act with action mechanisms other than the drugs currently in use (Coates & Hu, 2007; Payne et al., 2007). In this sense, natural antibiotics are an important source of research since they usually present complex chemical structures that are important for specific interactions and recognition by macromolecular targets in pathogenic bacteria (Walsh, 2003).

5. Conclusion

Different copaiba commercial oils tested (oils 1, 2 and 3) produced antibacterial action in standard strains and isolated mammoplasty. This antibacterial activity of the oils in question can be justified by its main chemical constituent, β-caryophyllene. The evaluation of bacterial susceptibility through the disc-diffusion assay has shown that oil 2 can produce a synergistic effect when associated with amoxicillin and may be a therapeutic option.

In view of the promising results, it is suggested to carry out toxicity studies in animal models and subsequently to evaluate the clinical efficacy in the postoperative period of patients undergoing mammoplasty.

Acknowledgments and Funding Information

The authors would like to thank Eloisa Schneider Silva, Isabel Cristina da Silva Caetano, and Carlos Emilio Weingartner for the inestimable help with experiments. They also wish to thank Universidade Paranaense (Protocol 33188/2018) and Fundação Araucária (Protocol 48414.506.48325.25052017) for providing the research grant and fellowships that enabled this study.

Conflicts of Interest

The authors have nothing to disclose.

References

Adams, R. P. (2012). Identification of essential oils components by gas chromatography/mass spectroscopy. (4a ed.), AlluredBussiness Media, USA, 804.

Arruda, C., Mejía, J. A. A., Ribeiro, V. P., Borges, C. A. G., Martins, C. H. G., & Veneziani, R. C. S. et al. (2009). Occurrence, chemical composition, biological activities and analytical methods on Copaifera genus – a review. Biomed.Pharmacother. 109, 1–20.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: a review. J. Pharm. Anal.6,71–79.

Barbosa, P. C. S., Medeiros, R. S., Sampaio, P. T. B., Vieira, G., Wiedemann, L. S. M., & Veiga-Junior, V. F. (2012). Influence of abiotic factors on the chemical composition of copaiba oil (Copaiferamultijuga Hayne): soil composition, seasonality and diameter at breast height. J. Braz. Chem. Soc.23, 10, 1823–1833.

Belletti, N., Ndagijimana, M., Sisto, C., Guerzoni, M. E., Lanciotti, R., & Gardini, F. (2004). Evaluation of the antimicrobial activity of citrus essences on Saccharomyces cerevisiae. J. Agric. Food Chem. 52, 6932–6938.

Cascon, V., & Gilbert, B. (2000). Characterization of the chemical composition of oleoresins of CopaiferaguianensisDesf., Copaiferaduckei Dwyer and Copaiferamultijuga Hayne. Phytochem.55, 773–778.

Chiavari-Frederico, M. O., Barbosa, L. N., dos Santos, I. C., da Silva, G. R., de Castro, A. F., Bortolucci, W. C., Barboza, L. N., Campos, C. F. A. A., Gonçalves, J. E., Menetrier, J. V., Jacomassi, E., Gazim, Z. C., Wietzikoski, S., Lívero, F. A. R., Wietzikoski Lovato, E. C. (2020) Antimicrobial activity of Asteraceae species against bacterial pathogens isolated from

postmenopausal women. Plos one. 5(1), e0227023. https://doi.org/10.137 1/journal.pone.0227023

CLSI. (2018). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. 4th ed. CLSI supplement VET08. Wayne, PA: Clinical and Laboratory Standards Institute. 1–170.

Coates, A. R. M., & Hu, Y. (2007). Novel approaches to developing new antibiotics for bacterial infections. Br. J.Pharmacol. 152, 1147–1154.

El-Kalamouni, C., Venskutonis, P. R., Zebib, B., Merah, O., Raynaud, C., & Talou, T. (2017). Antioxidant and antimicrobial activities of the essential oil of Achilleamillefolium L. grown in France. Medicines. 4, 1-10.

Fair, R. J., & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. Perspect.Medicin. Chem. 6, 25–64.

Feldman, E. M., Kontoyiannis, D. P., Sharabi, S. E., Lee, E., Kaufman, Y., & Heller, L. (2010). Breast implant infections: is cefazolin enough? Plast.Reconstr. Surg.126, 779–785.

Franco, D., Cardoso, F. L. L., & Franco, T. (2006). Antibiotic use in plastic surgery. Rev. Soc. Bras. Cir.Plast.21, 112–115.

Fung, H. B., Kirschenbaum, H. L., & Ojofeitimi, B. O. (2001). Linezolid: an oxazolidinone antimicrobial agent. Clin.Ther. 23, 356–391.

Gazim, Z. C., Amorim, A. C. L., Hovell, A. M. C., Rezende, C. M., & Nascimento, I. A. et al. (2010). Seasonal variation, chemical composition, and analgesic and antimicrobial activities of the essential oil from leaves of Tetradeniariparia(Hochst.) codd in southern Brazil. Mol.15, 5509–5524.

Goren, A. C., Piozz, F., Akcicek, E., Kiliç, T., Çarikçi, S., & Mozioglu, E. et al. (2011). Essential oil composition of twenty-two Stachysspecies (mountain tea) and their biological activities. Phytochem.Lett. 4, 448–453.

Gravante, G., Caruso, R., Araco, A., & Cervelli, V. (2008). Infections after plastic procedures: incidences, etiologies, risk factors, and antibiotic prophylaxis. Aesth.Plast. Surg.232, 243–251.

Gutwein, L. G., Panigrahi, M., Schultz, G. S., & Mast, B. A. (2012). Microbial barriers. Clin.Plast. Surg. 39, 229–238.

Hashim, S. E., Sirat, H. M., & Yen, K. H. (2014). Chemical compositions and antimicrobial activity of the essential oils of Hornstedtiahavilandii (Zingiberaceae). Nat. Prod.Commun. 9, 119–120.

Jawetz, E., Melnick, J. L., Adelberg, E. A., Jawetz, Melnick & Adelberg's. (1991). Med. Microbiol. 19ªed. Typopress, Beirut, Lebanon. 632.

Kaye, K. S., Anderson, D. J., Sloane, R., Chen, L. F., Choi, Y., & Link, K. et al. (2009). The impact of surgical site infection on older operative patients. J. Am.Geriatr. Soc. 57, 46–54.

Khan, U. D. (2010). Breast augmentation, antibiotic prophylaxis, and infection: comparative analysis of 1,628 primary augmentation mammoplasties assessing the role and efficacy of antibiotics prophylaxis duration. Aesth.Plast. Surg.34, 42–47.

Krumperman, P. H. (1983). Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. Appl.Environ. Microbiol.46, 165–170.

Leandro, L. M., Varas, F. S., Barbosa, P. C., Neves, J. K., Silva, J. A., & Veiga-Junior, V. F. (2012). Review: chemistry and biological activities of terpenoids from copaiba (Copaifera spp.) oleo-resins. Mol.17, 3866–3889.

Matsuo, Y., Sakagami, H., & Mimaki, Y. (2014). A rare type of sesquiterpene and β-santalol derivatives from Santalum album and their cytotoxic activities. Chem. Pharm. Bull. 62, 1192–1199.

Monzote, L., Scull, R., Cos, P., & Setzer, W. N. (2017). Essential oil from Piper aduncum: chemical analysis, antimicrobial assessment, and literature review. Medicines 4, 49.

Nascimento, P. F. C., Nascimento, A. C., Rodrigues, C. S., Antoniolli, A. R., Santos, P. O., & Barbosa Junior, A. M. et al. (2007). Antimicrobial activity of the Essentials oils: a multifactor approach of the methods. Rev. Bras.Farmacogn. 17, 108–113.

Negi, P. S. (2012). Plant extracts for the control of bacterial growth: efficacy, stability and safety issues for food application. Int. J. Food Microbiol. 156, 7–17.

Njume, C., Afolayan, A. J., Green, E., & Ndip, R. N. (2011). Volatile compounds in the stem bark of Sclerocaryabirrea (Anacardiaceae) possess antimicrobial activity against drug-resistant strains of Helicobacter pylori. Int J.Antimicrob. Agents. 38, 319–324.

Okdakowska-Jedynak, U., Paczek, L., Krawczyk, M., Zieniewicz, K., Nyckowski, P., & Pawlak, J. et al. (2003). Resistance of gram-positive pathogens to antibiotic is a therapeutic challenge after liver transplantation: clinical experience in one center with linezolid. Transplant. Proc. 35, 2304–2306.

Payne, D. J., & Gwynn, M. N., Holmes, D. J., Pompliano, D. L. (2007). Drugs for bad bugs: confronting the challenges of antibacterial discovery. Nat. Rev. Drug.Discov. 6, 29–40.

Pereira, A. S., Shitsuka, D. M., Pereira, F. J., Shitsuka, R. (2018) Metodologia do trabalho científico. Santa Maria: UAB / NTE / UFSM.

Pieri, F. A., Silva, V. O., Souza, C. F., Costa, J. C. M., Santos, L. F., & Moreira, M. A. S. (2012). Antimicrobial profile screeningoftwooilsofCopaifera genus. Arq. Bras. Med. Vet.Zootec.64, 241–244.

Saepou, S., Pohmakotr, M., Reutrakul, V., Yoosook, C., Kasisit, J., & Napaswad, C. et al. (2010). Anti-HIV-1 diterpenoids from leaves and twigs of Polyalthiasclerophylla. Planta Med.76, 721–725.

Santos, A. O., Ueda-Nakamura, T., Dias Filho, B. P., Veiga Junior, V. F., Pinto, A. C., & Nakamura, C. V. (2008). Antimicrobial activity of Brazilian copaiba oils obtained from different species of the Copaifera genus. Mem. Inst. Oswaldo Cruz. 103, 277–281.

Sebisubi, F. M., Odyek, O., Anokbonggo, W. W., Ogwal-Okeng, J., Carcache-Blanco, E. J., & Ma, C. et al. (2010). Antimalarial activity of Aspiliapruliseta, a medicinal plant from Uganda. Planta Med.76, 1870–1873.

Spear, S. L., & Seruya, M. (2010). Management of the infected or exposed breast prosthesis: a single surgeon's 15-year experience with 69 patients. Plast.Reconstr. Surg.125, 1074–1084.

Teng, Y., Yang, Q., Yu, Z., Zhou, G., Sun, Q., & Jin, H. et al. (2010). In vitro antimicrobial activity of the leaf essential oil of Spiraeaalpina Pall. World J.Microbiol.Biotechnol. 26, 9–14.

Valgas, C., Souza, S. M., Smânia, E. F. A., & Smânia, A. J. (2007). Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol. 38, 369–380.

Veiga Junior, V. F., & Pinto, A. C. (2002). The Copaifera L. genus. Quím. Nova. 25, 273–286.

Walsh, C. (2003). Where will new antibiotics come from? Nat. Rev.Microbiol. 1, 65–70.

Wietzikoski Lovato, E. C., Gurgel Velasquez, P. A., dos Santos Oliveira, C., Baruffi, C., Anghinoni, T., Machado, R. C., Lívero, F. A. R., Sato, S. W., Martins, L. A. (2018). High frequency equipment promotes antibacterial effects dependent on intensity and exposure time. Clin Cosmet Investig Dermatol. 11, 131-135. https://doi.org/10.2147/CCID.S156282

Zetola, N., Francis, J. S., Nuermberger, E. L., & Bishai, W. R. (2005). Community-acquired methicillin-resistant Staphylococcus aureus: an emerging threat. Lancet. Infect. Dis. 5, 275–286.

Percentage of contribution of each author in the manuscript

Raquel Costa Machado – 13,2%

Ana Karina Vargas Soares – 3,1%

Isabela Carvalho dos Santos – 3,1%

Wanessa de Campos Bortolucci – 3,1%

Luis Fernando Espinoza Luizar – 2,2%

Caio Franco de Araújo Almeida Campos – 3,1%

José Eduardo Gonçalves – 3,1%

Lidiane Nunes Barbosa – 13,2%

Samantha Wietzikoski – 3,1%

Lisiane de Almeida Martins – 13,2%

Zilda Cristiani Gazim – 13,2%

Francislaine Aparecida dos Reis Lívero – 13,2%

Evellyn Claudia Wietzikoski Lovato – 13,2%