

# Evaluation of antimicrobial activity of *Copaifera sp.*

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**Abstract**—The oil-resin of *Copaifera sp.* it is excreted from *copaibeira*, an endemic plant in Brazil. Its main medicinal properties are: anti-inflammatory activity, healing and antibacterial action. Its therapeutic potential occurs due to its chemical constituents sesquiterpenes and diterpenes, which are:  $\beta$ -karyophyllene,  $\alpha$ -copaene and copalic acid. The present work evaluates the activity of *Copaifera sp.* against the strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The technique used was the dilution in broth in Agar Müller Hinton medium, in concentrations 0.5%, 2.5%, 5.0%, 7.5% and 10.0%, aiming to analyze the oil inhibiting power in the bacterial growth. A comparison with conventional antimicrobials, aminoglycosides (gentamicin),  $\beta$ -lactams / cephalosporins (cephalothin), phenicols (chloramphenicol) and tetracyclines, was also performed. The preliminary antibacterial results were considered satisfactory against the *Staphylococcus aureus* strain, completely inhibiting its growth, whereas on the strains of *Pseudomonas aeruginosa* and *Escherichia coli*, the inhibition was considered weak to moderate.

**Keywords** —Oil-resin, Antibacterial, *Copaifera sp.*, Agar Müller Hinton.

## I. INTRODUCTION

Popular medicine is the basis of the knowledge acquired in relation to medicinal plants, known for centuries and practiced worldwide, being in some populations the only resource available in the treatment of diseases. Despite their chemical complexity, plant-based drugs are widely used in

modern medicine, based on therapeutic experiences gathered over the years (Maciel, Pinto, Veiga Junior, Grynberg, & Echevarria, 2002).

Brazil has a very rich flora, although, there is a lack of studies that prove the pharmacological action of these drugs. However, popular knowledge has been helping research

regarding pharmacological actions, such as copaiba oil and its anti-inflammatory and healing properties, which can be found in numerous places, such as fairs and pharmacies for natural products (Packer & Luz, 2007).

From the perspective of ethnopharmacology, the use of some plant inputs, with specific pharmacological actions, such as anti-inflammatory, analgesic, antimicrobial, among others, is crucial to indicate the path in the production of new plant-based drugs (Scudeller, Rosa, & Barbosa, 2007). Plants belonging to the *Copaifera* genus are popularly known as copaiba, pau-d'óleo and copaibeira (Plowden, 2004) represented by many species, more than 72 in the world and approximately 16 endemic in Brazil, as reported by Veiga Júnior and Pinto (2002).

Pharmacologically evaluating copaiba oil, it was found that its use is well used, since *in vitro* and *in vivo* studies demonstrate anti-inflammatory, healing, trypanosomicidal, antitumor and antiedematogenic activities produced by the oil-resin (Soares et al., 2003), other research demonstrates its role as an antimicrobial (Cotoras & Mendoza, 2004; Santos et al., 2008), as well as an edema inhibiting agent (Francisco, 2005).

Recent researches has demonstrated the composition of copaiba oils, which invariably contains sesquiterpenes and diterpenes. The sesquiterpenes  $\beta$ -karyophyllene,  $\alpha$ -copaene and copalic acid (diterpene), are undoubtedly the main compounds of the oil-resin. The authors also state that in studies with different species and from different regions of Brazil, they all have this acid in common, therefore being considered a biomarker (Veiga Junior & Pinto, 2005; Soares et al., 2003). Pereira et al. (2008), quantifies the portion of karyophyllene oxide that varies from 33, 72% to 38, 98% and reaffirms it as a major compound of *Copaifera langsdorffii* oil-resin. In addition, Maciel et al. (2002), reports that diterpenes have been proven to be responsible for most therapeutic properties.

Attention to antimicrobial substances has been promoting the search for plant extracts capable of providing efficient action against certain agents. Several studies report on this action, demonstrating effectiveness against sensitive and resistant microorganisms, emphasizing the potential of plants in drug therapy (Nascimento, Locateli, Freitas, & Silva, 2000). According to Cowan (1999), most of the drugs sold come from plant bases, however these resources are not commonly used for antibacterial therapy.

The worsening of bacterial resistance in recent years, mainly in relation to microorganisms isolated from patients with infection, has produced large-scale studies on the pharmacological actions of antimicrobials (Oliveira et al., 2006). It is known that bacterial resistance is frequent and

quite confusing, bacteria of great importance such as: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Campylobacter jejuni*, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Escherichia coli*, *Enterococcus sp.*, *Shigella sp.*, *Salmonella sp.* and *Vibrio cholerae*, have strains resistant to the most advanced antibiotic therapy (Gurgel & Carvalho, 2008).

It is important to measure the problem of this resistance at a global level, since it inevitably accompanies the studies that deal with the discovery of new drugs (Moellering, 2000). Currently, several types of combinations are being used in order to establish new therapeutic responses, relating antimicrobials to plant extracts (Yunes, Perosa, & Cechinel, 2001; Novais et al., 2003; Filoche, Soma, & Sissons, 2005; Oliveira et al., 2006; Rosato, Vitali, De Laurentis, Armenise, & Millo, 2007; Shahverdi, Abdolpour, Monsef-Esfahani, & Farson, 2007). Therefore, this study is important to add knowledge to the literature for medicinal plants in the Amazon, specifically to determine the antimicrobial activity performed by the oil-resin of *Copaifera sp.* and to observe the concentrations necessary to inhibit microbial growth.

## II. MATERIALS AND METHODS

The experiments were developed locally in the multi-analysis laboratory of a private college in the city of Belém-PA. To collect the material to be used, manual extraction of copaiba oil-resin was performed, obtained in the region of Cametá, in western Pará, was carried out, transported in an amber bottle and stored at room temperature until the development of the experiments.

In order to carry out the tests and later evaluate the inhibitory activity of copaiba oil, the following microbial standard strains were used: *Escherichia coli* - ATCC 8739; *Pseudomonas aeruginosa* - ATCC 9027; and, *Staphylococcus aureus* - ATCC 25,923.

The profile of susceptibility to copaiba oil was initially tested using the dilution technique of vegetable oil in a melting medium, as described by Nonato, Lameira and Oliveira (2009). This method is based on the cultivation of bacteria in a synthetic culture medium Agar Müller Hinton; the raising was carried out from 1 mm discs of the bacteria, obtained from the Cefar Diagnóstica culture collection, which guarantees a basic microbiological profile.

Then, the Agar Müller Hinton culture medium was dissolved in distilled water and autoclaved at 121 °C for 15 minutes. Copaiba oil-resin in concentrations of 0.5%; 2.5%; 5.0%; 7.5% and 10.0% were added to the previously sterilized Agar Müller Hinton culture medium. Then, it was homogenized to be discarded in sterile Petri dishes

(90x15mm), awaiting polymerization and subsequent inoculation of the bacterial discs.

The experiments were carried out in triplicate and the control was carried out with the culture medium in two phases, the positive with the value of the concentration established in its standard solvent, distilled water and the negative with the culture medium plus the determined concentration of copaiba oil-resin. In parallel, plates of Agar Müeller Hinton were sown with Gram negative bacteria: *Pseudomonas aeruginosa* and *Escherichia coli*, and Gram positive: *Staphylococcus aureus*, after which the gentamicin antimicrobial disks 10mcg, cephalothin 30mcg, chloramphenicol 30mcg and tetrin, 30mcg and chloramphenicol 30mg from the company LABORCLIN (Produtos para Laboratório LTDA), in order to provide parameters of efficiency or not of the plant product under analysis.

The data analysis was performed using a software for statistical calculations, called SISVAR (Program of statistical analysis and design of experiments), developed by

Table.1: Microbiological inhibition of copaiba oil-resin in a concentration of 0.5%

Concentration	Bacteria	Halo-cm (mean)	Significance level
0,5%	<i>P.aeruginosa</i>	6,76cm	a <sub>3</sub>
	<i>S. aureus</i>	<b>0,78cm</b>	<b>a<sub>1</sub></b>
	<i>E. coli</i>	3,45cm	a <sub>2</sub>

Source: authorship

The result of the 2.5% concentration was satisfactory and equally significant for all strains tested (Table 2).

Table.2: Microbiological inhibition of copaiba oil-resin at a concentration of 2.5%

Concentration	Bacteria	Halo-cm (mean)	Significance level
2,5%	<i>P.aeruginosa</i>	3,81cm	a <sub>1</sub>
	<i>S. aureus</i>	<b>3,57cm</b>	<b>a<sub>1</sub></b>
	<i>E. coli</i>	3,27cm	a <sub>1</sub>

Source: authorship

the Federal University of Lavras (UFLA), designed to perform analysis of variance preferably for balanced data. The studies were made from Analysis of Variance (ANAVA), with multiple comparisons between the means of the experiment, through the Tukey Test, considered rigorous since it is based on the minimum significant difference (DMS).

### III. RESULTS

The present study evaluated the antimicrobial action of *Copaifera sp.* oil-resin in different concentrations: 0.5%, 2.5%, 5.0%, 7.5% and 10.0%, against standard gram negative strains of *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739 and gram positive *Staphylococcus aureus* ATCC 25,923. The evaluation of the concentration at 0.5% between the strains found significant values for the gram positive bacterium *Staphylococcus aureus* with the average of its growth halo  $\leq 0.78$  cm (Table 1).

In the 5.0% concentration, only the growth halo formed by the *Staphylococcus aureus* was considered highly significant (Table 3).

Table.3: Microbiological inhibition of oil-resin from copaiba oil-resin at a concentration of 5%

Concentration	Bacteria	Halo-cm (mean)	Significance level
5,0%	<i>P.aeruginosa</i>	4,34cm	a <sub>2</sub>
	<i>S.aureus</i>	<b>0,27cm</b>	<b>a<sub>1</sub></b>
	<i>E.coli</i>	3,4cm	a <sub>2</sub>

Source: authorship

When the 7.5% copaiba oil concentration was evaluated, the significance was observed at different levels of the three strains evaluated, however the values of the growth halo for the microorganisms were considered significant, judging the values already found in the lower concentrations and mainly the action of oil on gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* (Table 4).

Table.4: Microbiological inhibition of copaiba oil-resin at a concentration of 7.5%.

Concentration	Bacteria	Halo-cm (mean)	Significance level
7,5%	<i>P.aeruginosa</i>	3,46cm	a <sub>2</sub>

<i>S.aureus</i>	0,30cm	a <sub>1</sub>
<i>E.coli</i>	2,5cm	a <sub>2</sub>

Source: authorship

The 10.0% concentration was found to be highly significant for only one of the strains used, gram positive *Staphylococcus aureus* (Table 5).

Table.5: Microbiological inhibition of copaiba oil-resin at 10.0% concentration

Concentration	Bacteria	Halo-cm (mean)	Significance level
10,0%	<i>P.aeruginosa</i>	3,73cm	a <sub>3</sub>
	<i>S.aureus</i>	0,00cm	a <sub>1</sub>
	<i>E.coli</i>	2,76cm	a <sub>2</sub>

Source: authorship

When this bacterium was evaluated against the antimicrobials: gentamicin and chloramphenicol, the response obtained was that *Staphylococcus aureus* - ATCC 25.923 produced a 13 mm halo, characterized as an intermediate performance for the evaluated antimicrobials, as established by the NCCLS (Clinical and Laboratory Standards Institute / NCCLS, 2005). The results found for the *Staphylococcus aureus* strain characterize the oil-resin of *Copaifera* sp. as an excellent antimicrobial, since at 10.0% concentration, it was able to totally inhibit the growth of the tested bacteria.

Table 6 demonstrates the performance of the gram positive bacterium *Staphylococcus aureus*, against the copaiba oil-resin, in all concentrations performed, allowing to assess the progression of microbial inhibition and to compare with the other tests, shown in tables 7 and 8.

Table.6: Performance of gram positive bacteria *Staphylococcus aureus*

Concentration	Bacteria	Halo-cm (mean)
0,5%	<i>Staphylococcus aureus</i>	0,78cm
2,5%		3,57cm
5,0%		0,27cm
7,5%		0,30cm
10,0%		0,00cm
Control		9,00cm

Source: authorship

Table.7: Performance of gram negative bacteria *Pseudomonas aeruginosa* before the oil-resin of *Copaiba*

Concentration	Bacteria	Halo-cm (mean)
0,5%	<i>Pseudomonas aeruginosa</i>	6,70cm
2,5%		3,81cm
5,0%		4,34cm
7,5%		3,46cm
10,0%		3,46cm
Control		9,00cm

Source: authorship

Table.8: Performance of the gram negative bacteria *Escherichia coli* against the oil-resin of *Copaiba*

Concentration	Bacteria	Halo-cm
0,5%	<i>Escherichia coli</i>	3,45cm
2,5%		3,27cm
5,0%		3,45cm
7,5%		2,50cm
10,0%		2,76cm
Control		9,00cm

Source: authorship

To facilitate the understanding of the study carried out, as well as to demonstrate the main results obtained, follow figures 1, 2 and 3, which show the inhibitions that occurred in the tests with the studied bacteria and the copaiba oil:

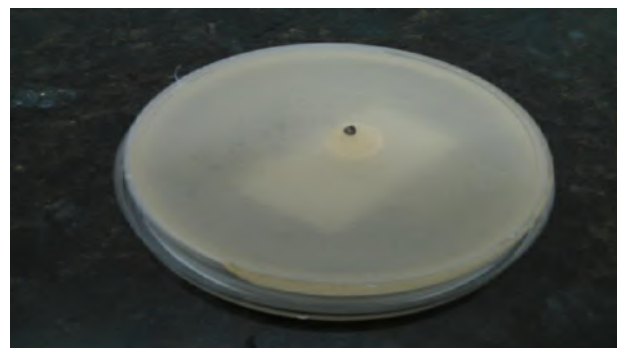


Fig.1: Photo showing the inhibition of gram positive bacteria *Staphylococcus aureus* in Agar Mueller Hinton culture medium with 7.5% copaiba oil.

Source: authorship



Fig.2: Photo showing the inhibition of Gram negative bacteria *Pseudomonas aeruginosa* in Agar Müller Hinton culture medium with 0.5% copaiba oil.

Source: authorship

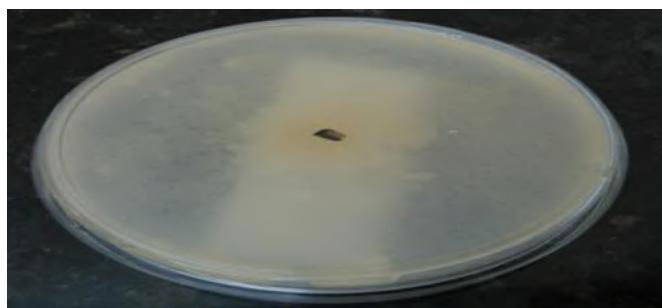


Fig.3: Photo showing the inhibition of Gram negative bacteria *Pseudomonas aeruginosa* in Agar Müller Hinton culture medium with 7.5% copaiba oil.

Source: authorship

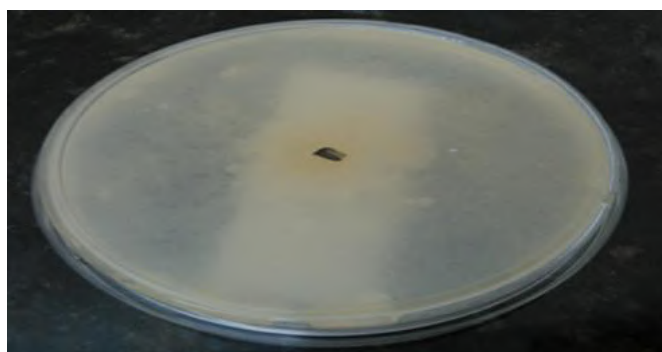


Fig.4: Photo showing the inhibition of Gram negative bacteria *Escherichia coli* in Agar Müller Hinton culture medium with 0.5% copaiba oil.

Source: authorship

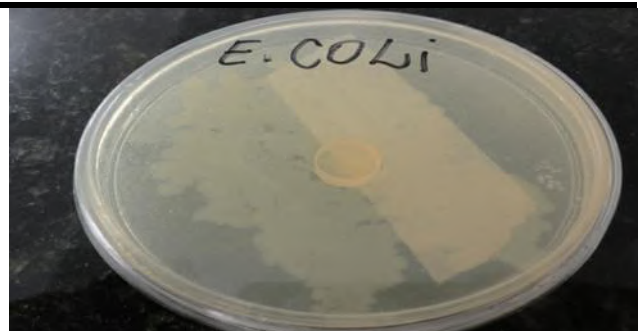


Fig.5: Photo showing the inhibition of gram negative bacteria *Escherichia coli* in Agar Müller Hinton culture medium with 7.5% copaiba oil.

Source: authorship

#### IV. DISCUSSION

It was observed that for copaiba oil-resin, in general, the antimicrobial action cannot be determined only by chemical constituents, a previous evaluation of the bacterium is important, so that the results obtained proved different inhibitory actions against the tested strains .

There are numerous studies that demonstrate the antibacterial action of copaiba oil in vitro as when it was tested on bacteria that form dental plaque, with excellent inhibitory results (Gilbert, Alves, & Ferreira, 2002; Simões et al., 2007). An in vitro study that evaluated the antimicrobial activity of *Copaifera officinalis* oil-resin on oral microorganisms: *Streptococcus pyogenes*, *Streptococcus salivarius*, *Streptococcus mutans*, *Enterococcus faecalis*, was effective among all tested strains (Pieri, Mussi, & Moreira, 2009), confirming the results obtained in this research, where Gram positive bacteria of the *Staphylococcus aureus* strain were inhibited by copaiba oil.

Santos et al. (2008), demonstrated the action of oil as an antimicrobial agent for several Gram positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), these events corroborate the results obtained in this work, where the Gram positive strain *Staphylococcus aureus* was the most significant among all tested strains, since the inhibition occurred completely by copaiba oil in 10.0% concentration.

It could also be associated with the results found those described by Mendonça and Onofre (2009), which differ in methodology and in the specification of the species of copaiba oil, similar results were obtained, where all the bacteria evaluated were inhibited by the copaiba oil-resin , and in the present study, the best performance of the bacterium *Escherichia coli* occurred in the concentration at 7.5%, with an inhibition halo of  $\pm 2.5$ cm, whereas in the

work previously mentioned, *Escherichia coli* showed prominence with minimum inhibitory concentration of 1.56%, both showing susceptibility to copaiba oil-resin.

Analyzing the results obtained in this study and comparing those achieved by Bloise (2003), where *Copaifera* spp oils were used, and different methodologies, equivalent answers were obtained, where the strains of *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*, showed sensitivity to copaiba oil. Biavatti, Dossin, Deschamps and Lima (2006), also tested the activity of *Copaifera multijuga* oil, the most satisfactory results happened for *Streptococcus mutans*, for *Staphylococcus aureus* and *Escherichia coli* the results were considered significant, in agreement with the results found in our research.

Nascimento et al. (2007), reports that in order to compare the results obtained in the studies with those available in the literature, several factors must be analyzed, such as the technique used, the plant material, the culture medium, the innocuous density and the diluent used, the variability of materials and techniques makes comparison difficult, making it impossible to reproduce new works.

Santos et al. (2008), evaluated the in vitro antibacterial action of oil-resin from several copaiba species on Gram positive and Gram negative bacteria, the antibacterial activity occurred only on Gram positive bacteria, Pacheco, Barata and Duarte (2006), also demonstrated inactivity of copaiba oil on Gram negative strains, results that are opposed to those found in this work, where the inhibitory bacterial response occurred for all strains tested, with the halves being more satisfactory for *Pseudomonas aeruginosa* and *Escherichia coli*, which occurred in the concentration at 7, 5%, respectively  $\pm 3.46$  cm and  $\pm 2.5$  cm, for the Gram positive strain *Staphylococcus aureus*, the most significant concentration was 10.0%, where copaiba oil was able to completely inhibit bacterial growth.

França and Kuster (2009), reports that the difference in susceptibility may be due to the structure of the external cell membrane of Gram negative bacteria, and not only due to the biological activity of the tested products. Santos et al. (2008), further suggests, that the susceptibility of the bacteria occurs by the rupture of the cell wall and release of the cytoplasmic content caused by the oil, which was observed through a detailed assessment of the morphology and structure of the cell wall of Gram positive bacteria.

In vivo studies were carried out with the intention of validating the oil's antimicrobial action, as described by Martins and Silva (2010), where the activity of *Copaifera* sp. pure in topical application of infected wound, producing reduction of local edema and cessation of

purulent secretion resulting from the infectious process. Masson (2011), carried out in vitro and in vivo studies with *Copaifera langsdorffii* oil-resin where he presented an antimicrobial response to gram positive microorganisms, demonstrated by the topical application in cream to 10.0% of copaiba oil in the infected ulcer model, producing reduction of the superficial microbial load and even of the deep tissue.

These data found in the literature strengthen the benefits of this work, which when evaluating different concentrations of *Copaifera* sp. against the pathogen *Staphylococcus aureus*, exhibited an efficient antimicrobial potential. In vivo tests guarantee credibility to the clinical use of copaiba oil (Gilbert, Alves, & Ferreira, 2002; Tincusi et al., 2002). Gonçalves, Alves Filho and Menezes (2005), in a diffusion test in Agar, tested the extract of the bark of *Copaiba* (*Copaifera officinalis*) on the strain of *Streptococcus pyogenes*, and the inhibition did not happen, suggesting that only the oil-resin of copaiba has constituents capable of inhibiting microbiological growth.

## V. CONCLUSION

Through the results obtained, it is concluded that the oil-resin of *Copaifera* sp., When evaluated in the concentration at 10.0%, presented a total inhibitory activity of the gram positive strain *Staphylococcus aureus*. Regarding the activity on the gram negative strains of *Pseudomonas aeruginosa* and *Escherichia coli*, the oil performed an inhibitory action, considered moderate, being possible to use it as a parameter to determine the use or not of the product. This fact is justified by the great diversity of chemical constituents in vegetable raw materials.

In view of the significant impact of the problems involved in the bacterial resistance process, copaiba oil-resin as an antimicrobial agent becomes a valuable adjunct for further studies, aiming to measure the true microbiological potential of the oil, its mechanism of action and its toxicity, ensuring its use. In addition, the results obtained show a significant contribution to the characterization of the antimicrobial activity of copaiba oil-resin, widely used in popular medicine.

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