

## Chemistry

# Synergistic effect of *Copaifera langsdorffii* oil with essential oils for anti-inflammatory activity

Efeito sinérgico do óleo de *Copaifera langsdorffii* com óleos essenciais para atividade anti-inflamatória

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## ABSTRACT

*Copaifera langsdorffii* essential oil belongs to the Leguminosae family and has anti-inflammatory, antibacterial, antifungal, analgesic, healing action, antiseptic, and antitumor potential, among others. Considering the importance of essential oils, this study aims to evaluate the total phenolic content, antioxidant and anti-inflammatory activity of *Copaifera langsdorffii* essential oil and its synergistic effect in association with essential oils of *Aniba duckei* Kostermans, *Syzygium aromaticum* (L.) Merr., *Citrus limonia* Osbeck, *Citrus limettioides* Tanaka, and *Eucalyptus globulus* Labill. Essential oils were extracted by hydrodistillation technique. The determination of the total phenolic compounds of the essential oil was performed by the Folin-Ciocalteu method. Antioxidant activity was assessed by the spectrophotometric method for eliminating hydroxyl radicals from salicylic acid, while anti-inflammatory activity was assessed by the protein denaturation method. The total phenolic content of *Copaifera langsdorffii* was 63.99 mg EAT g<sup>-1</sup>. The essential oil showed an antioxidant activity of EC<sub>50</sub> 16.67 mg L<sup>-1</sup> being classified as active, as well as the anti-inflammatory potential, with EC<sub>50</sub> 52.46 mg L<sup>-1</sup> also being classified as active. Finally, this study showed that the essential oil of *Copaifera langsdorffii*, as well as their synergies, have excellent antioxidant and anti-inflammatory potential.

**Keywords:** *Copaifera langsdorffii*; Synergism; Anti-inflammatory

## RESUMO

O óleo essencial de *Copaifera langsdorffii*, pertence à família Leguminosae, apresenta propriedades anti-inflamatórias, antibacteriana, antifúngica, analgésicas, ação cicatrizante, potencial antisséptico, antitumoral e entre outras. Com vista a considerar a importância dos óleos essenciais, o estudo tem por objetivo avaliar o conteúdo fenólico total, atividade antioxidante e anti-inflamatória do óleo

essencial de *Copaifera langsdorffii* e seu efeito sinérgico em associação aos óleos essenciais de *Aniba duckei* Kostermans, *Syzygium aromaticum* (L.) Merr., *Citrus limonia* Osbeck, *Citrus limettioides* Tanaka, *Eucalyptus globulus* Labill. Os óleos essenciais foram extraídos pela técnica de hidrodestilação. A determinação dos compostos fenólicos totais do óleo essencial foi realizada pelo método de Folin-Ciocalteu. A atividade antioxidante foi feita pelo método espectrofotométrico de eliminação de radicais hidroxila do ácido salicílico e a atividade anti-inflamatória foi avaliada pelo método de desnaturação proteica. O conteúdo fenólico total de *Copaifera langsdorffii* foi de 63,99 mg EAT g<sup>-1</sup>. O óleo essencial apresentou atividade antioxidante de CE<sub>50</sub> 16,67 mg L<sup>-1</sup> se classificando como ativo, da mesma forma com o potencial anti-inflamatório, com CE<sub>50</sub> 52,46 mg L<sup>-1</sup> também sendo classificado como ativo. Por fim, esse estudo mostrou que o óleo essencial de *Copaifera langsdorffii* assim como suas sinergias possuem ótimos potenciais antioxidantes e anti-inflamatórios.

**Palavras-chave:** *Copaifera langsdorffii*, Sinergismo, Anti-inflamatório

## 1 INTRODUCTION

In recent years, there has been a great scientific advance involving the chemical and pharmacological studies of medicinal plants, to obtain new compounds with therapeutic properties (Moraes, Correa e Vanin, 2018). Among the products obtained from medicinal plants, essential oils stand out. These consist of secondary metabolites, which are volatile, odoriferous, liquid substances with an oily appearance at room temperature that can be extracted from leaves, flowers, fruits, stems and roots of plants. Among the substances present in essential oils, the class of terpenes is mostly found, including their alcoholic and aldehyde derivatives (Aciole, 2009).

Another important point is the ability of oils to mix, known as synergy, which is a harmonic mixture of multiple oils. There are several essential oils with countless possibilities for combinations. In addition, the interaction potentiates the active principles and allows for a more accurate and adequate treatment (Spiassa, 2017).

The species *Copaifera langsdorffii* is one of the most studied plants. The name “copaíba” comes from the tupi kupa’ iwa which means “deposit tree” and refers to the fact that these species belong to the genus *Copaifera* L. This plant is highly valued in the market due to its multiple uses, such as in the pharmaceutical, cosmetics and perfume industries. Copaibas are also sources of raw materials for the timber industry, being

highly exploited (Martini et al., 1998). Brazil is the country with the highest production and exportation of copaiba oil, as the Amazon region is the main source of raw material and its main supplier (Santana, et al, 2014).

The oil of *C. langsdorffii* has a solid and non-volatile part of resin (volatile substances are easier to detach, that is, they evaporate faster) which is formed by diterpenes responsible for 55% to 60% of the resin, and an essential oil composed of sesquiterpenes, which can be divided into oxygenated sesquiterpenes and sesquiterpene hydrocarbons, favoring its use in the pharmaceutical, cosmetic and perfumery industries (Perfecto, 2020).

*Copaifera langsdorffii* essential oil consists of a set of forty terpenes and paraffins, with sesquiterpenes (96.36%) as the main components, the three principal ones being  $\alpha$ -trans-bergamotene (48.38%),  $\alpha$ -himachalene (11.17%) and  $\beta$ -caryophyllene (5.47%) (Gelmini et al., 2013). This oil also has scientifically proven effects such as diuretic, laxative, anti-tetanus, healing, tumor inhibitor and anti-inflammatory action. It can be used orally and topically, as each therapeutic action of the treatment differs according to the species of each copaiba tree where the oil-resin is extracted. (Rodrigues And Souza, 2017). Thus, this study aimed to evaluate the total phenolic content, antioxidant and anti-inflammatory activity of *Copaifera langsdorffii* essential oil and its synergistic effect in association with essential oils *Aniba duckei* Kostermans, *Syzygium aromaticum* (L.) Merr., *Citrus limonia* Osbeck, *Citrus limettioides* Tanaka, and *Eucalyptus globulus* Labil.

## 2 METHODOLOGY

### 2.1 Extraction of essential oils

To extract the EOs *Copaifera langsdorffii* (barks), *Aniba duckei* Kostermans (leaves), *Syzygium aromaticum* (L.) Merr. (shoots), *Citrus limonia* Osbeck (barks), *Citrus limettioides* Tanaka (barks), and *Eucalyptus globulus* Labill (barks), the hydrodistillation technique was used with a glass Clevenger extractor coupled to a round-bottom flask placed in an

electric blanket as a source of heat. 100g of plant materials were used, adding distilled water (1:10). Hydrodistillation was conducted at 100°C for 3h, collecting the extracted EO. Each EO was dried by percolation with anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) and centrifuged. These operations were performed in triplicate and the samples were stored in amber glass vials under refrigeration at 4°C, then subsequently submitted to analysis.

## 2.2 Synergies

The identification of samples for the tests of total phenolics, antioxidant action and anti-inflammatory action followed the description presented in Table 1.

Table 1 – Identification of samples and synergies for action tests

Samples	Identification	Species	Proportion
1	OB	<i>Copaifera langsdorffii</i> essential oil	
2	OAD	<i>Aniba duckei</i> Kostermans essential oil	
3	OSA	<i>Syzygium aromaticum</i> (L.) Merr. essential oil	1:0:0
4	OCL	<i>Citrus lemon</i> Osbeck essential oil	
5	OCT	<i>Citrus limettioides</i> Tanaka essential oil	
6	OEG	<i>Eucalyptus globulus</i> Labill essential oil	
7	S1	OB+OAD	
8	S2	OB+OSA	
9	S3	OB+OCL	1:1:0
10	S4	OB+OCT	
11	S5	OB+OEG	
12	S6	OB+OAD+OSA	
13	S7	OB+OAD+OCL	
14	S8	OB+OAD+OCT	
15	S9	OB+OAD+OEG	
16	S10	OB+OSA+OCL	1:1:1
17	S11	OB+OSA+OCT	
18	S12	OB+OSA+OEG	
19	S13	OB+OCL+OCT	
20	S14	OB+OCL+OEG	
21	S15	OB+OCT+OEG	

**Note:** OB- *Copaifera langsdorffii* essential oil; OAD- *Aniba duckei* Kostermans essential oil; OSA- *Syzygium aromaticum* (L.) Merr essential oil.; OCL- *Citrus limonia* Osbeck essential oil; OCT- *Citrus limettioides* Tanaka essential oil; OEG- *Eucalyptus globulus* Labill essential oil; S1- OB+OAD; S2- OB+OSA; S3- OB+OCL; S4- OB+OCT; S5- OB+OEG; S6- OB+OAD+OSA; S7- OB+OAD+OCL; S8- OB+OAD+OCT; S9- OB+OAD+OEG; S10- OB+OSA+OCL; S11- OB+OSA+OCT; S12- OB+OSA+OEG; S13- OB+OCL+OCT; S14- OB+OCL+OEG; S15- OB+OCT+OEG

Source: Autorship (2024)

### **2.3 Total Phenolics**

The determination of the total phenolic compounds of the oils and synergies was carried out with an adaptation of the Folin-Ciocalteu method (Waterhouse, 2002). 5 mg of samples diluted in 1 mL of ethanol were used. To this solution, 7 mL of distilled water, 800  $\mu$ L of Folin-Ciocalteu reagent and 2.0 mL of 20% sodium carbonate were added. After two hours, the reading was performed in triplicate by a UV-VIS spectrophotometer at a length of 760 nm. The standard curve was expressed in mg L<sup>-1</sup> of tannic acid.

### **2.4 Evaluation of antioxidant activity by scavenging hydroxyl radicals (R-OH·)**

Antioxidant activity was determined by the spectrophotometric method of elimination of hydroxyl radicals from salicylic acid, according to the methods described by Smirnoff & Cumbes (1989) and Sundararajan et al. (2016). The oils and synergies in different concentrations from 10-100 mg L were dissolved in phosphate buffered saline (PBS). To these concentrations were added 1 mL of salicylic acid (9 mM), 1 mL of ferrous sulfate (9 mM) and 1 mL of hydrogen peroxide (9 mM). Ascorbic acid was used as a positive standard. The reaction mixture was incubated for 60 min at 37 °C in a water bath; after incubation, the absorbance of the mixtures was measured at 510 nm in a UVVis spectrophotometer. The elimination of hydroxyl radicals was expressed in percentage and the Efficient Concentration 50% (IC<sub>50</sub>) and 90% (IC<sub>90</sub>) able to inhibit 50% and 90%, respectively, of the elimination was expressed in mg L.

### **2.5 Anti-inflammatory activity by albumin protein denaturation**

The anti-inflammatory activity was evaluated by the albumin protein denaturation method by thermal degradation (Padmanabhan & Jangle, 2012). The reaction mixture (4000  $\mu$ L) consisted of 1000  $\mu$ L of different concentrations of oils and synergies (100-500 mg L<sup>-1</sup>) diluted in PBS and 2000  $\mu$ L of a 10% albumin solution diluted in PBS and incubated at (37 $\pm$ 1) ° C for 15 minutes. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 minutes. After cooling, absorbance was

measured at 660 nm in a UV Vis spectrophotometer. Inhibition of protein denaturation was expressed in percentage and the 50% Efficient Concentration ( $IC_{50}$ ) capable of inhibiting 50% of denaturation was expressed in  $mg L^{-1}$ .

### 3 RESULTS AND DISCUSSION

#### 3.1 Total Phenolics

Table 2 – Total phenolic content (mg EAT g<sup>-1</sup>) for the tested oils and synergies

Identification	CFT (mg EAT/g)	Equation	R <sup>2</sup>
OB	63.99		
OAD	58.08		
OSA	179.26		
OCL	217.34		
OCT	228.54		
OEG	124.86		
S1	177.54	$y=0.0586x+0.06$	0.9999
S2	200.00		
S3	221.63		
S4	231.22		
S5	213.88		
S6	328.43		
S7	249.05		
S8	286.57		
S9	250.56		
S10	332.27		
S11	342.14		
S12	319.40		
S13	251.73		
S14	268.00		
S15	279.43		

**Note:** OB- *Copaifera langsdorffii* essential oil; OAD- *Aniba duckei* Kostermans essential oil; OSA- *Syzygium aromaticum* (L.) Merr essential oil.; OCL- *Citrus limonia* Osbeck essential oil; OCT- *Citrus limettioides* Tanaka essential oil ; OEG- *Eucalyptus globulus* Labill essential oil ; S1- OB+OAD; S2- OB+OSA; S3- OB+OCL; S4- OB+OCT; S5- OB+OEG; S6- OB+OAD+OSA; S7- OB+OAD+OCL; S8- OB+OAD+OCT; S9-OB+OAD+OEG; S10- OB+OSA+OCL; S11- OB+OSA+OCT; S12- OB+OSA+OEG; S13- OB+OCL+OCT; S14- OB+OCL+OEG; S15- OB+OCT+OEG

Source: Autorship (2024)

The result of the quantification of the total phenolic content of the essential oils is shown in Table 1. The total phenolic content (TPC) was expressed as tannic acid equivalents (mg EAT g<sup>-1</sup> of plant material) the equation of the straight line obtained was  $y = 0.0586x + 0.06$  ( $R^2 = 0.9999$ ), where  $y$  represents the absorbance and  $x$  equivalent concentration of tannic acid.

With the end of the analysis, it was observed that the result with the highest individual phenolic content was the essential oil of *Citrus limettioides* Tanaka (OCT) of 228.54 mg EAT g<sup>-1</sup> and in synergy were from *Copaifera langsdorffii*, *Syzygium aromaticum* (L.) Merr. and *Citrus limettioides* Tanaka (S11), of 342.14 mg EAT g<sup>-1</sup>. Lower results for total phenolics of *Copaifera langsdorffii* oil were found by Vigo (2019) who presented values of 0.49 mg EAT g<sup>-1</sup> when analyzing oil in the province of Coronel Portillo, Ucayali. Similarly, Penido et al. (2017) found a lower value of 48.03 mg EAT g<sup>-1</sup>. Regarding *Aniba duckei* Kostermans essential oil, Zieniuk & Betkowska (2021) showed a lower value of 0.11 mg EAG g<sup>-1</sup> in Warsaw, Poland. Likewise, Sankomkai et al. (2017) also observed a lower value of 23.65 mg EAG g<sup>-1</sup>. Regarding *Syzygium aromaticum* (L.) Merr essential oil; values were found with better efficacy by Turgay & Esen (2015), being 560 mg EAG g<sup>-1</sup>. Ramadan (2013) found a result of 5.9 mg EAG g<sup>-1</sup> which had a lower efficacy. As for the essential oil of *Citrus limonia* Osbeck, a lower value was found by Gravena et al. (2009) who presented 14.5 mg EAG g<sup>-1</sup>. Consistently, Mehmood et al. (2020) also presented a lower value of 138.66 mg EAG g<sup>-1</sup>.

In regard to the essential oil of *Citrus limettioides* Tanaka, a higher value was observed by Moraes Barros et al. (2012) which reported 310.18 mg EAG g<sup>-1</sup>. However, Sankomkai et al. (2017) indicated 12.13 mg EAG g<sup>-1</sup> being a lower result. Superior results for total phenolics of *Eucalyptus globulus* Labill oil were mentioned per Dezsi et al. (2015) which presented 235.87 mg EAG g<sup>-1</sup>. It also reported an inferior finding by Lin et al. (2009) which presented 6.79 mg EAG g<sup>-1</sup>.

### 3.2 Antioxidant activity

Table 3 presents the results of the oils' antioxidant activities and synergies.

Table 3 – Efficient Concentration 50% ( $EC_{50}$ - $IC_{50}$ ) for antioxidant action of oils and synergies

Identification	$CE_{50}$ mg L <sup>-1</sup>	Equation	R <sup>2</sup>
OB	16.67	$y = 27.651x + 16.212$	0.9934
OAD	22.17	$y = 26.226x + 14.704$	0.9968
OSA	20.53	$y = 18.855x + 25.255$	0.9984
OCL	67.21	$y = 21.142x + 11.365$	0.9952
OCT	20.38	$y = 21.359x + 22.037$	0.9919
OEG	14.00	$y = 33.036x + 12.136$	0.9969
S1	13.94	$y = 30.008x + 15.663$	0.9934
S2	15.03	$y = 27.246x + 17.93$	0.9971
S3	14.66	$y = 31.851x + 12.854$	0.9914
S4	21.06	$y = 31.533x + 8.2674$	0.9921
S5	32.29	$y = 30.915x + 3.3487$	0.9911
S6	11.38	$y = 23.943x + 24.713$	0.9905
S7	10.61	$y = 25.352x + 23.988$	0.991
S8	12.55	$y = 28.833x + 18.317$	0.9937
S9	14.09	$y = 23.034x + 23.535$	0.9934
S10	46.88	$y = 25.272x + 7.7696$	0.9993
S11	28.86	$y = 27.616x + 9.669$	0.9948
S12	46.36	$y = 13.672x + 27.22$	0.9997
S13	66.73	$y = 20.419x + 12.748$	0.9958
S14	17.31	$y = 40.049x + 0.3999$	0.9917
S15	28.33	$y = 30.151x + 6.2093$	0.9947

**Note:** OB- *Copaifera langsdorffii* essential oil; OAD- *Aniba duckei* Kostermans essential oil; OSA- *Syzygium aromaticum* (L.) Merr essential oil.; OCL- *Citrus limonia* Osbeck essential oil; OCT- *Citrus limettioides* Tanaka essential oil ; OEG- *Eucalyptus globulus* Labill essential oil ; S1- OB+OAD; S2- OB+OSA; S3- OB+OCL; S4- OB+OCT; S5- OB+OEG; S6- OB+OAD+OSA; S7- OB+OAD+OCL; S8- OB+OAD+OCT; S9-OB+OAD+OEG; S10- OB+OSA+OCL; S11- OB+OSA+OCT; S12- OB+OSA+OEG; S13- OB+OCL+OCT; S14- OB+OCL+OEG; S15- OB+OCT+OEG.

Source: Autorship (2024)

According to Table 3, all analyzed bioproducts and synergies were classified as active, according to the criteria of Campos et al. (2003). Since they have a concentration of less than 500 mg L<sup>-1</sup>. According to Sousa et al. (2011), the lower the  $EC_{50}$  value, the greater the activity of the plant compound, as a lower concentration of oil is required to reduce the radical by 50%.

With the end of the analyzes it was observed that the result of the best individual antioxidant activity was the essential oil of *Eucalyptus globulus* Labill of 22.17  $\mu\text{g mL}^{-1}$  and in synergy were the oils of *Copaifera langsdorffii*, *Aniba duckei* Kostermans and *Citrus lemon* Osbeck (S7) of 10.61  $\mu\text{g mL}^{-1}$ .

With regard to the essential oil of *Copaifera langsdorffii*, when evaluating the antioxidant activity was found by Carmo et al. (2016) a more effective value of  $\text{EC}_{50}$  3.95  $\mu\text{g mL}^{-1}$ . Like Costa et al. (2015) who also presented a more efficient result of 10.52  $\mu\text{g mL}^{-1}$ . In reference to *Aniba duckei* Kostermans essential oil, Teles et al. (2020) when evaluating the antioxidant activity, found a more efficient value of  $\text{EC}_{50}$  15.46  $\mu\text{g mL}^{-1}$ . However, Ferreira et al. (2020) found a lower value of  $\text{EC}_{50}$  40.06  $\mu\text{g mL}^{-1}$ .

When evaluating the antioxidant activity of the essential oil of *Syzygium aromaticum*, Selles et al. (2020) results in  $\text{EC}_{50}$  4.82  $\mu\text{g mL}^{-1}$  which is a more efficient result. However, Alfikri et al. (2020) found a less efficient  $\text{EC}_{50}$  value of 22.2  $\mu\text{g mL}^{-1}$ . About the essential oil *Citrus limonia* Osbeck, Bhuvanewari et al. (2020) ends up finding a less efficient value of 150  $\mu\text{g mL}^{-1}$  when analyzing the antioxidant activity. Like Osanloo et al. (2021) who had 1280  $\mu\text{g mL}^{-1}$  as a result.

About the essential oil of *Citrus limettioides* Tanaka, in the analyzes of the antioxidant activity Barros et al. (2012) found a more effective  $\text{EC}_{50}$  value of 7.44  $\mu\text{g mL}^{-1}$ . Like Janoti et al. (2014) who also had a more efficient result of  $\text{EC}_{50}$  15.35  $\mu\text{g mL}^{-1}$ . Regarding the antioxidant activity of the essential oil of *Eucalyptus globulus* Labill, inferior results were found by Dezsi et al. (2015) who showed  $\text{EC}_{50}$  15.27  $\mu\text{g mL}^{-1}$ . However, Lin et al. (2009) found a more efficient  $\text{EC}_{50}$  value of 12.53  $\mu\text{g mL}^{-1}$ .

The observed antioxidant actions may represent the presence of compounds capable of delaying or inhibiting the oxidation process through the inactivation of radicals. Phenolic compounds, for example, determined in the previous item, fit into this category, acting as switches in reaction chains, making them stable or creating antioxidant lipid complexes (Luzia & Jorge, 2009).

*Copaifera langsdorffii* essential oil, used in all synergies consists of a set of forty terpenes and paraffins, consisting of sesquiterpenes (96.36%), the three main ones being  $\alpha$ -trans-bergamotene (48.38%),  $\alpha$ -himachalene (11.17%),  $\beta$ -caryophyllene (5.47%). These properties are not due to the presence of phenolic, polyphenolic species, but to various volatile sesquiterpenes and the concomitant presence of unsaturated diterpene acid components (of which copalic acid is the most abundant in *Copaifera langsdorffii*, followed by abietic acid, polyaltic acid, kaurenoic acid and diterpene and sesquiterpene species (Gelmini et al. 2013).

Finally, synergism as an improvement in antioxidant activity is stated by Halliwell & Gutteridge (2015) when they define antioxidant synergism as the effect of the interaction between different bioactive compounds and/or macronutrients, which provide an antioxidant effect greater than the sum of the expected antioxidant effects for individual species.

### 3.3 Anti-inflammatory activity

The results obtained for the anti-inflammatory action regarding the analyzed oils and synergies are shown in Table 4.

According to Table 4, the essential oil was classified as active, according to the criteria of Jonville et al. (2011): very active  $EC_{50} < 50 \text{ mg L}^{-1}$ ;  $EC_{50}$  active between 50-130  $\text{mg L}^{-1}$  and moderate  $EC_{50}$  greater than 130  $\text{mg L}^{-1}$ . At the end of the analysis, it was observed that the essential oil of *Citrus limettioides* Tanaka (OCT) of 28.02  $\mu\text{g mL}^{-1}$  had the best individual anti-inflammatory activity, while the essential oils of *Copaifera langsdorffii* and *Syzygium aromaticum* (S2) 31.91  $\mu\text{g mL}^{-1}$  had the best synergy.

The anti-inflammatory potential of essential oils, as observed in this study, is attributed to their ability to scavenge free radicals, since the inflammatory response normally involves oxidative stress, and to their interactions with signaling cascades involving cytokines and regulatory transcription factors, and to the expression of pro-inflammatory genes (Miguel, 2010; Boscardin, 2012).

Table 4 – Efficient Concentration 50% (EC<sub>50</sub>-IC<sub>50</sub>) for anti-inflammatory action of oils and synergies

Identification	CE <sub>50</sub> mg L <sup>-1</sup>	Equation	R <sup>2</sup>
OB	52.46	y = 1.6891x + 47.095	0.9991
OAD	153.32	y = 15.275x + 16.615	0.9951
OSA	86.30	y = 16.643x + 17.779	0.9985
OCL	42.44	y = 3.1036x + 44.948	0.9951
OCT	28.02	y = 3.7209x + 44.614	0.9967
OEG	100.50	y = 4.0481x + 41.895	0.9999
S1	198.39	y = 2.6768x + 43.85	0.9986
S2	31.91	y = 5.6581x + 41.491	0.9963
S3	47.86	y = 2.6638x + 45.525	0.996
S4	171.31	y = 3.7x + 41.735	0.995
S5	372.27	y = 13.385x + 12.913	0.9947
S6	117.53	y = 19.642x + 9.3384	1
S7	57.23	y = 39.364x - 19.188	0.9933
S8	177.92	y = 15.118x + 15.981	0.9982
S9	252.64	y = 6.4083x + 34.604	0.999
S10	133.95	y = 3.7053x + 42.119	0.9914
S11	275.54	y = 5.3098x + 37.188	0.9985
S12	91.27	y = 5.3098x + 37.188	0.9958
S13	36.69	y = 5.3098x + 37.188	0.9973
S14	258.75	y = 5.3098x + 37.188	0.9982
S15	36.41	y = 2.5805x + 45.971	0.9996

**Note:** OB- *Copaifera langsdorffii* essential oil; OAD- *Aniba duckei* Kostermans essential oil; OSA- *Syzygium aromaticum* (L.) Merr essential oil.; OCL- *Citrus limonia* Osbeck essential oil; OCT- *Citrus limettioides* Tanaka essential oil ; OEG- *Eucalyptus globulus* Labill essential oil ; S1- OB+OAD; S2- OB+OSA; S3- OB+OCL; S4- OB+OCT; S5- OB+OEG; S6- OB+OAD+OSA; S7- OB+OAD+OCL; S8- OB+OAD+OCT; S9-OB+OAD+OEG; S10- OB+OSA+OCL; S11- OB+OSA+OCT; S12- OB+OSA+OEG; S13- OB+OCL+OCT; S14- OB+OCL+OEG; S15- OB+OCT+OEG

Source: Autorship (2024)

Among the most common chemical groups of natural origin with possible anti-inflammatory effect we can mention phenolic compounds, lignans, terpenes (sesquiterpenes, diterpenes, triterpenes, clerodanes and saponins), some phytosteroids and alkaloids (Calixto et al. 2004).

The component of copaiba oil responsible for the anti-inflammatory effect has not yet been definitively confirmed, but several studies attribute this activity mainly to  $\beta$ - caryophyllene (Leandro et al., 2012; Veiga Júnior et al., 2007). Likewise,

the anti-inflammatory mechanism of copaiba oil has not yet been adequately proven. However, Gelmini et al. (2013) analyzing the action of the chemical components of copaiba oil on the secretion of cytokines, demonstrated that there is an inhibition of the translocation of the NFκB protein complex from the cytosol to the nucleus, thus explaining the health benefits resulting from topical or upon oil administration. Finally, an additional mechanism that contributes to the anti-inflammatory activity of phenolic compounds is their ability to regulate the cellular redox state through antioxidant action by capturing free radicals, thus reducing the inductive signal in the production of cytokines and factors transcriptional (NFκB) (González-Gallego et al., 2007).

## 4 CONCLUSIONS

This study showed a great action of *Copaifera langsdorffii* essential oil, where a great result was obtained individually, mainly in the antioxidant activity, being the second-best result. Antioxidant activity obtained excellent results, mainly since all tests were classified as active. However, the anti-inflammatory action, even showing only 12 active results from the 21 samples, obtained promising results.

Therefore, based on the results, it was possible to conclude that the good anti-inflammatory action observed can be justified both due to the phenolic compounds that regulate the redox state through the antioxidant action by capturing free radicals, as well as the presence of the various volatile sesquiterpenes and the concomitant presence of unsaturated diterpene acid components of which copalic acid is the most abundant in *Copaifera langsdorffii*.

## REFERENCES

Aciole, S. D. G. (2009). *Avaliação da Actividade Insecticida Dos Óleos Essenciais Nas Plantas Amazônicas Annonaceae, Boraginaceae e de Mata Atlântica Myrtaceae como Alternativa De Controle às Larvas de Aedes aegypti (Linnaeus, 1762) (Diptera: Culicidae)* [Master's thesis, Universidade de Lisboa (Portugal)]. [https://repositorio.ul.pt/bitstream/10451/1418/1/20478\\_ulfc080628\\_tm.pdf](https://repositorio.ul.pt/bitstream/10451/1418/1/20478_ulfc080628_tm.pdf)

- Alfikri, F. N., Pujiarti, R., Wibisono, M. G., & Hardiyanto, E. B. (2020). Yield, quality, and antioxidant activity of clove (*Syzygium aromaticum* L.) bud oil at the different phenological stages in young and mature trees. *Scientifica*, 2020(1), 9701701. doi: <https://doi.org/10.1155/2020/9701701>
- Bhuvanewari, G., Thirugnanasampandan, R., & Gogulramnath, M. (2020). Effect of colchicine induced tetraploidy on morphology, cytology, essential oil composition, gene expression and antioxidant activity of Citrus limon (L.) Osbeck. *Physiology and Molecular Biology of Plants*, 26, 271-279. doi: <https://doi.org/10.1007/s12298-019-00718-9>
- Boscardin, P. M. D. (2012). *Avaliação anti-inflamatória e citotóxica do óleo essencial de Eucalyptus Benthamii Maiden et Cambage*. <https://hdl.handle.net/1884/28038>
- Calixto, J. B. (2004). Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. *Planta Med*, 70, 93-103.
- Campos, K. E., Diniz, Y. S., Cataneo, A. C., Faine, L. A., Alves, M. J. Q. F., & Novelli, E. L. B. (2003). Hypoglycaemic and antioxidant effects of onion, *Allium cepa*: dietary onion addition, antioxidant activity and hypoglycaemic effects on diabetic rats. *International journal of food sciences and nutrition*, 54(3), 241-246. doi: <https://doi.org/10.1080/09637480120092062>
- Campos, M. G., Webby, R. F., Markham, K. R., Mitchell, K. A., & Da Cunha, A. P. (2003). Age-induced diminution of free radical scavenging capacity in bee pollens and the contribution of constituent flavonoids. *Journal of agricultural and food chemistry*, 51(3), 742-745. doi: <https://doi.org/10.1021/jf0206466>
- Carmo, J. F., Miranda, I., Quilhó, T., Sousa, V. B., Cardoso, S., Carvalho, A. M., & Pereira, H. (2016). *Copaifera langsdorffii* bark as a source of chemicals: Structural and chemical characterization. *Journal of Wood Chemistry and Technology*, 36(5), 305-317. doi: <https://doi.org/10.1080/02773813.2016.1140208>
- Costa, A. R., Freitas, L. A., Mendiola, J., & Ibáñez, E. (2015). *Copaifera langsdorffii* supercritical fluid extraction: Chemical and functional characterization by LC/MS and in vitro assays. *The Journal of Supercritical Fluids*, 100, 86-96. doi: <https://doi.org/10.1016/j.supflu.2015.02.028>
- Dezsi, Ş., Bădărău, A. S., Bischin, C., Vodnar, D. C., Silaghi-Dumitrescu, R., Gheldiu, A. M., ... & Vlase, L. (2015). Antimicrobial and antioxidant activities and phenolic profile of *Eucalyptus globulus* Labill. and *Corymbia ficifolia* (F. Muell.) KD Hill & LAS Johnson leaves. *Molecules*, 20(3), 4720-4734. doi: <https://doi.org/10.3390/molecules20034720>
- Ferreira, A. M., Mouchrek Filho, V. E., Mafra, N. S. C., Sales, E. H., Júnior, P. S. S., & Everton, G. O. (2020). Constituintes químicos, toxicidade, potencial antioxidante e atividade larvicida frente a larvas de *Aedes aegypti* do óleo essencial de *Aniba rosaeodora* Ducke. *Research, Society and Development*, 9(8), e520985663-e520985663. doi: <https://doi.org/10.33448/rsd-v9i8.5663>
- Gelmini, F., Beretta, G., Anselmi, C., Centini, M., Magni, P., Ruscica, M., Cavalchini, A., & Facino, R. M. (2013). GC-MS profiling of the phytochemical constituents of the oleoresin from *Copaifera langsdorffii* Desf. and a preliminary in vivo evaluation of its antipsoriatic effect. *International journal of pharmaceutics*, 440(2), 170-178. doi: <https://doi.org/10.1016/j.ijpharm.2012.08.021>

- González-Gallego, J., Sánchez-Campos, S., & Tunon, M. J. (2007). Anti-inflammatory properties of dietary flavonoids. *Nutrición hospitalaria*, 22(3), 287-293. <https://www.redalyc.org/pdf/3092/309226717002.pdf>
- Gravena, R., Victoria Filho, R., Alves, P. L. C., Mazzafera, P., & Gravena, A. R. (2009). Low glyphosate rates do not affect Citrus limonia (L.) Osbeck seedlings. *Pest Management Science: Formerly Pesticide Science*, 65(4), 420-425. doi: <https://doi.org/10.1002/ps.1694>
- Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford university press. doi:
- Janoti, D. S., Rana, M., & Rawat, A. K. S. (2014). Comparative antioxidant activity of essential oil of leaves of Citrus limettoides and Citrus pseudolimon of Nainital District. *Journal of Pharmacognosy and Phytochemistry*, 2(5), 24-26. doi: <https://doi.org/10.1016/j.jep.2007.03.005>
- Jonville, M. C., Kodja, H., Strasberg, D., Pichette, A., Ollivier, E., Frédérick, M., ... & Legault, J. (2011). Antiplasmodial, anti-inflammatory and cytotoxic activities of various plant extracts from the Mascarene Archipelago. *Journal of ethnopharmacology*, 136(3), 525-531. doi: <https://doi.org/10.1016/j.jep.2010.06.013>
- Leandro, L. M., de Sousa Vargas, F., Barbosa, P. C. S., Neves, J. K. O., Da Silva, J. A., & da Veiga-Junior, V. F. (2012). Chemistry and biological activities of terpenoids from copaiba (*Copaifera* spp.) oleoresins. *Molecules*, 17(4), 3866-3889. doi: <https://doi.org/10.3390/molecules17043866>
- Lin, C. W., Yu, C. W., Wu, S. C., & Yih, K. H. (2009). DPPH free-radical scavenging activity, total phenolic contents and chemical composition analysis of forty-two kinds of essential oils. *Journal of food and drug analysis*, 17(5), 9. doi: <https://doi.org/10.38212/2224-6614.2594>
- Luzia, D. M. M., & Jorge, N. (2009). Atividade antioxidante do extrato de sementes de limão (*Citrus limon*) adicionado ao óleo de soja em teste de estocagem acelerada. *Química Nova*, 32, 946-949. doi: <https://doi.org/10.1590/S0100-40422009000400022>
- Martini, A., Rosa, N. A., & Uhl, C. (1998). Espécies madeireiras da Amazônia potencialmente ameaçadas. *Série Amazônia*, (11). <https://api.semanticscholar.org/CorpusID:163137411>
- Mehmood, T., Afzal, A., Anwar, F., Memon, N., Memon, A. A., & Qadir, R. (2020). Variation in phenolic acids and antibacterial attributes of peel extracts from ripe and unripe [*Citrus limon* (L.) Osbeck] fruit. *Journal of Food Measurement and Characterization*, 14, 1325-1332. doi: <https://doi.org/10.1007/s11694-020-00380-w>
- Miguel, M. G. (2010). Antioxidant and anti-inflammatory activities of essential oils: a short review. *Molecules*, 15(12), 9252-9287. doi: <https://doi.org/10.3390/molecules15129252>
- Moraes Barros, H. R., de Castro Ferreira, T. A. P., & Genovese, M. I. (2012). Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. *Food chemistry*, 134(4), 1892-1898. doi: <https://doi.org/10.1016/j.foodchem.2012.03.090>

- Moraes, R. P., Correa, I. C., & Vanin, A. B. (2018). Toxicidade dos óleos essenciais de endro (*Anethum graveolens*) e nim (*Azadirachta indica* A. Juss) através do ensaio de bioletalidade utilizando *Artemia salina*. *Seminário de Iniciação Científica e Seminário Integrado de Ensino, Pesquisa e Extensão (SIEPE)*. <https://periodicos.unoesc.edu.br/siepe/article/view/17928>
- Osanloo, M., Ghanbariasad, A., Taghinezhad, A. (2021). Antioxidant and anticancer activities of *Anethum graveolens* L., *Citrus limon* (L.) Osbeck and *Zingiber officinale* Roscoe essential oils. *Traditional and Integrative Medicine*, 333-347. doi: <https://doi.org/10.18502/tim.v6i4.8266>
- Padmanabhan, P., & Jangle, S. N. (2012). Evaluation of in-vitro anti-inflammatory activity of herbal preparation, a combination of four medicinal plants. *International journal of basic and applied medical sciences*, 2(1), 109-116. <https://api.semanticscholar.org/CorpusID:111380163>
- Perfecto, R. (2020). Um produto natural de possível suporte para o tratamento da periodontite: revisão bibliográfica. *Av Odontoestomatol*, 36(3). doi: <https://dx.doi.org/10.4321/s0213-12852020000300003>
- Penido, A. B., De Moraes, S. M., Ribeiro, A. B., Alves, D. R., Rodrigues, A. L. M., Dos Santos, L. H., & de Menezes, J. E. S. A. (2017). Medicinal plants from northeastern Brazil against Alzheimer's disease. *Evidence-Based Complementary and Alternative Medicine*, 2017(1), 1753673.
- Ramadan, M. F. (2013). Healthy blends of high linoleic sunflower oil with selected cold pressed oils: Functionality, stability and antioxidative characteristics. *Industrial Crops and Products*, 43, 65-72. doi: <https://doi.org/10.1016/j.indcrop.2012.07.013>
- Rodrigues, R., & Souza, J. (2017). Utilização do óleo de copaíba no tratamento e cicatrização de lesões. *Revista Interdisciplinar de Ciências Médicas-Anais*, 1(4), 168.
- Sankomkai, W., Suttisunsanee, U., Somsong, P., & Srichamnong, W. (2018, August). Effect of storage temperature on antioxidant activities of *Citrus medica* L. var. *limetta*. In 4th Asia Symposium on Quality Management in Postharvest Systems (pp. 257-262). *International Society for Horticultural Science*. doi: 10.17660/ActaHortic.2018.1210.36
- Santana, S., Bianchini-Pontuschka, R., Bay Hurtado, F., de Oliveira, C. A., Rodrigues Melo, L. P., & dos Santos, G. J. (2014). Uso medicinal do óleo de copaíba (*Copaifera* sp.) por pessoas da melhor idade no município de Presidente Médici, Rondônia, Brasil. *Acta Agronômica*, 63(4), 361-366. doi: <https://doi.org/10.15446/acag.v63n4.39111>
- Selles, S. M. A., Kouidri, M., Belhamiti, B. T., & Ait Amrane, A. (2020). Chemical composition, in-vitro antibacterial and antioxidant activities of *Syzygium aromaticum* essential oil. *Journal of Food Measurement and Characterization*, 14(4), 2352-2358. doi: <https://doi.org/10.1007/s11694-020-00482-5>
- Smirnoff, N., & Cumbes, Q. J. (1989). Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry*, 28(4), 1057-1060. doi: [https://doi.org/10.1016/0031-9422\(89\)80182-7](https://doi.org/10.1016/0031-9422(89)80182-7)

- Sousa, M. S. B., Vieira, L. M., & Lima, A. D. (2011). Fenólicos totais e capacidade antioxidante in vitro de resíduos de polpas de frutas tropicais. *Brazilian Journal of Food Technology*, 14, 202-210. doi: <https://doi.org/10.4260/BJFT2011140300024>
- Spiassa, V. Aromaterapia: Uma abordagem sistêmica e multidisciplinar na estética e bemestar. Óleos essenciais. 2017. <https://pt.scribd.com/document/426136045/Aromaterapia-Uma-Abordagem-Sistemica-e-Multidisciplinar-Na-Estetica-e-Bem-Estar-Vanessa-Spiassa>
- Sundararajan, B., Mahendran, G., Thamaraiselvi, R., & Ranjitha Kumari, B. D. (2016). Biological activities of synthesized silver nanoparticles from *Cardiospermum halicacabum* L. *Bulletin of Materials Science*, 39, 423-431. doi: <https://doi.org/10.1007/s12034-016-1174-2>
- Teles, A. M., Silva-Silva, J. V., Fernandes, J. M. P., Calabrese, K. D. S., Abreu-Silva, A. L., Marinho, S. C., ... & Almeida-Souza, F. (2020). Aniba rosaeodora (Var. amazonica Ducke) essential oil: Chemical composition, antibacterial, antioxidant and antitrypanosomal activity. *Antibiotics*, 10(1), 24. doi: <https://doi.org/10.3390/antibiotics10010024>
- Turgay, O., & Esen, Y. (2015). Antioxidant, total phenolic and antimicrobial characteristics of some species. *Bulgarian Journal of Agricultural Science*, 21(3), 498-503. <https://www.agrojournal.org/21/03-05.pdf>
- Veiga Junior, V., Rosas, E. C., Carvalho, M. V. D., Henriques, M. G. M. O., & Pinto, A. C. (2007). Chemical composition and anti-inflammatory activity of copaiba oils from *Copaifera cearensis* Huber ex Ducke, *Copaifera reticulata* Ducke and *Copaifera multijuga* Hayne—A comparative study. *Journal of ethnopharmacology*, 112(2), 248-254. doi: <https://doi.org/10.1016/j.jep.2007.03.005>
- Vigo, S. L. (2019). *Compuestos bioactivos y capacidad antioxidante de extractos de hoja de sachaculantro (Eryngium foetidum L.) y de aceite de copaiba (Copaifera paupera) procedentes de la provincia de Coronel Portillo, Ucayali*. <https://hdl.handle.net/20.500.12805/741>
- Waterhouse, A. L. (2002). Determination of total phenolics. *Current protocols in food analytical chemistry*, 6(1), 11-1. doi: <https://doi.org/10.1002/0471142913.fai0101s06>
- Zieniuk, B., & Bętkowska, A. (2021, October). Mixture design as a tool for optimization of antimicrobial activity of selected essential oils. *In Biology and Life Sciences Forum* (Vol. 6, No. 1, p. 98). MDPI. doi: <https://doi.org/10.3390/Foods2021-11018>

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