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QUÍMICA LICENCIATURA

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EFEITO SINÉRGICO DO ÓLEO DE *Copaifera langsdorfii* COM ÓLEOS ESSENCIAIS PARA ATIVIDADE ANTI-INFLAMATÓRIA.

SÃO LUÍS – MA

2022

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Monografia apresentada ao Curso de Química da Universidade Federal do Maranhão como requisito para a obtenção do título de licenciado em Química.

Orientador: Prof. Dr. Victor Elias Mouchrek Filho.

Co-orientador: Prof. Me. Gustavo Oliveira Everton.

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Trabalho de Conclusão de Curso apresentado ao Curso de Química da Universidade Federal do Maranhão como requisito para a obtenção do título de Licenciado em Química.

Aprovada em ___/___/___

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"Não sou baixinho. O mundo é que é grande demais."

(Fullmetal Alchemist - Edward Elric)

APRESENTAÇÃO

A pesquisa conta com o óleo essencial de Copaifera langsdorfii onde vai ser analisada em sinergismo com outros óleos essenciais para avaliar o seu conteúdo fenólico total, atividade antioxidante e a atividade anti-inflamatória. O trabalho está em conformidade com o Regulamento interno para os Trabalhos de Conclusão de Curso (TCC) da Licenciatura em Química (Art. 9°), na medida em que submete para apreciação do Colegiado e da Comissão Examinadora, um artigo científico, aceito para publicação em uma revista indexada na área de Química, cuja temática é na área de formação do discente, e formatado em consonância com as normas da revista escolhida.

TABELA DE SIGLA

CE_{50} - Concentração Eficiente 50%
OB - Óleo essencial de *Copaifera langsdorffii*
OAD - Óleo essencial de *Aniba duckei* Kostermans
OSA - Óleo essencial de *Syzygium aromaticum* (L.) Merr.
OCL - Óleo essencial de *Citrus lemon* Osbeck
OCT - Óleo essencial de *Citrus limettioides* Tanaka
OEG - Óleo essencial de *Eucalyptus globulus* Labill
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

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^{-1}. O óleo essencial apresentou atividade antioxidante de CE_{50} 16,67 mg L^{-1} se classificando como ativo, da mesma forma com o potencial anti-inflamatório, com CE_{50} 52,46 mg L^{-1} 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.

ABSTRACT

Copaifera langsdorffii essential oil, belongs to the Leguminosae family, has anti-inflammatory, antibacterial, antifungal, analgesic, healing action, antiseptic, antitumor potential, among others. In order to consider the importance of essential oils, the 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 *Aniba duckei* Kostermans, *Syzygium aromaticum* (L.) Merr., *Citrus limonia* Osbeck, *Citrus limettioides* Tanaka, *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 of eliminating hydroxyl radicals from salicylic acid and anti-inflammatory activity was assessed by the protein denaturation method. The total phenolic content of *Copaifera langsdorffii* was 63.99 mg EAT g^{-1}. The essential oil showed an antioxidant activity of EC_{50} 16.67 mg L^{-1} being classified as active, as well as the anti-inflammatory potential, with EC_{50} 52.46 mg L^{-1} also being classified as active. Finally, this study showed that the essential oil of *Copaifera langsdorffii* as well as their synergies, they have excellent antioxidant and anti-inflammatory potential.

Keywords: *Copaifera langsdorffii*, synergism, anti-inflammatory.

SUMÁRIO

1. INTRODUÇÃO

Nos últimos anos têm-se verificado um grande avanço científico envolvendo os estudos químicos e farmacológicos de plantas medicinais, a fim de obter novos compostos com propriedades terapêuticas (MORAES *et al*., 2018). Dentre os produtos obtidos de plantas medicinais, têm-se destaque aos óleos essenciais. Estes são constituídos por metabólitos secundários, que são substâncias voláteis, odoríferas, líquidas e de aparência oleosa à temperatura ambiente, podendo ser extraídos de folhas, flores, frutos, caules e raízes das plantas. Dentre as substâncias presentes nos óleos essenciais, a classe dos terpenos é encontrada majoritariamente, incluindo seus derivados alcoólicos e aldeídicos (ACIOLE, 2009).

Outro ponto importante é a capacidade dos óleos se misturarem, conhecida como sinergia: onde trata-se da mistura harmônica de mais de um óleo. Há diversos óleos essenciais e com inúmeras possibilidades de combinações. Além disso, a interação potencializa os princípios ativos e permite um tratamento mais preciso e adequado (SPIASSA, 2017).

A espécie *Copaifera langsdorffii* é uma das plantas mais estudadas. O nome “copaíba” vem do tupi kupa’ iwa que significa “árvore deposito” está referido ao fato de que essas espécies pertencem ao gênero *Copaifera*, essa planta é muito valorizada no mercado devido seus múltiplos usos como na indústria farmacêutica, de cosméticos e na de perfumarias, as copaíbas também são fontes de matérias-primas para a indústria madeireira e a mesma encontra-se sobre alta exploração (MARTINI *et al*, 1998). O Brasil é o país com maior produção e exportação do óleo da copaíba e região amazônica é a principal fonte de matéria prima, fornecedora do mesmo (SANTANA, *et al*,2014).

O óleo de *C. langsdorffii* possui uma parte sólida e não volátil de resina (substâncias voláteis tem um desprendimento mais fácil, ou seja, evapora mais rápido) é formado por diterpenos responsáveis por 55% a 60% da resina e um óleo essencial composto por sesquiterpenos, pode-se dividir em sesquiterpenos oxigenados e em hidrocarbonetos sesquiterpênicos, o que favorece sua utilização na indústria farmacêutica, cosmética e de perfumaria (PERFECTO *et al*, 2020).

O óleo essencial de *Copaifera langsdorffii* é constituído majoritariamente pelo β-cariofileno - 33,72% (SOUSA, 2011). O óleo essencial de *Aniba duckei* Kostermans tem majoritariamente o Linalol - 71,2% (PIMENTEL, 2015). O óleo essencial de *Syzygium aromaticum* (L.) Merr. já apresenta o eugenol – 70% (SRIVASTAVA, 2005).

O óleo essencial de *Citrus limonia* Osbeck tem o Limoneno – 31,5% como majoritário (OWOLABI, 2018). Já o óleo essencial de *Citrus limettioides* Tanaka tem o dl-Limoneno - 89,08% (LOPES *et al*., 2018) e o óleo essencial de *Eucalyptus globulus* Labill apresenta o 1,8-Cineol – 36,8% (SILVESTRE *et al*., 1997).

O óleo de copaíba também possui efeitos comprovados cientificamente como; ação diurética, laxante, antitetânico, cicatrizante, inibidor tumoral e anti-inflamatória. Podendo ser utilizado por via oral e tópica, cada ação terapêutica do tratamento se difere de acordo com as espécies de cada copaibeira onde se extrai o óleo-resina (RODRIGUES e SOUZA, 2017). Desta forma, este estudo teve 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.

2. OBJETIVOS

2.1. Objetivo Geral

- Avaliar o conteúdo fenólico total, atividade antioxidante, anti-inflamatória e efeito sinérgico do óleo essencial de *Copaifera langsdorffii*.

2.2. Objetivos Específicos

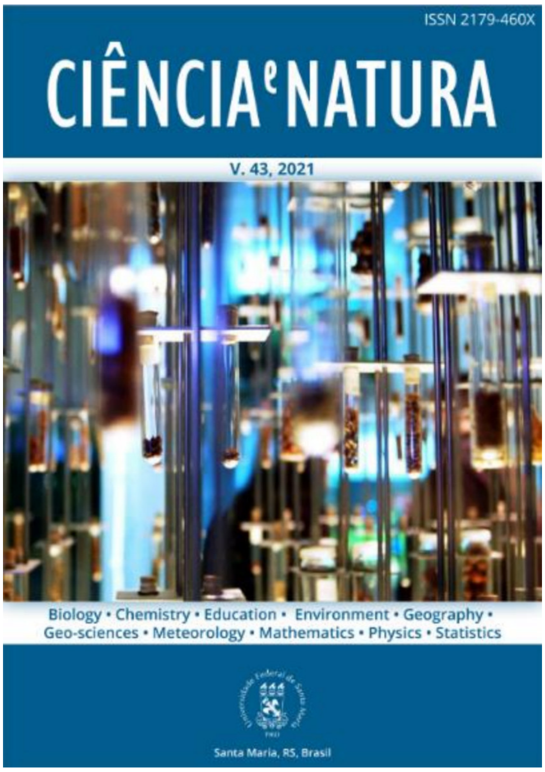
- Quantificar o teor de fenólicos totais do óleo essencial de *Copaifera langsdorffii* e de suas sinergias pelo método de Folin-Ciocalteu com óleos essenciais de *Aniba duckei* Kostermans, *Syzygium aromaticum* (L.) Merr., *Citrus limonia* Osbeck, *Citrus limettioides* Tanaka, *Eucalyptus globulus* Labill;
- Determinar a Concentração Eficiente 50% para atividade antioxidante do óleo de *Copaifera langsdorffii* e de suas sinergias pelo método espectrofotométrico de eliminação de radicais hidroxila do ácido salicílico com óleos essenciais de *Aniba duckei* Kostermans, *Syzygium aromaticum* (L.) Merr., *Citrus limonia* Osbeck, *Citrus limettioides* Tanaka, *Eucalyptus globulus* Labill;
- Determinar a Concentração Eficiente 50% para atividade anti-inflamatória do óleo de *Copaifera langsdorffii* e de suas sinergias pelo método de desnaturação proteica com óleos essenciais de *Aniba duckei* Kostermans, *Syzygium aromaticum* (L.) Merr., *Citrus limonia* Osbeck, *Citrus limettioides* Tanaka, *Eucalyptus globulus* Labill.

3. CAPÍTULO 1 - ARTIGO

ARTIGO A SER SUBMETIDO NA REVISTA CIÊNCIA E NATURA

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

Área: Química



Synergistic effect of *Copaifera langsdorfii* oil with essential oils for anti-inflammatory activity
Efeito sinérgico do óleo de *Copaifera langsdorfii* com óleos essenciais para atividade anti-inflamatória

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^{-1}. O óleo essencial apresentou atividade antioxidante de CE_{50} 16,67 mg L^{-1} se classificando como ativo, da mesma forma com o potencial anti-inflamatório, com CE_{50} 52,46 mg L^{-1} 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.

Abstract
Copaifera langsdorffii essential oil, belongs to the Leguminosae family, has anti-inflammatory, antibacterial, antifungal, analgesic, healing action, antiseptic, antitumor potential, among others. In order to consider the importance of essential oils, the 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 *Aniba duckei* Kostermans, *Syzygium aromaticum* (L.) Merr., *Citrus limonia* Osbeck, *Citrus limettioides* Tanaka, *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 of eliminating hydroxyl radicals from salicylic acid and anti-inflammatory activity was assessed by the protein denaturation method. The total phenolic content of *Copaifera langsdorffii* was 63.99 mg EAT g^{-1}. The essential oil showed an antioxidant activity of EC_{50} 16.67 mg L^{-1} being classified as active, as well as the anti-inflammatory potential, with EC_{50} 52.46 mg L^{-1} also being classified as active. Finally, this study showed that the essential oil of *Copaifera langsdorffii* as well as their synergies, they have excellent antioxidant and anti-inflammatory potential.
Keywords: *Copaifera langsdorffii*, synergism, anti-inflammatory.

Introduction

In recent years, there has been a great scientific advance involving the chemical and pharmacological studies of medicinal plants, in order to obtain new compounds with therapeutic properties (MORAES *et al*., 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, which 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: where it is the harmonic mixture of more than one oil. There are several essential oils and with countless possibilities for combinations. In addition, the interaction potentiates the active principles and allows for a more accurate and adequate treatment (SPIASSA, 2017).

Copaifera langsdorffii species 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 industry, cosmetics and in the perfumeries, copaibas are also sources of raw materials for the timber industry and it is under high exploitation (MARTINI *et al*, 1998). Brazil is the country with the highest production and export of copaiba oil and the Amazon region is the main source of raw material, supplier of the same. (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) it is formed by diterpenes responsible for 55% to 60% of the resin and an essential oil composed of sesquiterpenes can be divided into oxygenated sesquiterpenes and sesquiterpene hydrocarbons, which favors their use in the pharmaceutical, cosmetic and perfumery industries (PERFECTO *et al*, 2020).

The essential oil of *Copaifera langsdorffii* consists mainly of β-caryophyllene - 33.72% (SOUSA, 2011). The essential oil of *Aniba duckei* Kostermans has mostly Linalool - 71.2% (PIMENTEL, 2015). The essential oil of *Syzygium aromaticum* (L.) Merr. already presents eugenol – 70% (SRIVASTAVA, 2005). The essential oil of *Citrus limonia* Osbeck has Limonene – 31.5% as the majority (OWOLABI, 2018). The essential oil of *Citrus limettioides* Tanaka has dl-Limonene - 89.08% (LOPES *et al*., 2018) and the essential oil of *Eucalyptus globulus* Labill presents 1,8-Cineol - 36.8% (SILVESTRE *et al*., 1997).

Copaiba oil also has scientifically proven effects like; diuretic, laxative, anti-tetanus, healing, tumor inhibitor and anti-inflammatory action. It can be used orally and topically; each therapeutic action of the treatment differs according to the species of each copaiba tree where the oil-resin is extracted. (RODRIGUES&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, *Eucalyptus globulus* Labil.

Methodology

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), *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) (m/v).

Hydrodistillation was conducted at 100°C for 3h, collecting the extracted EO. Each EO was dried by percolation with anhydrous sodium sulfate (Na_2SO_4) and centrifuged. These operations were performed in triplicate and the samples were stored in amber glass vials under refrigeration at 4°C. Subsequently submitted to analysis.

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	Acronym	Species	Proportion
1	OB	*Copaifera langsdorffii* essential oil	1:0:0
2	OAD	*Aniba duckei* Kostermans essential oil	
3	OSA	*Syzygium aromaticum* (L.) Merr. essential oil	
4	OCL	*Citrus lemon* Osbeck essential oil	
5	OCT	*Citrus limettioides* Tanaka essential oil	
6	OEG	*Eucalyptus globulus* Labill essential oil	
7	S1	OB+OAD	1:1:0
8	S2	OB+OSA	
9	S3	OB+OCL	
10	S4	OB+OCT	
11	S5	OB+OEG	
12	S6	OB+OAD+OSA	1:1:1
13	S7	OB+OAD+OCL	
14	S8	OB+OAD+OCT	
15	S9	OB+OAD+OEG	
16	S10	OB+OSA+OCL	
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	

Total Phenolics

The determination of the total phenolic compounds of the oils and synergies were 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 μL 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^{-1} of tannic acid.

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 ppm 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 UV/VIS spectrophotometer. The elimination of hydroxyl radicals was expressed in percentage and the

Efficient Concentration 50% (EC_{50} /IC_{50}) and 90% (EC_{90} /IC_{90}) able to inhibit 50% and 90%, respectively, of the elimination was expressed in ppm.

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 µL) consisted of 1000 µL of different concentrations of oils and synergies (100-500 ppm) diluted in PBS and 2000 µL of a 10% albumin solution diluted in PBS and incubated at (37±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 (EC_{50} /IC_{50}) capable of inhibiting 50% of denaturation was expressed in ppm.

Results and discussion

Total Phenolics

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^{-1} 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.

Table 2– Total phenolic content (mg EAT g^{-1}) for the tested oils and synergies.

Acronym	TFC (mg EAT g^{-1})	Equity straight	R^2
OB	63.99		
OAD	58.08		
OSA	179.26		
OCL	217.34		
OCT	228.54		
OEG	124.86		
S1	177.54		
S2	200.00		
S3	221.63		
S4	231.22		
S5	213.88	y=0.0586x+0.06	0.9999
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.

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^{-1} and in synergy were from *Copaifera langsdorffi, Syzygium aromaticum* (L.) Merr. and *Citrus limettioides* tanaka (S11), of 342.14 mg EAT g^{-1}. Lower results for total phenolics of *Copaifera langsdorffi* oil were found by VIGO (2019) who presented values of 0.49 mg EAT g^{-1} when analyzing oil in the province of Coronel Portillo, Ucayali. Like PENIDO *et al*. (2017) found a lower value of 48.03 mg EAT g^{-1}. Regarding *Aniba duckei* Kostermans essential oil, ZIENIUK&BETKOWSKA (2021) showed a lower value of 0.11 mg EAG g^{-1} in Warsaw, Poland. Just like SANKOMKAI *et al*. (2017) where a lower value of 23.65 mg EAG g^{-1} was also observed.

Regarding *Syzygium aromaticum* (L.) Merr essential oil; values were found with better efficacy by TURGAY&ESEN (2015), being 560 mg EAG g^{-1}. RAMADAN (2013) found a result of 5.9 mg EAG g^{-1} which had a lower efficacy. With regard to the essential oil of *Citrus limonia* Osbeck, a lower value was found by GRAVENA *et al*. (2009) who presented 14.5 mg EAG g^{-1}. Like MEHMOOD *et al*. (2020) also presented a lower value of 138.66 mg EAG g^{-1}.

With regard to the essential oil of *Citrus limettioides* Tanaka, a higher value was observed by MORAES BARROS *et al*. (2012) which exposed 310.18 mg EAG g^{-1}. However, SANKOMKAI *et al*. (2017) indicated 12.13 mg EAG g^{-1} 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^{-1}. It also reported an inferior finding by LIN *et al*. (2009) which presented 6.79 mg EAG g^{-1}.

Antioxidant activity

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

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 ppm. According to SOUSA *et al*. (2007), 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 (OAD) of 22.17 µg mL^{-1} and in synergy were the oils of *Copaifera langsdorffi, Aniba duckei* Kostermans and *Citrus lemon* Osbeck (S7) of 10.61 µg 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 EC_{50} 3.95 µg mL^{-1}. Like COSTA *et al*. (2015) who also presented a more efficient result of 10.52 µg 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 EC_{50} 15.46 µg mL^{-1}. However, FERREIRA *et al*. (2020) found a lower value of EC_{50} 40.06 µg mL^{-1}.

When evaluating the antioxidant activity of the essential oil of *Syzygium aromaticum*, SELLES *et al*. (2020) results in EC_{50} 4.82 µg mL^{-1} which is a more efficient result. However, ALFIKRI *et al*. (2020) found a less efficient EC_{50} value of 22.2 µg mL^{-1}. With regard to the essential oil *Citrus limonia* Osbeck, BHUVANESWARI *et al*. (2020) ends up finding a less efficient value of 150 µg mL^{-1} when analyzing the antioxidant activity. Like OSANLOO *et al*. (2022) who had 1280 µg mL^{-1} as a result.

With regard to the essential oil of *Citrus limettioides* Tanaka, in the analyzes of the antioxidant activity BARROS *et al*. (2012) found a more effective EC_{50} value of 7.44 µg mL^{-1}.

Like JANOTI *et al*. (2014) who also had a more efficient result of EC_{50} 15.35 µg 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 EC_{50} 15.27 µg mL^{-1}. However, Lin *et al*. (2009) found a more efficient EC_{50} value of 12.53 µg mL^{-1}.

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

Acronym	CE $_{50}$ ppm	Equity straight	R^2
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.

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 α-trans-bergamotene (48.38%), α-himachalene (11.17%), β-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 (2007) when they define antioxidant synergism as the effect of the interaction between different bioactive compounds and/or and macronutrients, which provide an antioxidant effect greater than the sum of the expected antioxidant effects for individual species.

Anti-inflammatory activity

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

Table 4 –Efficient Concentration 50% (EC_{50} /IC_{50}) for anti-inflammatory action of oils and synergies.

Acronym	CE $_{50}$ ppm	Equity straight	R^2
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 ; EEG- *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

According to Table 4, the essential oil was classified as active, according to the criteria of JOINVILLE *et al*. (2011): very active EC_{50} < 50 ppm; EC_{50} active between 50-130 ppm and moderate EC50 greater than 130 ppm. With the end of the analyzes it was observed that the result of the best individual anti-inflammatory activity was the essential oil of *Citrus limettioides* Tanaka (OCT) of 28.02 µg mL^{-1} and in synergy were the essential oils of *Copaifera langsdorffi* and *Syzygium aromaticum* (S2) 31.91 µg mL^{-1}.

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 in the expression of pro-inflammatory genes (MIGUEL, 2010; BOSCARDIN, 2012).

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 β-caryophyllene (LEANDRO *et al*., 2012; VEIGA *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 oral administration in live on oil.

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).

Final considerations

This study showed an excellent action of the essential oil of Copaifera langsdorffii, where an individualized result was obtained, mainly in the antioxidant activity, being the second best result.

Antioxidant activity obtained extraordinary results, mainly due to all tests being classified as active. However, an anti-inflammatory action even showing only 12 active results of the 21 samples, obtained promising results where the best results were classified as very active.

Therefore, through the results obtained, it was possible to conclude that the 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 presence concomitant use of unsaturated diterpene cutting components of which copalic acid is the most abundant in Copaifera langsdorffii.

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4. CONSIDERAÇÕES FINAIS

Esse estudo mostrou uma excelente ação do óleo essencial de *Copaifera langsdorffii*, onde obteve-se um resultado satisfatório individualmente principalmente na atividade antioxidante sendo o segundo melhor resultado.

A atividade antioxidante obteve resultados extraordinários, principalmente devido a todos os testes terem sidos classificados como ativos. No entanto, a ação anti-inflamatória mesmo apresentando apenas 12 resultados ativos das 21 amostras, obteve resultados promissores onde os melhores resultados foram classificados como muito ativos.

Portanto, através dos resultados obtidos, foi possível concluir que a ação anti-inflamatória observada pode ser justificada tanto devido aos compostos fenólicos que regulam o estado redox através da ação antioxidante ao captar os radicais livres, quanto pela presença dos vários sesquiterpenos voláteis e à presença concomitante de componentes ácidos diterpenos insaturados dos quais o ácido copálico é o mais abundante em *Copaifera langsdorffii*.

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ANEXO A - NORMAS DA REVISTA

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As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

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