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**PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS**

**MOZANIEL SANTANA DE OLIVEIRA**

# ESTUDO DA COMPOSIÇÃO QUÍMICA E ATIVIDADES BIOLÓGICAS DE ÓLEOS ESSENCIAIS DE *Piper divaricatum, Syzygium aromaticum* e *Siparuna guianensis*

**Belém**
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Tese de doutorado, apresentada para o Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos da Universidade Federal do Pará - UFPA.

Orientador: Prof. Dr. Raul Nunes de Carvalho Junior.

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# TERMO DE JULGAMENTO DE DEFESA DE TESE DE DOUTORADO

Aos dezessete dias do mês de dezembro de 2018, às 10:00 h, nas dependências da sala de aula PPGCTA/UFPA, (Campus Belém), presente a comissão julgadora integrada pelos Senhores Professores Doutores **Raul Nunes de Carvalho Junior** (Orientador), **Lúcia de Fátima Henriques Lourenço** (Membro Interno), **Rosinelson da Silva Pena** (Membro Interno), **Eloisa Helena de Aguiar Andrade** (Membro Externo), **Daniel Santiago Pereira** (Membro Externo), **Alessandra Santos Lopes** (Membro Suplente) e **Antônio Pedro da Silva Souza Filho** (Membro Suplente), iniciou-se a **Defesa de Tese de Doutorado** do discente MOZANIEL SANTANA DE OLIVEIRA.

**Título da Tese:** "ESTUDO DA COMPOSIÇÃO QUÍMICA E ATIVIDADES BIOLÓGICAS DE ÓLEOS ESSENCIAIS DE *Piper divaricatum, Syzygium aromaticum* e *Siparuna guianessis*"

Concluída a argüição, procedeu-se ao julgamento na forma regulamentar, tendo a Comissão Julgadora considerado o candidato:

| | |
|---|---|
| **Raul Nunes de Carvalho Junior** | ( APROVADO ) |
| **Lúcia de Fátima Henriques Lourenço** | ( APROVADO ) |
| **Rosinelson da Silva Pena** | ( APROVADO ) |
| **Eloisa Helena de Aguiar Andrade** | ( Aprovado ) |
| **Daniel Santiago Pereira** | ( APROVADO ) |
| **Alessandra Santos Lopes** | ( ) |
| **Antônio Pedro da Silva Souza Filho** | ( ) |

Para constar, é lavrado o presente termo, que vai assinado pela Comissão Julgadora e pela Coordenadora do Programa.

A Comissão Julgadora: Raul Nunes de Carvalho Junior

Coordenadora do Programa: _____

Belém 17 de 12 de 2018

Obs: Imediatamente após o encerramento da argüição da dissertação cada examinador expressará seu julgamento em sessão secreta, considerando o(a) condidato(a) APROVADO ou REPROVADO.

Dedico este trabalho primeiramente a Deus que se faz presente em todos os momentos da minha vida.

Aos meus pais Maria e Manoel, minha esposa Joyce que amo muito, meus irmãos, amigos e outros familiares pela compreensão de minha ausência e pela paciência.

Também dedico esse trabalho ao amigo Marcos que se foi tão cedo do LABEX. E a todos aqueles que de forma direta e indireta me auxiliaram no desenvolvimento

desta árdua tarefa acadêmica.

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“Ninguém que é curioso é idiota. As pessoas que não fazem perguntas permanecem ignorantes para o resto de suas vidas” (**Neil DeGrasse Tyson**).

## RESUMO

A presente tese traz três estudos de revisão de literaturas, onde mostramos o uso do $CO_2$ supercrítico para a obtenção de óleos essenciais de diferentes plantas aromáticas, além disso, são mostradas as diferentes aplicações biológicas de óleos essenciais, como antibacterianas, antifúngicas, antioxidantes, anticâncer, antiparasitária, anti-inflamatória e fitotóxica. Com base nesses estudos foram feitos três manuscritos de pesquisas. O primeiro reporta o uso de $CO_2$ supercrítico em diferentes combinações de temperatura e pressão: 35 e 55 °C e 100, 300 e 500 bar. Também relatamos o uso do processo de hidrodestilação para obtenção de frações do óleo essencial *Piper divaricatum*. Os rendimentos em massa da extração, composição química, atividade antioxidante e atividade inibitória da acetilcolinesterase (AChE) foram analisados. A extração supercrítica de $CO_2$ apresentou melhor eficiência na obtenção de óleo essencial em comparação à hidrodestilação. A isoterma de 55/500 bar conduziu ao maior rendimento em massa de 7,40 0,08%. O metil eugenol foi o composto com maior concentração variando de 48,01 a 61,85%, sendo a fração obtida na condição de 35ºC / 300 bar a mais efetiva em relação à atividade antioxidante, com valores de 34,69 ± 1,38% (DPPH) e 296,86 ± 8,96 (mgTrolox / mL) (ABTS), respectivamente. Ligantes, após acoplamento molecular, exibiram posições moleculares que promoveram interações com diferentes resíduos de aminoácidos que são importantes para a catálise enzimática com His447. O segundo artigo fala sobre a atividade citotóxica, antimicrobiana e o mecanismo de ação do componente majoritário do óleo essencial de *Syzygium aromaticum* obtido por CO2 supercrítico. Neste trabalho, fibroblastos gengivais foram expostos ao óleo essencial em diferentes concentrações por uma hora: 5 μL / ml, 7,5 μL / ml e 10 μL / ml. O meio de cultura foi usado como controle. A análise de citotoxicidade foi realizada utilizando o método do brometo de 3- (4,5-dimetiltiazol-2-il) -2,5-difeniltetrazólio (MTT®). A suscetibilidade foi avaliada em três microrganismos *Candida albicans*, Escherichia coli e *Staphylococcus aureus*. As análises estatísticas mostraram diferença significativa na viabilidade celular para a concentração de 10 μL / mL, em relação ao grupo controle. Como resultado, o extrato da planta não apresentou citotoxicidade em concentrações abaixo de 10 μL / mL nos fibroblastos gengivais humanos. O modo de interação do eugenol, principal composto e principal componente responsável pela atividade biológica do óleo essencial, foi avaliado. A ancoragem molecular do eugenol com proteínas importantes da via metabólica dos microorganismos *C. albicans*, E. coli e S. aureus foram realizadas. Os resultados demonstraram que o composto é capaz de interagir com resíduos catalíticos das enzimas e formar um sistema energeticamente favorável com essas proteínas. Os resultados da ligação da energia livre obtida demonstram essa capacidade. Para o sistema eugenol-N-miristoiltransferase (*C. albicans*), o valor de ΔGbin foi -19,01 kcal / mol, para a Enoil redutase (*E. Coli*) ΔGbind foi igual a -11,31 kcal / mol e para a SarA (S. aureus) ΔGbind foi de -13,58 kcal / mol. E no terceiro artigo falamos sobre o óleo essencial de *Siparuna guianensis* esse óleo foi obtido por hidrodestilação. A identificação dos compostos químicos foi realizada por cromatografia gasosa acoplada a espectrometria de massa (GC / MS). A atividade antimicrobiana foi realizada em quatro microorganismos: *Streptococcus mutans*, (ATCC 3440), *Enterococcus faecalis* (ATCC 4083), *Escherichia coli* (ATCC 25922) e *Candida albicans* (ATCC-10231). Os estudos de docagem e dinâmica molecular foram realizados com a molécula que apresentou a maior concentração de proteínas alvo-droga, 1ILA (*C. albicans*), 1C14 (*E. coli*), 2WE5 (*E. faecalis*) e 4TQX (*S. mutans*). Os principais compostos identificados foram: Elemeno (7,58%), Curzereno (7,62%), Germacreno D (8,17%), β-Elemenone (12,76%) e Atractilona (18,96%). Bactérias e fungos Gram-positivos

foram os mais suscetíveis aos efeitos do óleo essencial. Os resultados obtidos na simulação mostraram que o principal composto atractilona interage com os sítios catalíticos das proteínas alvo, formando sistemas energeticamente favoráveis e permanecendo estáveis durante o período de dinâmica molecular. Os resultado apresentados pelos óleos essenciais das três espécies estudas na presente tese, mostram que eles possuem aplicações em várias áreas de conhecimento como para o controle de microrganismos e como conservante na indústria de alimentos pois tem ação antioxidantes, controle de radicais livres, e como possíveis agentes promotores de atividade neuroprotetora sendo usado principalmente para a inibição da acetilcolinesterase, retardando a hidrolise da acetilcolina, com isso pode melhorar as manifestações colinérgicas nas fendas sinápticas do cérebro humano.

**PALAVRAS-CHAVE**: Produtos naturais, *Piper divaricatum*, *Siparuna guianessis*, *Syzygium aromaticum*, óleo essencial, compostos bioativos.

## ABSTRACT

The present thesis brings three literature review studies, where we show the use of supercritical $CO_2$ to obtain essential oils from different aromatic plants. In addition, the different biological applications of essential oils such as antibacterial, antifungal, antioxidants, anticancer, antiparasitic, anti-inflammatory and phytotoxic. Based on these studies, three research manuscripts were made. The first reports the use of supercritical $CO_2$ in different combinations of temperature and pressure: 35 and 55 °C and 100, 300 and 500 bar. We also report the use of the hydrodistillation process to obtain fractions of the essential oil Piper divaricatum. Mass extracts, chemical composition, antioxidant activity and acetylcholinesterase inhibitory activity (AChE) were analyzed. Supercritical $CO_2$ extraction showed better efficiency in obtaining essential oil compared to hydrodistillation. The 55/500 bar isotherm resulted in the highest bulk yield of 7.40 0.08 %. Methyl eugenol was the compound with the highest concentration ranging from 48.01 to 61.85%, the fraction obtained in the condition of 35 ºC / 300 bar being the most effective in relation to the antioxidant activity, with values of 34.69 ± 1.38 % (DPPH) and 296.86 ± 8.96 (mgTrolox / mL) (ABTS), respectively. Ligands, following molecular coupling, exhibited molecular positions that promoted interactions with different amino acid residues that are important for enzymatic catalysis with His447. The second article discusses the cytotoxic, antimicrobial activity and mechanism of action of the major component of the essential oil of *Syzygium aromaticum* obtained by supercritical $CO_2$. In this work, gingival fibroblasts were exposed to the essential oil in different concentrations for one hour: 5 μL / ml, 7.5 μL / ml and 10 μL / ml. The culture medium was used as control. Cytotoxicity analysis was performed using the 3(4,5dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT®) method. Susceptibility was evaluated in three microorganisms *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus*. Statistical analyzes showed a significant difference in cell viability for the concentration of 10 μL / mL, in relation to the control group. As a result, the plant extract showed no cytotoxicity at concentrations below 10 μL / mL in human gingival fibroblasts. The interaction mode of eugenol, the main compound and main component responsible for the biological activity of the essential oil, was evaluated. Molecular anchoring of eugenol with important metabolic pathway proteins of *C. albicans*, *E. coli* and *S. aureus* microorganisms were performed. The results demonstrated that the compound is capable of interacting with catalytic residues of the enzymes and forming an energetically favorable system with such proteins. The results of the free energy binding obtained demonstrate this ability. For the eugenol-N-myristoyltransferase system (*C. albicans*), the ΔGbin value was -19.01 kcal / mol, for the Enoil reductase (*E. Coli*) ΔGbind was equal to -11.31 kcal / mol and for the SarA (S. aureus) ΔGbind was -13.58 kcal / mol. And in the third article we talked about the essential oil of *Siparuna guianensis* that oil was obtained by hydrodistillation. Identification of the chemical compounds was performed by gas chromatography coupled to mass spectrometry (GC/MS). The antimicrobial activity was performed in four microorganisms: *Streptococcus mutans*, (ATCC 3440), *Enterococcus faecalis* (ATCC 4083), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC-10231). The docking and molecular dynamics studies were performed with the highest concentration of target-drug proteins, 1ILA (*C. albicans*), 1C14 (*E. coli*), 2WE5 (*E. faecalis*) and 4TQX (*S. mutans*). The main compounds identified were: Elemene (7.58%), Curzereno (7.62%), Germacrene D (8.17%), β-Elemenone (12.76%) and Atratylone (18.96%). Gram-positive bacteria and fungi were the most susceptible to the effects of essential oil. The results obtained in the simulation showed that the main compound atratylone interacts with the catalytic sites of the target proteins, forming energetically favorable systems and remaining stable during the period of

molecular dynamics. The results presented by the essential oils of the three species studied in the present thesis show that they have applications in several areas of knowledge as for the control of microorganisms and as a preservative in the food industry because it has antioxidant action, free radical control, and as possible agents promoting neuroprotective activity being used primarily for the inhibition of acetylcholinesterase, retarding the hydrolysis of acetylcholine, thereby improving cholinergic manifestations in the synaptic clefts of the human brain

KEY WORDS: Natural products, *Piper divaricatum*, *Siparuna guianensis*, *Syzygium aromaticum*, essential oil, bioactive compounds.

# SUMÁRIO

# 1 CAPÍTULO I

## 1.1 Introdução Geral

Plantas ricas em óleos essenciais (OE) podem representar uma fonte viável para o uso nas mais variadas atividades humanas como, por exemplo, na indústria de alimentos, pois as atividades biológicas, como antioxidantes, antimicrobiana, fitotoxica, neuroprotetora, anti-inflamatória, entre outras, são importantes para esse segmento industrial; Além disso, (OE) apresentam baixa citotoxicidade, o que diminuí os riscos de intoxicação. Os (OEs) são frações voláteis naturais extraídas de plantas aromáticas, e são formados no metabolismo secundário das plantas. Diversas classes de substâncias voláteis podem ser encontradas na sua composição química, como ésteres de ácidos graxos, mono e sesquiterpenos, fenilpropanóides, álcoois, aldeídos e em alguns casos, hidrocarbonetos alifáticos. Essa variação de composição, depende da fisiologia, das condições ambientais, das variações geográficas, da sazonalidade, do horário da coleta, de fatores genéticos e evolução da planta. Como isso, propriedades físico-químicas de (OE) podem ser alteradas, e as concentrações de óleos nas partes de plantas como caule, folhas, flores e frutos, pode aumentar ou diminuir.

A extração de óleos essencial geralmente ocorre pelo uso de técnicas convencionais, como a hidrodestilação, usando um extrator tipo Clevenger original ou modificado, sendo esta a técnica mais difundida para o isolamento de óleos voláteis de planta. No entanto, outras técnicas de extração também se mostram eficientes como, por exemplo, extração com dióxido de carbono supercrítico (SC-$CO_2$). Esse tipo de extração é uma técnica considerada limpa e não provoca mudança nas estruturas químicas das moléculas, pois geralmente trabalha em baixas temperaturas de extração. O uso do (SC-$CO_2$) pode representar uma alternativa viável, para a extração de óleos essenciais de diferentes matrizes vegetais. Essa técnica tem mostrado grandes vantagens em relação aos métodos convencionais, como o fato de ser seletiva, dependendo das condições operacionais (temperatura, pressão e densidade) empregadas durante o processo de extração, além de ser considerada uma "técnica verde" para a obtenção de princípios ativos de origem vegetal.

Com o objetivo de contribuir com para a difusão do conhecimento relacionado ao potencial biotecnológico de três plantas produtoras de óleos essenciais: *Piper divaricatum, Syzygium aromaticum e Siparuna guianensis*, o presente estudo avaliou duas técnicas de extração, como a hidrodestilação e o dióxido de carbono supercrítico para obtenção de diferentes frações de óleo essencial. Além disso, são mostradas as composições químicas,

para correlacionar com as atividades antioxidante, potencial antimicrobiano, potencial inibitório da acetilcolinesterase. Com isso, a presente tese foi estruturada em 7, capítulos contando com o texto integrador, como pode ser observado abaixo.

A revisão da literatura é composta por três capítulos de livros, apresentados no capítulo II, capítulo III e capítulo IV. Com seus respectivos títulos descritos abaixo, neles são descritos o uso de técnicas de extração de óleos essenciais, dando ênfase a extração com $CO_2$ supercrítico, também são apresentadas diferentes atividades biológicas de óleos essenciais.

O capítulo II. Esse capítulo é composto por um capítulo de livro intitulado de Aplicação de CO2 supercrítico na extração de óleo essencial. (Título do livro: "Aplicações Industriais de Solventes Verdes", a ser publicado por Materials Research Forum LLC. The USA.

O capítulo III. Esse capítulo é composto por um capítulo de livro "Potencial de uso medicinal de óleos essenciais de plantas aromáticas" (Título do livro: Essential Oils), publicado pela Editora internacional IntechOpen.

O capítulo IV. Esse capítulo é composto por um capítulo de livro intitulado: "Compostos químicos potencialmente fitotóxico de presentes no óleo essencial para controle de plantas invasoras" - Uma mini-revisão (Título do livro: Abordagens biológicas para o controle). Publicado pela Editora internacional IntechOpen.

Em relação aos resultados da pesquisa realizada durante o período do doutoramento, são apresentados três capítulos no formato de artigos científicos, os seus respectivos títulos estão descritos nos capítulos V, VI e VII. Nesses artigos é mostrado o uso do $CO_2$ supercrítico e a hidrodestilação para a obtenção de diferentes frações de óleos essenciais, e são descritas as composições químicas e também que os óleos essenciais das plantas estudadas, possuem diversas atividades biológicas benéficas para a manutenção da saúde humana.

O capítulo V apresenta o artigo publicado no **The Journal of Supercritical Fluids** QUALIS A1 (COM FATOR DE IMPACTO 3.1 (2017)). "Perfil fitoquímico, atividade antioxidante, inibição da acetilcolinesterase e mecanismo de interação dos principais componentes do óleo essencial de *Piper divaricatum* obtido por $CO_2$ Supercrítico".

O capítulo VI. apresenta o artigo submetido: “Atividade citotóxica antimicrobiana do óleo essencial de *Syzygium aromaticum*, ancoramento molecular e estudos moleculares da dinâmica de seus principais constituintes químicos”

O capítulo VII. apresenta o artigo submetido: “Composição química, propriedades antimicrobianas do óleo essencial de *Siparuna guianensis* e um estudo de docagem molecular e dinâmica molecular de seu principal constituinte químico”

Todos os capítulos de livro e artigos publicados ou submetidos, estão formatados seguindo as diretrizes para autores dos livros ou das revistas cientificas.

# 2 Objetivos da Pesquisa

## 2.1 Geral

Avaliar técnicas de extração para obtenção de metabolitos secundários de plantas aromáticas, com objetivo de obter óleos essenciais ricos em compostos com aplicação antioxidante, farmacológica, medicinal. Além disso, realizar simulação através de química computacional para analisar a inibição de sítios catalíticos de enzimas.

### 2.1.1 Específicos

- ✓ Realizar um estudo da arte sobre o uso do $CO_2$ supercrítico para o isolamento de óleos essenciais de diferentes matrizes vegetais. CAPÍTULO II.
- ✓ Revisar o estado da arte sobre as potências aplicação biológicas de óleos essenciais e seus constituintes químicos. CAPÍTULO III e IV.
- ✓ Investigar o perfil químico e as atividades biológicas de diferentes frações de óleos essenciais de *Piper divaricatum*. CAPÍTULO V.
- ✓ Avaliar o potencial citotóxico e antimicrobiano do óleo essencial de cravo da índia e seu composto majoritário. CAPÍTULO VI.
- ✓ Avaliar o perfil químico e antimicrobiana do óleo essencial de *Siparuna guianensis.* CAPÍTULO VII.

# 3 CAPÍTULO II

## 3.1 Supercritical $CO_2$ application in the essential oil extraction.

Thank for publishing: Industrial Applications of Green Solvents

De: Inamuddin (inamuddin@zhcet.ac.in)

Para: mozaniel.oliveira@yahoo.com.br

Data: segunda-feira, 25 de março de 2019 05:55 BRT

# Note: Kindly Discard my previous email It was sent by Mistake.

Dear Prof./Dr. Mozaniel Oliveira,

This is to inform you that the book edition **"Industrial Applications of Green Solvents"** has been submitted to the publisher. I am very thankful to all the contributing authors and their co-authors for their esteemed contribution to this book and heartedly congratulate for the acceptance and publication of **Chapter** entitled **"Supercritical CO2 application in the essential oil extraction"** in the book edition entitled **"Industrial Applications of Green Solvents"** **Volume Number 2** which will be published by MRF, U.S.A.; a highly reputed and one of the largest International Publishers.

I would also like to express my deep sense of gratitude to my friends, family members, copyright holders, my fellow colleagues and others for their valuable suggestions, guidance and constant inspiration.

I am again very much thankful for your nice contribution. I hope for your contribution in the near future also.

Thanking you.

With best regards,

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CHAPTER 1

# Supercritical $CO_2$ application in the essential oil extraction

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## Abstract

The supercritical $CO_2$ has demonstrated efficiency and selectivity for the extraction of essential oils from vegetable matrices. This technique is based on the solvation power of the supercritical $CO_2$, and the solute-solvent interactions can be altered depending on the operating parameters used. These changes can result in an increase in the essential oil solubility and consequent increase in its yield. Supercritical $CO_2$ combines properties of gases and liquids, and one of its advantages is that it leaves no residues in the essential oil.

.

**Keywords:** Natural products, Essential oils, $CO_2$ extraction.

## 1. Introduction

Natural products of plant origin play an important role in human life, as they offer various nutritional, therapeutic and aromatic benefits, which can be applied in different segments, such as in cosmetics, food and pharmaceutical industries [1–4]. Among the natural products, the essential oils are composed of a complex mixture of volatile substances such as oxygenated and non-oxygenated sesquiterpenes, benzenoids, phenylpropanoids, and other compounds [5–7], and also exhibit biological activities relating to their chemical composition [8,9]. Among these classes of compounds, the terpenes are the most found in several plants species [10], and are formed and classified by their isoprene units, each one with five carbons, as shown in Figure 1

There are lots of methods used for extracting essential oils, such as supercritical fluid extraction, subcritical liquid extraction, microwave assisted extraction and the conventional techniques (hydrodistillation, steam distillation, organic solvent extraction). Each technique presents certain advantages and disadvantages. The main drawbacks refer to alterations in the biological and physicochemical properties of extracted oils [11]. Fornari et al. [12], takes into consideration extraction with supercritical carbon dioxide as an innovative, clean and ecologically correct technology, represents a viable alternative for the extraction of essential oils. Other reports in the literature have demonstrated supercritical $CO_2$ as selective extractant, depending on the operating parameters of extraction [13–15]; and at the end of the dynamic extraction period, there is no need for residues treatment associated with a toxic solvent [16]. In addition, when compared to conventional extraction techniques, such as hydrodistillation or extraction with organic solvents, the essential oils obtained with supercritical $CO_2$ present superior quality and yield [17–20]. These advantages make supercritical technology an excellent tool, for use on industrial scale, to obtain essential oils.

The (Figure 2) illustrates some applications of supercritical $CO_2$ in the extraction of bioactive compounds from natural products. In this sense, the present chapter aims to show, through a literature review, that supercritical $CO_2$ is an unconventional "green" technology that can help researchers in the formulation of natural products from essential oils.

## 2. Supercritical fluid extraction (SFE)

### *2.1 SFE principles*

In natural products, obtaining extracts requires faster efficient separation techniques. The isolation of substances (analytes) from a plant sample requires a method that can present high efficiency and that does not cause qualitative and/or quantitative degradation of the chemically active components in the extracts, and finally that does not generate toxic residues that could cause harm to the environment [21].

#### *2.1.1 Supercritical fluid concept*

Extraction with supercritical fluid can be defined as a combination of different types of unit operations that exploits, the solvation power of the fluid [22]. For a substance to be considered a supercritical fluid, its pressure and temperature should exceed to this critical values, as shown in the phase diagram (Figure 3). At this point, a supercritical fluid (SCF) may behave as a liquid or a gas. Among all the gases that can be used in SFE, $CO_2$ is considered the best option for extraction of bioactive compounds due to its low critical point (31.02 ºC, 73.6 bar - see Table 1), and low reactivity. In addition, it is relatively cheaper when compared to other solvents, non-flammable and is easy to recycle. Because it a solvent of low polarity, $CO_2$ presents higher interaction with apolar or low polarity compounds, however when incorporating a polar cosolvent, such as water

or ethanol, its solvation power can be improved for extraction of molecules of greater polarity [22–25].

### *2.2 Process parameters in supercritical $CO_2$ extraction*

#### *2.2.1 Diffusivity*

The supercritical solvent usually presents high diffusivity and this parameter is affected by operating conditions, such as temperature and pressure [22,26,27]. The effect of diffusion is present in the mass transfer phenomena, which describe the supercritical process at higher pressures, and this effect is mathematically represented by the diffusivity term, obtained from physical parameters that originate in Fick's second law. Over the years, the study of mass transfer has been used as a tool to understand the supercritical extraction applied to the production of essential oils of aromatic plants [28,29], and this has justified the development of mathematical models capable of predicting the value of diffusivity, an important parameter in the equations developed to determine extraction kinetics [30–33]. According to the literature [34,35], when there is an increase in diffusivity, there is a decrease in the solvent viscosity, and this allows greater penetration of the supercritical $CO_2$ in the raw material, which can result in an increase in the mass transfer rate in the extraction process, and consequent increase in the mass yield [36–38]. However, the diffusivity of the supercritical $CO_2$ can be altered according to the chemical composition [39], and polarity of the solute molecules, since they can cause different physico-chemical interactions [40–45]. These interactions also depend on thermodynamic properties such as energy, pressure, thermal pressure coefficient, coefficient of thermal expansion, isothermal and adiabatic compressibility, isobaric and isochoric heat capacities, Joule-Thomson coefficient, and vapor pressure [46,47].

Savage *et al*. [48] pointed out a number of advantages that are associated with transport reactions under supercritical conditions, and Krishna *et al.* [49], showed the dependency supercritical fluid extraction technology on the adequate estimation of the solute diffusion in the supercritical fluid phase. In their work [49], they correlated Peng–Robinson equation of state [50] combined with the Maxwell-Stefan equation, and analyzed the effect of thermodynamic equilibrium on diffusion effects applied at high pressures. They [49] also verified that the proposed diffusivity presented precision in the results when the compressibility factor was added in binary mixtures with density lower than 10 kmol.m$^3$.

It is also possible to find articles in the literature that indicating the plant structure as an influent factor in the diffusive effect, and consequently in the extraction yield [51–53]. Different models have been proposed [54–57] to verify the diffusive effect of plant particles in the supercritical solvent, since in the models developed from mass balance equations, one stage of the diffusive process may prevail over the other stages. For example, when the diffusion process is much slower than the transfer of the extract from the surface of the sample particle to the extractor outlet, the developed model can be simplified, so it can only describe the limiting step of the process. In this context, the diffusivity study comprises the study of the adsorption process of the supercritical solvent in the solute, a process that is represented by five steps: diffusion of $CO_2$ through the plant particles; diffusion of $CO_2$ into the pores and adsorption on the surface of the solid; transport of the essential oil to the outer layer of the pore; formation of a thin liquid film around the solid particles; dissolution of the oil in the supercritical $CO_2$; and after that, the convective transport of the solute within the fluid. The internal mass transfer resistance in the pores is supposedly low, and, therefore, the extraction rate depends mainly on the adsorption

equilibrium. The internal mass transfer resistance is completely neglected in the models of particle desorption [58].

Pourmortazavi and Hajimirsadeghi. [59] presented some applications of the supercritical fluid technology in the isolation of essential oils from plant matrices, in which they verified that the diffusion through the solid plant matrix affects the extraction yield and, according to Brunner. [22], this effect may occur due to temperature and pressure conditions, as well as due to the uniqueness of each plant tissue, which causes different values for solvent diffusivity. According to Brunner. [22], the cell membrane of the plant consists of three layers of lipid molecules and pores, in which the absence of water can cause the pores to close and make the membrane impermeable. This situation allows the identification of the initial extraction period, which consists of a rapid extraction, and the final extraction period, which is characterized by a slow extraction obtaining.

Reis-Vasco *et al*. [60] obtained pennyroyal essential oil by supercritical fluid extraction, and concluded that the largest amount of essential oil obtained was from the leaves surface present in hairy trichomes. They proved this result stating that the oil of the trichomes was adsorbed on the leaves. They also used the equilibrium model to describe the process in the initial part of the extraction curve. The final part of the process was calculated as the extraction controlled by the internal mass transfer resistance.

Gaspar *et al*. [61] obtained oregano bracts essential oil and used a model taking into consideration the oil fraction already available on the leave surface, with slow diffusion in the final extraction step . They assumed that the easily accessible oil was released from the glands

during the mechanical pretreatment step and, thus, the essential oil obtained with supercritical fluid was either inside the glands or retained in the internal tissues.

### *2.2.2 Effect of temperature and pressure*

In the process of supercritical fluid extraction, pressure and temperature are variables that influence the essential oil yield, and the increase in pressure and decrease in temperature results in an increase in the solvent density, and consequently also in the solubility between solute and solvent [59,62]. Oliveira *et al*. [63] obtained clove essential oil (*Syzygium aromaticum*) and found that, maintaining the operating pressure at 100 bar, the extraction yield was higher at 40 °C (13.14%) than at 50 °C (12.70%) [64]. In some situations, the increase in pressure can compact the solid matrix, which leads to unfavorable extraction results [65–67]. However, the effect of pressure on certain extraction isotherms has advantages because it allows the selective production of bioactive phytochemicals, such as thymol obtained from rosemary pepper (*Lippie sidoides*), and eugenol obtained from holy basil (*Ocimum sanctum* L.), and clove (*Syzygium aromaticum*) [64,68,69].

The temperature is another parameter that influence the extraction process. It can affect the extraction yield, the composition of the essential oil, the supercritical $CO_2$ density, and the solubility between solute and solvent. Decreasing the extraction temperature may cause a change in solute vapor pressure, resulting in a phenomenon called "cross-over effect", in which high temperatures produce low yields, while low temperatures produce high yields. Scopel *et al*. [70] verified this effect when studying extraction of clove essential oil, when the phenomenon of retrograde condensation was reported. Oliveira *et al*. [64] also verified this effect when extracting clove essential oil (*Syzygium aromaticum*) at temperatures of 40 °C and 50 °C; an inflection point

at 150 bar was also observed. Ghosh *et al.* [68] verified a similar effect on yield isotherms when studying the essential oil obtained from holy basil (*Ocimum sanctum*); it was identified that the increase in temperature resulted in a decrease in the amount of eugenol, at lower pressures.

### *2.3 SC–$CO_2$ as a solvent for essential oil extraction*

Supercritical $CO_2$ extraction is an innovative and green methodology used to obtain various extracts and essences from several matrices, such as essential oils [71]. This technique plays an important role in the food and pharmaceutical industries [20,71]. Extracts or essential oils obtained by supercritical $CO_2$ did not contain any type of substance different from those present in the composition of the extracted material, which means that the oil or extract obtained is pure, and of high quality. This is due to the critical point of $CO_2$ being reached at low pressure and temperature, as previously reported, so it does not degrade or cause transformations in the thermolabile components [72,73].

Extraction of essential oil with supercritical $CO_2$ is much faster than traditional methods of extraction since this gas has ability to mix with the botanical material, and offers little resistance to flow in the extraction cell. Essential oils are mostly composed of nonpolar components, so the extraction with supercritical $CO_2$ is very efficient since it is a solvent of low polarity [71].

The supercritical fluid extraction system basically consists of a carbon dioxide cylinder, cooler, high-pressure pump, oven, an extraction cell, collecting vessel, air compressor, flow meter and flow control valves [64,71]. For the process, the gas ($CO_2$) passes through the cooler where it is liquefied, then it is pressurized by the pump to reach pressures higher than its critical pressure. During the static equilibrium step, the pressurized $CO_2$ goes through the extraction column, positioned inside the oven, with a temperature control. These processes guarantee the thermodynamic conditions of temperature and pressure to occur the isolation of the essential oil

from the plant material [71]. The supercritical extraction has been, over the years, an important alternative for the extraction of essential oils. As evident from Table 2. In Figures 4 and 5 we observed two supercritical extraction plants one on industrial scale (Figure 4) and another on analytical scale (Figure 5), are depicted.

Frohlich *et al.* [74] used supercritical $CO_2$ to extract eugenol, the main bioactive compound present in clove leaves, and verified the effect of temperature and pressure on the yield of this secondary metabolite. The authors used conditions of pressure (150-220) bar and temperatures (40-60 °C), and found that the highest yield (1.08 %), and higher content of eugenol (29.73 %) were obtained at 40 °C and 220 bar. In this combination of temperature and pressure, $CO_2$ has a higher density (858.11 kg/m$^3$) in relation to the other experiments, which means that this parameter also influences the extraction.

### *2.4 Conventional methods of essential oils extraction*

Among the several conventional methods of obtaining essential oils, hydrodistillation, steam distillation, and extraction with organic solvents stand out [72,95,96]. Hydrodistillation (HD) is the methodology mostly used in the extraction of essential oils. It is old, simpler, and vastly used in the industries, does not require expensive equipment, and is a method of easy implementation with great selectivity [72,97]. The HD process began to be performed in alembics [72,73], however, from the third edition of the European Pharmacopoeia, the use of the Clevenger system was recommended in the hydrodistillation, since this system allow the recycling of the condensates [72]. HD is based on azeotropic distillation and the extraction system requires a heating source, a container to place the biomass (still or volumetric flask), condenser and a decanter to collect the mixture of oil and water [72].

The (Figure 6), describes the HD process. The plant material to be extracted (leaves, branches, barks, roots, fruits, flowers or seeds) is in direct contact with the distilled water in a certain container (in this case, a volumetric flask), which is connected to a Clevenger, and coupled to a cooling system, for the maintenance of water condensation. Subsequently, heating of the solid-liquid mixture until the boiling temperature of water occurs under atmospheric pressure, so that the odoriferous molecules of the cells of the plant material are evaporated together with the water, thus forming an azeotrope mixture. This mixture enters the condenser, which liquefies and is collected at the end of the extraction. Since the terpene molecules of the oil and water are immiscible, the organic phase is separated from the aqueous phase by decantation. In addition to decantation, centrifugation is used with anhydrous $Na_2SO_4$ to make the EO completely free of water [73,98–100].

Although hydrodistillation is a classic procedure, free of chemical compounds, and recommended by the French Pharmacopoeia for the extraction of essential oils [73], it presents some negative points, such as long extraction time, formation of artifacts, chemical changes in the terpenic molecules due to the high temperature used, and loss of some polar molecules that are solubilized in water [72,97,102,103].

Another conventional extraction method is steam hydrodistillation and steam distillation (Figure 7 A and B) respectively, which is widely used by the industry to obtain essential oil for commercialization. It is a simple process, applicable to the processing of significant quantities of botanical material [102,104,105]. Steam distillation occurs with the same principles of HD, but the fundamental difference lies in not putting the plant biomass in contact with water. In HD, the vegetal material to be extracted is in the same container, whereas in the steam distillation, the

vapors responsible for the extraction process are produced outside the extraction vessel, and reach the plant material through a serpentine coil [72,106].

In this process, the passage of vapors through the plant material causes the rupture of the specialized secretory structures (glands) responsible for the production of essential oil, which are released and carried with the water vapor towards the condenser, where the cooling of the mixture occurs, and then it is collected and separated, just as it is done in hydrodistillation [72,73]. Usually, steam distillation is performed in a shorter period of time, when compared to hydrodistillation, and the raw material does not come into direct contact with water. This helps to reduce essential oil composition, artifact production, and the loss of polar molecules [72].

## 3. Comparisons among extraction methods

As previously reported, depending on the extraction methods used, there may be differences in the mass yields and in the phytochemical profile of essential oils. In this sense, Danh *et al*. [107] studied the effects of different parameters on the yield and chemical composition of the essential oil extracted from *Lavandula angustifolia* L. In terms of mass yield, the extraction with supercritical $CO_2$ showed higher efficiency with a result of 6.7% (on dry basis), compared to of hydrodistillation were the yield was 4.6%. Regarding the chemical composition, it was observed that the linalool content was relatively higher when extracted by hydrodistillation (52.59 ± 0.70% against 42.82 ± 0.19%, obtained by supercritical $CO_2$ extraction), however, Linalyl acetate was obtained in higher concentration with supercritical $CO_2$ (23.40 ± 0.23 % against 9.27 ± 0.21 % obtained by hydrodistillation).

Costa *et al*. [108], studied the chemical profiles of the extracts of *Lavandula viridis* L obtained by hydrodistillation and extraction with supercritical fluid. The results showed that the highest mass yields were obtained by supercritical $CO_2$, whereas hydrodistillation favored the extraction of other substances also. More compounds were isolated by HD, and camphor was the main component identified in the essential oil, representing around (31.59 ± 1.32 %) of the total extract. In Table 3, the differences in yield and chemical composition, regarding the operating conditions of supercritical $CO_2$ extraction, hydrodistillation, microwave hydrodiffusion and gravity; hexane extraction; and steam distillation can be observed.

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## 4. Conclusions

The supercritical $CO_2$ technology allows the extraction of different classes of secondary metabolites from different plant species. In addition, this is a green technique for extraction of natural compounds. Supercritical $CO_2$ does not leave toxic residues and is chemically inert, which means that it does not react with the compounds present in the essential oils. Studies on supercritical extraction begin on analytical scale, and are optimized for larger scales, for higher yields. In this technique, temperature and pressure can be manipulated to allow greater selectivity to obtain essential oils rich in certain classes of chemical compounds. cknowledgments

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## 5. Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper

## References

[1] F. Shahidi, P. Ambigaipalan, Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects - A review, J. Funct. Foods. 18 (2015) 820–897.

[2] K. Leslie, M.L. Allen, E.C. Hessian, P.J. Peyton, J. Kasza, A. Courtney, P.A. Dhar, J. Briedis, S. Lee, A.R. Beeton, D. Sayakkarage, S. Palanivel, J.K. Taylor, A.J. Haughton, C.X. O'Kane, Safety of sedation for gastrointestinal endoscopy in a group of university-affiliated hospitals: A prospective cohort study, Br. J. Anaesth. 118 (2017) 90–99.

[3] C. López-Alarcón, A. Denicola, Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays, Anal. Chim. Acta. 763 (2013) 1–10.

[4] A. Filly, X. Fernandez, M. Minuti, F. Visinoni, G. Cravotto, F. Chemat, Solvent-free microwave extraction of essential oil from aromatic herbs: From laboratory to pilot and industrial scale, Food Chem. 150 (2014) 193–198.

[5] F. V. Silva, A.G. Guimarães, E.R.S. Silva, B.P. Sousa-Neto, F.D.F. Machado, L.J. Quintans-Júnior, D.D.R. Arcanjo, F.A. Oliveira, R.C.M. Oliveira, Anti-Inflammatory and Anti-Ulcer Activities of Carvacrol, a Monoterpene Present in the Essential Oil of Oregano, J. Med. Food. 15 (2012) 984–991.

[6] I.P. Sauter, G.E. Rossa, A.M. Lucas, S.P. Cibulski, P.M. Roehe, L.A.A. da Silva, M.B. Rott, R.M.F. Vargas, E. Cassel, G.L. von Poser, Chemical composition and amoebicidal activity of Piper hispidinervum (Piperaceae) essential oil, Ind. Crops Prod. 40 (2012) 292–295.

[7] R.R. Junker, D. Tholl, Volatile Organic Compound Mediated Interactions at the Plant-Microbe Interface, J. Chem. Ecol. 39 (2013) 810–825.

[8] G.İ. Tanrıkulu, Ö. Ertürk, C. Yavuz, Z. Can, H.E. Çakır, Chemical Compositions, Antioxidant and Antimicrobial Activities of the Essential Oil and Extracts of Lamiaceae Family (Ocimum basilicum and Thymbra spicata) from Turkey, Int. J. Second. Metab. 4 (2017) 340–348.

[9] C.M.O. Azeredo, T.G. Santos, B.H.L. de N.S. Maia, M.J. Soares, In vitro biological evaluation of eight different essential oils against Trypanosoma cruzi, with emphasis on Cinnamomum verum essential oil, BMC Complement. Altern. Med. 14 (2014) 1–8.

[10] A. Aharoni, M.A. Jongsma, H.J. Bouwmeester, Volatile science? Metabolic engineering of terpenoids in plants, Trends Plant Sci. 10 (2005) 594–602.

[11] Z.A.A. Aziz, A. Ahmad, S.H.M. Setapar, A. Karakucuk, M.M. Azim, D. Lokhat, M.A. Kamal, G.M. Ashraf, Essential Oils: Extraction techniques, Pharmaceutical and Therapeutic potential - A review, Curr. Drug Metab. 19 (2018).

[12] T. Fornari, G. Vicente, E. Vázquez, M.R. García-Risco, G. Reglero, Isolation of essential oil from different plants and herbs by supercritical fluid extraction, J. Chromatogr. A. 1250 (2012) 34–48.

[13] L.T. Danh, N.D.A. Triet, L.T.N. Han, J. Zhao, R. Mammucari, N. Foster, Antioxidant activity, yield and chemical composition of lavender essential oil extracted by supercritical CO2, J. Supercrit. Fluids. 70 (2012) 27–34.

[14] I.L.M. Barzotto, K.A. Santos, E.A. da Silva, A.C. Sene, N.S. da Silva, L. Vieira, Supercritical extraction of Eugenia involucrata leaves: Influence of operating conditions on yield and A-tocopherol content, J. Supercrit. Fluids. 143 (2019) 55–63.

[15] S. Bilgiç-Keleş, N. Şahin-Yeşilçubuk, A. Barla-Demirkoz, M. Karakaş, Response surface optimization and modelling for supercritical carbon dioxide extraction of Echium vulgare seed oil, J. Supercrit. Fluids. 143 (2019) 365–369.

[16] R.P. Cuco, L. Cardozo-Filho, C. da Silva, Simultaneous extraction of seed oil and active compounds from peel of pumpkin (Cucurbita maxima) using pressurized carbon dioxide as solvent, J. Supercrit. Fluids. 143 (2019) 8–15.

[17] P.C. Frohlich, K.A. Santos, F. Palú, L. Cardozo-Filho, C. da Silva, E.A. da Silva, Evaluation of the effects of temperature and pressure on the extraction of eugenol from clove (Syzygium aromaticum) leaves using supercritical CO2, J. Supercrit. Fluids. 143 (2019) 313–320.

[18] I. Lemonis, D. Tsimogiannis, V. Louli, E. Voutsas, V. Oreopoulou, K. Magoulas, Extraction of Dittany (Origanum dictamnus) using supercritical CO2and liquid solvent, J. Supercrit.

Fluids. 76 (2013) 48–53.

[19] H. Sovova, M. Sajfrtova, M. Topiar, Supercritical CO2extraction of volatile thymoquinone from Monarda didyma and M. fistulosa herbs, J. Supercrit. Fluids. 105 (2015) 29–34.

[20] M.S. de Oliveira, J.N. da Cruz, S. Gomes Silva, W.A. da Costa, S.H.B. de Sousa, F.W.F. Bezerra, E. Teixeira, N.J.N. da Silva, E.H. de Aguiar Andrade, A.M. de Jesus Chaves Neto, R.N. de Carvalho, Phytochemical profile, antioxidant activity, inhibition of acetylcholinesterase and interaction mechanism of the major components of the Piper divaricatum essential oil obtained by supercritical CO2, J. Supercrit. Fluids. 145 (2019) 74–84.

[21] P.. Sapkale, G.N. & Patil, S.M. & Surwase, U.S. & Bhatbhage, Supercritical fluid extraction - a review, Int. J. Chem. Sci. 8 (2010) 729–743. https://www.researchgate.net/publication/290484039_Supercritical_fluid_extraction_-_a_review.

[22] G. Brunner, Gas Extraction An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes, Steinkopff, Heidelberg, 1994.

[23] Y. Naudé, W.H.J. De Beer, S. Jooste, L. Van Der Merwe, S.J. Van Rensburg, Comparison of supercritical fluid extraction and Soxhlet extraction for the determination of DDT , DDD and DDE in sediment, Water South Africa. 24 (1998) 205–214.

[24] M. Herrero, J.A. Mendiola, A. Cifuentes, E. Ibáñez, Supercritical fluid extraction: Recent advances and applications, J. Chromatogr. A. 1217 (2010) 2495–2511.

[25] R. Parhi, P. Suresh, SUPERCRITICAL FLUID TECHNOLOGY: A REVIEW, J. Adv. Pharm. Sci. Technol. 1 (2013) 13–36.

[26] G. Brunner, Applications of Supercritical Fluids, Annu. Rev. Chem. Biomol. Eng. 1 (2010) 321–342.

[27] G. Brunner, Industrial Process Development Countercurrent Multistage Gas Extraction (SFE) Processes, J. Supercrit. Fluids. 13 (1998) 283–301.

[28] E. Reverchon, Supercritical fluid extraction and fractionation of essential oils and related products, J. Supercrit. Fluids. 10 (1997) 1–37.

[29] C. Grosso, J.P. Coelho, F.L.P. Pessoa, J.M.N.A. Fareleira, J.G. Barroso, J.S. Urieta, A.F. Palavra, H. Sovová, Mathematical modelling of supercritical CO2 extraction of volatile oils from aromatic plants, Chem. Eng. Sci. 65 (2010) 3579–3590.

[30] E.R. Gilliland, Diffusion Coefficients in Gaseous Systems, Ind. Eng. Chem. 26 (1934) 681–685.

[31] S. Chapman, T.G. Cowling, D. Burnett, The mathematical theory of non-uniform gases: an account of the kinetic theory of viscosity, thermal conduction and diffusion in gases, Ed 3°, Cambridge university press, Cambridge, United Kingdom, 1990.

[32] C.R. Wilke, P. Chang, Correlation of diffusion coefficients in dilute solutions, AIChE J. 1 (1955) 264–270.

[33] M.A. Siddiqi, K. Lucas, Correlations for prediction of diffusion in liquids, Can. J. Chem. Eng. 64 (1986) 839–843.

[34] C.-C. Lai, C.-S. Tan, Measurement of molecular Diffusion Coefficient in Supercritical Carbon Dioxide Using a Coated Capillary Column, Ind. Eng. Chem. Res. 34 (1995) 674–680.

[35] T. Funazukuri, C.Y. Kong, N. Murooka, S. Kagei, Measurements of Binary Diffusion Coefficients and Partition Ratios for Acetone, Phenol, alpha-Tocopherol, and beta-Carotene in Supercritical Carbon Dioxide with a Poly(ethylene glycol)-Coated Capillary Column, Ind. Eng. Chem. Res. 39 (2000) 4462–4469.

[36] A.A. Clifford, J.R. Williams, Introduction to supercritical fluids and their applications, Methods Biotechnol. 13 (2000) 1–16.

[37] R.L. Mendes, B.P. Nobre, M.T. Cardoso, A.P. Pereira, A.F. Palavra, Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae, Inorganica Chim. Acta. 356 (2003) 328–334.

[38] S.S. Petrović, J. Ivanović, S. Milovanović, I. ŽIžović, Comparative analyses of the diffusion coefficients from thyme for different extraction processes, J. Serbian Chem. Soc. 77 (2012) 799–813.

[39] R. V. Vaz, J.R.B. Gomes, C.M. Silva, Molecular dynamics simulation of diffusion coefficients and structural properties of ketones in supercritical CO2 at infinite dilution, J. Supercrit. Fluids. 107 (2016) 630–638.

[40] H. Higashi, Y. Iwai, H. Uchida, Y. Arai, Di ff usion coe ffi cients of aromatic compounds in supercritical carbon dioxide using molecular dynamics simulation, J. Supercrit. Fluids. 13 (1998) 93–97.

[41] H. Higashi, Y. Iwai, Y. Arai, Calculation of Self-Diffusion and Tracer Diffusion Coefficients near the Critical Point of Carbon Dioxide Using Molecular Dynamics Simulation, Ind. Eng. Chem. Res. 39 (2000) 4567–4570.

[42] J. Wang, H. Zhong, H. Feng, W. Qiu, L. Chen, Molecular dynamics simulation of diffusion coefficients and structural properties of some alkylbenzenes in supercritical carbon dioxide at infinite dilution, J. Chem. Phys. 140 (2014) 104501.

[43] R. Ravi, V. Guruprasad, Lennard-jones fluid and diffusivity: Validity of the hard-sphere model for diffusion in simple fluids and application to CO2, Ind. Eng. Chem. Res. 47 (2008) 1297–1303.

[44] H. Higashi, K. Tamura, Calculation of diffusion coefficient for supercritical carbon dioxide and carbon dioxide+naphthalene system by molecular dynamics simulation using EPM2 model, Mol. Simul. 36 (2010) 772–777.

[45] J.-H. Yoo, A. Breitholz, Y. Iwai, K.-P. Yoo, Diffusion coefficients of supercritical carbon dioxide and its mixtures using molecular dynamic simulations, Korean J. Chem. Eng. 29 (2012) 935–940.

[46] T.M. Yigzawe, R.J. Sadus, Intermolecular interactions and the thermodynamic properties of supercritical fluids, J. Chem. Phys. 138 (2013) 194502.

[47] A. Laesecke, C.D. Muzny, Reference correlation for the viscosity of carbon dioxide, J. Phys. Chem. Ref. Data. 46 (2017).

[48] P.E. Savage, S. Gopalan, T.I. Mizan, C.J. Martino, E.E. Brock, Reactions at supercritical conditions: Applications and fundamentals, AIChE J. 41 (1995) 1723–1778.

[49] R. Krishna, J.M. van Baten, Describing diffusion in fluid mixtures at elevated pressures by combining the Maxwell–Stefan formulation with an equation of state, Chem. Eng. Sci. 153 (2016) 174–187.

[50] D.-Y. Peng, D.B. Robinson, A New Two-Constant Equation of State, Ind. Eng. Chem. Fundam. 15 (1976) 59–64.

[51] A. Rai, K.D. Punase, B. Mohanty, R. Bhargava, Evaluation of models for supercritical fluid extraction, Int. J. Heat Mass Transf. 72 (2014) 274–287.

[52] V. Abrahamsson, N. Andersson, B. Nilsson, C. Turner, Method development in inverse modeling applied to supercritical fluid extraction of lipids, J. Supercrit. Fluids. 111 (2016) 14–27.

[53] M. Poletto, E. Reverchon, Comparison of Models for Supercritical Fluid Extraction of Seed and Essential Oils in Relation to the Mass-Transfer Rate, Ind. Eng. Chem. Res. 35 (1996) 3680–3686.

[54] X. Han, L. Cheng, R. Zhang, J. Bi, Extraction of safflower seed oil by supercritical CO2, J. Food Eng. 92 (2009) 370–376.

[55] A.G. Egorov, A.A. Salamatin, Bidisperse Shrinking Core Model for Supercritical Fluid Extraction, Chem. Eng. Technol. 38 (2015) 1203–1211.

[56] H. Sovová, Steps of supercritical fluid extraction of natural products and their characteristic times, J. Supercrit. Fluids. 66 (2012) 73–79.

[57] Z. Zeković, S. Filip, S. Vidović, S. Jokić, S. Svilović, Mathematical Modeling of Ocimum basilicum L. Supercritical CO 2 Extraction, Chem. Eng. Technol. 37 (2014) 2123–2128.

[58] A.A. Salamatin, Detection of Microscale Mass-Transport Regimes in Supercritical Fluid Extraction, Chem. Eng. Technol. 40 (2017) 829–837.

[59] S.M. Pourmortazavi, S.S. Hajimirsadeghi, Supercritical fluid extraction in plant essential and volatile oil analysis, J. Chromatogr. A. 1163 (2007) 2–24.

[60] E.M.. Reis-Vasco, J.A.. Coelho, A.M.. Palavra, C. Marrone, E. Reverchon, Mathematical modelling and simulation of pennyroyal essential oil supercritical extraction, Chem. Eng.

Sci. 55 (2000) 2917–2922.

[61] F. Gaspar, T. Lu, R. Santos, B. Al-Duri, Modelling the extraction of essential oils with compressed carbon dioxide, J. Supercrit. Fluids. 25 (2003) 247–260.

[62] M. Nejad-Sadeghi, S. Taji, I. Goodarznia, Optimization of supercritical carbon dioxide extraction of essential oil from Dracocephalum kotschyi Boiss: An endangered medicinal plant in Iran, J. Chromatogr. A. 1422 (2015) 73–81.

[63] M.S. de Oliveira, W.A. da Costa, D.S. Pereira, J.R.S. Botelho, T.O. de Alencar Menezes, E.H. de Aguiar Andrade, S.H.M. da Silva, A.P. da Silva Sousa Filho, R.N. de Carvalho, Chemical composition and phytotoxic activity of clove (Syzygium aromaticum) essential oil obtained with supercritical CO2, J. Supercrit. Fluids. 118 (2016) 185–193.

[64] M.S. de Oliveira, W.A. da Costa, D.S. Pereira, J.R.S. Botelho, T.O. de Alencar Menezes, E.H. de Aguiar Andrade, S.H.M. da Silva, A.P. da Silva Sousa Filho, R.N. de Carvalho, Chemical composition and phytotoxic activity of clove (Syzygium aromaticum) essential oil obtained with supercritical CO2, J. Supercrit. Fluids. 118 (2016) 185–193.

[65] A. Mouahid, C. Crampon, S.-A.A. Toudji, E. Badens, Effects of high water content and drying pre-treatment on supercritical CO2 extraction from Dunaliella salina microalgae: Experiments and modelling, J. Supercrit. Fluids. 116 (2016) 271–280.

[66] J.T. Paula, L.C. Paviani, M.A. Foglio, I.M.O. Sousa, F.A. Cabral, Extraction of anthocyanins from Arrabidaea chica in fixed bed using CO2 and CO2/ethanol/water mixtures as solvents, J. Supercrit. Fluids. 81 (2013) 33–41.

[67] M. Bimakr, R.A. Rahman, A. Ganjloo, F.S. Taip, L.M. Salleh, M.Z.I. Sarker, Optimization of Supercritical Carbon Dioxide Extraction of Bioactive Flavonoid Compounds from Spearmint (Mentha spicata L.) Leaves by Using Response Surface Methodology, Food Bioprocess Technol. 5 (2012) 912–920.

[68] S. Ghosh, D. Chatterjee, S. Das, P. Bhattacharjee, Supercritical carbon dioxide extraction of eugenol-rich fraction from Ocimum sanctum Linn and a comparative evaluation with other extraction techniques: Process optimization and phytochemical characterization, Ind. Crops Prod. 47 (2013) 78–85.

[69] T.T. Garmus, L.C. Paviani, C.L. Queiroga, F.A. Cabral, Extraction of phenolic compounds from pepper-rosmarin (Lippia sidoides Cham.) leaves by sequential extraction in fixed bed extractor using supercritical CO2, ethanol and water as solvents, J. Supercrit. Fluids. 99 (2015) 68–75.

[70] R. Scopel, M.A. Falcão, A.M. Lucas, R.N. Almeida, P.H.K. Gandolfi, E. Cassel, R.M.F. Vargas, Supercritical fluid extraction from Syzygium aromaticum buds: Phase equilibrium, mathematical modeling and antimicrobial activity, J. Supercrit. Fluids. 92 (2014) 223–230.

[71] G. Sodeifian, S.A. Sajadian, N. Saadati Ardestani, Experimental optimization and mathematical modeling of the supercritical fluid extraction of essential oil from Eryngium billardieri: Application of simulated annealing (SA) algorithm, J. Supercrit. Fluids. 127

(2017) 146–157.

[72] A. El Asbahani, K. Miladi, W. Badri, M. Sala, E.H.H.A. Addi, H. Casabianca, A. El Mousadik, D. Hartmann, A. Jilale, F.N.R. Renaud, A. Elaissari, Essential oils: From extraction to encapsulation, Int. J. Pharm. 483 (2015) 220–243.

[73] Y. Li, A.-S. Fabiano-Tixier, F. Chemat, Essential Oils: From Conventional to Green Extraction, in: Springer International Publishing, Cham, 2014: pp. 9–20.

[74] P.C. Frohlich, K.A. Santos, F. Palú, L. Cardozo-Filho, C. da Silva, E.A. da Silva, Evaluation of the effects of temperature and pressure on the extraction of eugenol from clove (Syzygium aromaticum) leaves using supercritical CO2, J. Supercrit. Fluids. 143 (2019) 313–320.

[75] A. Zermane, O. Larkeche, A.-H. Meniai, C. Crampon, E. Badens, Optimization of essential oil supercritical extraction from Algerian Myrtus communis L. leaves using response surface methodology, J. Supercrit. Fluids. 85 (2014) 89–94.

[76] S. Zhao, D. Zhang, Supercritical CO2 extraction of Eucalyptus leaves oil and comparison with Soxhlet extraction and hydro-distillation methods, Sep. Purif. Technol. 133 (2014) 443–451.

[77] N.P. Povh, M.O.M. Marques, M.A.A. Meireles, Supercritical CO2 extraction of essential oil and oleoresin from chamomile (Chamomilla recutita [L.] Rauschert), J. Supercrit. Fluids. 21 (2001) 245–256.

[78] A. Nematollahi, H. Kamali, N. Aminimoghadamfarouj, E. Golmakani, The optimization of essential oils supercritical CO 2 extraction from Lavandula hybrida through static-dynamic steps procedure and semi-continuous technique using response surface method, Pharmacognosy Res. 7 (2015) 57.

[79] T. Suetsugu, M. Tanaka, H. Iwai, T. Matsubara, Y. Kawamoto, C. Saito, Y. Sasaki, M. Hoshino, A.T. Quitain, M. Sasaki, J. Sakamoto, M. Goto, Supercritical CO2 extraction of essential oil from Kabosu (Citrus sphaerocarpa Tanaka) peel, Flavour. 2 (2013) 18.

[80] L.A. Conde-Hernández, J.R. Espinosa-Victoria, J.Á. Guerrero-Beltrán, Supercritical extraction of essential oils of Piper auritum and Porophyllum ruderale, J. Supercrit. Fluids. 127 (2017) 97–102.

[81] O. Larkeche, A. Zermane, A.-H. Meniai, C. Crampon, E. Badens, Supercritical extraction of essential oil from Juniperus communis L. needles: Application of response surface methodology, J. Supercrit. Fluids. 99 (2015) 8–14.

[82] N.M. Shrigod, N.R. Swami Hulle, R. V. Prasad, Supercritical fluid extraction of essential oil from mint leaves ( mentha spicata ): Process optimization and its quality evaluation, J. Food Process Eng. 40 (2017) e12488.

[83] Y.Y. Andrade-Avila, J. Cruz-Olivares, C. Pérez-Alonso, C.H. Ortiz-Estrada, M. del C. Chaparro-Mercado, Supercritical Extraction Process of Allspice Essential Oil, J. Chem. 2017 (2017) 1–8.

[84] N. Herzi, S. Camy, J. Bouajila, P. Destrac, M. Romdhane, J.-S. Condoret, Supercritical CO2 extraction of Tetraclinis articulata: Chemical composition, antioxidant activity and mathematical modeling, J. Supercrit. Fluids. 82 (2013) 72–82.

[85] N.S.M. Norodin, L.M. Salleh, Hartati, N.M. Mustafa, Supercritical carbon dioxide (SC-CO 2 ) extraction of essential oil from Swietenia mahagoni seeds, IOP Conf. Ser. Mater. Sci. Eng. 162 (2016) 012030.

[86] A. Donelian, L.H.C. Carlson, T.J. Lopes, R.A.F. Machado, Comparison of extraction of patchouli (Pogostemon cablin) essential oil with supercritical CO2 and by steam distillation, J. Supercrit. Fluids. 48 (2009) 15–20.

[87] Z. Zekovic, Z. Lepojevic, S. Milic, D. Adamovic, I. Mujic, Supercritical CO2 extraction of mentha (Mentha piperita L.) at different solvent densities, J. Serbian Chem. Soc. 74 (2009) 417–425.

[88] A.L. Chassagnez-Méndez, N.T. Machado, M.E. Araujo, J.G. Maia, M.A.A. Meireles, Supercritical CO 2 Extraction of Curcumins and Essential Oil from the Rhizomes of Turmeric ( Curcuma longa L.), Ind. Eng. Chem. Res. 39 (2000) 4729–4733.

[89] G. Sodeifian, N. Saadati Ardestani, S.A. Sajadian, S. Ghorbandoost, Application of supercritical carbon dioxide to extract essential oil from Cleome coluteoides Boiss: Experimental, response surface and grey wolf optimization methodology, J. Supercrit. Fluids. 114 (2016) 55–63.

[90] L.A. Conde-Hernández, J.R. Espinosa-Victoria, A. Trejo, J.Á. Guerrero-Beltrán, CO 2 - supercritical extraction, hydrodistillation and steam distillation of essential oil of rosemary ( Rosmarinus officinalis ), J. Food Eng. 200 (2017) 81–86.

[91] H. Bagheri, M.Y. Bin Abdul Manap, Z. Solati, Antioxidant activity of Piper nigrum L. essential oil extracted by supercritical CO2 extraction and hydro-distillation, Talanta. 121 (2014) 220–228.

[92] G. Sodeifian, S.A. Sajadian, N. Saadati Ardestani, Optimization of essential oil extraction from Launaea acanthodes Boiss: Utilization of supercritical carbon dioxide and cosolvent, J. Supercrit. Fluids. 116 (2016) 46–56.

[93] A. Zermane, O. Larkeche, A.-H. Meniai, C. Crampon, E. Badens, Optimization of Algerian rosemary essential oil extraction yield by supercritical CO2 using response surface methodology, Comptes Rendus Chim. 19 (2016) 538–543.

[94] E. Uquiche, N. Cirano, S. Millao, Supercritical fluid extraction of essential oil from Leptocarpha rivularis using CO 2, Ind. Crops Prod. 77 (2015) 307–314.

[95] L. Pistelli, S. Giovanelli, P. Margari, C. Chiappe, Considerable effect of dimethylimidazolium dimethylphosphate in cinnamon essential oil extraction by hydrodistillation, RSC Adv. 6 (2016) 52421–52426.

[96] E.S.S. Abdel-Hameed, M.S. Salman, M.A. Fadl, A. Elkhateeb, M.M. Hassan, Chemical Composition and Biological Activity of Mentha longifolia L. Essential Oil Growing in Taif,

KSA Extracted by Hydrodistillation, Solvent Free Microwave and Microwave Hydrodistillation, J. Essent. Oil-Bearing Plants. 21 (2018) 1–14.

[97] A. Filly, X. Fernandez, M. Minuti, F. Visinoni, G. Cravotto, F. Chemat, Solvent-free microwave extraction of essential oil from aromatic herbs: From laboratory to pilot and industrial scale, Food Chem. 150 (2014) 193–198.

[98] P.L.B. Figueiredo, R.C. Silva, J.K.R. da Silva, C. Suemitsu, R.H. V. Mourão, J.G.S. Maia, Chemical variability in the essential oil of leaves of Araçá (Psidium guineense Sw.), with occurrence in the Amazon, Chem. Cent. J. 12 (2018) 52.

[99] J.G.S. Maia, E.H.A. Andrade, Database of the Amazon aromatic plants and their essential oils, Quim. Nova. 32 (2009) 595–622.

[100] F. Augusto, A. Leite e Lopes, C.A. Zini, Sampling and sample preparation for analysis of aromas and fragrances, TrAC Trends Anal. Chem. 22 (2003) 160–169.

[101] M. Vinatoru, An overview of the ultrasonically assisted extraction of bioactive principles from herbs, Ultrason. Sonochem. 8 (2001) 303–313.

[102] E.-S.S. Abdel-Hameed, M.S. Salman, M.A. Fadl, A. Elkhateeb, M.M. Hassan, Chemical Composition and Biological Activity of Mentha longifolia L. Essential Oil Growing in Taif, KSA Extracted by Hydrodistillation, Solvent Free Microwave and Microwave Hydrodistillation, J. Essent. Oil Bear. Plants. 21 (2018) 1–14.

[103] M. Moridi Farimani, F. Mirzania, A. Sonboli, F.M. Moghaddam, Chemical composition and antibacterial activity of Dracocephalum kotschyi essential oil obtained by microwave extraction and hydrodistillation, Int. J. Food Prop. 20 (2017) 306–315.

[104] S. Burt, Essential oils: their antibacterial properties and potential applications in foods—a review, Int. J. Food Microbiol. 94 (2004) 223–253.

[105] E. Cassel, R.M.F. Vargas, Experiments and Modeling of the Cymbopogon winterianus Essential Oil Extraction by Steam Distillation Article, Chem. Soc. 50 (2006) 126–129. http://www.scielo.org.mx/pdf/jmcs/v50n3/v50n3a12.pdf.

[106] N.V. Busato, J.C. Silveira, A.O.S. da Costa, E.F. da Costa Junior, Estratégias de modelagem da extração de óleos essenciais por hidrodestilação e destilação a vapor, Ciência Rural. 44 (2014) 1574–1582.

[107] L.T. Danh, L.N. Han, N.D.A. Triet, J. Zhao, R. Mammucari, N. Foster, Comparison of Chemical Composition, Antioxidant and Antimicrobial Activity of Lavender (Lavandula angustifolia L.) Essential Oils Extracted by Supercritical CO2, Hexane and Hydrodistillation, Food Bioprocess Technol. 6 (2013) 3481–3489.

[108] P. Costa, C. Grosso, S. Gonçalves, P.B. Andrade, P. Valentão, M. Gabriela Bernardo-Gil, A. Romano, Supercritical fluid extraction and hydrodistillation for the recovery of bioactive compounds from Lavandula viridis L'Hér, Food Chem. 135 (2012) 112–121.

[109] N. Bousbia, M. Abert Vian, M.A. Ferhat, E. Petitcolas, B.Y. Meklati, F. Chemat,

Comparison of two isolation methods for essential oil from rosemary leaves: Hydrodistillation and microwave hydrodiffusion and gravity, Food Chem. 114 (2009) 355–362.

[110] M.C. Mesomo, M.L. Corazza, P.M. Ndiaye, O.R. Dalla Santa, L. Cardozo, A.D.P. Scheer, Supercritical CO2extracts and essential oil of ginger (Zingiber officinale R.): Chemical composition and antibacterial activity, J. Supercrit. Fluids. 80 (2013) 44–49.

[111] H. Bagheri, M.Y. Bin Abdul Manap, Z. Solati, Antioxidant activity of Piper nigrum L. essential oil extracted by supercritical CO2 extraction and hydro-distillation, Talanta. 121 (2014) 220–228.

[112] M. Khajeh, Y. Yamini, S. Shariati, Comparison of essential oils compositions of Nepeta persica obtained by supercritical carbon dioxide extraction and steam distillation methods, Food Bioprod. Process. 88 (2010) 227–232.

[113] E. Ghasemi, F. Raofie, N.M. Najafi, Application of response surface methodology and central composite design for the optimisation of supercritical fluid extraction of essential oils from Myrtus communis L. leaves, Food Chem. 126 (2011) 1449–1453.

[114] F. Menichini, R. Tundis, M. Bonesi, B. de Cindio, M.R. Loizzo, F. Conforti, G.A. Statti, R. Menabeni, R. Bettini, F. Menichini, Chemical composition and bioactivity of Citrus medica L. cv. Diamante essential oil obtained by hydrodistillation, cold-pressing and supercritical carbon dioxide extraction, Nat. Prod. Res. 25 (2011) 789–799.

[115] J. Ivanović, D. Mišić, M. Ristić, O. Pešić, I. Žižović, Supercritical CO2extract and essential oil of bay (Laurus nobilis L.) - Chemical composition and antibacterial activity, J. Serbian Chem. Soc. 75 (2010) 395–404.

[116] H. Bendif, K. Adouni, M.D. Miara, R. Baranauskienė, P. Kraujalis, P.R. Venskutonis, S.M. Nabavi, F. Maggi, Essential oils (EOs), pressurized liquid extracts (PLE) and carbon dioxide supercritical fluid extracts (SFE-CO2) from Algerian Thymus munbyanus as valuable sources of antioxidants to be used on an industrial level, Food Chem. 260 (2018) 289–298.

**Figures**

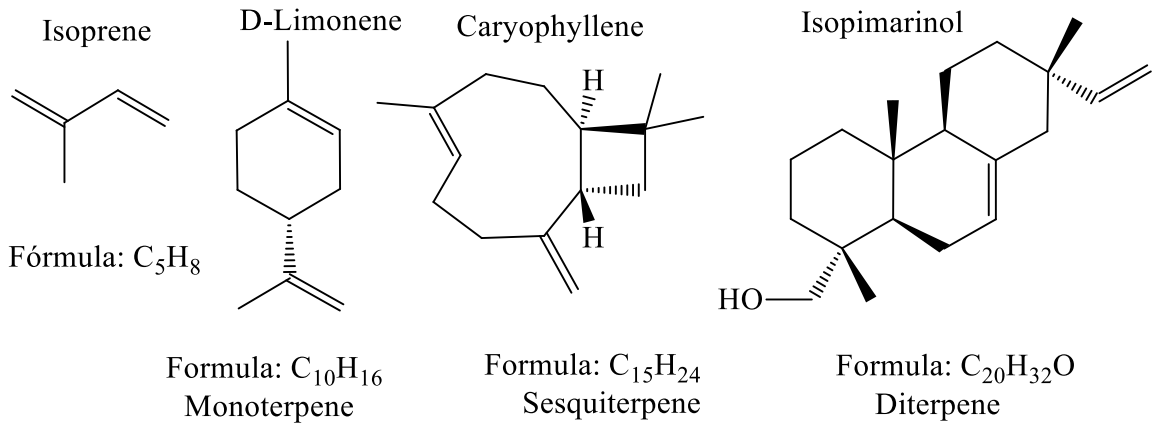


Figure 1 Isoprene units in the formation of different types of terpenoids


Figure 2 Applications of supercritical $CO_2$ in the extraction of bioactive compounds from natural products.

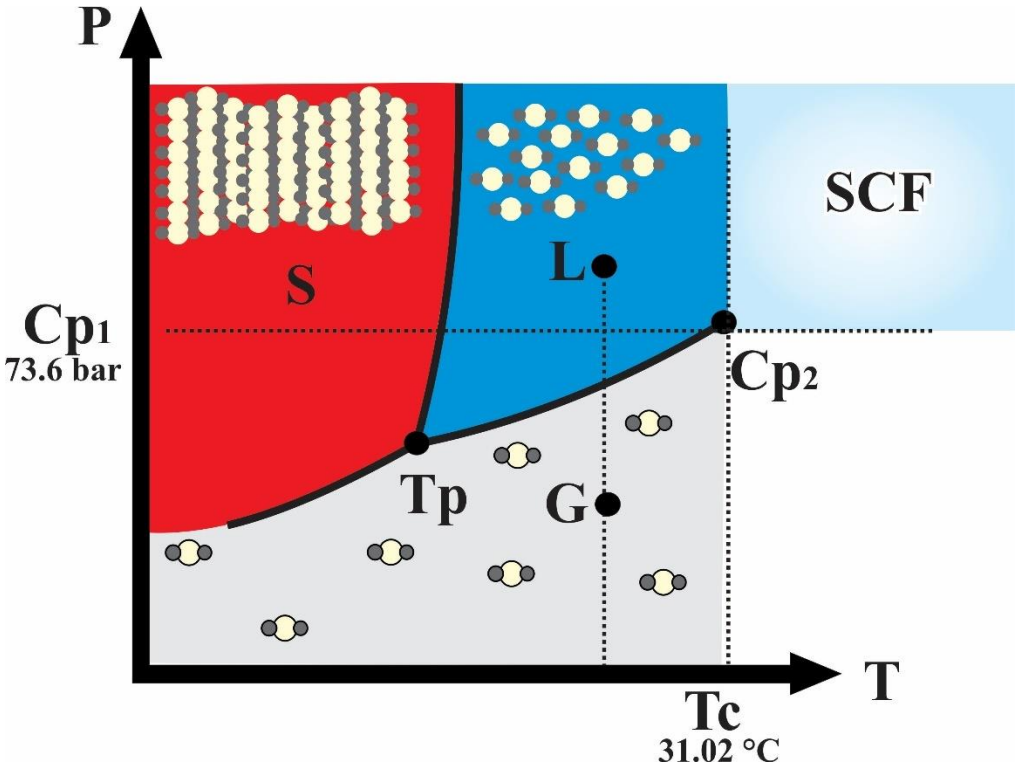


Figure 3. Phase diagram of $CO_2$ equilibrium states. The solid line (S), gass (G), liquid (L) does not require the phenomenon of condensation to cross the liquid-vapor coexistence curve at constant temperature. Triple point (TP), critical pressure ($Cp_1$), critical temperature (Ct), critical point ($Cp_2$), pressure (P), temperature (T).

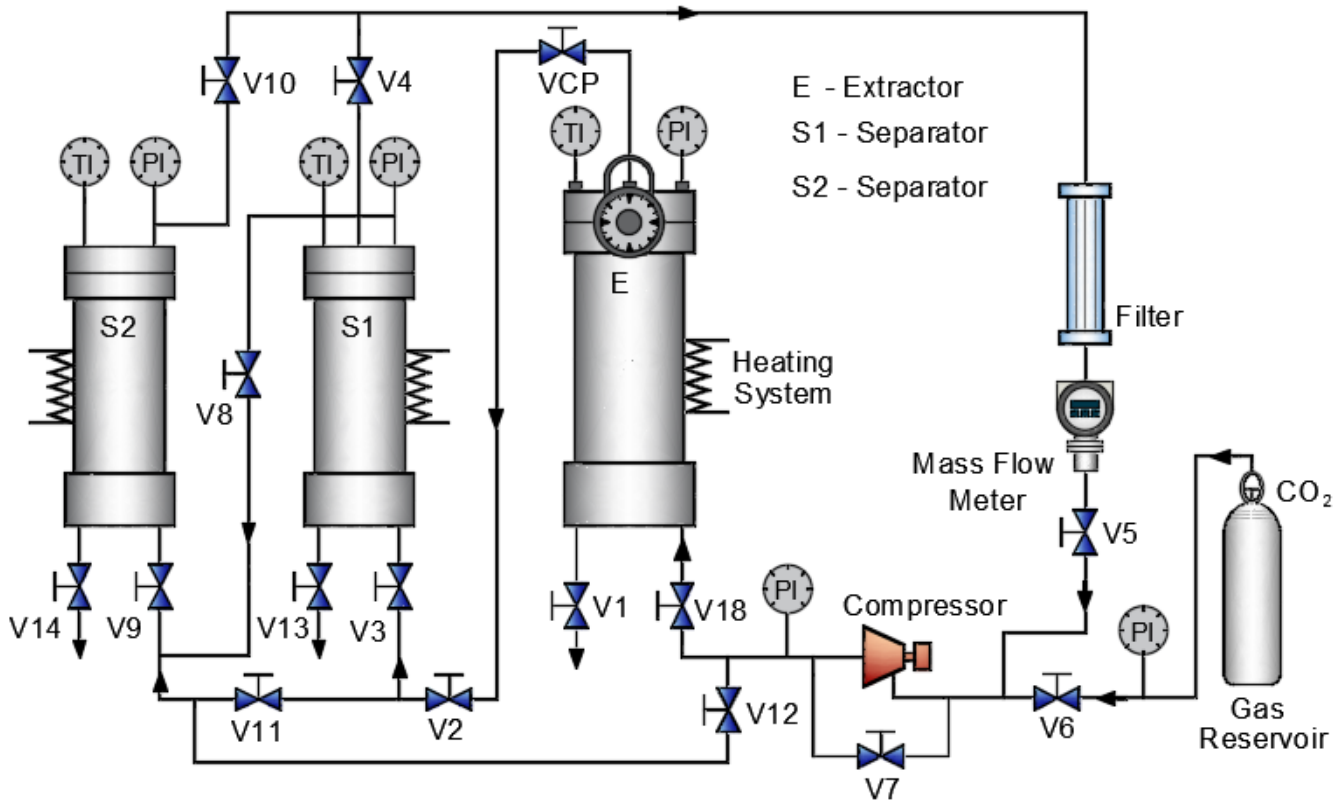


Figure 4. Scheme of an industrial supercritical fluid extraction plant.

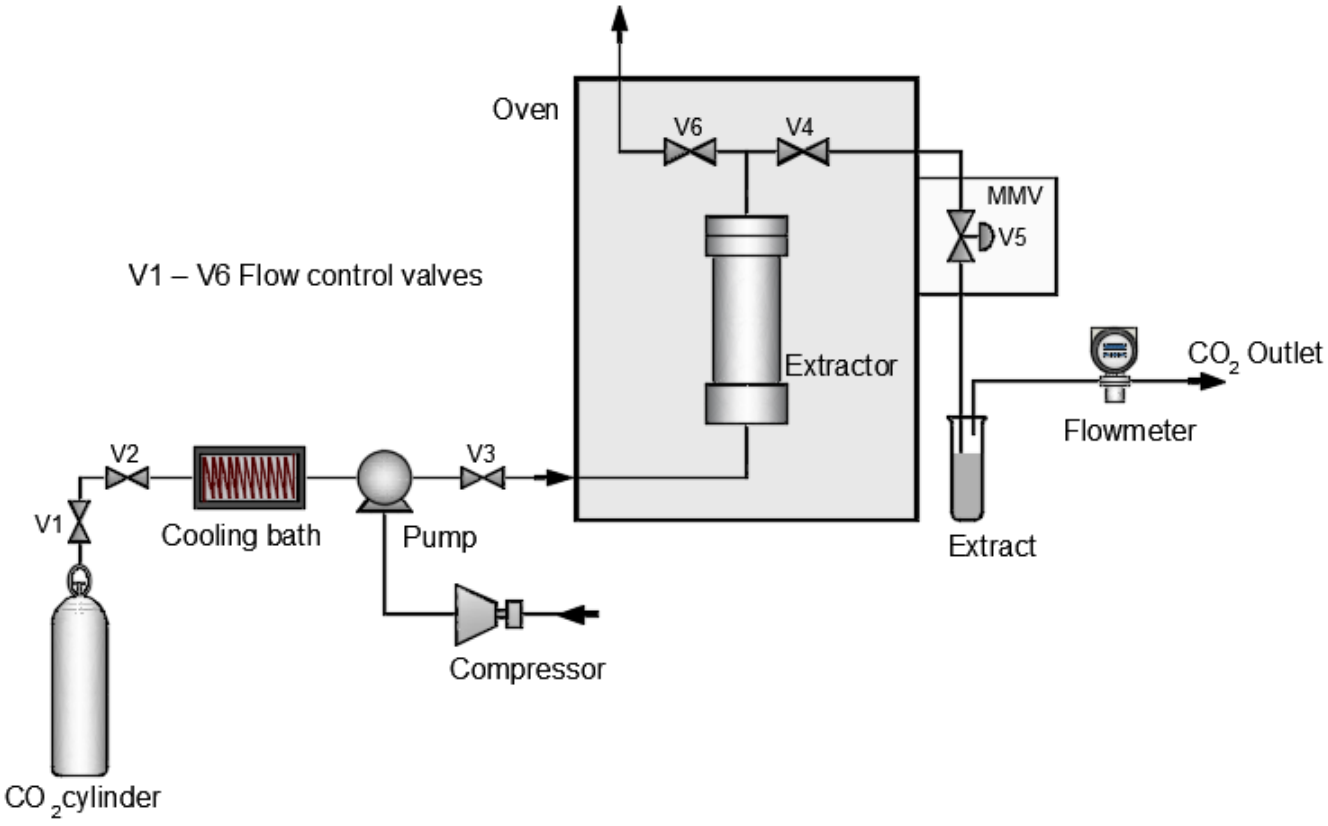


Figure 5. Scheme of supercritical fluid extraction plant in analytical scale.

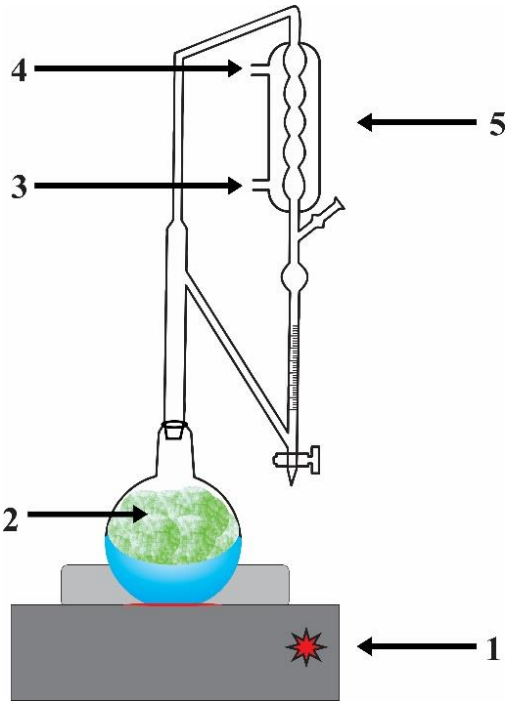


Figure 6. Hydrodistillation system, 1 = heating mantle, 2 = water + plant sample 3 = cold water inlet, 4 = water outlet, 5 = condenser. Adapted from [101].

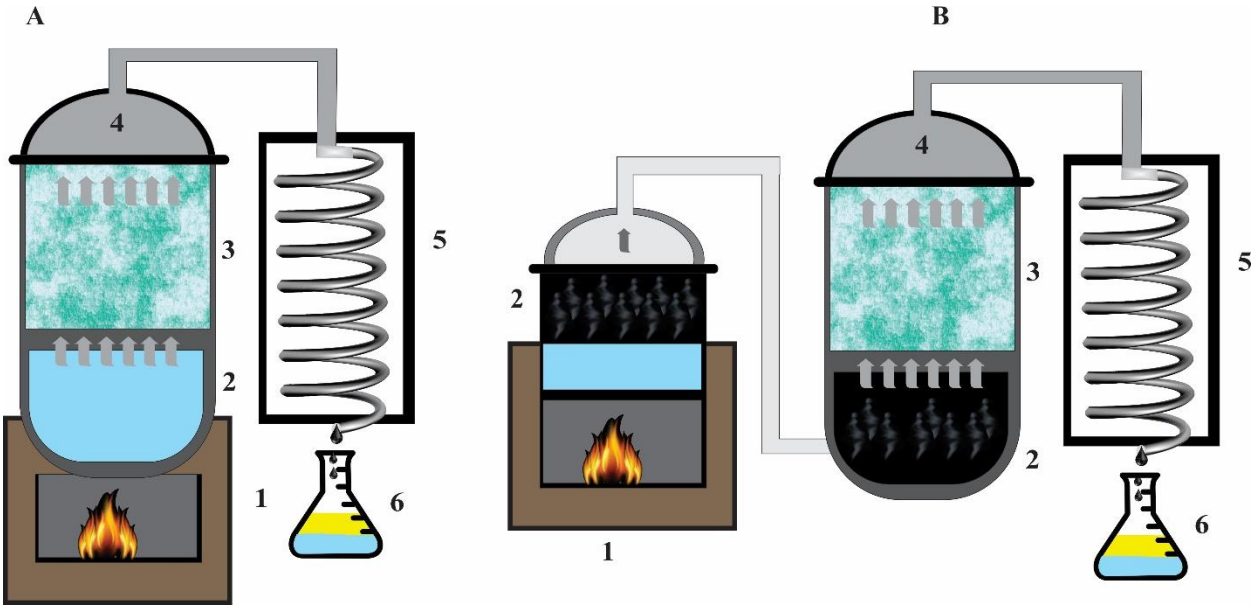


Figure 7. Representative flow diagram representative diagram of steam hydrodistillation (A) 1 = temperature controller, 2 = water, 3 = vegetable sample, 4 = water vapor + essential oil, 5 = condensers, 6 = collection flask. Steam distillation (B). 1 = temperature controller, 2 = water vapor, 3 = sample, 4 = essential oil vapor and water vapor, 5 = condenser, 6 = collection vial.

## Tables

Table 1 Critical Properties of Different Compounds.

| Critical Properties | Formula | Tc (°C) | Pc (bar) |
|---|---|---|---|
| Ethylene | $C_2H_4$ | 9.35 | 51 |
| Carbon dioxide | $CO_2$ | 31.02 | 73.6 |
| Methanol | $CH_3OH$ | 240.55 | 79 |
| Benzene | $C_6H_6$ | 289.05 | 49 |
| Water | $H_2O$ | 374.45 | 221 |

Table 2 Use of supercritical $CO_2$ to extract essential oils from different plant matrices.

| Raw material | T (ºC) and P (bar) | Yield (%) | Most concentrated compound | Reference |
|---|---|---|---|---|
| *Myrtus communis* | 40/300 | 4.89 | Not reported | [75] |
| *Eucalyptus loxophleba* | 70/400 | 4.78 | 1,8-Cineole | [76] |
| *Chamomilla recutita* | 40/200 | 4.33 | β-farnesene | [77] |
| *Lavandula hybrida* | 50/111.6 | 4.62 | 1,8-Cineole | [78] |
| *Citrus sphaerocarpa* | 80/200 | 1.55 | Limonene | [79] |
| *Piper auritum / Porophyllum ruderale* | 172/50 | 3.09 / 1.35 | Safrole / isosafrole | [80] |
| *Uniperus communis* | 55/300 | 6.55 | Germacrene D | [81] |
| *Mentha spicata* | 48/151 | 1.4 | Carvone | [82] |
| *Pimenta dioica* . | 45/360 | 68.47 | Eugenol | [83] |
| *Tetraclinis articulata* | 40/1000 | 25.5 | α-Pinene | [84] |
| *Swietenia mahagoni* | 60/300 | 20.68 | Not reported | [85] |
| *Pogostemon cablin* | 40/140 | 5.07 | Patchoulol | [86] |
| *Mentha piperita* | 40/100 | 3.57 | Menthol | [87] |

| | | | | |
|---|---|---|---|---|
| *Curcuma Longa*. | 45/300 | 6.51 | (Z)-γ-atlantona | [88] |
| *Cleome coluteoides* | 35/220 | 0.65 | α-Cadinol | [89] |
| *Rosmarinus officinalis* | 40/172 | 1.41 | Camphor | [90] |
| *Piper nigrum* | 50/300 | 2.88 | β-Caryophyllene | [91] |
| *Launaea acanthodes* | 54.85/240 | 1.02 | γ-Phenylbutyric acid | [92] |
| *Algerian rosemary* | 40/220 | 3.52 | Camphor | [93] |
| *Leptocarpha rivularis* | 50/120 | 4.60 | Caryophyllene oxide | [94] |

*T = temperature, P = pressure.

Table 3 Influence of the extraction method on yield and chemical composition of different plant species

| Raw material | Condition of extraction | | Yield (%) | | Majority compound | | Ref |
|---|---|---|---|---|---|---|---|
| | CE | SCE | CE | SCE | CE | SCE | |
| Rosemary leaves | **HD-** t= 180 min; **MHG-** t= 15 min | T = 40°C; P = 300 bar; t = 300 min | **HD-** 0.35±0.07%; **MHG-** 0.33±0.09% | 4.15% | **HD-** α-Pinene (44.05%); **MHG-** α-Pinene (43.60%) | 1,8-Cineole (59.20%). | [109] |
| Ginger roots (*Zingiber officinale* R.) | **HD-** t= 180 min | T = 60 °C; P = 250 bar; t = 180 min | **HD-** 1.79%; | 2.62% | **HD-** α-Curcumene (11.32%) | α-Zingiberene (19.77%) | [110] |
| *Piper nigrum* L. | **HD-** t= 270 min | T = 50 °C; P = 300 bar; t = 80 min | **HD-** 2.88 ± 0.07% | 2.16±0.02% | **HD-** β-Caryophyllene (18.64±0.84%) | β-Caryophyllene (25.38±0.62%) | [111] |
| Lavender (*Lavandula angustifolia* L.) | **HD-** t= 300 min; **HE-** t= 180 min | T= 45°C; P= 140 bar; t= 50 min | **HD-** 4.57±0.13%; **HE-** 7.57±0.11% | 6.68±0.57% | **HD-** Linalool (52.59±0.70%) **HE-** Linalool (33.35±8.26%) | Linalool (42.82±0.19%) | [107] |
| *Nepeta persica* **aerial parts** | **SD-** t= 90 min | T= 35°C; P= 355 bar | **SD-** **0.08%** | **8.90%** | **SD-** 4αβ,7α,7aα-nepetalactone (26.50%) | 4αβ,7α,7aα-nepetalactone (48.10%) | [112] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | t= 50 min | | | | | |
| *Myrtus communis* L. leaves | **HD-** t= 300 min | P= 350 bar; t= 25 min | **HD-**0.47% | 6.3**0%** | **HD-** α-Pinene (31.80%) | α-Pinene (38.60%) | [113] |
| *Citrus medica* L. cv. Diamante fruits | **HD-** t= 180 min | T= 40 °C; P= 100 bar; t= 360 min | **HD-** 0.10% (v/w); | 2.80**%** (v/w) | **HD-** Limonene (35.4±1.5%); | Citropten (84.50± 3.70%) | [114] |
| Bay leaves (*Laurus nobilis* L.) | **HD-** t= 240 min | T= 40 °C; P= 100 bar; t= 84 min | **HD-** 1.43% | 1.37**%** (v/w) | **HD-** 1,8-Cineole (33.40%) | Methyl linoleate (16.18%) | [115] |
| *Thymus munbyanus* subsp. coloratus | **HD-** t= 240 min | T= 70 °C; P= 450 bar; t = 180 min | **HD-** 0.11% | 0.35±0.0% | Camphor (11.70%) | Squalene (10.80%) | [116] |
| *Thymus munbyanus* subsp. munbyanus | **HD-** t= 240 min | T= 70 °C; P= 450 bar; t = 180 min | **HD-** 0.09% | 0.43±0.0% | (*E*)-Nerolidol (13.7%) | Squalene (11.40%) | |

**CE –** Conventional extraction; **SCE –** Supercritical Extraction, **t -** time; **T –** temperature; **P -** Pressure; **HD –** Hydrodistillation; **MHG -** Microwave Hydrodiffusion and Gravity; **HE –** Hexane extraction; **SD -** Steam distillation.

# 4 CAPÍTULO III.

4.1 Potential of medicinal use of essential oils from aromatic plants (Book title: Essential Oils).

Chapter 1

# Potential of Medicinal Use of Essential Oils from Aromatic Plants

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Additional information is available at the end of the chapter

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**Abstract**

The use of medicinal plants rich in essential oils can represent a viable source for the control of some diseases, being able to constitute a possible therapeutic alternative due to its effectiveness. Essential oils are natural volatile fractions extracted from aromatic plants and formed by classes of substances such as esters of fatty acids, mono and sesquiterpenes, phenylpropanoids, aldehyde alcohols and, in some cases, aliphatic hydrocarbons, among others. Essential oils have been used by mankind for medicinal purposes for several centuries, with reports coming from Ancient Egypt. In this sense, the present work aims to approach the biological activities of essential oils such as antioxidant, anticancer, antiprotozoal, antifungal, antibacterial and anti-inflammatory activities of different plant matrices rich in essential oils.

**Keywords:** natural products, essential oils, medicinal application, biological activity

## 1. Introduction

The essential oils are formed by volatile substances and generally have low molecular weight, these substances are formed in the secondary metabolism of aromatic plants [1, 2]. However, some natural factors such as physiological variations, environmental conditions, geographic variations, genetic factors and plant evolution can alter the chemical composition of these oils as well as their yield [3].

The extraction of essential oils usually occurs with the use of conventional techniques such as hydrodistillation using a Clevenger type extractor, which is the most widespread technique for the isolation of volatile plant oils [4, 5], however, other extraction techniques are also efficient such as extraction with supercritical $CO_2$ [6, 7], this type of extraction is a technique considered clean and does not cause change in the chemical structures of the molecules, since it usually works at low operating temperatures [8].

In nature, essential oils play an important role in plants as protection and communication, chemical protections that these secondary metabolites present, also is decisive in plant resistance against pathogens and herbivores [9]. In the communication the plant can use a chemical agent that travels through the atmosphere and activate defensive genes of other plants, such as the methyl jasmonate of *Solanaceae* and *Fabaceae* [10].

In the industry these oils are widely studied, mainly for their potential applications as agents promoting biological activities. The volatile compounds have presented over the years several pharmacological applications, such as antioxidant, anticancer, antiprotozoal, antimicrobial and anti-inflammatory activities [11–15]. In recent work [16] demonstrated that species like *Ocimum basilicum* and *Thymbra spicata* have good antioxidant and antimicrobial activity against *Staphylococcus aureus*, *Streptomyces murinus*, *Micrococcus luteus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Proteus vulgaris*, *Candida albicans* and *Aspergillus niger*. Jeena et al. [17] revealed that ginger oil has significant antioxidant, anti-inflammatory and antinociceptive activities and Xiang et al. [18] evidenced that the essential oils of *Curcuma herbs* have anticarcinogenic actions against LNCaP and HepG2 cells. In this sense, this work aims to approach different biological activities of essential oils that may be important for the maintenance of human health.

## 2. Biological activities of essential oils

### 2.1. Antibacterial and antifungal activity of essential oils (EO)

The antimicrobial action of essential oils is not yet fully understood, but can be attributed to their permeability to the cell wall of microorganisms due to their diverse chemical and synergistic composition. The hydrophobic characteristic of the essential oils acts in the partition of the lipids of the cellular membrane and the mitochondria, making them more permeable, in this way, the critical ions and molecules (lipids, proteins and nucleic acids) are extravasated, leading them to death. EOs generally have less action on gram-positive bacteria than on gram-negative bacteria due to the interaction of the hydrophobic components of the essential oils and the cell membrane [19–21].

Different methods are used to evaluate the antibacterial and antifungal properties. The most used are: the method of disc diffusion of Agar, Minimal Inhibition Concentration (MIC), Minimum Bacteria Concentration (MBC) and Minimum Fungicide Concentration (MFC). Since the use of the disc diffusion method in agar is limited by the hydrophobic nature of essential oils and plant extracts that prevents its uniform diffusion through the agar medium, most authors report the results obtained with MIC and MBC [22].

In recent years, different microbial species of medical interest have been tested, from which encouraging results have emerged. **Table 1** shows data on the antimicrobial activity of essential oils on fungi and bacteria, also showing the main components of essential oil.

The potential antimicrobial activity of essential oils of the *Hedychium coronarium* Koen rhizome from different locations in Eastern India was studied in gram-positive, gram-negative bacteria and fungal strains. The study revealed that the essential oils presented more satisfactory effects to the antifungal action than to the antibacterial activity. In addition, the gram-positive bacteria are more sensitive to oil than gram-negative due to the peptidoglycan layer did not selectively act on essential oil compounds. The antimicrobial action of the essential oils was attributed to its constituents in an isolated way, as well as synergistically, additive or antagonistic to each other [23].

Essential oils isolated from *Nepeta leucophylla*, *Nepeta ciliaris*, *Nepeta clarkei* and *Calamintha umbrosa* showed significant antifungal activity *in vitro* against phytopathogenic fungi responsible for plant diseases. Essential oils have the potential to be used as a possible biofungicide (as an alternative to synthetic products) that may contribute to an increase in the pre and post harvest storage life of food crops [25].

The good results obtained encourage future research aimed at a possible application of these substances in food, pharmaceutical and cosmetology fields. **Table 1** presents the main chemical components of essential oils of several plants with antimicrobial potential.

### 2.2. Antioxidant activity

The interest in the study of the antioxidant substances of essential oils has become more and more intensified and is now indispensable for the prevention of diverse pathologies [27]. In the literature, it is reported the presence of antioxidant activity in several essential oils [28–30].

This property acts at different levels in the microorganism protection and plays a key role in some of the biological activities of essential oils, being able to combat the development of oxidative stress that causes damage to health, increasing the risk of diseases such as Alzheimer's, Parkinson's and inflammation associated with atherosclerosis and rheumatoid arthritis. Some studies point out that these diseases may be consequences of damages caused by free radicals, besides oxygen and reactive nitrogen species that act as mediators of inflammation as messenger molecules. This shows that essential oils can also act as an anti-inflammatory agent [31–33].

Essential oils have great potential in the nutrition industry in view of their antioxidant properties, they are use as feed additives for farm animals, for example, and that may be fundamental to the quality of food products from these animals, since essential oils can improve nutritional value, oxidative stability and increase the shelf life of these products such as meats

| Plant source | Main components | Microorganism | *MIC | Reference |
|---|---|---|---|---|
| *Hedychium coronarium* Koen. | β-Pinene; eucalyptol; linalool; coronarin-E; α-pinene; p-cymene; γ-terpinene and 10-*epi*-γ-eudesmol | *Candida albicans* and *Fusarium oxysporum* | 3.12–400 μg/ml | [23] |
| *Laportea aestuans* (Gaud) | Methyl salicylate; fenchol; 1,2-cyclohexanedione dioxime; 1,4-octadiene and linalool | *E. coli*; *S. aureus*, *B. subtilis*; *P. aeruginosa*; *K. pneumoniae*; *S. typhi*; *C. albicans*; *R. stolon*; *A. niger* and *P. notatum* | 50–200 mg/ml | [24] |
| *C. umbrosa* | β-caryophyllene | *F. oxysporum* | 1500–3000 μg/ml | [25] |
| | Germacrene D | *H. maydis* | | |
| | Spathulenol | *A. solani* | | |
| *N. leucophylla* | Caryophyllene oxide | *F. oxysporum* | 1000–3000 μg/ml | |
| | Iridodial β-monoenol | *H. maydis* | | |
| | Acetate | *A. solani* | | |
| *N. ciliaris* | β-Caryophyllene | *F. oxysporum* | 1000–3000 μg/ml | |
| | β-Sesquiphellandrene | *H. maydis* | | |
| | Caryophyllene oxide | *A. solani* | | |
| *N. clarkei* | β-Sesquiphellandrene | *F. oxysporum* | 1000–3000 μg/ml | |
| | Actinidine | *H. maydis* | | |
| | Germacrene D | *A. solani* | | |
| *Juglans regia* L. | α-Pinene | *S. aureus* | 15.62–62.50 μg/ml | [26] |
| | β-Pinene | *E. coli* | | |
| | β-Caryophyllene germacrene D limonene | *S. typhi* | | |
| | | *S. dysenteriae* | | |
| | | *K. pneumonia* | | |
| | | *B. subtilis* | | |
| | | *S. epidermidis* | | |
| | | *P. vulgaris* | | |
| | | *P. aeruginosa* | | |

*Minimum Inhibitory Concentrations.

**Table 1.** Main components of essential oils with antimicrobial potential.

and eggs. In addition, they are often treated as foods to enhance the taste and organoleptic properties, and even has the function of decreasing the process of deterioration of food. The latter is mainly due to its antimicrobial and antioxidants activities [31, 34, 35].

The interest in extracts rich in natural antioxidants has recently increased, especially the antioxidant activity of essential oils. Most of them confirm the assumption that essential oils are promising as natural antioxidants, which can replace synthetic additives such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) that are potentially harmful to

human health [36–38]. In this context, **Table 2** presents some more recent studies found in the literature based on the antioxidant activity of essential oils, highlighting its main constituents and antioxidant performance evaluation methods.

### 2.3. Anticancer activity

Essential oils from aromatic plants have been treated as a product containing anticancer properties because they have the ability to inhibit cell proliferation and decrease the spread of cancer, improving the quality of life of cancer patients and reducing the level of their agony. Mediated therapy with essential oils can be used in combination with conventional therapies in the treatment of cancer (quimioterapia e radioterapia) [44–46].

According to the World Health Organization [47] cancer is a generic term used for a large group of diseases that can affect any part of the body, is characterized by the growth of abnormal cells beyond their usual limits in the body. Other common terms used are malignant tumors and neoplasms, the latter process or stage of the disease is called metastasis. Cancer is a major public health problem and is considered the second leading cause of death worldwide, accounting for 8.8 million deaths by 2015, where nearly 1 in 6 deaths is caused by cancer. Ref. [48] reported that the American Cancer Society reported in the year 2017 approximately 1,688,780 new cases of cancer and 600,920 deaths from cancer in the United States. According to [49–51] the most common causes of cancer death are melanoma, leukemia, followed by lung, liver, prostate, breast, cervical, colorectal, and endometrial cancers.

| Plant source | Main constituents | Biological activity | Reference |
|---|---|---|---|
| *Pinus* (*P. tabulaeformis*, *P. tabulaeformis* f. *shekanensis*, *P. tabulaeformis* var. *mukdensis*, *P. tabulaeformis* var. *umbraculifera*, *P. henryi* and *P. massoniana*) | α-Pinene, bornyl acetate, β-caryophyllene, α-guaiene, germacrene D | *Pinus* were evaluated for antioxidant potential by three methods (DPPH, FRAP and ABTS) | [39] |
| *Ocimum basilicum* L. | Linalool, methyl chavicol, 1,8-cineole | The free radical scavenging activity of the oil was measured by the DPPH method | [40] |
| *Ocimum basilicum*, *Mentha spicata*, *Pimpinella anisum* and *Fortunella margarita* | Carvone, methyl chavicol, trans-anethole, limonene | The evaluation of the ability to eliminate the free radicals of the oils was by the DPPH and ABTS methods | [41] |
| *Salvia lavandulifolia* | Camphor, 1,8-cineole, camphene, α-pinene | The *S. lavandulifolia* were evaluated for antioxidant potential by three methods (DPPH, FRAP and ABTS) | [42] |
| *Rosmarinus officinalis* | α-Pinene, 1,8-cineole, Camphor | The antioxidant activity was evaluated in 7 samples of rosemary oil based on the measurement of the antioxidant reduction capacity in relation to the DPPH radical | [43] |

**Table 2.** Antioxidant activity of essential oils.

The sharp increase in the number of cancer cases can be attributed to eating habits, since foods contain many chemicals such as preservatives and dyes, making people more susceptible to cancer, which can also be accentuated with the use of tobacco and alcohol, chronic infections, exposure to harmful radiation, or due to change in lifestyle and environmental pollution [45, 52]. Previous studies have reported that oxidative stress increases the onset of different chronic diseases, including cancer. Reactive oxygen species (ROS) are highly unstable compounds that have the ability to attack cells and tissues in the human body, followed by destructive effects that lead to the beginning of cancer [46, 53].

Therefore, there has been a recent increase in the use of natural products such as spices and plants to replace or accompany common treatments for cancer because of their high costs, side effects and the development of resistance of patients against anticancer drugs [44, 52].

Thus, essential oils from different aromatic plants have anticancer potential against mouth, breast, lung, prostate, liver, kidney, colon, bone, ovary, pancreas, uterus and brain cancer and even in leukemia, glioblastoma, melanoma [45, 54]. Thus [52] have shown that essential oil extracted from cloves (*Syzygium aromaticum* L.) is an ideal natural source as a chemopreventive agent against breast cancerbetulinic acid and other triterpenes, can be indicated as constituents responsible for anticancer properties [55] which determined that the essential oil of eucalyptus (*Pulicaria inuloides*) presented anticancer activity against breast, liver and colorectal/colon cancer due to the abundant presence of citronellol, pulegol and citronelil acetate.

The myrtle essential oil (*Myrtus communis* L.) shows anticancer activity against blood cancer (leukemia) due to the presence of 1,8-cineole, linalool, myrtenyl acetate, and myrtenol [56]. However, [46] have shown that orange peel oil (*Citrus sinensis*) has anticancer properties against colorectal/colon, prostate and lung cancer, with D-limonene being the predominant chemical constituent. Therefore, the results of studies justify the use of essential oils, as a possible alternative medicine in the treatment of cancer.

Essential oils act in the chemoprevention and suppression of cancer, which involve apoptosis, cell cycle retention, antimetastatic and antiangiogenic, increased levels of reactive oxygen and nitrogen species (ROS/RNS), modulation of DNA repair and others that demonstrate their antiproliferative cancer cell activity [53, 57]. In addition, the lipophilic nature of the EOs allows them to cross cell membranes and enter easily within the cell [45, 54], in **Table 3** we can observe the anticancer activities of different aromatic plants.

### 2.4. Antiparasitic activity

Current treatment media control most diseases of protozoan origin mainly through chemotherapy, where synthetic drugs are generally used, but they show several side effects of cytotoxicity in humans. Due to the hydrophobic and bioactivities nature of its components, essential oils (EO) can be considered important sources of development of agents against intracellular pathogens such as protozoa, which cause parasitic diseases [64].

The EO of leaves of *Artemisia indica* showed antimalarial activity in vitro, being a prophylactic potential of malaria, which is a disease caused by the protozoan of the genus *Plasmodium*. The oil inhibited at least two recombinant enzymes from the biosynthesis of plasmid fatty acids and showed low cytotoxicity in mammals [65].

| Plant source | Main constituents | Biological activity | Reference |
|---|---|---|---|
| *Rosa damascena* | Nerol, kaempferol and geraniol | Liver cancer, human breast cancer, prostate cancer | [58] |
| *Pulicaria inuloides* | 4,5-dimetiltiazol-2-il and 2,5-difeniltetrazólio | Breast cancer | [55] |
| *Citrus sinensis* | ɒ-Limonene and alcohol perylic (oxygenated monoterpene) | Colorectal/colon cancer, prostate cancer, lung cancer | [46] |
| *Aquilaria crassna* | β-Caryophyllene, 1-phenanthrenecarboxylic acid, α-caryophyllene and azulene benzenedicarboxylic acid | Colorectal/colon carcinoma, pancreatic cancer | [59] |
| *Myrtus communis* L. | 1,8-cineole, linalool, myrtenyl acetate and myrtenol | Blood cancer (leukemia) | [56] |
| *Eucalyptus citriodora* Hook | Pulegol, citronellol and citronellil acetate | Breast cancer, liver cancer, colorectal/colon cancer | [53] |
| *Cinnamon cassia* spp. | Cinnamic aldehyde, cinnamyl aldehyde and tannins | Head and neck cancer | [57] |
| *Syzygium aromaticum* L. | Betulinic acid and triterpenes | Human breast cancer | [52] |
| *Trachyspermum ammi* L. | γ-Terpinene, timol and P-cymene | Liver cancer | [60] |
| *Commiphora myrrha* | 2-cyclohexen-1-one and 4-ethynyl-4-hydroxy-3,5,5-trimethyl | Liver cancer, cervical cancer | [61] |
| *Salvia officinalis* | Hydrocarbons, monoterpene, oxygenated monoterpenes sesquiterpene and diterpenes | Human breast cancer, prostate cancer, kidney cancer | [62] |
| *Tagetes minuta* L. | cis-β-ocimene, cis-tagetone and trans-tagetenone | Breast cancer, blood cancer (leukemia) | [63] |

**Table 3.** Anticancer activity of essential oils.

Another EO that presents the antimalarial effect is that obtained from *Piper aduncum* leaves, with camphor (17.1%), viridiflorol (14.5%) and piperitone (23.7%) being the main components found in this oil [66]. The EO of the leaves of *Aniba canelilla* (HBK) Mez presented a trypanocidal effect, being considered a potential for the natural treatment to trypanosomosis, which is caused by the protozoan *Trypanosoma evansi*, since it proved its action *in vivo*. Its antiprotozoal activity is related to the compounds 1-nitro-2-phenylethane (83.68%) and methyleugenol (14.83%), the latter being slightly more active than the first in the treatment of the disease [67].

The EO of the leaves of *Tetradenia riparia* presented antileishmanial effect *in vivo* and *in vitro*, being effective in the fight against the protozoan of the species *Leishmania (Leishmania) amazonenses*, without showing toxicity to human erythrocytes. The main compound responsible for this therapeutic effect is the 6,7-dehydroroyleanone, which was also tested in isolation and showed a similar effect to the EO [68]. EO from *Lippia alba*, was investigated *in vitro* and *in vivo* assays to evaluate antiparasitic effects and histopathological changes of tambaqui (*Colossoma macropomum*). Concentrations of 1280 and 2560 mg/L showed 100% efficacy after 20 min of oil exposure in (*Anacanthorus spathulatus*, *Notozothecium janauachensis* and *Mymarothecium boegeri*) [69].

The antiparasitic activity of *Lavandula stoechas* oil was investigated in *Leishmania major*, *Leishmania tropica* and *Leishmania infantum*. The evaluation of the antileishmanial activity of *Lavandula stoechas* EO presented a greater effect in comparison to the drug Glucantime. The bioactive compounds present in this oil are: fenchone (31.81%), camphor (29.60%), terpineol (13.14%), menthone (8.96%) and eucalyptol [70].

The anthelmintic activity of *Thymus vulgaris* L. EO was investigated in *in vitro* and *in vivo* tests to evaluate the effect on *Haemonchus contortus* parasites present in the gastrointestinal system of sheep. Thymol is the major compound corresponding to 50.22% of the oil from the *Thymus vulgaris* species. Results showed that EO inhibited 96.4% of egg incubation, 90.8% of larval development and 97% of larval mobility [71]. Other essential oils, their chemical constituents and biological antiparasitic activities are shown in **Table 4**.

### 2.5. Anti-inflammatory activity

Essential oils have complex mixtures of chemicals that are present in different concentrations, these oils are used in medicine to treat a myriad of diseases because they present potential for anti-inflammatory activity [78, 79].

Inflammation is typically a protective mechanism that can be stimulated by a variety of harmful agents, which may be chemical, physical or biological. Living and vascular tissues respond to stimuli that are considered irritating to the body. These irritations can usually be linked to pain, redness (erythema), heat, tumor (edema), tissue loss or organic function [80, 81].

In recent years the anti-inflammatory potential of essential oils and their chemical position has become the object of study of several researchers in the search for new drugs of natural origin [82–84], as well as a study of the synergistic anti-inflammatory effect of the chemical constituents of essential oils and synthetic drugs, showing a possible association between

| Plant source | Main constituents | Biological activity | Reference |
|---|---|---|---|
| *Chenopodium ambrosioides* | Ascaridole, carvacrol and caryophyllene oxide | Antileshmanial, antimalarial and antitrypanosoma | [72] |
| *Cinnamomum verum* | (E)-cinnamaldehyde and eugenol | Antitrypanosoma | [73] |
| *Eugenia uniflora* L. | Sesquiterpenes, curzerene, γ-elemene and trans-β-elemenone | Antileshmanial | [74] |
| *Lavandula angustifolia* | Borneol, epi-ɒ-muurolol, ɒ-bisabolol, precocene I and eucalyptol | Antischistosomatic | [75] |
| *Piper hispidinervum* (Piperaceae) | Safrole | Antiamoebicidal | [76] |
| *Teucrium ramosissimum* | δ-Cadinene, δ-cadinol, β-eudesmol, γ-gurjunene and cedrene | Antiamoebicidal | [77] |

**Table 4.** Anti-parasitic activity of essential oils.

| Plant source | Main constituents | Biological activity | Reference |
|---|---|---|---|
| *Globba sessiliflora* Sims. | β-Eudesmol, (E)-β-caryophyllene, caryophyllene oxide, T-muurolol | Anti-inflammatory | [91] |
| *Piper glabratum* | β-Pinene, longiborneol, α-pinene, (E)-caryophyllene | Anti-inflammatory | [84] |
| *Phyllanthus muellerianus* | Isoelemicinb, caryophyllene oxide, α-Cadinol, 2-isopropyl benzoic acid | Anti-inflammatory | [92] |
| *Salvia officinalis* | 1,8-Cineole, camphor, β-pinene, E-β-caryophyllene | Anti-inflammatory | [93] |
| *Lippia gracilis* Schauer | Thymol, carvacrol, p-cymene, α-pinene | Anti-inflammatory and healing activity | [94] |
| *Citrus limon* | Limonene, β-pinene, γ-terpinene, sabinene | Anti-inflammatory | [95] |
| *Cymbopogon citratus* | Geranial, neral, β-myrcene, geranyl acetate | Anti-inflammatory | [96]. |
| *Anethum graveolens* L. | α-Phellandrene, limonene, dill ether, α-pinene | Anti-inflammatory | [97] |
| *Citrus aurantium* L. | Linalool, linalylacetate, nerolidol, Z,E-farnesol | Anti-inflammatory | [98] |
| *Blumea balsamifera* (L.) DC. | Borneol, caryophyllene, ledol, caryophyllene oxide | Anti-inflammatory | [99] |

**Table 5.** Anti-inflammatory activity of essential oils.

clinical remedies with natural products as a pharmacological alternative and avoiding adverse reactions caused by synthetic products [85]. In vivo tests performed on rats confirm the potential of these essential oils as natural products, helping to advance research [86, 87].

The knowledge of the chemical composition and the chemootype of the aromatic plants are important factors in studies of the anti-inflammatory activity, since the concentration of the compounds diverge due to this biological variation, in this way researchers have evaluated both aspects [88, 89]. Evaluating the specific constituents of a particular essential oil may help in understanding the performance of these compounds in the anti-inflammatory action [90]. **Table 5** shows the anti-inflammatory potential of different essential oils.

## 3. Conclusion

Essential oils may play an important role in the maintenance of human health, since they have several biological properties, and may become a natural alternative for the control of several diseases, however, the great majority of published works present the results of these oils based on its chemical composition complex and not only based on a substance, because the biological effects of these oils can be related to a synergism and/or an antagonism between the chemically active substances that are part of its composition.

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## References

[1] Aharoni A, Jongsma MA, Bouwmeester HJ. Volatile science? Metabolic engineering of terpenoids in plants. Trends in Plant Science. 2005;**10**(12):594-602

[2] Hartmann T. From waste products to ecochemicals: Fifty years research of plant secondary metabolism. Phytochemistry. 2007;**68**(22-24):2831-2846

[3] FigueiredoAC, BarrosoJG, PedroLG, SchefferJJC. Factors affecting secondary metabolite production in plants: Volatile components and essential oils. Flavour and Fragrance Journal. 2008;**23**(4):213-226. Available from: %5C%5CRobsrv-05%5Creference manager%5CArticles%5C8230.pdf

[4] Roby MHH, Sarhan MA, Selim KAH, Khalel KI. Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare* L.) and chamomile (*Matricaria chamomilla* L.). Industrial Crops and Products. 2013;**44**:437-445. DOI: 10.1016/j.indcrop.2012.10.012

[5] Diao W-R, Hu Q-P, Zhang H, Xu J-G. Chemical composition, antibacterial activity and mechanism of action of essential oil from seeds of fennel (*Foeniculum vulgare* Mill.). Food Control. 2014;**35**(1):109-116. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0956713513003393

[6] Danh LT, Triet NDA, Han LTN, Zhao J, Mammucari R, Foster N. Antioxidant activity, yield and chemical composition of lavender essential oil extracted by supercritical $CO_2$. Journal of Supercritical Fluids. 2012;**70**(February):27-34. DOI: 10.1016/j.supflu.2012.06.008

[7] Zermane A, Larkeche O, Meniai A-H, Crampon C, Badens E. Optimization of essential oil supercritical extraction from Algerian *Myrtus communis* L. leaves using response

surface methodology. Journal of Supercritical Fluids. 2014;**85**(March):89-94. Available from: http://www.sciencedirect.com/science/article/pii/S0896844613003665

[8] Fornari T, Vicente G, Vázquez E, García-Risco MR, Reglero G. Isolation of essential oil from different plants and herbs by supercritical fluid extraction. Journal of Chromatography A. 2012;**1250**:34-48. DOI: 10.1016/j.chroma.2012.04.051

[9] Wink M. Plant breeding: Importance of plant secondary metabolites for protection against pathogens and herbivores. Theoretical and Applied Genetics. 1988;**75**(2):225-233

[10] Farmer EE, Ryan CA. Interplant communication: Airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proceedings of the National Academy of Sciences of the United States of America. 1990;**87**(19):7713-7716. DOI: 10.1073/pnas.87.19.7713

[11] Wang W, Li N, Luo M, Zu Y, Efferth T. Antibacterial activity and anticancer activity of *Rosmarinus officinalis* L. essential oil compared to that of its main components. Molecules. 2012;**17**(3):2704-2713

[12] Benavides S, Villalobos-Carvajal R, Reyes JE. Physical, mechanical and antibacterial properties of alginate film: Effect of the crosslinking degree and oregano essential oil concentration. Journal of Food Engineering. 2012;**110**(2):232-239. DOI: 10.1016/j.jfoodeng.2011.05.023

[13] Leal SM, Pino N, Stashenko EE, Martínez JR, Escobar P. Antiprotozoal activity of essential oils derived from Piper spp. grown in Colombia. Journal of Essential Oil Research. 2013;**25**(6):512-519

[14] Silva FV, Guimarães AG, Silva ERS, Sousa-Neto BP, Machado FDF, Quintans-Júnior LJ, Arcanjo DDR, Oliveira FA, Oliveira RCM. Anti-inflammatory and anti-ulcer activities of carvacrol, a monoterpene present in the essential oil of oregano. Journal of Medicinal Food. 2012;**15**(11):984-991. DOI: http://online.liebertpub.com/doi/abs/10.1089/jmf.2012.0102

[15] Noori S, Zeynali F, Almasi H. Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (*Zingiber officinale*) essential oil and its effect on safety and quality attributes of chicken breast fillets. Food Control. 2018;**84**:312-320. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0956713517304103

[16] Tanrıkulu Gİ, Ertürk Ö, Yavuz C, Can Z, Çakır HE. Chemical compositions, antioxidant and antimicrobial activities of the essential oil and extracts of Lamiaceae Family (*Ocimum basilicum* and *Thymbra spicata*) from Turkey. International Journal of Secondary Metabolite. 2017;**4**(2):340-348. DOI: http://dergipark.gov.tr/doi/10.21448/ijsm.373828

[17] Jeena K, Liju VB, Kuttan R. Antioxidant, anti-inflammatory and antinociceptive activities of essential oil from ginger. Indian Journal of Physiology and Pharmacology. 2013;**57**(1):51-62

[18] Xiang H, Zhang L, Xi L, Yang Y, Wang X, Lei D, Zheng X, Liu X. Phytochemical profiles and bioactivities of essential oils extracted from seven Curcuma herbs. Industrial Crops and Products. 2018;**111**(October):298-305

[19] Calo JR, Crandall PG, O'Bryan CA, Ricke SC. Essential oils as antimicrobials in food systems—A review. Food Control. 2015;**54**:111-119. DOI: 10.1016/j.foodcont.2014.12.040

[20] Akthar MS, Birhanu Degaga TA. Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms: A review antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms: A review. Issues in Biological Sciences and Pharmaceutical Research. 2014;**2**(1):001-007

[21] Solórzano-Santos F, MG M-N. Essential oils from aromatic herbs as antimicrobial agents. Current Opinion in Biotechnology. 2012;**23**(2):136-141

[22] Bilia AR, Santomauro F, Sacco C, Bergonzi MC, Donato R. Essential Oil of Artemisia annua L.: An Extraordinary Component with Numerous Antimicrobial Properties. Evidence-Based Complement Altern Med [Internet]. 2014;**2014**:1-7. Available from: http://www.hindawi.com/journals/ecam/2014/159819/

[23] Ray A, Jena S, Dash B, Kar B, Halder T, Chatterjee T, Ghosh B, Panda PC, Nayak S, Mahapatra N. Chemical diversity, antioxidant and antimicrobial activities of the essential oils from Indian populations of Hedychium coronarium Koen. Industrial Crops and Products. 2018;**112**(December):353-362. Available from: https://www.sciencedirect.com/science/article/pii/S0926669017308622

[24] Oloyede GK. Toxicity, antimicrobial and antioxidant activities of methyl salicylate dominated essential oils of *Laportea aestuans* (Gaud). Arabian Journal of Chemistry. 2016 Sep;**9**:S840-S845. DOI: 10.1016/j.arabjc.2011.09.019

[25] Kumar V, Mathela CS, Tewari AK, Bisht KS. In vitro inhibition activity of essential oils from some Lamiaceae species against phytopathogenic fungi. Pesticide Biochemistry and Physiology. 2014;**114**(1):67-71. DOI: 10.1016/j.pestbp.2014.07.001

[26] Rather MA, Dar BA, Dar MY, Wani BA, Shah WA, Bhat BA, Ganai BA, Bhat KA, Anand R, Qurishi MA. Chemical composition, antioxidant and antibacterial activities of the leaf essential oil of *Juglans regia* L. and its constituents. Phytomedicine. 2012;**19**(13):1185-1190. DOI: 10.1016/j.phymed.2012.07.018

[27] Ye C-L, Dai D-H, Hu W-L. Antimicrobial and antioxidant activities of the essential oil from onion (*Allium cepa* L.). Food Control. 2013;**30**(1):48-53. Available from: http://linkinghub.elsevier.com/retrieve/pii/S095671351200429X

[28] Teixeira B, Marques A, Ramos C, Batista I, Serrano C, Matos O, Neng NR, Nogueira JMF, Saraiva JA, Nunes ML. European pennyroyal (*Mentha pulegium*) from Portugal: Chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil. Industrial Crops and Products. 2012;**36**(1):81-87

[29] Ćavar S, Maksimović M, Vidic D, Parić A. Chemical composition and antioxidant and antimicrobial activity of essential oil of *Artemisia annua* L. from Bosnia. Industrial Crops and Products. 2012;**37**(1):479-485

[30] Moradi M, Tajik H, Razavi Rohani SM, Oromiehie AR, Malekinejad H, Aliakbarlu J, Hadian M. Characterization of antioxidant chitosan film incorporated with Zataria multiflora Boiss essential oil and grape seed extract. LWT—Food Science and Technology. 2012;**46**(2):477-484. DOI: 10.1016/j.lwt.2011.11.020

[31] Pérez-Rosés R, Risco E, Vila R, Peñalver P, Cañigueral S. Biological and nonbiological antioxidant activity of some essential oils. Journal of Agricultural and Food Chemistry. 2016;**64**(23):4716-4724

[32] Amorati R, Foti MC, Valgimigli L. Antioxidant activity of essential oils. Journal of Agricultural and Food Chemistry. 2013;**61**(46):10835-10847. Available from:. DOI: http://www.ncbi.nlm.nih.gov/pubmed/24156356%5Cnhttp://pubs.acs.org/doi/abs/10.1021/jf403496k

[33] Hsu F-L, Li W-H, Yu C-W, Hsieh Y-C, Yang Y-F, Liu J-T, Shih J, Chu Y-J, Yen P-L, Chang S-T, Liao VH-C. In vivo antioxidant activities of essential oils and their constituents from leaves of the Taiwanese Cinnamomum osmophloeum. Journal of Agricultural and Food Chemistry. 2012;**60**(12):3092-3097. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0378874101002732

[34] Wang HF, Yih KH, Yang CH, Huang KF. Anti-oxidant activity and major chemical component analyses of twenty-six commercially available essential oils. Journal of Food and Drug Analysis. 2017;**25**(4):881-889. DOI: 10.1016/j.jfda.2017.05.007

[35] Mimica-DukićN, OrčićD, LesjakM, ŠibulF. Essential oils as powerful antioxidants: Misconception or scientific fact? In: ACS Symposium Series [Internet]. 1st ed. Washington, DC; 2016. p. 187-208. Available from: http://pubs.acs.org/doi/abs/10.1021/bk-2016-1218.ch012

[36] Rashid S, Rather MA, Shah WA, Bhat BA. Chemical composition, antimicrobial, cytotoxic and antioxidant activities of the essential oil of *Artemisia indica* Willd. Food Chemistry. 2013;**138**(1):693-700. DOI: 10.1016/j.foodchem.2012.10.102

[37] Miri A, Monsef-Esfahani HR, Amini M, Amanzadeh Y, Hadjiakhoondi A, Hajiaghaee R, Ebrahimi A. Comparative chemical composition and antioxidant properties of the essential oils and aromatic water from Teucrium persicum Boiss. Iranian Journal of Pharmaceutical Research. 2012;**11**(2):573-581

[38] Taghvaei M, Jafari SM. Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. Journal of Food Science and Technology. 2015;**52**(3):1272-1282

[39] Xie Q, Liu Z, Li Z. Chemical composition and antioxidant activity of essential oil of six Pinus taxa native to China. Molecules. 2015;**20**(5):9380-9392

[40] Chenni M, El Abed D, Rakotomanomana N, Fernandez X, Chemat F. Comparative study of essential oils extracted from Egyptian basil leaves (*Ocimum basilicum* L.) using hydrodistillation and solvent-free microwave extraction. Molecules. 2016;**21**(1):113. Available from: http://www.mdpi.com/1420-3049/21/1/113

[41] Fitsiou E, Mitropoulou G, Spyridopoulou K, Tiptiri-Kourpeti A, Vamvakias M, Bardouki H, Panayiotidis MI, Galanis A, Kourkoutas Y, Chlichlia K, Pappa A. Phytochemical profile and evaluation of the biological activities of essential oils derived from the Greek aromatic plant species *Ocimum basilicum*, *Mentha spicata*, *Pimpinella anisum* and *Fortunella margarita*. Molecules. 2016;**21**(8):1069. Available from: http://www.mdpi.com/1420-3049/21/8/1069

[42] Cutillas A-B, Carrasco A, Martinez-Gutierrez R, Tomas V, Tudela J. Composition and antioxidant, antienzymatic and antimicrobial activities of volatile molecules from Spanish *Salvia lavandulifolia* (Vahl) essential oils. Molecules. 2017;**22**(8):1382. Available from: http://www.mdpi.com/1420-3049/22/8/1382

[43] Bajalan I, Rouzbahani R, Pirbalouti AG, Maggi F. Antioxidant and antibacterial activities of the essential oils obtained from seven Iranian populations of *Rosmarinus officinalis*. Industrial Crops and Products. 2017;**107**(February):305-311. DOI: 10.1016/j.indcrop.2017.05.063

[44] Bayala B, Bassole IH, Scifo R, Gnoula C, Morel L, Lobaccaro J-MA, Simpore J. Anticancer activity of essential oils and their chemical components—A review. American Journal of Cancer Research. 2014;**4**(6):591-607. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25520854%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4266698

[45] Gautam N, Mantha AK, Mittal S. Essential Oils and Their Constituents as Anticancer Agents: A Mechanistic View. Biomed Res Int [Internet]. 2014;**2014**:1-23. Available from: http://www.hindawi.com/journals/bmri/2014/154106/

[46] Yang C, Chen H, Chen H, Zhong B, Luo X, Chun J. Antioxidant and anticancer activities of essential oil from gannan navel orange peel. Molecules. 2017;**22**(8):1-10

[47] (WHO) world HO. Câncer. http://www.who.int/mediacentre/factsheets/fs297/en/. 2017. pp. 1-7

[48] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA: A Cancer Journal for Clinicians. 2017;**67**(1):7-30. Available from:. DOI: http://doi.wiley.com/10.3322/caac.21387

[49] Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JWW, Comber H, Forman D, Bray F. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. European Journal of Cancer. 2013;**49**(6):1374-1403. DOI: 10.1016/j.ejca.2012.12.027

[50] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA: A Cancer Journal for Clinicians. 2016;**66**(1):7-30. Available from:. DOI: http://doi.wiley.com/10.3322/caac.21332

[51] Deshmukh SK, Azim S, Ahmad A, Zubair H, Tyagi N, Srivastava SK, Bhardwaj A, Singh S, Rocconi RP, Singh AP. Biological basis of cancer health disparities: Resources and challenges for research. American Journal of Cancer Research. 2017;**7**(1):1-12

[52] Kumar P, Febriyanti R, Sofyan F, Luftimas D, Abdulah R. Anticancer potential of *Syzygium aromaticum* L. in MCF-7 human breast cancer cell lines. Pharmacognosy Research. 2014;**6**(4):350. Available from: http://www.phcogres.com/text.asp?2014/6/4/350/138291

[53] Ghareeb MA, Refahy LA, Saad AM, Ahmed WS. Chemical composition, antioxidant and anticancer activities of the essential oil from *Eucalyptus citriodora* (Hook.) leaves. Der Pharma Chemica. 2016;**8**(1):192-200

[54] Nieto G. Biological activities of three essential oils of the Lamiaceae Family. Medicines [Internet]. 2017;**4**(3):63. Available from: http://www.mdpi.com/2305-6320/4/3/63

[55] Qaid M, Al-Hajj N, Algabr MN, Ali Omar K, Wang H. Anticancer, antimicrobial and antioxidant activities of the essential oils of some aromatic medicinal plants (*Pulicaria inuloides*-Asteraceae). Journal of Food and Nutrition Research. 2017;**5**(7):490-495. Available from: http://pubs.sciepub.com/jfnr/5/7/6/index.html

[56] Romeilah RM. Chemical compositions, antioxidant, anticancer activities and biological effects of *Myrtus communis* L. and *Origanum vulgare* essential oils. Asian Journal of Biochemistry. 2016;**11**(2):104-117. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-84958057346&partnerID=tZOtx3y1

[57] Yang X, Zheng H, Ye Q, Li R, Chen Y. Essential oil of cinnamon exerts anti-cancer activity against head and neck squamous cell carcinoma via attenuating epidermal growth factor receptor—tyrosine kinase. 2015;**20**(134):1518-1525

[58] Sehgal K, Singh M. Essentials to kill the cancer. Cancer Therapy & Oncology International Journal. 2017;**4**(5):4-7. Available from: https://juniperpublishers.com/ctoij/CTOIJ.MS.ID.555650.php

[59] Dahham SS, Hassan LEA, Ahamed MBK, Majid ASA, Majid AMSA, Zulkepli NN. In vivo toxicity and antitumor activity of essential oils extract from agarwood (*Aquilaria crassna*). BMC Complementary and Alternative Medicine. 2016;**16**(1):1-11. DOI: 10.1186/s12906-016-1210-1

[60] Abdel-Hameed E-SS, Bazaid SA, Al Zahrani O, El-Halmouch Y, El-Sayed MM, El-Wakil AE. Chemical composition of volatile components , antimicrobial and anticancer activity of n-hexane extract and essential oil from *Trachyspermum ammi* L. seeds. Oriental Journal of Chemistry. 2014;**30**(4):1653-1662

[61] Chen Y, Zhou C, Ge Z, Liu Y, Liu Y, Feng W, Li S, Chen G, Wei T. Composition and potential anticancer activities of essential oils obtained from myrrh and frankincense. Oncology Letters. 2013;**6**(4):1140-1146

[62] Fu Z, Wang H, Hu X, Sun Z, Han C. The pharmacological properties of salvia essential oils. Journal of Applied Pharmaceutical Science. 2013;**3**(7):122-127

[63] Mahmoud G. Biological effects, antioxidant and anticancer activities of marigold and basil essential oils. Medicinal Plants Research. 2013;**7**(10):561-572. Available from: http://www.academicjournals.org/JMPR/PDF/pdf2013/10Mar/Mahmoud.pdf

[64] García M, Scull R, Satyal P, Setzer WN, Monzote L. Chemical characterization, antileishmanial activity, and cytotoxicity effects of the essential oil from leaves of *Pluchea carolinensis* (Jacq.) G. Don. (Asteraceae). Phytotherapy Research: PTR. 2017;**31**(9):1419-1426. DOI: http://doi.wiley.com/10.1002/ptr.5869

[65] Tasdemir D, Tierney M, Sen R, Bergonzi M, Demirci B, Bilia A, Baser K, Brun R, Chatterjee M. Antiprotozoal Effect of Artemisia indica Extracts and Essential Oil. Planta Med [Internet]. 2015 Jun 17;**81**(12/13):1029-1037. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-0035-1565826

[66] Monzote L, Scull R, Cos P, Setzer W. Essential oil from *Piper aduncum*: Chemical analysis, antimicrobial assessment, and literature review. Medicines. 2017;**4**(3):49. Available from: http://www.mdpi.com/2305-6320/4/3/49

[67] Giongo JL, Vaucher RA, Da Silva AS, Oliveira CB, de Mattos CB, Baldissera MD, Sagrillo MR, Monteiro SG, Custódio DL, Souza de Matos M, Sampaio PT, Teixeira HF, Koester LS, da Veiga Junior VF. Trypanocidal activity of the compounds present in *Aniba canelilla* oil against *Trypanosoma evansi* and its effects on viability of lymphocytes. Microbial Pathogenesis. 2017;**103**(2017):13-18. DOI: 10.1016/j.micpath.2016.12.006

[68] Demarchi IG, Thomazella MV, de Souza Terron M, Lopes L, Gazim ZC, Cortez DAG, Donatti L, Aristides SMA, Silveira TGV, Lonardoni MVC. Antileishmanial activity of essential oil and 6,7-dehydroroyleanone isolated from *Tetradenia riparia*. Experimental Parasitology. 2015;**157**:128-137. DOI: 10.1016/j.exppara.2015.06.014

[69] Soares BV, Neves LR, Oliveira MSB, Chaves FCM, Dias MKR, Chagas EC, Tavares-Dias M. Antiparasitic activity of the essential oil of Lippia alba on ectoparasites of Colossoma macropomum (tambaqui) and its physiological and histopathological effects. Aquaculture. 2016;**452**:107-114. DOI: 10.1016/j.aquaculture.2015.10.029

[70] Bouyahya A, Et-Touys A, Abrini J, Talbaoui A, Fellah H, Bakri Y, Dakka N. Lavandula stoechas essential oil from Morocco as novel source of antileishmanial, antibacterial and antioxidant activities. Biocatalysis and Agricultural Biotechnology. 2017;**12**:179-184. DOI: 10.1016/j.bcab.2017.10.003

[71] Ferreira LE, Benincasa BI, Fachin AL, França SC, Contini SSHT, Chagas ACS, Beleboni RO. *Thymus vulgaris* L. essential oil and its main component thymol: Anthelmintic effects against Haemonchus contortus from sheep. Veterinary Parasitology. 2016;**228**:70-76. DOI: 10.1016/j.vetpar.2016.08.011

[72] Monzote L, García M, Pastor J, Gil L, Scull R, Maes L, Cos P, Gille L. Essential oil from *Chenopodium ambrosioides* and main components: Activity against Leishmania, their mitochondria and other microorganisms. Experimental Parasitology. 2014;**136**(1):20-26. DOI: 10.1016/j.exppara.2013.10.007

[73] Azeredo CMO, Santos TG, de Noronha Sales Maia BHL, Soares MJ. In vitro biological evaluation of eight different essential oils against *Trypanosoma cruzi*, with emphasis on Cinnamomum verum essential oil. BMC Complementary and Alternative Medicine. 2014;**14**(1):1-8

[74] Rodrigues KAF, Amorim LV, de Oliveira JMG, Dias CN, DFC M, Andrade EHA, JGS M, SMP C, Carvalho FAA. *Eugenia uniflora* L. Essential oil as a potential anti-Leishmania agent: Effects on Leishmania amazonensis and possible mechanisms of action. Evidence-Based Complementary and Alternative Medicine. 2013;**2013**:279726. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23533469%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3590759%5Cnhttp://www.hindawi.com/journals/ecam/2013/279726/

[75] Mantovani ALL, Vieira GPG, Cunha WR, Groppo M, Santos RA, Rodrigues V, Magalhães LG, Crotti AEM. Chemical composition, antischistosomal and cytotoxic effects of the essential oil of *Lavandula angustifolia* grown in southeastern Brazil. Brazilian Journal of Pharmacognosy. 2013;**23**(6):877-884. DOI: 10.1590/S0102-695X2013000600004

[76] Sauter IP, Rossa GE, Lucas AM, Cibulski SP, Roehe PM, da Silva LAA, Rott MB, Vargas RMF, Cassel E, von Poser GL. Chemical composition and amoebicidal activity of *Piper hispidinervum* (Piperaceae) essential oil. Industrial Crops and Products. 2012;**40**(1):292-295. DOI: 10.1016/j.indcrop.2012.03.025

[77] Ghazouani N, Sifaoui I, Bachrouch O, Abderrabba M, E. Pinero J, Lorenzo-Morales J. Essential oil composition and anti Acanthamoeba studies of Teucrium ramosissimum. Exp Parasitol [Internet]. 2017 Dec;**183**:207-211. Available from: http://dx.doi.org/10.1016/j.exppara.2017.09.010

[78] de Cássia da Silveira e Sá R, Andrade L, dos Reis Barreto de Oliveira R, de Sousa D. A review on anti-inflammatory activity of phenylpropanoids found in essential oils. Molecules. 2014;**19**(2):1459-80. Available from: http://www.mdpi.com/1420-3049/19/2/1459/

[79] De Lima VT, Vieira MC, Kassuya CAL, Cardoso CAL, Alves JM, Foglio MA, De Carvalho JE, Formagio ASN. Chemical composition and free radical-scavenging, anticancer and anti-inflammatory activities of the essential oil from *Ocimum kilimandscharicum*. Phytomedicine. 2014;**21**(11):1298-1302. DOI: 10.1016/j.phymed.2014.07.004

[80] Choi JH, Cha DS, Jeon H. Anti-inflammatory and anti-nociceptive properties of *Prunus padus*. Journal of Ethnopharmacology. 2012;**144**(2):379-386. DOI: 10.1016/j.jep.2012.09.023

[81] Nwaehujor CO, Ezeja MI, Udeh NE, Okoye DN, Udegbunam RI. Anti-inflammatory and anti-oxidant activities of *Mallotus oppositifolius* (Geisel) methanol leaf extracts. Arabian Journal of Chemistry. 2014;**7**(5):805-810. DOI: 10.1016/j.arabjc.2012.03.014

[82] Chou S-T, Lai C-P, Lin C-C, Shih Y. Study of the chemical composition, antioxidant activity and anti-inflammatory activity of essential oil from *Vetiveria zizanioides*. Food Chemistry. 2012;**134**(1):262-268. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0308814612003469

[83] Rodrigues V, Cabral C, Évora L, Ferreira I, Cavaleiro C, Cruz MT, Salgueiro L. Chemical composition, anti-inflammatory activity and cytotoxicity of *Thymus zygis* L. subsp. sylvestris (Hoffmanns. &link) Cout. essential oil and its main compounds. Arabian Journal of Chemistry. Sep 2015. Available from: http://linkinghub.elsevier.com/retrieve/pii/S187853521500266X

[84] Branquinho LS, Santos JA, Cardoso CAL, Mota J da S, Junior UL, Kassuya CAL, Arena AC. Anti-inflammatory and toxicological evaluation of essential oil from *Piper glabratum* leaves. Journal of Ethnopharmacology. 2017;**198**:372-378. DOI: 10.1016/j.jep.2017.01.008

[85] Macedo EMA, Santos WC, Sousa Neto BP, Lopes EM, Piauilino CA, Cunha FVM, Sousa DP, Oliveira FA, Almeida FRC. Association of terpinolene and diclofenac presents anti-nociceptive and anti-inflammatory synergistic effects in a model of chronic inflammation. Brazilian Journal of Medical and Biological Research. 2016;**49**(7):1-10

[86] Rodrigues LB, Oliveira Brito Pereira Bezerra Martins A, Cesário FRAS, Ferreira e Castro F, de Albuquerque TR, Martins Fernandes MN, Fernandes da Silva BA, Quintans Júnior LJ, da Costa JGM, Melo CoutinhoHD, Barbosa R, Alencar de Menezes IR. Anti-inflammatory and antiedematogenic activity of the *Ocimum basilicum* essential oil and its main compound estragole: In vivo mouse models. Chemico-Biological Interactions. 2016;**257**:14-25

[87] Rodrigues LB, Martins AOBPB, Ribeiro-Filho J, Cesário FRAS, e Castro FF, de Albuquerque TR, Fernandes MNM, da Silva BAF, Quintans Júnior LJ, Araújo AA de S, Menezes P dos P, Nunes PS, Matos IG, Coutinho HDM, Goncalves Wanderley A, de Menezes IRA. Anti-inflammatory activity of the essential oil obtained from *Ocimum basilicum* complexed with β-cyclodextrin (β-CD) in mice. Food and Chemical Toxicology. 2017;**109**:836-46. DOI: 10.1016/j.fct.2017.02.027

[88] Wang Y-T, Zhu L, Zeng D, Long W, Zhu S-M. Chemical composition and anti-inflammatory activities of essential oil from *Trachydium roylei*. Journal of Food and Drug Analysis. 2016;**24**(3):602-609. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1021949816300308

[89] Mogosan C, Vostinaru O, Oprean R, Heghes C, Filip L, Balica G, Moldovan R. A Comparative Analysis of the Chemical Composition, Anti-Inflammatory, and Antinociceptive Effects of the Essential Oils from Three Species of Mentha Cultivated in Romania. Molecules [Internet]. 2017 Feb 10;**22**(2):263-274. Available from: http://www.mdpi.com/1420-3049/22/2/263

[90] Lee SC, Wang SY, Li CC, Liu CT. Anti-inflammatory effect of cinnamaldehyde and linalool from the leaf essential oil of *Cinnamomum osmophloeum* Kanehira in endotoxin-induced mice. Journal of Food and Drug Analysis. 2017:1-10. DOI: 10.1016/j.jfda.2017.03.006

[91] Kumar R, Om P, Anil KP, Mahesh K, Valary AI, Lech S. Chemical composition and anti-inflammatory, anti-nociceptive and antipyretic activity of rhizome essential oil of Globba sessiliflora Sims. collected from Garhwal region of Uttarakhand. Journal of Herbal Drugs. 2017;**8**(1):59-69. Available from: http://jhd.iaushk.ac.ir/article_25761.html

[92] Boakye YD, Agyare C, Abotsi WKM, Ayande PG, Ossei PPS. Anti-inflammatory activity of aqueous leaf extract of *Phyllanthus muellerianus* (Kuntze) Exell. and its major constituent, geraniin. Journal of Ethnopharmacology. 2016;**187**:17-27. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0378874116302112

[93] Abu-Darwish MS, Cabral C, Ferreira IV, Gonçalves MJ, Cavaleiro C, Cruz MT, Al-bdour TH, Salgueiro L. Essential Oil of Common Sage ( Salvia officinalis L.) from Jordan: Assessment of Safety in Mammalian Cells and Its Antifungal and Anti-Inflammatory Potential. Biomed Res Int [Internet]. 2013;**2013**:1-9. Available from: http://www.hindawi.com/journals/bmri/2013/538940/

[94] Riella KR, Marinho RR, Santos JS, Pereira-Filho RN, Cardoso JC, Albuquerque-Junior RLC, Thomazzi SM. Anti-inflammatory and cicatrizing activities of thymol, a monoterpene of the essential oil from *Lippia gracilis*, in rodents. Journal of Ethnopharmacology. 2012;**143**(2):656-663. DOI: 10.1016/j.jep.2012.07.028

[95] Amorim JL, Simas DLR, Pinheiro MMG, Moreno DSA, Alviano CS, Da Silva AJR, Fernandes PD. Anti-inflammatory properties and chemical characterization of the essential oils of four Citrus species. PLoS One. 2016;**11**(4):1-18

[96] Boukhatem MN, Ferhat MA, Kameli A, Saidi F, Kebir HT. Lemon grass (Cymbopogon citratus) essential oil as a potent anti-inflammatory and antifungal drugs. Libyan J Med [Internet]. 2014 Jan 19;**9**(1):25431. Available from: https://www.tandfonline.com/doi/full/10.3402/ljm.v9.25431

[97] Kazemi M. Phenolic profile, antioxidant capacity and anti-inflammatory activity of *Anethum graveolens* L. essential oil. Natural Product Research. 2015;**29**(6):551-553. Available from: http://www.tandfonline.com/doi/full/10.1080/14786419.2014.951934

[98] Khodabakhsh P, Shafaroodi H, Asgarpanah J. Analgesic and anti-inflammatory activities of *Citrus aurantium* L. blossoms essential oil (neroli): Involvement of the nitric oxide/cyclic-guanosine monophosphate pathway. Journal of Natural Medicines. 2015; **69**(3):324-331

[99] Pang Y, Wang D, Hu X, Wang H, Fu W, Fan Z, Chen X, Yu F. Effect of volatile oil from *Blumea Balsamifera* (L.) DC. leaves on wound healing in mice. Journal of Traditional Chinese Medical Sciences. 2014;**34**(6):716-724. Available from:. DOI: http://linkinghub.elsevier.com/retrieve/pii/S025462721530087X

# 5 CAPÍTULO IV.

5.1 Potentially phytotoxic of chemical compounds present in essential oil for invasive plants control - A mini-review (Book title: Biological Approaches for Controlling).

Chapter 5

# Potentially Phytotoxic of Chemical Compounds Present in Essential Oil for Invasive Plants Control: A Mini-Review

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Additional information is available at the end of the chapter

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**Abstract**

The control of invasive plants is still carried out with the use of synthetic chemical agents that may present high toxicity and, consequently, be harmful to humans and animals. In Brazil, especially in the Amazon, small producers use this kind of technique in a rustic way, with brushcutters or fire. In this sense, the search for natural agents with bioherbicide potential becomes necessary. Examples of these agents are the essential oils that over the years have been shown to be a viable alternative to weed control. Thus, this review aims to show the potentially phytotoxic activity of allelochemicals present in essential oils of different aromatic plants.

**Keywords:** natural products, essential oils, allelochemicals, allelopathy

## 1. Introduction

The performance of agricultural activity in tropical regions, both in fertile and in low fertility soils, has been limited by the occurrence of a series of extremely aggressive and diverse plants, called weeds. The main consequence of crop infestation by these plants is increasing costs to maintain the crops and reduction of productivity and its consequent competitive

capacity. These plants may also represent an additional problem for farmers either because they are often toxic to different animals or because they are permanent sources for the spread of diseases to crop plants [1]. In this context, weed management and control become crucial both from the point of view of crop productivity and the profitability of the farming system.

In modern agriculture, where high yields are expected, in the face of increasing demands for food – due to the increasing world population – the control of these plants has been made, basically, by the use of chemical herbicides. However, such a procedure may not be sustainable over time, especially because it conflicts with the interests of modern society, which is increasingly concerned with the quality of food and with the preservation of natural resources. At the same time, the reduction in the efficiency of the current products available in the market has been observed as a consequence of the appearance of resistant plants [2, 3], leading to an increase in the use of herbicides or the contractions employed, which only increases the problem. All these factors point to the need of science to make available new and revolutionary methods of weed control.

A viable alternative to this challenge are the numerous chemically diverse compounds produced by plants that may offer new chemical structures capable of efficiently replace those already available in the market. In this line, crude extracts and isolated or associated chemical substances can be an excellent strategy to partially or totally replace the use of herbicides.

Over the last decades, different chemical compounds with bioherbicidal properties have been isolated and identified in different plants [4–7]. Among the many chemical classes with potential use in weed management, the secondary metabolites present in essential oils can be highlighted, since the different chemical classes of volatile compounds are notable for the wide potential of use in different activities of interest for humanity and specifically in the management of weeds.

## 2. Allelopathy history

Allelopathy is the chemical interaction between plants and other living organisms [8]. There are two types of interactions between plants: a phytotoxic one, which inhibits the germination of seeds and the development of the radicle and hypocotyl [9], and a stimulatory effect, which favors the development of the plant [10]. The chemical substances responsible for the allelopathic effect are called allelochemicals [11].

The allelopathy is a relatively new science, having its basic concepts established over the last 8 decades. However, chemical interactions among plants are not exactly new, since reports on the subject are found in old references. [12–16]. In the 1800s, several phenomena were attributed to the chemical interaction among plants [17]. In the early 1900's, [18] reported the presence of toxic compounds produced by plants that could be extracted from the soil. The first reports proving the interference promoted by chemical compounds were developed in the 1960's [19], showing that the volatile compounds were affecting the dynamics among plants.

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## 3. Control of invasive plants

Currently, the chemical control method is the most used to inhibit the growth of invasive plants, which includes the use of synthetic herbicides, in large quantities, mainly by large producers, as reported by some authors [20, 21]. The use of synthetic and toxic chemical herbicides in management areas promotes the death of weeds in a selective way and, consequently, it ends the competition among the plants, helping to increase the production of green mass in the pasture [22]. The increasing use of agrochemicals may represent an unsustainable practice because these pesticides can pollute the environment and promote the contamination of various animal species. Also, new insecticide-resistant insects are appearing and invasive plants that are tolerant to modern herbicides are becoming more frequent [23].

Weed resistance to herbicides may be related to an evolutionary process; however, some developments of resistant weed biotypes are imposed by agriculture through selection pressure caused by the intensive use of herbicides. Weed resistance to herbicides may result from biochemical, physiological, morphological or phenological changes of certain invasive plant biotypes. Many cases of resistance to herbicides result from either the alteration of the site of action of the herbicide or the increase of its metabolism, or the departmentalization and compartmentalization of the herbicide in the plant [24, 25]. This way, allelopathy can be a natural alternative for the control of invasive plants.

## 4. Volatile allelochemicals

Weeds promote two basic types of interference in agricultural crops: allelospoly and allelopathy. Allelospoly is the type of interference promoted by competition for essential factors to the species survival, such as water, nutrients and physical space. Allelopathy involves the production of allelochemicals and subsequent release into the environment [26]. Almost all allelochemicals exist in conjugated, non-toxic forms. The toxic fragment can be released after exposure to stress or after tissue death [27].

The use of allelopathy for weed control may be an ecologically viable alternative [28]. Thus, the use of essential oils with phytotoxic potential is becoming widespread, since the allelochemicals present in these oils generally have low cytotoxicity. For example, [29] evaluated the effect of *Carum carvis* essential oils rich in carvone (71.08%) and limonene (25.42%), and verified that this oil has a strong phytotoxic activity on seed germination and radicle elongation of *Linum usitatissimum*, *Phalaris canariensis* and *Triticum aestivum*.

Another example is the eucalypt essential oil that has a rich chemical composition in 1,8-cineole (58.3%), $\alpha$-pinene (17.3%) and $\alpha$-thujene (15.5%), which significantly inhibited seed germination of *Sinapis arvensis*, *Diplotaxis harra* and *Trifolium campestre*, in different intensities according to the recipient species, demonstrating that each species has a different specificity. In addition, the application of post-emergence oil causes inhibition of chlorophyll production, leading to injuries such as chlorosis, necrosis and even complete wilting of plants [30].

Plant species such as *Origanum onites L.* and *Rosmarinus officinalis L.* also show strong allelopathic activity on species of *Poaceae* and invasive plants, by suppressing germination rate and elongation of radicle and hypocotyl [31]. The phytotoxic effects related to these two species of aromatic plants may be related to their rich chemical composition in the oxygenated monoterpenes 1,8-cineole, linalool, camphor and carvacrol and the monoterpene hydrocarbon p-cymene [32–35], however, compounds found in lower concentrations as methyl phenylpropanoids have also demonstrated good allelopathic activity [36].

In the case of essential oils for the control of invasive plants, it is usually analyzed the effects of individual form, attributing the phytotoxic activity to only one component [37, 38]. However, the effects of volatile oils can also be related to the mixture of compounds, such as *Artemisia scoparia* oil which has a mixture of compounds such as monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, aliphatic compounds and other aromatic compounds [39]. The chemical composition of the essential oils depends on the biosynthetic path of the different classes of compounds, as can be observed in **Figure 1**, which brings the biosynthesis of some classes of volatile compounds.

Compounds such as eucalyptol, β-phellandrene, hexyl butanoate, *p*-cymene, α-ionone, (z)-3-octen-1-ol, theaspirane a, vitispirane, dihydro-(−)-neoclovene, β-caryophyllene, (e)-2-octen-1-ol, a-terpineol, dehydro-ar-ionene, methyl salicylate, (z)-b-damascenone, (z)-dehydro-ar-ionene,

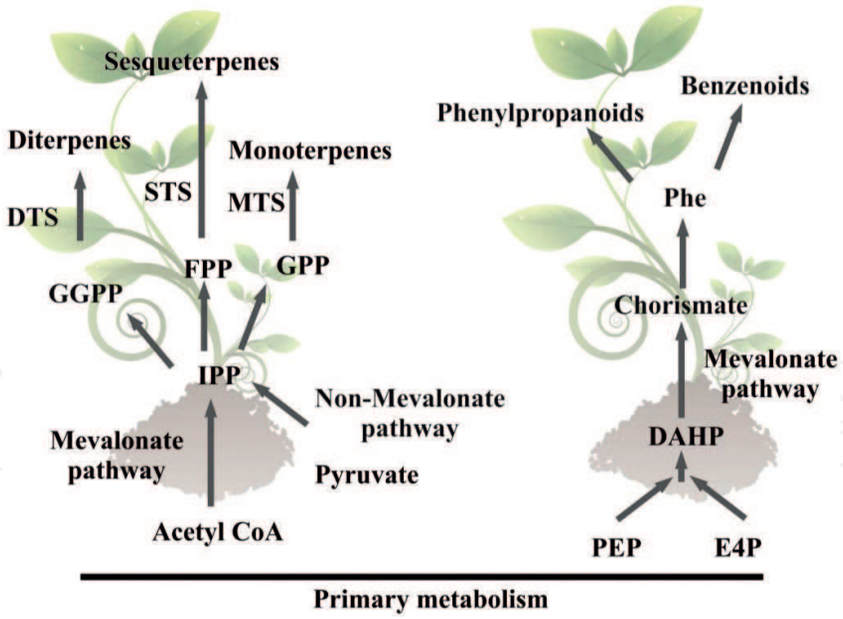


**Figure 1.** Biosynthesis of plant volatiles. Overview of biosynthetic pathways leading to the emission of plant volatile organic compounds. The plant precursors originate from primary metabolism. Abbreviations: DTS: Diterpene synthase; FPP: farnesyldiphosphate; GGPP: geranylgeranyldiphosphate; GLVs: green-leaf volatiles; GPP: geranyldiphosphate; IPP: isopentenyl pyrophosphate; MTS: Monoterpene synthase; STS: Sesquiterpene synthase; DAHP: 3-deoxy-D-arabinoheptulosonate-7 phosphate; E4P: erythrose 4-phosphate; PEP: phosphoenolpyruvate; Phe: phenylalanine. This flowchart was adapted from [40] and [41].

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10-(tetrahydro-pyran-2-yloxy)-tricyclo[4.2.1(2,5)]decan-9-ol,(−)-caryophylleneoxide,dihydro-β-ionone, viridiflorol, cubenol, caryophyllene, α-bisabolol oxide-b, tetracosane and *n*-hexadecane can be found in *Anisomeles indica* essential oil and also present good phytotoxic activity against invasive plants [42]. As well as *P. heyneanus Benth* essential oils, rich in patchouli alcohol, α-bulnesene, α-guaiene, seichelene and α-patchulene, and *P. hispidinervium* C. DC oils, rich in safrole, terpinolene, (E)-β-ocimene, δ-3-carene and pentadecane [43].

### 4.1. Monoterpenes

The monoterpenes have presented good phytotoxic activity, and reports of the use of these compounds to control plants refer to the 1960s [44]. This activity depends on the structural

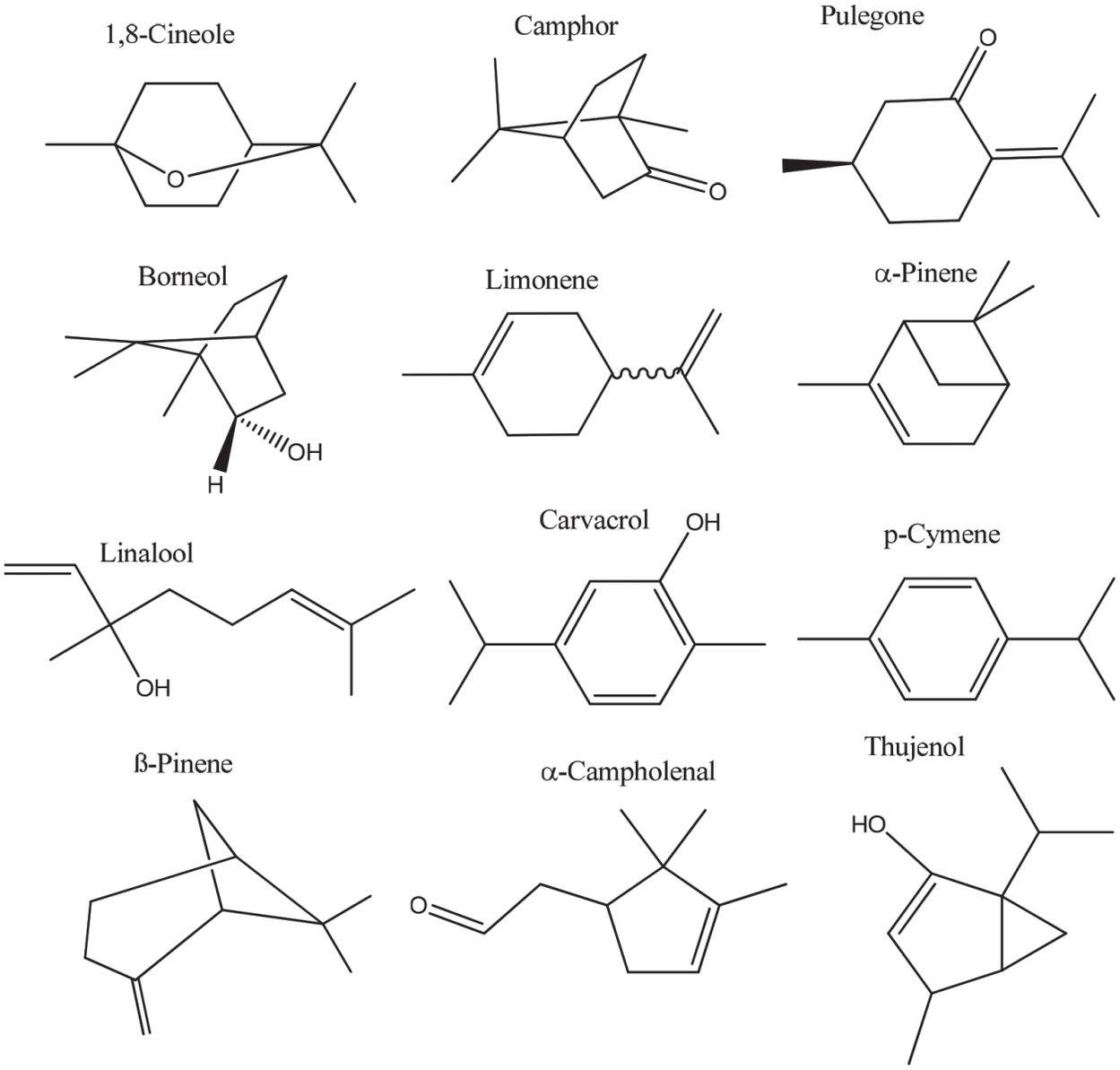


**Figure 2.** Chemical structures of oxygenated and non-oxygenated monoterpenes with bioherbicidal action.

characteristics of the molecules; for example, oxygenated monoterpenes exhibit different effects on germination and seedling development, and also alter cellular respiration, which impairs energetic metabolism [33, 34]. However, these phytotoxic effects promoted by a chemical species depend on its concentration, for example, *Lactuca sativa* essential oil composed essentially of $\alpha$-pinene (16.00%), 1,8-cineole (66.93%) and pimonene (10.04%) presents different rates of germination inhibition [45].

In general, oxygenated monoterpenes have the highest phytotoxic effects over non-oxygenated [46]. However, there are non-oxygenated volatile molecules such as limonene which also have good phytotoxic activity [47]. Some monoterpenes had high inhibitory activity on germination and radicle elongation, and this may be related to the anatomical and physiological changes in the host plants, as well as to the reduction in some organelles such as mitochondria, and accumulation of lipid globules in the cytoplasm [48]. In **Figure 2**, the chemical structures of some monoterpenes with phytotoxic activity can be observed.

### 4.2. Sesquiterpenes

Bioassays have demonstrated that the sesquiterpenic allelochemicals β-cariofilene, β-copaene, spathulenol, germacrene B, bicyclogermacrene, globulol, viridiflorol, a-guaiene, and g-elemene have presented phytotoxity against various invasive plants and, in some cases, promote inhibition of other plants development, when they are close to species that produce these secondary metabolites [49–51]. Authors compared the effects of essential oils rich in sesquiterpenes and others rich in monoterpenes and found that the effects presented by sesquiterpenes, in some cases, may be smaller in relation to the affections exhibited by monoterpenes [52]. **Figure 3** shows the chemical structures of oxygenated and non-oxygenated sesquiterpenes with phytotoxic action.

However, this depends largely on the presence of oxygenated and non-oxygenated, cyclic or acyclic molecules, because depending on the molecular conformation the allelopathic effect may be higher or lower [53, 54]. This justifies the results obtained by other authors [55], who analyzed the effects of fractions of essential oils of *E. adenophorum,* of the inflorescence region, rich in sesquiterpenes, and its root rich in monterpenes. When the oils were tested at the same concentration (1 μL/mL), they inhibited germination and seedling elongation at the same ratio.

### 4.3. Phenylpropanoids

Phenylpropanoids are a class of secondary metabolites that are also naturally present in plants, and have exhibited strong phytotoxic activity against invasive plants. In 2016, [9] demonstrated that eugenol is the main active ingredient of clove essential oil and is also the agent possibly promoting phytotoxic activity against the invasive plants *Mimosa pudica* and *Senna obtusifolia*. Other authors also report the potentially allelopathic activity

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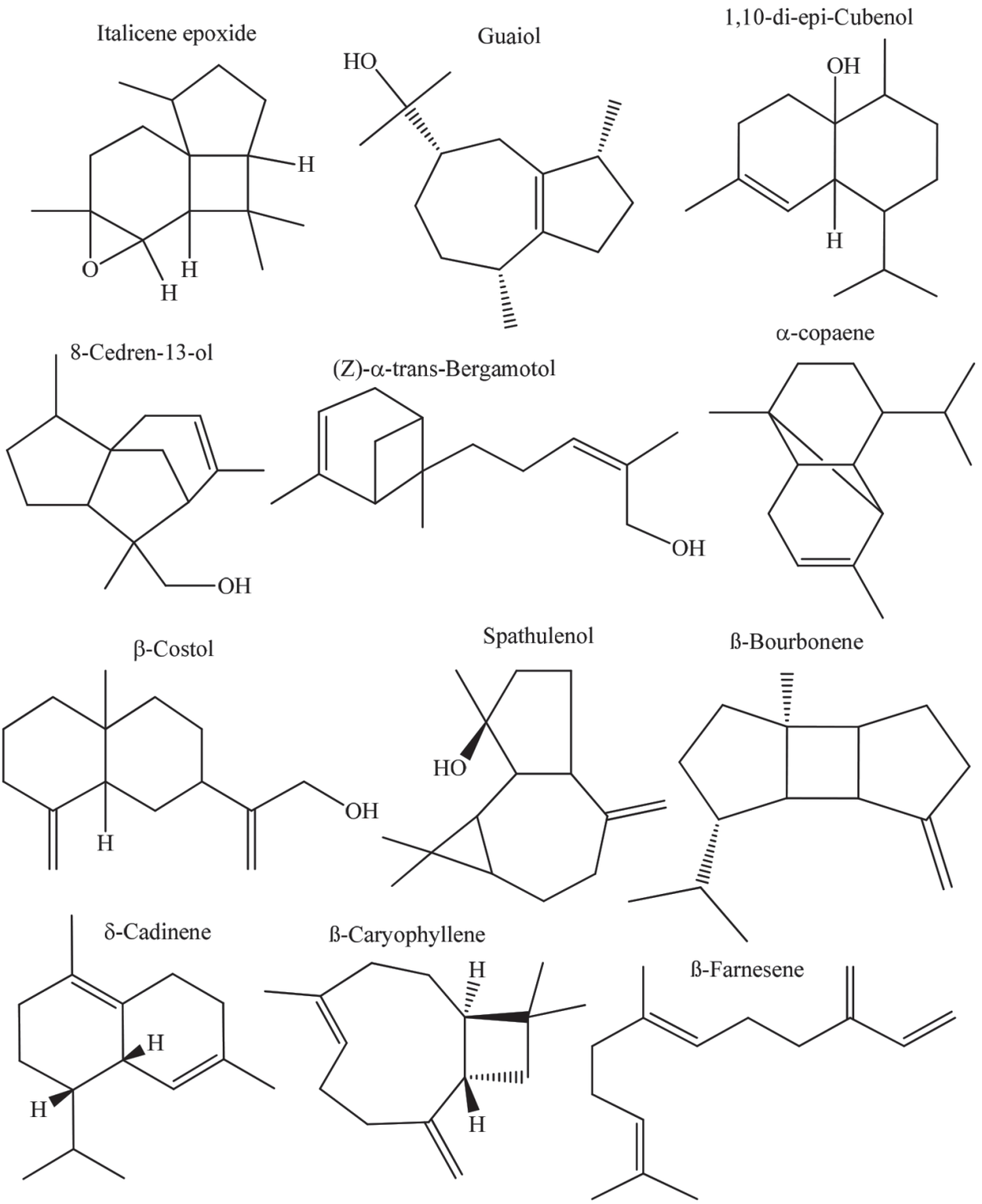


**Figure 3.** Chemical structures of oxygenated and non-oxygenated sesquiterpenes with bioherbicidal action.

of clove essential oil *Syzygium aromaticum* [56–58]. In addition to eugenol, other phenylpropanoids present in essential oils with phytotoxic activity are eugenyl acetate, safrole, methyl eugenol, anethole, myristicin, estragole, anethole and trans-anethole [36, 59–64]. **Figure 4**

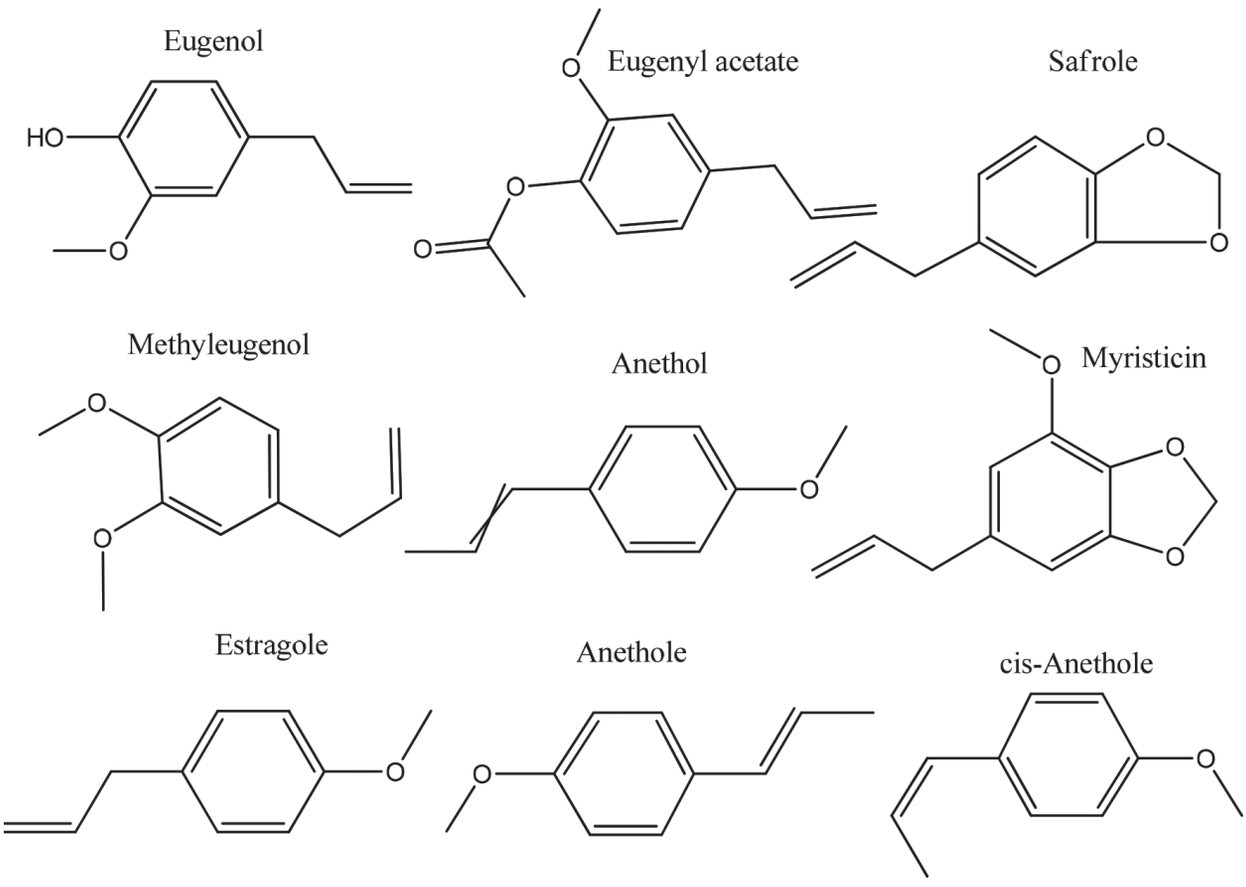


**Figure 4.** Chemical structures of phenylpropanoids with bioherbicidal action.

shows the chemical structures of the phenylpropanoids with potential use for control of invasive plants.

## 5. Conclusion

For essential oils to have good phytotoxic activity, some factors such as chemical composition, concentration and host plants may be taken into account. Among the monoterpene allelochemicals we can highlight the 1,8 cineole, among the sesquiterpenes or β-caryophyllene and among phenylpropanoids, eugenol. On the other hand, one of the difficulties that can appear for the use in large scale of essential oils is the volatility of their components.



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## References

[1] Poletti M, Omoto C. Resistência de Inimigos Naturais a Pesticidas. Rev Biotecnol Ciência e Desenvolv. 2003;**30**:1-17

[2] Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M. Reducing the risks of herbicide resistance: Best management practices and recommendations. Weed Science [Internet]. Jan 20, 2012;**60**(SP1):31-62. DOI: http://www.bioone.org/doi/full/10.1614/WS-D-11-00155.1

[3] Devine MD, Shukla A. Altered target sites as a mechanism of herbicide resistance. Crop Protection [Internet]. Sep 2000;**19**(8-10):881-889. Available from: http://linkinghub.elsevier.com/retrieve/pii/S026121940000123X

[4] Souza Filho APS. In: da Silva Sousa Filho AP, editor. Ecologia química: A experiência brasileira. 1st ed. Belém, PA: Embrapa Amazônia Oriental, Belém; 2008. 150 p

[5] Santos JCF, Edilene GM, Marchi CS. In: de Miranda FVC, do Nascimento FE, de Oliveira JF, editors. Daninhas do Café. 1st ed. Planaltina, DF- Brazil: Empresa Brasileira de Pesquisa Agropecuária Embrapa Cerrados Ministério da Agricultura, Pecuária e Abastecimento; 2008

[6] Filho AJC, Santos LS, Guilhon GMSP, Moraes RPC, dos Santos RA, Filho AP da SS, Felizzola JF. Identified substances from the leaves of *Tephrosia cinerea* (Leguminoseae) crude extracts and their phytotoxic effects. International Journal of Life-Sciences Scientific Research [Internet]. Jul 6, 2017;**3**(4):1137-1141. Available from: http://ijlssr.com/currentissue/Tephrosia cinerea (Leguminoseae) Crude Leaves Extracts and their Phytotoxic Effects.pdf

[7] Pereira SG, Soares AM dos S, Guilhon G, Santos LS, Pacheco LC, Souza Filho AP da S. Phytotoxic potential of piperine and extracts from fruits of *Piper tuberculatum* Jaq. on Senna obtusifolia and *Mimosa pudica* plants. Allelopathy Journal 2016;**38**(1):91-102

[8] Bostan C, Butnariu M, Butu M, Ortan A, Butu A, Rodinoc S, Parvue C. Allelopathic effect of *Festuca rubra* on perennial grasses. Romanian Biotechnology Letters. 2013;**18**(2):8190-8196

[9] de Oliveira MS, da Costa WA, Pereira DS, Botelho JRS, de Alencar Menezes TO, de Aguiar Andrade EH, da Silva SHM, da Silva Sousa Filho AP, de Carvalho RN. Chemical composition and phytotoxic activity of clove (*Syzygium aromaticum*) essential oil obtained with supercritical $CO_2$. Journal of Supercritical Fluids [Internet]. 2016;**118**:185-193. DOI: http://dx.doi.org/10.1016/j.supflu.2016.08.010

[10] Batista C de CR, de Oliveira MS, Araújo ME, Rodrigues AMC, Botelho JRS, da Silva Souza Filho AP, Machado NT, Carvalho RN. Supercritical CO2 extraction of açaí (*Euterpe oleracea*) berry oil: Global yield, fatty acids, allelopathic activities, and determination of phenolic and anthocyanins total compounds in the residual pulp. Journal of Supercrit Fluids [Internet]. Jan 2016;**107**:364-369. DOI: http://dx.doi.org/10.1016/j.supflu.2015.10.006

[11] Latif S, Chiapusio G, Weston LA. Allelopathy and the role of allelochemicals in plant defence. In: Becard G, editor. Advances in Botanical Research [Internet]. 82nd ed. 2017;**82**: 19-54. Available from: https://www.sciencedirect.com/science/article/pii/S0065229616301203

[12] Pavlychenko TK, Harrington J. Competitive efficiency of weeds and cereal crops. Canadian Journal of Research. 1934;**10**:77-94

[13] Lucas CE. The ecological effects of external metabolites. Biological Reviews of the Cambridge Philosophical Society [Internet]. 1947;**22**(3):270-295. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20253195

[14] Rice EL. Allelopathy – An update. The Botanical Review. 1979;**45**(1):15-109

[15] Rice EL. Allelopathy. In: Rice EL, editor. 2nd ed. New York: Academic Press; 1984. 422 p

[16] Lee IK, Monsi M. Ecological studies on Pinus densiflora forest. Effect of plant substances in the floristic composition of undergrowth. Bot Manager. 1963;**76**:400-413

[17] Stickney JS, Hoy PR. Toxic action of black walnut. Transactions of the Wisconsin State Horticultural Society. 1881;**11**:166-167

[18] Schreiner O, Reed HS. The production of deleterious excretions by roots. Bull Torrey Bot Club [Internet]. 1907 Jun;**34**(6):279-303. Available from: http://www.jstor.org/stable/2479157?origin=crossref

[19] Muller CH. Inhibitory terpenes volatilized from Salvia shrubs. Bulletin of the Torrey Botanical Club. 1965:38-45

[20] Bueno MR, Alves GS, Paula ADM, Cunha JPAR. Volumes de calda e adjuvante no controle de plantas daninhas com glyphosate. Planta Daninha [Internet]. 2013;**31**(3): 705-713 Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-83582013000300022&lng=pt&nrm=iso&tlng=en

[21] Gazziero DLP. Misturas de agrotóxicos em tanque nas propriedades agrícolas do Brasil. Planta Daninha [Internet]. Mar 2015;**33**(1):83-92 Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-83582015000100083&lng=pt&nrm=iso&tlng=en

[22] Ferreira E, Procópio S, Galon L, Franca A, Concenço G, Silva A, Aspiazu I, Silva A, Tironi S, Rocha PR. Manejo de plantas daninhas em cana-crua. Planta Daninha [Internet]. 2010 Dec;**28**(4):915-925 Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-83582010000400025&lng=pt&tlng=pt

[23] Souza Filho AP, Alves S de M. Alelopa tia em ecossistema de pastagem cultivada [Internet]. 1st ed. Belém: Embrapa; 1998. 71 p. Available from: http://www.infoteca.cnptia.embrapa.br/handle/doc/388173

[24] Christoffoleti PJ, Victoria R, Filho S, Da CB. Resistência de planta s daninhas aos herbicidas. Planta Daninha. 1994;**12**(1990):13-20

[25] LeBaron HM, McFarland J. Herbicide resistance in weeds and crops. In: Society AC, editor. Herbicides – Current Research and Case Studies in Use [Internet]. InTech; 1990. pp. 336-352. Available from: http://www.intechopen.com/books/herbicides-current-research-and-case-studies-in-use/herbicide-resistant-weeds-the-technology-and-weed-management

[26] Spiassi A, Konopatzki MRS, Nóbrega LHP. Estratégias de manejo de plantas invasoras. Revista Varia Scientia Agrárias. [Internet]. 2010;**1**:177-188 Available from: http://e-revista.unioeste.br/index.php/variascientiaagraria/article/viewArticle/3493

[27] Putnam AR. Allelochemicals from plants as herbicides. Weed Science Society of America [Internet]. May 1, 1988;**2**(4):510-518 Available from: http://www.jstor.org/stable/3987390

[28] Macías FA, Molinillo JM, Varela RM, Galindo JC. Allelopathy – A natural alternative for weed control. Pest Management Science [Internet]. 2007 Apr;**63**(4):327-348. DOI: http://doi.wiley.com/10.1002/ps.1342

[29] Marichali A, Hosni K, Dallali S, Ouerghemmi S, Bel Hadj Ltaief H, Benzarti S, Kerkeni A, Sebei AH. Allelopathic effects of *Carum carvi* L. essential oil on germination and seedling growth of wheat, maize, flax and canary grass. Allelopathy Journal [Internet]. 2014;**34**(1):81-94. Available from: http://search.proquest.com/openview/1742af81c669e6bce54668881b849f28/1?pq-origsite=gscholar

[30] Grichi A, Nasr Z, Khouja ML. Phytotoxic effects of essential oil from *Eucalyptus lehmanii* against weeds and its possible use as a bioherbicide. Bulletin of Environment, Pharmacology and Life Sciences [Internet]. 2016;**5**(March):17-23. Available from: http://bepls.com/beplsmarch2016/4.pdf

[31] Atak M, Mavi K, Uremis I. Bio-Herbicidal Effects of oregano and rosemary essential oils on germination and seedling growth of bread wheat cultivars and weeds. Romanian Biotechnology Letters. 2015;**21**(1):11149-11159

[32] Romagni JG, Allen SN, Dayan FE. Allelopathic effects of volatile cineoles on two weedy plant species. Journal of Chemical Ecology. 2000;**26**(1):303-313

[33] Singh HP, Batish daizy R, Kaur S, Ramezani H, Kohli RK. Comparative phytotoxicity of four monoterpenes against *Cassia occidentalis*. Annals of Applied Biology [Internet]. Oct 2002;**141**(2):111-116. DOI: http://doi.wiley.com/10.1111/j.1744-7348.2002.tb00202.x

[34] Kordali S, Cakir A, Ozer H, Cakmakci R, Kesdek M, Mete E. Antifungal, phytotoxic and insecticidal properties of essential oil isolated from *Turkish Origanum* acutidens and its three components, carvacrol, thymol and p-cymene. Bioresource Technology [Internet]. Dec 2008;**99**(18):8788-8795. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0960852408003696

[35] Jassbi AR, Zamanizadehnajari S, Baldwin IT. Phytotoxic volatiles in the roots and shoots of *Artemisia tridentata* as detected by headspace solid-phase microextraction and gas chromatographic-mass spectrometry analysis. Journal of Chemical Ecology. 2010;**36**(12):1398-1407

[36] De Almeida LFR, Frei F, Mancini E, De Martino L, De Feo V. Phytotoxic activities of Mediterranean essential oils. Molecules. 2010;**15**(6):4309-4323

[37] Singh HP, Batish DR, Kaur S, Kohli RK, Arora K. Phytotoxicity of the volatile monoterpene citronellal against some weeds. Zeitschrift fur Naturforsch – Section C Journal of Biosciences 2006;**61**(5-6):334-340

[38] Batish DR, Singh HP, Setia N, Kaur S, KRK. Chemical composition and phytotoxicity of volatile essential oil from intact and fallen leaves of *Eucalyptus citriodora*. Zeitschrift fur Naturforsch – Section C Journal of Biosciences. 2006;**61**(7-8):465-471

[39] Kaur S, Singh HP, Mittal S, Batish DR, Kohli RK. Phytotoxic effects of volatile oil from *Artemisia scoparia* against weeds and its possible use as a bioherbicide. Industrial Crops and Products [Internet]. 2010;**32**(1):54-61. DOI: http://dx.doi.org/10.1016/j.indcrop.2010.03.007

[40] Kant MR, Bleeker PM, Wijk M Van, Schuurink RC, Haring MA. Chapter 14: Plant volatiles in defence. In: Advances in Botanical Research [Internet]. 1st ed. Elsevier Ltd.; 2009. pp. 613-666. DOI: http://dx.doi.org/10.1016/S0065-2296(09)51014-2

[41] Dudareva N, Klempien A, Muhlemann JK, Kaplan I. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. New Phytologist [Internet]. Apr 2013;**198**(1):16-32. DOI: http://doi.wiley.com/10.1111/nph.12145

[42] Batish DR, Singh HP, Kaur M, Kohli RK, Singh S. Chemical characterization and phytotoxicity of volatile essential oil from leaves of *Anisomeles indica* (Lamiaceae). Biochemical Systematics and Ecology [Internet]. Apr 2012;**41**:104-109. DOI: http://dx.doi.org/10.1016/j.bse.2011.12.017

[43] Souza Filho APDS, de Vasconcelos MAM, Zoghbi MDGB, Cunha RL. Efeitos potencialmente alelopáticos dos óleos essenciais de *Piper hispidinervium* C. DC. e *Pogostemon heyneanus* Benth sobre plantas daninhas. Acta Amazonica [Internet]. 2009;**39**(2):389-395. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0044-59672009000200018&lng=pt&tlng=pt

[44] Asplund RO. Some quantitative aspects of the phytotoxicity of monoterpenes. Weed Science Society of America. 1969;**17**(4):454-455

[45] de Oliveira CM, Cardoso M das G, Figueiredo AC da S, de Carvalho MLM, Miranda CASF de, Marques Albuquerque LR, Lee Nelson D, de Souza Gomes M, Silva LF, de Andrade Santiago J, Teixeira ML, Brandão RM. Chemical composition and allelopathic activity of the essential oil from *Callistemon viminalis* (Myrtaceae) *Blossoms on Lettuce* (*Lactuca sativa* L.) seedlings. American Journal of Plant Sciences [Internet]. 2014;**5**(24):3551-3557. Available from: http://www.scirp.org/journal/PaperInformation.aspx?PaperID=51896

[46] Mancini E, Arnold NA, de Feo V, Formisano C, Rigano D, Piozzi F, Senatore F. Phytotoxic effects of essential oils of *Nepeta curviflora* Boiss. and *Nepeta nuda* L. subsp. albiflora growing wild in Lebanon. Journal of Plant Interactions. 2009;**4**(4):253-259

[47] Ibrahim MA, Kainulainen P, Aflatuni A, Tiilikkala K, Holopainen JK. Insecticidal, repellent, antimicrobial activity and phytotoxicity of essential oils: With special reference to limonene and its suitability for control of insect pests. Agricultural and Food Science in Finland. 2001;**10**(April):243-259

[48] De Martino L, Mancini E, De Almeida LFR, De Feo V. The antigerminative activity of twenty-seven monoterpenes. Molecules. 2010;**15**(9):6630-6637

[49] Hiltpold I, Turlings TCJ. Belowground chemical signaling in maize: When simplicity rhymes with efficiency. Journal of Chemical Ecology [Internet]. May 29, 2008;**34**(5):628-635. Available from:. DOI: http://link.springer.com/10.1007/s10886-008-9467-6

[50] Kobaisy M, Tellez MR, Dayan FE, Duke SO. Phytotoxicity and volatile constituents from leaves of *Callicarpa japonica* Thunb. Phytochemistry [Internet]. 2002 Sep;**61**(1):37-40 Available from: http://linkinghub.elsevier.com/retrieve/pii/S0031942202002078

[51] Wang R, Peng S, Zeng R, Ding LW, Xu Z. Cloning, expression and wounding induction of p-caryophyllene synthèse gene from *Mikania micrantha* H. B. K. and allelopathie potential of ß-caryophyllene. Allelopathy Journal. 2009;**24**(1):35-44

[52] Verdeguer M, Blázquez MA, Boira H. Phytotoxic effects of Lantana camara, *Eucalyptus camaldulensis* and *Eriocephalus africanus* essential oils in weeds of Mediterranean summer crops. Biochemical Systematics and Ecology [Internet]. Oct 2009;**37**(4):362-369. DOI: http://dx.doi.org/10.1016/j.bse.2009.06.003

[53] Macías FA, Galindo JCG, Molinillo JMG, Cutler HG. Allelopathy: Chemistry and mode of action of allelochemicals [Internet]. In: Macías FA, Galindo JCG, Molinillo JMG, Cutler HG, editors. Allelopathy - Chemsitry and Mode of Action and Allelochemicals. New York, Washington, D.C: CRC Press, Taylor & Francis; 2003. 392 p. Available from: https://books.google.com.br/books/about/Allelopathy.html?id=B0937BBWXjQC&redir_esc=y

[54] Silva MP, Piazza LA, López D, López Rivilli MJ, Turco MD, Cantero JJ, Tourn MG, Scopel AL. Phytotoxic activity in *Flourensia campestris* and isolation of (−)-hamanasic acid A as its active principle compound. Phytochemistry [Internet]. 2012;**77**:140-148. DOI: http://dx.doi.org/10.1016/j.phytochem.2011.09.020

[55] Ahluwalia V, Sisodia R, Walia S, Sati OP, Kumar J, Kundu A. Chemical analysis of essential oils of *Eupatorium adenophorum* and their antimicrobial, antioxidant and phytotoxic properties. Journal of Pest Science (2004). 2014;**87**(2):341-349

[56] Mazzafera P. Efeito alelopático do extrato alcoólico do cravo-da-índia e eugenol. Revista Brasileira de Botânica [Internet]. Jun 2003;**26**(2):231-238 Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-84042003000200011

[57] Bainard LD, Isman MB, Upadhyaya MK. Phytotoxicity of clove oil and its primary constituent eugenol and the role of leaf epicuticular wax in the susceptibility to these essential oils. Weed Science [Internet]. Oct 20, 2006;**54**(5):833-837 Available from: https://www.cambridge.org/core/product/identifier/S0043174500015599/type/journal_article

[58] Ajayi OE, Appel AG, Henry Y. Phytotoxicity of some essential oil components to cowpea (*Vigna unguiculata* (L.) Walp.) seeds. International Journal of Plant Biology & Research. 2014;**2**(4):2-9

[59] Cruz GS, Wanderley-Teixeira V, Oliveira J V., Correia AA, Breda MO, Alves TJ, Cunha FM, Teixeira AA, Dutra KA, Navarro DM. Bioactivity of *Piper hispidinervum* (Piperales: Piperaceae) and *Syzygium aromaticum* (Myrtales: Myrtaceae) oils, with or without formulated Bta on the biology and immunology of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Journal of Economic Entomology [Internet]. 2014;**107**(1):144-153. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24665696

[60] Shreeya A, Batish DR, Singh HP. Research paper alleopathic effect of aromatic plants: Role of volatile. Journal of Global Biosciences 2016;**5**(7):4386-4395

[61] Rolli E, Marieschi M, Maietti S, Sacchetti G, Bruni R. Comparative phytotoxicity of 25 essential oils on pre- and post-emergence development of *Solanum lycopersicum* L.: A multivariate approach. Industrial Crops and Products [Internet]. 2014;**60**:280-90. DOI: http://dx.doi.org/10.1016/j.indcrop.2014.06.021

[62] Andrés MF, Rossa GE, Cassel E, Vargas RMF, Santana O, Díaz CE, González-Coloma A. Biocidal effects of *Piper hispidinervum* (Piperaceae) essential oil and synergism among its main components. Food and Chemical Toxicology [Internet]. 2017;**109**:1086-1092. DOI: http://dx.doi.org/10.1016/j.fct.2017.04.017

[63] Meepagala KM, Sturtz G, Wedge DE, Schrader KK, Duke SO. Phytotoxic and antifungal compounds from two Apiaceae species, *Lomatium californicum* and *Ligusticum hultenii*, rich sources of Z-ligustilide and apiol, respectively. Journal of Chemical Ecology. 2005;**31**(7):1567-1578

[64] Vasilakoglou I, Dhima K, Paschalidis K, Ritzoulis C. Herbicidal potential on *Lolium rigidum* of nineteen major essential oil components and their synergy. Journal of Essential Oil Research. 2013;**25**(1):1-10

# 6 CAPÍTULO V.

## 6.1 “Phytochemical profile, antioxidant activity, inhibition of acetylcholinesterase and interaction mechanism of the major components of the *Piper divaricatum* essential oil obtained by Supercritical $CO_2$”

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# Phytochemical profile, antioxidant activity, inhibition of acetylcholinesterase and interaction mechanism of the major components of the *Piper divaricatum* essential oil obtained by supercritical $CO_2$

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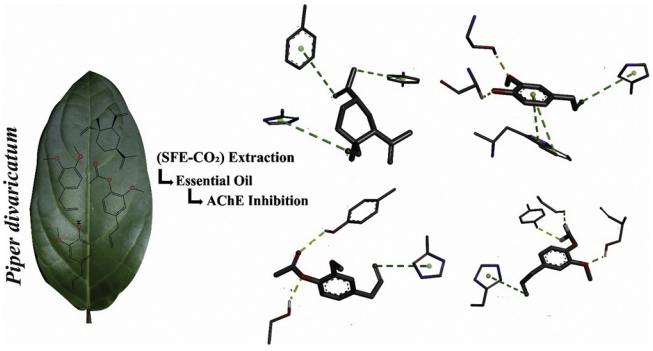
## GRAPHICAL ABSTRACT

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## ABSTRACT

The present study reports the use of supercritical $CO_2$ at different combinations of temperature and pressure: (35 and 55) °C, and (100, 300, and 500) bar to obtain fractions of the essential oil *Piper divaricatum*. The mass yields from extraction, chemical composition, antioxidant activity, and acetylcholinesterase (AChE) inhibitory activity were analyzed. The supercritical $CO_2$ extraction showed better efficiency for obtaining essential oil compared with hydrodistillation. The isotherm of 55 °C/ 500 bar led to the highest mass yield (7.40 ± 0.08) %. methyl eugenol was the compound with the highest concentration ranging from (48.01–61.85) %, and the fraction obtained in the condition of 35 °C/ 300 bar was the most effective regarding the antioxidant activity, with values of (34.69 ± 1.38) % (DPPH) and (296.86 ± 8.96) mgTrolox/mL (ABTS), respectively. Ligands, after molecular

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docking, exhibited molecular poses that promoted interactions with different residues of amino acids that are important for the enzymatic catalysis with His447.

## 1. Introduction

Alzheimer's disease (AD), recognized as a progressive neurodegenerative disease and the main cause of dementia in adults [1], is characterized by the deficit of β-amyloid peptides (Aβ) in the cholinergic system [2], which may lead to neuroinflammation and neurodegeneration [3]. It is estimated that dementia will affect 13.8 million people in the U.S. alone [4]. According to the World Health Organization [5], by 2030, the number of people with dementia in the world will be 71.2 million, and it will be 106.8 million in 2050.

Over the years, advances in knowledge of the disease pathogenesis have inspired researchers to seek pharmacological therapies to inhibit the action of acetylcholinesterase [6]. The enzymatic inhibition of acetylcholinesterase (AChE) is still an important target for the decrease in AD progression [7]. This inhibition is performed with the help of drugs such as galantamine, rivastigmine, donepezil, and huperzine. However, such AChE inhibitors may have adverse effects, such as hepatotoxicity, gastrointestinal disorders, insomnia, fatigue, syncope, and bradycardia [8,9], and in some cases their application may result in an increase in the mortality rate [10].

The molecular interactions of AChE inhibitors can be explained with the aid of computational chemistry, using docking and molecular dynamics. Both tools are important because they reduce the positive or negative errors that may exist in the experimental work and can be considered a reliable alternative [11]. In order to develop new drugs, it is also necessary to elucidate the catalytic sites responsible for the intermolecular interactions between the drug and the enzyme [12,13].

There is interest in identifying new AChE inhibitors from natural products that are available in the market [14,15]. In the literature, plants rich in essential oils have been proven to be a potential source of chemically active AChE-inhibitory molecules [16,17], mainly because of the presence of monoterpenic and sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and phenylpropanoids [18,19].

Essential oils, such as the ones extracted from Piperaceae, have also been shown to be an important source of natural antioxidants and can be used to inhibit or retard possible free radical actions [20]. These oils can reduce the oxidation caused by other molecules, reducing the risk of developing diseases such as cancer, cardiovascular diseases, Alzheimer's disease and Parkinson's disease, caused by free radicals [21].

Among the aromatic plants, the Piperaceae family includes 5 genera with approximately 2000 species, which are characterized as herbs, lianas, shrubs, and trees. The genus *Piper* is the largest of this family and covers about 700 species. Its occurrence is reported in tropical and subtropical regions around the world, and its chemical composition may vary according to several factors due to the environment [22–24]. In the *Piper divaricatum* species, two classes of secondary metabolites can be found (terpenoids and phenylpropanoids), which have been shown to present biological activities as antioxidants [23]. They may also be acetylcholinesterase inhibitors [25].

The extraction of essential oils from vegetable matrices can be performed in several ways, including microwave extraction without solvents, entrainment by water steam, steam-hydrodistillation, steam-distillation, hydrodiffusion, organic solvent extraction, and cold pressing [26]. Among them, hydrodistillation (HD) is widely used in the recovery of essential oils; however, this method has some disadvantages such as thermal degradation, oxidation, and hydrolysis of compounds. Extraction with supercritical $CO_2$ is an alternative, since it yields more pure products, with no organic solvent. It also involves the use of low critical temperature and pressure 31.1 °C and 73.7 bar, respectively, and supercritical $CO_2$ is non-toxic, non-flammable, odorless, and easily separated from the extract [19,27].

In this context, the present work aimed to analyze the mass yields, chemical composition, antioxidant, and acetylcholinesterase inhibitory activities of *Piper divaricatum* essential oils obtained by different extraction methods and to explain the mechanism of action of the major compounds by docking and molecular dynamics. The resulting products could be used in new studies aimed at improving the clinical manifestations related to Alzheimer's disease.

## 2. Materials and methods

### 2.1. Plant material

The *Piper divaricatum* leaves used in the extraction processes were collected in Belém (Pará, Brazil) in the first semester of 2017. This specimen was identified by comparison with an authentic sample, with incorporation of an *exsicata* in the Herbarium of Emílio Goeldi Museum, in the city of Belém, Pará, Brazil, under the registration number *MG-165214*.

### 2.2. Preparation and characterization of the raw material

The samples were dried in a forced air circulation oven for 5 days at a temperature of 32 °C and then ground in a knife mill (Tecnal, model TE-631/3, Brazil) at speed of 2251 rpm for 10 min. The sieving was performed with a standard series of 6 stainless steel TYLER type mesh screens (24-mesh to 48-mesh). The moisture content was analyzed in a moisture analyzer (model IV2500 - GEHAKA, Duquesa de Góias, Real Parque, São Paulo - Brazil). The apparent density was calculated by relating the sample mass used in the extraction to the volume of the extraction cell. The true density was evaluated at the analytical center of the University of Campinas-SP (UNICAMP) using a helium pycnometer. The bed porosity was calculated using the mathematical relation between the apparent density and the true density. All experiments were performed in triplicate.

### 2.3. Soxhlet extraction

In order to compare the mass yields, an extraction with organic solvent was carried out. The experiment was conducted using a Soxhlet extractor of 0.250 L, with n-hexane as solvent. The mass of crushed leaves of *P. divaricatum* used in the extraction was 13 g (in wet basis), according to the method described by Adolfo Lutz *i*nstitute [28]. For the extraction process, a volume of 0.130 L of solvent was used. The extraction was carried out by reflux with an extraction time of 10,800 s. The extract was concentrated with the aid of a rotary evaporator, and then dried in an oven, at approximately 100 °C. The yield was determined from the mathematical relationship between the extract mass and the sample mass, similarly to the calculation shown in Eq. (1).

### 2.4. Hydrodistillation

Hydrodistillation was performed on a Clevenger type apparatus, using 176.29 ± 0.1 g of sample. The extraction period was 10,800 s at a temperature of 100 °C [29]. After extraction, anhydrous sodium sulfate ($Na_2SO_4$) was added, and the essential oil was centrifuged to make it moisture-free. The mass yield of the essential oil was calculated on dry basis (db) by relating the oil mass obtained in hydrodistillation to the dry mass used in the extraction process.

$$\%\, yield\ oil\left(\frac{w}{w}\right)db = \frac{m_{oil}}{m_{sample} - (humidity\ (\%))} x\ 100 \quad (1)$$

*db = dry basis, $m_{oil}$ = mass of essential oil, $m_{sample}$ = sample mass used in the extraction.

### 2.5. Extraction procedures: supercritical carbon dioxide ($SC-CO_2$) extraction

SFE was performed using a Spe-ed™ SFE system (model 7071, Applied Separations, Allentown, PA, USA), coupled to a compressor (model CSA 7.8, Schulz S/A, Joinville, Brazil), a $CO_2$ tank (99.9) % purity, White Martins, Belém, PA, Brazil, a recirculator (model F08400796, Polyscience, Nilles, Illinois, USA), and a $CO_2$ flow meter at the outlet (model M 5SLPM, Alicat Scientific system, Tucson, AZ, USA). The extractions were performed in triplicate. Global yield isotherms were determined using (10.66 ± 0.1) g in wet basis (wb) of ground *P. divaricatum*. The temperatures used were (35 and 55) °C, and the pressures used were 100 bar, 300 bar, and 500 bar, Extraction was performed in two stages: a static period (supercritical $CO_2$ and *P. divaricatum* leaves were in closed operation conditions in the extraction vessel) of 1800s and a dynamic period (system was opened and just $CO_2$ and essential oil were released continuously from the vessel to the collector flask) of 10,800 s. All extractions were carried out in a 0.05 L extraction cell. The $CO_2$ mass flow rate was $8.85 \times 10^{-5}$ kg/s. The global yield was calculated using Eq. (1). The thermophysical properties of the supercritical $CO_2$ systems were calculated using the software NIST Chemistry WebBook [30].

### 2.6. Analysis of volatile compounds

The chemical composition of the essential oils was evaluated by gas chromatography/mass spectrometry (Shimadzu, QP-2010 plus system), under the following conditions: silica capillary column Rtx-5MS (30 m × 0.25 mm, 0.25 µm film thickness); program temperature of (60–240) °C 3 °C / min; injector temperature of 250 °C; carrier gas: helium (linear velocity of 32 cm/s, measured at 100 °C); splitless injection (1 µl of a 2:1000 hexane solution). Ionization was obtained by the electronic impact technique at 70 eV, and the temperature of the ions source and other parts was 200 °C. The quantification of volatile compounds was determined by gas chromatography with a flame ionization detector (FID) (Shimadzu, QP 2010 system), under the same conditions as gas chromatography coupled to mass spectrometry (GC–MS), except that hydrogen was used as the carrier gas. The retention index was calculated for all volatile constituents using a homologous series of n-alkanes (C8 - C20), and they were identified by comparison of their mass spectra and retention indices to those in the literature [31,32].

### 2.7. Antioxidant activity

#### 2.7.1. Radical-scavenging (DPPH)

Assessment of the free radical scavenging (2,2-diphenyl-1-picrylhydrazyl) of the essential oil of *P. divaricatum* was conducted as described by Dar et al. [33], with adaptations, using a UV–vis spectrophotometer (Thermo Scientific, Evolution 60S). The different fractions of *P. divaricatum* essential oil obtained by hydrodistillation and with supercritical $CO_2$ were diluted in methanol at concentrations of (100, 70, 60, 50, and 30) $\mu g{\cdot}mL^{-1}$. Then, 100 µL of each concentration was added to 3.9 mL of the solution containing the radical DPPH (10 mg/L). After 30 min of reaction, the samples were read at 515 nm. Solutions with concentrations of (10–60) µM of DPPH were used to construct the analytical curve. Methanol was used as a control. The assays were performed in triplicate, and the calculation of the percentage of DPPH inhibition was performed as described [34].

#### 2.7.2. ABTS radical-scavenging assay

The antioxidant capacity determined by the Trolox equivalent antioxidant capacity (TEAC) method was assessed according to the procedure proposed by Re et al. [35], with modifications. The radical ABTS was obtained from the aqueous reaction of 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) at 7 µM with a stock solution of potassium persulfate at 140 µM. The mixture was held in the dark for (12–16) h. After formation of the radical ABTS, dilution in methanol was performed until a solution with an absorbance of (0.7 ± 0.05) at 734 nm was obtained.

The dilutions of the oils used in this experiment were maintained at the same concentrations as in the DPPH assays. Subsequently, 35 µL of each solution was mixed and 3.5 mL of the solution containing ABTS was added, and after 6 min of reaction, the absorbance was measured at 734 nm [36]. As a reference, an analytical curve was prepared with Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at concentrations of (0.01 to 0.20) mg/mL, and the results were calculated and expressed as Trolox mg/mL. All experiments were performed in triplicate.

### 2.8. Acetylcholinesterase bioassay

The assay was based on the previously described methodology [37]. Acetylcholinesterase (500 U) was dissolved in Tris − HCl buffer (pH 7.8) and stabilized by the addition of bovine serum albumin (0.1) % w/v. The essential oils were loaded onto TLC plates (0.01–1000) ng/spot. Physostigmine was used as positive control. The plates were sprayed with acetylcholinesterase solution (3.33 U/ml), carefully dried, and incubated at 37 °C for 20 min (room temperature). The enzymatic activity was detected by spraying with a (0.25) % solution of 1-naphthyl acetate in ethanol and (0.25) % aqueous solution of fast blue B salt (20 mL). Potential acetylcholinesterase inhibitors appeared as bright zones on a purple background.

### 2.9. Molecular docking and molecular dynamics simulation

#### 2.9.1. Ligand preparation and molecular docking

The molecular structures of methyl eugenol, eugenyl acetate, eugenol, and β-elemene were designed with the software GaussView 5.5 e optimized with Gaussian 09 [38], using the Density Functional Theory (DFT) and B3LYP/6-31G* [39].

The molecular docking method was used to predict the binding mode of the molecules to the active site of the AChE enzyme. The docking was performed with the software Molegro Virtual Docker (MVD) 5.5 [40], and the target crystal AChE structure can be found in the Protein Data Bank (http://www.rcsb.org) with the following ID: 4M0E [41].

The MolDock Score (GRID) scoring function was used with a Grid resolution of 0.30 Å and a radius of 7 Å, encompassing the entire connection cavity that has the center located at X: -18.23, Y: -41.70, and Z: 24.35, with a volume of 81.408 $Å^3$ and a surface area of 308.48 $Å^2$. The MolDock SE algorithm was used with a number of runs equal to 10.15 max interactions, and the max population size was equal to 50. The maximum evaluation of 300 steps with a neighbor distance factor equal to 1 and energy threshold equal to 100 were used during the simulation of molecular docking. The RMSD limit for multiple cluster poses was set to < 1.00 Å.

#### 2.9.2. System preparation for molecular dynamics (MD) simulations

The molecular atomic charges were obtained with the Restrained Electrostatic Potential (RESP) protocol using the Hartree-Fock method with the 6–31 G * base set [42,43]. The parameters for each molecule were constructed using the *Antechamber* module [44] and are described by the General Amber Force Field (GAFF) [45]. The modules *sander* and *pmemd.CUDA* of the Amber 16 package were used for the MD simulations [46,47], and the protonation state of the amino acid residues was

determined from the results obtained with the PDB2PQR server (http://nbcr-222.ucsd.edu/pdb2pqr_2.0.0) [48]. The ff14SB force field [49] was used for all MD simulations. The absent hydrogens in the protein crystal were added by the tLEaP module during the process of building the complexes. The systems were solvated in an octahedron periodic box containing explicit water molecules described by the TIP3P model [50]. The distance chosen for the shear radius was 12 Å for all directions of the solvent from the solute.

The Particle Mesh Ewald method was used for the calculation of electrostatic interactions [51], and bonds involving hydrogen atoms were restricted with the SHAKE algorithm [52]. The simulation of MD was divided into stages of energy minimization, heating, equilibrium, and production. The sander module was used for both steps of energy minimization, where the steepest descent method and conjugate gradient algorithm were employed to perform 1500 cycles divided among the steps. In the first step, the solute was restricted with a constant harmonic force of 100 kcal/mol·Å$^{-2}$, while the water and anti-ion molecules were free. In the second stage, the complexes were totally free to move.

Then, the systems were gradually heated for 600 ps until the temperature reached 300 K. The heating was divided into five stages, where the collision frequency was 3.0 ps$^{-1}$ and the Langevin thermostat was used for temperature control [53]. The heavy atoms were restricted with a constant harmonic force of 50 kcal/mol·Å$^{-2}$ during the initial four steps. In the last heating step, the constant harmonic force was removed. These simulations were performed at constant volume (NVT). In the equilibrium stage, the systems were submitted to a simulation of 5 ns (ns) with a temperature of 300 K and constant pressure. Before starting production simulation, the systems using accessory software such as Visual Molecular Dynamics (VMD) [54] and UCSF Chimera [55], along with Root Mean Square Deviation (RMSD) of the structures were observed. The systems maintained an adequate three-dimensional structure without drastic conformational changes. During the production stage, 100 ns of MD simulations were generated.

#### 2.9.3. Free energy calculations using the MM/GBSA approach

The free energy of each complex was obtained from the last 5 ns of the trajectory corresponding to 500 snapshots. In the MM-GBSA approach, binding free energy is calculated from the free energy of a linker interacting with a receptor to form the complex [56]. Eq. (2) is related to this phenomenon:

$$\Delta G_{bind} = \Delta G_{complex} - \Delta G_{receiver} - \Delta G_{lingand} \quad (2)$$

In each state, the free energy is calculated through the following expression:

$$\Delta G_{bind} = \Delta G_{MM} + \Delta G_{solv} - T\Delta S \quad (3)$$

$\Delta E_{MM}$ is the energy of the total molecular mechanics in the gas phase, $\Delta G_{solv}$ is the free energy of solvation, and $T\Delta S$ is the entropy of the system.

$E_{MM}$ represents the sum of the internal energy contributions ($\Delta E_{internal}$, sum of the binding energies, angles and dihedrals), eletrostatic interactions ($\Delta E_{electrostatic}$), and contributions of van der Waals ($\Delta E_{vdw}$), according to the equation:

$$\Delta E_{MM} = \Delta E_{internal} + \Delta E_{eletrotatic} + \Delta E_{vdw} \quad (4)$$

The free energy of solvation ($\Delta G_{solv}$), in Eq. (3), is composed by polar ($\Delta G_{GB}$) and non-polar ($\Delta G_{SASA}$) contributions. Polar contributions are approximated by the Generalized Born (GB) method, and the non-polar contributions are determined from the calculation of the solvent-accessible surface area (SASA):

$$\Delta G_{solv} = \Delta G_{GB} + \Delta G_{SASA} \quad (5)$$

#### 2.9.4. Per-residue free energy decomposition analysis

To analyze the energy contribution of the residues responsible for the interaction AChE-ligands, the binding energy was decomposed into van der Waals ($E_{vdW}$), electrostatic ($\Delta E_{elec}$), polar ($\Delta E_{pol}$), and non-polar ($\Delta E_{np}$) contributions, with the approach of MM/GBSA [57]. For each of the last 500 snapshots of the MD trajectory, the energy contributions were calculated by:

$$\Delta G_{MM} - GB_{SA} = \Delta E_{vdW} + \Delta E_{elec} + \Delta E_{pol} + \Delta E_{np} \quad (6)$$

### 2.10. Statistical analysis

The determinations were performed in triplicate, and the results are expressed as the mean of three independent replicates (n = 3). In order to verify the existence of a significant difference among the different fractions of essential oil of *P. divaricatum* obtained by hydrodistillation and with supercritical $CO_2$, the means of the results were submitted to analysis of variance and when significant, compared by the Tukey test at 95% confidence with the software Statistica® (version 7., Statsof, Inc. Tulsa, USA).

## 3. Results and discussion

### 3.1. Characterization of the raw material

The moisture content of the raw material was of (9 ± 0.1) % the mean diameter was $3.27 \times 10^{-4}$ m, the apparent density was (305 ± 0.1) kg/m$^3$, the true density was (1360 ± 10) kg/m$^3$, and the bed porosity was 0.776.

**Table 1**
Means of the mass yields of isolated essential oil fractions from *P. divaricatum* obtained by Hydrodistillation (HD) with the mass flow was constant, Soxhlet extraction (SE) and Supercritical carbon dioxide (SC − $CO_2$) extraction.

| HD, Soxhlet Extraction and SC-$CO_2$ | Mass of EO (g) / ExSE (g) | Yield % (wb) | Yield % (db) | Density (kg/m$^3$) SC-$CO_2$ | Viscosity (uPa s) SC-$CO_2$ | Joule-Thomson (K/bar) $CO_2$ |
|---|---|---|---|---|---|---|
| HD | 4.86 | 2.76 | 3.03 | | | |
| SE | 1.03 ± 0.15 | 7.92 ± 0.01 | 8.7 ± 0.02$^a$ | | | |
| 35 (°C) /100 (bar) | 0.45 ± 0.06 | 4.26 ± 0.57 | 4.68 ± 0.62$^e$ | 712.8 | 57.69 | 0.172 |
| 35 (°C) /300 (bar) | 0.58 ± 0.03 | 5.49 ± 0.23 | 6.03 ± 0.25$^d$ | 929.1 | 98.86 | 0.014 |
| 35 (°C) /500 (bar) | 0.62 ± 0.01 | 5.81 ± 0.06 | 6.39 ± 0.04$^d$ | 1005 | 123.01 | −0.007 |
| Global means SC-$CO_2$ (35 °C) | 0.55 ± 0.08 | 5.18 ± 0.81 | 5.7 ± 0.9$^c$ | | | |
| 55 (°C) /100 (bar) | 0.46 ± 0.04 | 4.28 ± 0.37 | 4.70 ± 0.41$^e$ | 325 | 25.31 | 0.612 |
| 55 (°C) /300 (bar) | 0.69 ± 0.0 | 6.51 ± 0.0 | 7.15 ± 0.0$^b$ | 850.2 | 80.65 | 0.034 |
| 55 (°C) /500 (bar) | 0.72 ± 0.01 | 6.73 ± 0.07 | 7.40 ± 0.08$^b$ | 947.9 | 104.01 | 0.001 |
| Global means SC-$CO_2$ (55 °C) | 0.62 ± 0.14 | 5.84 ± 1.13 | 6.41 ± 1.4$^c$ | | | |

* Grouping Information Using the Tukey Method and 95% Confidence, means that do not share a letter are significantly different. *(wb) wet basis; (db) dry base; (EO) Essential Oil·.

### 3.2. Global yields of Soxhlet extraction, hydrodistillation and SC−$CO_2$ extraction

As shown in Table 1, the operating conditions of temperature, pressure, and density influenced the extraction, as well as the property of transport viscosity. Another thermophysical property that can change the mass yield is the Joule-Thomson's coefficient, since it can cause cooling in the outlet valve during isenthalpic expansion.

The Soxhlet extraction was the one that presented the highest mass yield (8.7 ± 0.02) %. This result was approximately (15) % higher than the highest mass yield obtained in the supercritical $CO_2$ extraction, as can be seen in Table 1. However, this extraction was performed with organic solvent, which enables the total extraction of lipids and other compounds such as chlorophyll. The images of the extracts obtained by this technique can be observed in the supplementary material S1.

In the hydrodistillation of the essential oil of P. divaricatum, there was (3.03) % (db) mass yield. This result is close to that found in the literature [58]; this may be related to some factors such as the site, period, and time of collection of the raw material, as these are factors that modify the essential oil yield of aromatic plants as well as their chemical composition in qualitative and quantitative terms [59]. Compared to species of the same genus [60], Piper divaricatum showed a high concentration of essential oil. The images of the oil fractions obtained by hydrodistillation and supercritical $CO_2$ are given in the supplementary material, S2 and S3, respectively. We can also observe that the coloration of the essential oil obtained with supercritical $CO_2$ and stronger than that obtained by hydrodistillation.

By analyzing the effect of pressure in Table 1, it was observed that for the two isotherms, when pressure increased, there was an increase in the density of the supercritical $CO_2$, and consequently there was an increase in the essential oil yield. Regarding the temperature effect, it was observed that the density values for the isotherm of 55 °C are lower than those for the isotherm of 35 °C in their respective pressures 100 bar, 300 bar and 500 bar. However, the yields at the isotherm of 55 °C are higher than those at the isotherm of 35 °C. This can be explained by the solute P. divaricatum essential oil vapor pressure, which has greater influence than the density of the supercritical $CO_2$.

The highest yields were obtained at the isotherms of 55 °C and pressures of 300 with density of 850.2 (kg/m$^3$) and 500 bar with density of 947.9 (kg/m$^3$), presenting mass yield values of (7.15) % and (7.40 ± 0.08) % on dry basis, respectively. These results were not statistically different. The yields of (6.03 ± 0.25) % and (6.39 ± 0.04) % were obtained in relation to the isotherm of 35 °C under conditions of higher pressures 300 bar and 500 bar, respectively. Also, there was no statistical difference between them. The lowest extraction yields were obtained at 35 °C / 100 bar with density of 712.8 (kg/m$^3$) and 55 °C / 100 bar with density of 325 (kg/m$^3$), corresponding to (4.68 ± 0.62) % and (4.7 ± 0.41) %, respectively, and these results were statistically significant ($p < 0.05$). The overall mean of the 35 °C and 55 °C isotherms presented statistically similar results for the recovery of P. divaricatum essential oil, as can be observed in Table 1, with values of (5.7 ± 0.9) % and (6.41 ± 1.4) % (db), respectively.

The results of the isotherms of 35 °C / 300 bar and density of 929.1 (kg/m$^3$) and 55 °C / 500 bar with density of 947.9 (kg/m$^3$) were approximately twice as high as those obtained in hydrodistillation. In our results, it was observed that the change in temperature caused an increase in the extraction yield of the essential oil of *P. divaricatum*, however, there are reports that this parameter is not always the one that most influences the supercritical extraction process, as observed in the work by Zermane et al. [61]. However, supercritical $CO_2$ extraction has been shown to be a viable alternative for extracting essential oils from vegetable matrices, mainly because it is not toxic and allows a selective extraction, in comparison to the organic solvents such as n-hexane used in Soxhlet extraction.

### 3.3. Chemical composition

The chemical compositions of the different essential oil fractions are given in Table 2. In general, 18 components were identified, being mainly formed by sesquiterpene hydrocarbons and phenylpropanoids, in both extraction techniques and all combinations of temperature and pressure used in the supercritical $CO_2$ technique. However, small qualitative and quantitative differences in the chemical composition among

**Table 2**
Chemical composition of the essential oil of *P. divaricatum* obtained with and Supercritical carbon dioxide (SC−$CO_2$) and Hydrodistillation (HD) with the mass flow was constant.

| RI | Compounds | Molecular Formula | 35 (°C) | | | 55 (°C) | | | (HD) |
|---|---|---|---|---|---|---|---|---|---|
| | | | 100 (bar) | 300 (bar) | 500 (bar) | 100 (bar) | 300 (bar) | 500 (bar) | |
| | | | ρ 712.8 (kg/m$^3$) | ρ 929.1 (kg/m$^3$) | ρ 1005 (kg/m$^3$) | ρ 325 (kg/m$^3$) | ρ 850.2 (kg/m$^3$) | ρ 947.9 (kg/m$^3$) | |
| 1042 | (E)-β-Ocimene | $C_{10}H_{16}$ | | 0.36 | | | 0.45 | 0.92 | 0.38 |
| 1333 | δ-Elemene | $C_{15}H_{24}$ | 0.08 | | | | 0.06 | 0.05 | |
| 1351 | Eugenol | $C_{10}H_{12}O_2$ | 10.52 | 13.27 | 11.01 | 11.31 | 11.2 | 12.08 | 21.7 |
| 1374 | α-Copaene | $C_{15}H_{24}$ | 0.08 | 0.04 | 0.09 | 0.1 | 0.09 | 0.11 | |
| 1388 | β- Elemene | $C_{15}H_{24}$ | 7.38 | 6.39 | 6.8 | 6.88 | 7.35 | 6.53 | 5.32 |
| 1403 | Methyl eugenol | $C_{11}H_{14}O_2$ | 48.01 | 57.02 | 49.16 | 49.63 | 50.96 | 51.75 | 61.9 |
| 1417 | (E)-Caryophyllene | $C_{15}H_{24}$ | 2.66 | 2.86 | 2.74 | 2.86 | 3.19 | 3.24 | 1.99 |
| 1428 | β- Copaene | $C_{15}H_{24}$ | 0.46 | 0.17 | 0.32 | 0.42 | 0.42 | 0.31 | 0.26 |
| 1452 | (E)- β-Farnesene | $C_{15}H_{24}$ | 0.43 | | 0.29 | 0.3 | 0.19 | 0.17 | 0.1 |
| 1453 | α-Humulene | $C_{15}H_{24}$ | | | 0.1 | 0.09 | 0.08 | 0.13 | 0.08 |
| 1479 | Germacrene D | $C_{15}H_{24}$ | 5.24 | 4.95 | 6.02 | 5.49 | 5.61 | 6.3 | 3.21 |
| 1493 | Bicyclogermacrene | $C_{15}H_{24}$ | 0.26 | | 0.31 | 0.26 | 0.17 | 0.23 | 0.19 |
| 1496 | α-Muurolene | $C_{15}H_{24}$ | 0.16 | | 0.11 | 0.13 | 0.08 | 0.07 | |
| 1504 | Germacrene A | $C_{14}H_{22}$ | | | 0.24 | | | 0.13 | |
| 1516 | Eugenyl acetate | $C_{12}H_{14}O_3$ | 22.55 | 14.75 | 21.26 | 20.72 | 19.14 | 17.3 | 4.35 |
| 1546 | Elemicin | $C_{12}H_{16}O_3$ | 0.68 | 0.07 | 0.58 | 0.58 | 0.35 | 0.29 | 0.38 |
| 1696 | Pm = 236 | No found | 0.77 | 0.06 | 0.62 | 0.56 | 0.38 | 0.26 | |
| 2130 | Phytolderivative | No found | 0.38 | | 0.3 | 0.56 | 0.18 | 0.09 | |
| Monoterpene hydrocarbons | | | | 0.36 | | | 0.45 | 0.92 | 0.38 |
| Sesquiterpene hydrocarbons | | | 16.75 | 14.41 | 17.02 | 16.53 | 17.24 | 17.27 | 11.2 |
| Phenylpropanoids | | | 81.76 | 85.11 | 82.01 | 82.24 | 81.65 | 81.42 | 88.3 |
| Others | | | 1.15 | 0.06 | 0.92 | 1.12 | 0.56 | 0.35 | |
| Total | | | 99.66 | 99.94 | 99.95 | 99.89 | 99.9 | 99.96 | 99.8 |

*RI = Retention index (DB-5ms column). ρ = Density of Supercritical $CO_2$.

the fractions of essential oils of *P. divaricatum* obtained with supercritical $CO_2$ and hydrodistillation were observed. Qualitatively, the compounds δ-elemene, α-copaene, α-muurolene, germacrene A, and phytol derivative were obtained only on extraction with supercritical $CO_2$ and in some cases, only under specific temperature and pressure conditions. In quantitative terms, it is possible to show this difference among the contents of the major constituents obtained by the two extraction techniques. The chemical structures of the components identified in the different fractions of the essential oil of *P. divaricatum* can be observed in Fig. 1, and the chromatogram ions in the supplementary material S4.

The quantitative and qualitative variations of chemical constituents of the essential oil fractions obtained with supercritical $CO_2$ can be related to temperature, pressure, and density used in the extraction process. In relation to the major compounds, it was observed in Table 2, that the isotherm of 35 °C / 300 bar with density of 929.1 (kg/m$^3$) was the best condition for obtaining methyl eugenol and eugenol, however, in this condition, there was a decrease in the extraction of eugenyl acetate and β- elemene with values of (13.27) %, (57.02) %, (14.75) % and (6.39) %, respectively. The best yield of eugenyl acetate (22.55) % and β-elemene (7.38) % was observed in the isotherm of 35 °C / 100 bar with density of 712.8 (kg/m$^3$). Also, in this operating condition, the lowest concentrations of methyl eugenol and eugenol were obtained (48.01% and 10.52%, respectively), demonstrating that the process can be selective depending on the operating parameters. However, there are no data in the literature on supercritical extraction of essential oil of *P. divaricatum* for comparison. Table 2 also shows the results of hydrodistillation, in which it can be observed that with this technique of extraction, it was possible to obtain the highest concentrations of methyl eugenol and eugenol (61.85) % and (21.7) %, respectively, and also the lowest concentrations of eugenyl acetate and β-elemene (4.35) % and (5.32) %, respectively. These results were relatively close to those obtained previously [62] for the same plant species. Supercritical $CO_2$ also demonstrated efficiency for sesquiterpene hydrocarbons extraction, such as β-elemene, (*E*)-caryophyllene, and germacrene D. This may be related to the fact that supercritical $CO_2$ has a higher affinity for extraction of apolar or low polarity compounds [63], and the low temperature used in the extraction process is one of the factors that prevents thermal degradation of the chemical compounds in essential oil.

**Table 3**
Free radical scavenging activity and antioxidant capacity of *P. divaricatum* essential oil obtained by Hydrodistillation (HD) with the mass flow was constant and Supercritical carbon dioxide (SC − $CO_2$).

| HD and (SC-$CO_2$) | DPPH inhibition (%) | TEAC (mg Trolox/mL) |
|---|---|---|
| HD | 28.91 ± 1.15$^b$ | 209.88 ± 5.06$^c$ |
| 35 (°C) / 100 (bar) | 20.48 ± 2.70$^c$ | 116.17 ± 11.55$^d$ |
| 35 (°C) / 300 (bar) | 34.69 ± 1.38$^a$ | 296.86 ± 8.96$^a$ |
| 35 (°C) / 500 (bar) | 22.37 ± 0.68$^c$ | 201.80 ± 5.12$^c$ |
| 55 (°C) / 100 (bar) | 20.90 ± 0.24$^c$ | 195.07 ± 5.67$^c$ |
| 55 (°C) / 300 (bar) | 29.11 ± 0.89$^b$ | 200.42 ± 13.66$^c$ |
| 55 (°C) / 500 (bar) | 30.81 ± 0.52$^b$ | 233.67 ± 6.67$^b$ |

*All data are mean ± standard deviation (n = 3). Mean in the same column with the same letter are not statistically different at 95% confidence, by the Tukey test.

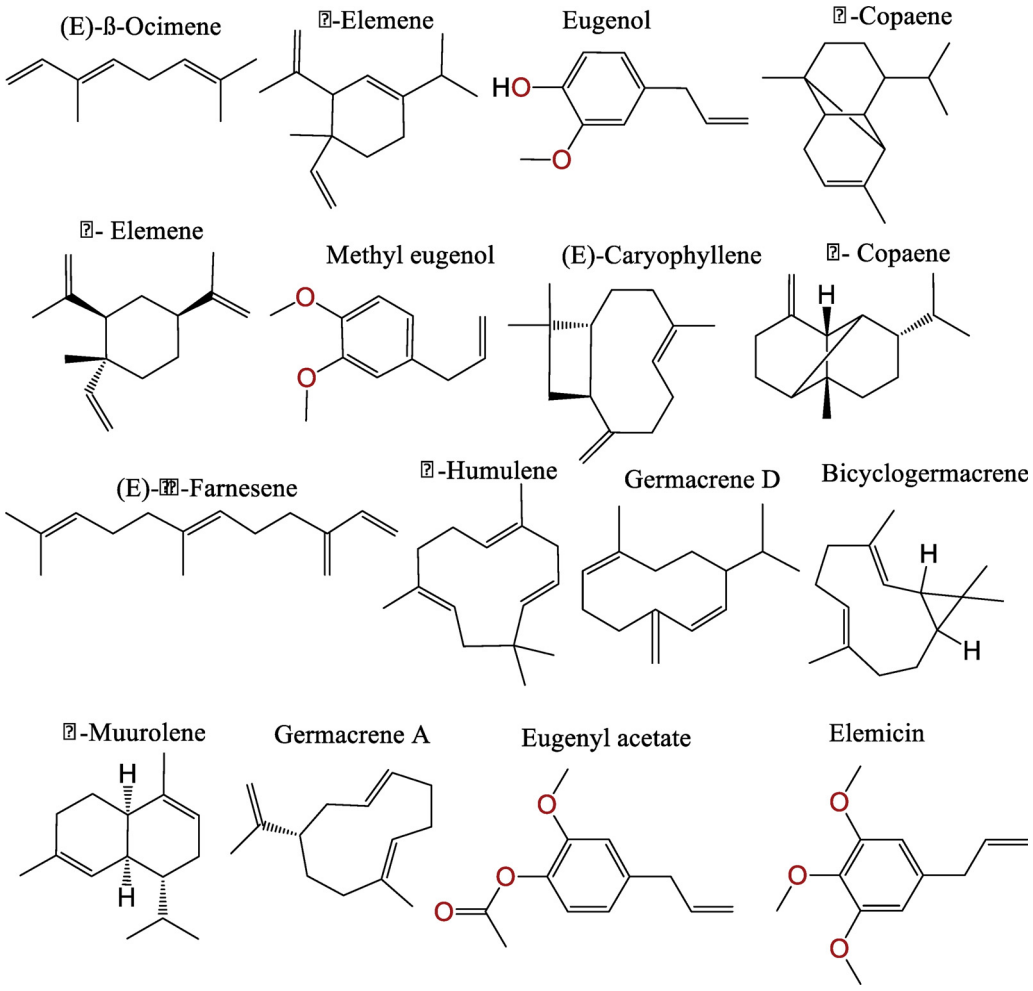


**Fig. 1.** Chemical structures of the different components identified in the fractions of *P. divaricatum* essential oil obtained with supercritical $CO_2$ and hydrodistillation.

### 3.4. Antioxidant activity

According to the results shown in Table 3, the essential oil fractions of *P. divaricatum* obtained by hydrodistillation and supercritical $CO_2$ exhibited the ability to reduce the radical DPPH. However, the percentage of anti-radical inhibition showed a significant variation between (20.48) % and (34.69) %. Therefore, among the conditions evaluated in the study, the fraction of essential oil obtained at 35 °C / 300 bar exhibited a significant inhibition percentage of (34.69) %. Therefore, it is the fraction that presented the greatest capacity for sequestration of the radical DPPH.

The antioxidant capacity of the essential oils of *P. divaricatum* by TEAC shows that the fractions obtained by hydrodistillation and supercritical $CO_2$ were also efficient in sequestering the radical ABTS; however, this action was differentiated, with significant variations between (116) mg Trolox/mL and (296.86) mg Trolox/mL. This difference can be related to the variations in chemical composition of the essential oil fractions presented in the previous item, with the results shown in Table 2, indicating that the antioxidant activities presented by the *P. divaricatum* EO fractions may not be directly associated with the isolated action of the secondary metabolites present in higher concentrations such as eugenol, methyl eugenol, and eugenyl acetate, and the sesquiterpene β-elemene. The different responses observed in Table 2 can be related to the synergistic effect among the chemical constituents of the oils. In this way, each compound can contribute in a different way to the results of antioxidant activity. Other studies have shown that essential oils rich in bioactive compounds such as phenylpropanoids have good antioxidant activity [64]. The highest antioxidant activity was observed for the essential oil obtained at 35 °C / 300 bar (296.86 mg TE/mL), which was close to that reported by Silva et al. [65] (306.1) mg Trolox/mL for the essential oils of the species *Piper aleryreanum* (412.2 mg Trolox/mL), *Piper anonifolium* (148.6) mg Trolox/mL, and *Piper hispidum* (303.1) mg Trolox/mL. Both methods used to evaluate the in vitro antioxidant capacity of essential oils of *P. divaricatum* exhibited high values for the hydrodistillation and supercritical $CO_2$ extractions, with potential application in neutralization and a possible protective effect against reactive species.

### 3.5. Experimental inhibition of acetylcholinesterase

The method that uses TLC is considered effective and quick for the determination of extracts with AChE inhibitory potential [66]. The sample obtained by HD produced inhibition halos at concentrations of (0.01, 1, and 100) ng/site, while the sample obtained by SFE produced halos at (0.01, 100, and 1000) ng/site. These results showed that both HD and SFE fractions are strong AChE inhibitors, with a detection limit of 0.01 ng, equivalent to physostigmine, the alkaloid used as a positive control [65] performed a TLC-silver anti-cholinesterase assay of the essential oil of three Piper species, which were *P. anonifoliu, P. hispidum*, and *P. aleyreanum.* The first two species presented the same detection limit (DL) equal to 0.01 ng/site and *P. aleyreanum* presented a DL = 10.0 ng/site, a DL a thousand times smaller than the first two. In the literature, there are reports that the phenylpropanoids eugenol, methyl eugenol [67,68], and sesquiterpene hydrocarbon β-elemene [69] exhibit a potential inhibition of AChE; however, their mechanism of action is unknown.

### 3.6. Mechanism of acetylcholinesterase inhibition

#### 3.6.1. Identifying binding modes

The catalytic triad of AChE, formed by the residues Ser203, Glu337, and His447, is located in a deep and narrow cavity of approximately 20 Å [70]. Within this cavity, there are also subsites that are important for enzymatic catalysis, such as the anionic subsite (Trp86, Tyr133, Tyr337, and Phe338), acyl pocket (Phe295 and Phe297), and oxyanion hole (Gly121, Gly122, and Ala204) [71]. The binding energies obtained with MVD 5.5 for the interactions of methyl eugenol, eugenyl acetate, eugenol, and β-elemene with AChE are summarized in Table 4.

**Table 4**
Moldock scores obtained from the docking protocol using MVD 5.5.

| Molecule | MolDock Score | Rerank Score |
|---|---|---|
| β-Elemene | −96.36 | −76.64 |
| Eugenol | −87.13 | −75.82 |
| Eugenyl acetate | −99.17 | −86.42 |
| Methyl eugenol | −90.44 | −75.05 |

The conformations obtained by molecular docking studies demonstrate that the molecules became buried in the AChE binding pocket and that they interact with the residues belonging to the catalytic triad and the anionic site (Fig. 2).

The interactions between ligands and catalytic cavity residues are essential for understanding their binding and inhibition mechanism; therefore, the nature of each interaction of the compounds with AChE was investigated and described. The analyses revealed that β-elemene bound to the binding pocket mainly by hydrophobic interactions. It exhibited hydrophobic interaction with His447 (triad catalytic), where the C7 carbon interacted with the imidazole ring of the residue at a distance of 4.12 Å. This molecule also interacted with the benzene ring of residues Tyr124 and Phe338 (anionic site), where there were π-alkyl-type interactions with distances of 4.1 and 3.99 Å, respectively.

Eugenol showed hydrophobic interactions between its C9 carbon atom and the imidazole ring of His447 (triad catalytic) at a distance of 3.97 Å. Hydrogen bonds were also formed with Gly126 and Ser125. Eugenol was the acceptor of hydrogen belonging to the alpha carbon of Gly126, and this interaction occurred at a distance of 2.52 Å, while with Ser125 the distance was 2.12 Å. In the same way, eugenol was an acceptor of hydrogen, receiving the atom of the hydroxyl group of Ser125. With the Trp86 (anionic site), two hydrophobic π-π interactions were established at distances of 3.98 and 4.21 Å.

Eugenyl acetate exhibited three interactions: one hydrophobic with His447 (triad catalytic) and two hydrogen bonds. The C12 carbon interacted hydrophobically with Hist447 (triad catalytic), and the oxygen atom O1 of the ligand was an acceptor of hydrogen H5 from the Ser125 hydroxyl, which was at a distance of 2.14 Å. The second hydrogen bond was established at a distance of 2.73 Å between the carbon O2 and the hydrogen H9 that belongs to the Trp337 phenol hydroxyl (anionic site).

Methyl eugenol was able to form one hydrophobic and three hydrogen bonds. The hydrogen bond formed with Ser125 was the closest of the three, and formed at a distance of 2.08 Å. With Gly120, the hydrogen bond was at 2.53 Å, and with the residue Tyr133, it occurred at 2.68 Å. An important hydrophobic interaction was established between the atom carbon C11 and His447 imidazole ring belonging to the catalytic triad of AChE. This interaction was of the π-alkyl-type occurring at a distance of 3.97 Å.

#### 3.6.2. Conformational stability of systems during MD simulation

The verification of the stability and convergence of the structures were evaluated through the RMSD graphs, where the Cα atoms were used to plot the protein backbone graphs, and the heavy atoms were used to plot the ligand graphs. The time series of RMSD values can be seen in Fig. 3.

During the simulations, the protein backbone in all systems reached stability. Throughout the simulation, according to the plots of RMSD, it was possible to observe that the three-dimensional structure of the protein did not undergo drastic conformational changes. The mean values of RMSD for the system backbone were as follows: AChE-methyl eugenol: 1.72, AChE-eugenyl acetate: 1.63, AChE-eugenol: 1.98, and AChE-β-elemene: 1.81 Å. During the simulation, the ligands remained interacting with the enzyme site, exhibiting small conformational changes relative to their respective starting structures.

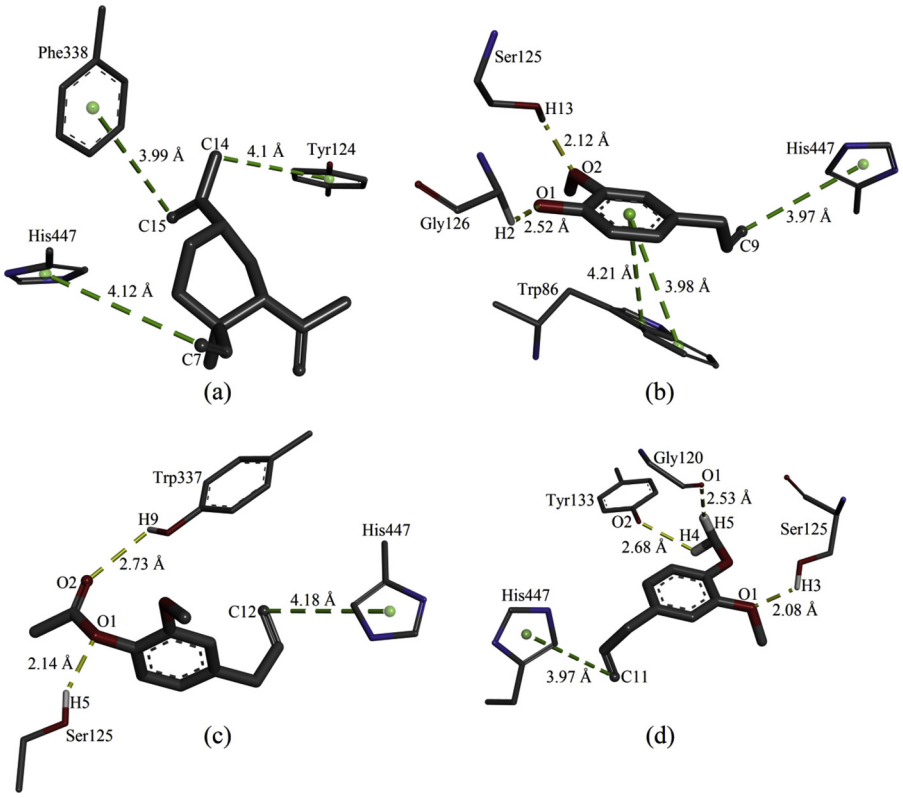


**Fig. 2.** Docked conformation of molecules in the binding cavity of AChE. The carbon atoms are represented in gray color, nitrogen in blue, oxygen in red and hydrogen in white. The hydrophobic interactions are represented by dashed lines of green color and the hydrogen bonds are represented in yellow color. The distance from each interaction was demonstrated in angstroms. The amino acid residues and their atoms involved in the interactions are named and numbered. (a) Predicted binding mode of β-elemene. (b) Predicted binding mode of eugenol. (c) Predicted binding mode of eugenyl acetate. (d) Predicted binding mode methyl eugenol.

*3.6.3. Analysis of free energy components and mechanism of binding interaction*

The free binding energy of the systems was calculated using the MM-GBSA method using the last 500 snapshots of each MD path, and the results can be observed in Table 5.

According to Table 5, the free energy values obtained were as follows: AChE-methyl eugenol: -22.22, AChE-eugenol: -23.20, AChE-eugenyl acetate: -24.26, and AChE-β-elemene: -27.54 kcal/mol. These results demonstrate that the interaction of these inhibitors with acetylcholinesterase is favorable for the complexes formation.

The main energy responsible for the interaction of inhibitors with AChE is the sum of non-polar interactions ($\Delta E_{non\text{-}polar}$). $\Delta E_{non\text{-}polar}$ is the sum of van der Waals interactions ($\Delta E_{vdW}$) with non-polar solvation free energy ($\Delta G_{NP}$), which is determined from the calculation of the solvent accessible surface area (SASA). β-elemene was the compound that presented more hydrophobic interactions, which may be related to its molecular structure, formed basically by carbon and hydrogen atoms.

Electrostatic contributions ($\Delta E_{ele}$) also favor ligand-receptor interactions, although they are weaker contributions. However, this energy is counterbalanced by the polar solvation free energy ($\Delta G_{GB}$), which is

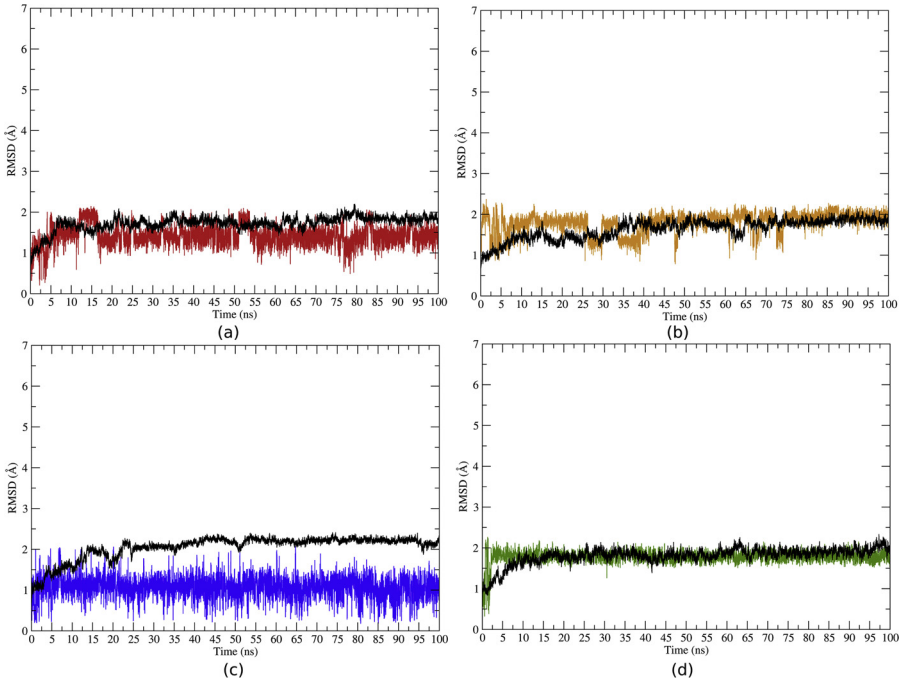


**Fig. 3.** Analysis of the conformational stability of the systems over 100 ns of MD simulation. The protein backbone is represented in black in all graphs while the colors to represent the binders vary. The RMSD graphs were plotted in relation to the systems obtained after the steps of minimization, heating and equilibrium. AChE-methyl eugenol in red (a), AChE-eugenyl acetate in yellow (b), AChE-eugenol in blue(c) and AChE-β-elemene (d) in green.

**Table 5**
Prediction of the free energy values of the systems and their components (values in kcal/mol). Non-polar and polar contributions were calculated, respectively, by: $\Delta E_{non-polar} = \Delta E_{vdW} + \Delta G_{NP}$ and $\Delta E_{polar} = \Delta E_{ele} + \Delta G_{GB}$

| Molecule | $\Delta E_{vdW}$ | $\Delta E_{ele}$ | $\Delta G_{GB}$ | $\Delta G_{NP}$ | $\Delta E_{non\text{-}polar}$ | $\Delta E_{polar}$ | $\Delta G_{MM\text{-}GBSA}$ |
|---|---|---|---|---|---|---|---|
| Methyl eugenol | −29.13 | −4.74 | 15.50 | −3.85 | −32.98 | 10.76 | -22.22 |
| Eugenyl acetate | −30.73 | −0.67 | 11.30 | −4.15 | −34.88 | 10.63 | −24.26 |
| Eugenol | -26.91 | −12.64 | 20.26 | -3.90 | −30.81 | 7.62 | -23.20 |
| β-Elemene | −34.71 | −4.31 | 16.12 | -4.64 | −39.35 | 11.81 | -27.54 |

unfavorable. The sum of these two contributions results in unfavorable total polar energy ($\Delta E_{polar}$) for the systems.

From the free energy results obtained by the MM-GBSA method, it was possible to observe that the contributions of van der Waals forces were the main contributions that directed the inhibitor interactions. Given this observation, from the results of the pre-residue free energy decomposition, the van der Waals contribution values were extracted. The residues important for the interactions had their energy values plotted on the graph that can be observed in Fig. 4.

In general, the spectrum of van der Waals interactions is similar for the systems. In all complexes, the highest interaction of van der Waals occurs with Trp86, belonging to the enzyme's anionic subsite. The indole ring of Trp86 performed stacking π interactions with the benzene ring of the inhibitor's eugenol, eugenyl acetate, and methyl eugenol. It is possible that the π-alkyl interaction between Trp86 and β-elemene also occurred. The interactions with this residue have been reported in different studies of drug design as being of great importance for the catalytic activity [72–74].

All inhibitors performed productive interactions with Ser203 and His447, belonging to the catalytic site of AChE. With His447, the interaction value was -0.59 kcal/mol for methyl eugenol, -0.65 kcal/mol for eugenyl acetate, -1.02 kcal/mol for eugenol, and -1.12 kcal/mol for β-elemene. With Ser203, methyl eugenol, eugenol, and β-elemene interacted, respectively, with the following values of affinity energy: -0.59, -0.53, and -0.61 kcal/mol. Terpenoids and phenylpropanoids exhibit different interactions; however, these active compounds can act as AChE inhibitors.

## 4. Conclusion

The different techniques of extraction of supercritical $CO_2$ and hydrodistillation were efficient for the isolation of the essential oils of *P. divaricatum* leaves. However, the supercritical $CO_2$ showed higher extraction power mainly under the operating condition of 55 °C and 500 bar. Small quantitative and qualitative differences in their chemical composition were observed, and the phenylpropanoids class presented the highest concentration, with emphasis on methyl eugenol. All 7 fractions of essential oils presented antioxidant activity for the radicals ABTS and DPPH. However, the antioxidant capacity of the *P. divaricatum* essential oil fractions may not be entirely associated with the isolated action of the phenylpropanoids, but possibly with the effects among the different groups of bioactive compounds such as monoterpenes, sesquiterpene hydrocarbons, and other compounds. And the fractions experimentally tested also showed AChE inhibitory potential. The molecular docking revealed that β-elemene, eugenol, eugenyl acetate, and methyl eugenol are capable of interacting with different residues belonging to the active site of AChE, such as His447. The RMSD plots showed that the molecules remained balanced at the binding site. The binding free energy values demonstrated that the receptor-ligand interaction is favorable for the systems. The results of per-residue free energy decomposition demonstrated that the molecules, during the simulation, performed interactions with residues of the active site that are important for the enzymatic activity inhibition.

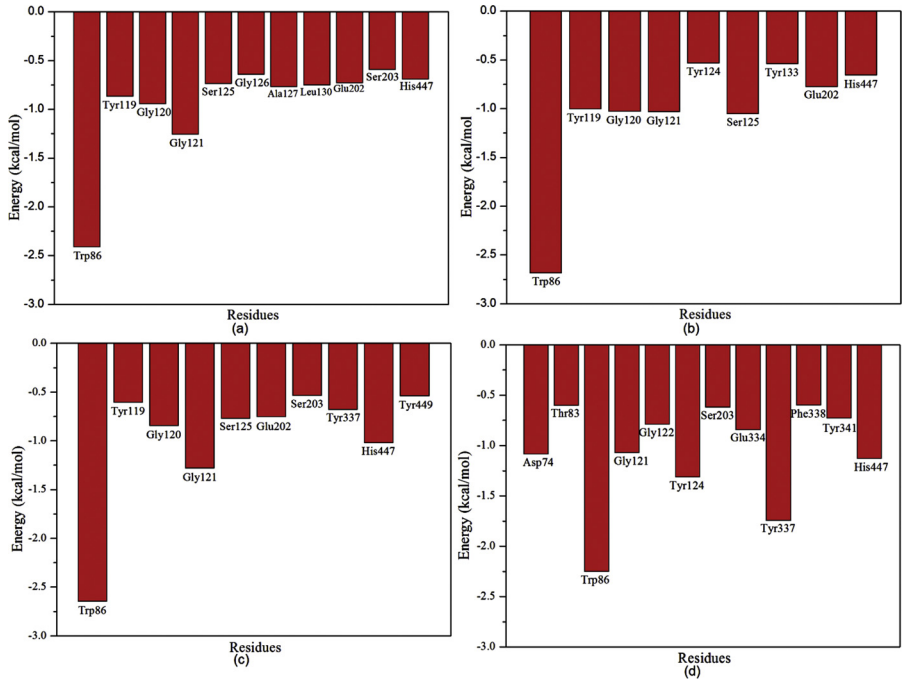


**Fig. 4.** van der Waals interactions between residues-ligands: (a) AChE-methyl eugenol, (b) AChE-eugenyl acetate, (c) AChE-eugenol and (d) AChE-β-elemene.

## Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.supflu.2018.12.003.

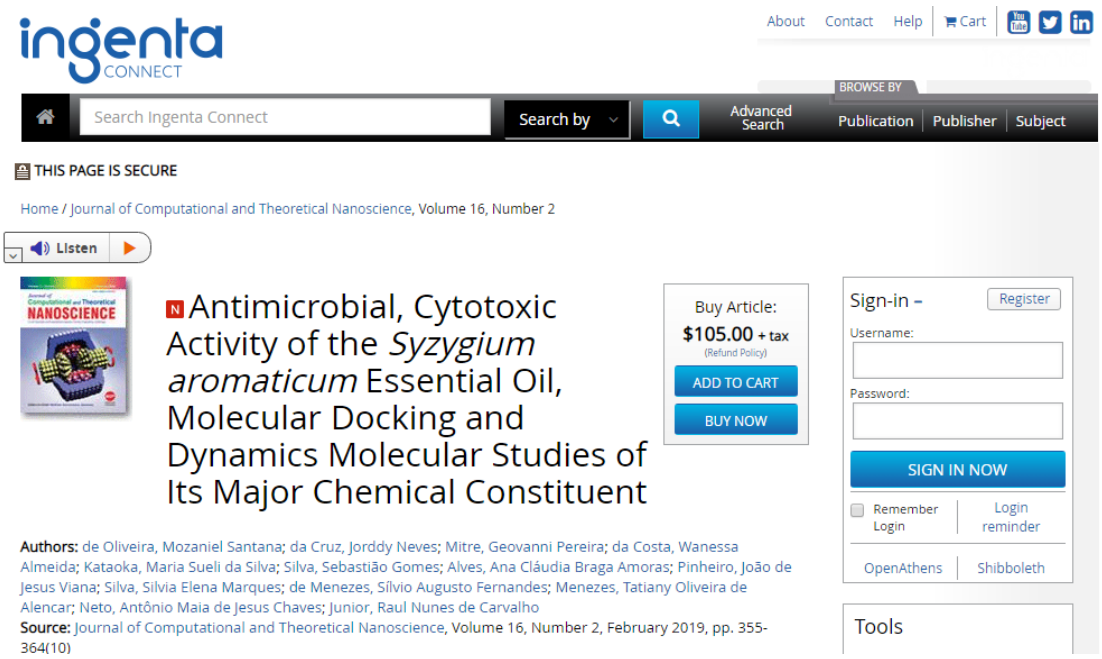
## References

[1] A. Kumar, A. Singh, Ekavali, A review on Alzheimer's disease pathophysiology and its management: an update, Pharmacol. Rep. 67 (2015) 195–203, https://doi.org/10.1016/j.pharep.2014.09.004.
[2] R.H. Takahashi, T. Nagao, G.K. Gouras, Plaque formation and the intraneuronal accumulation of β-amyloid in Alzheimer's disease, Pathol. Int. 67 (2017) 185–193, https://doi.org/10.1111/pin.12520.
[3] S. Piirainen, A. Youssef, C. Song, A.V. Kalueff, G.E. Landreth, T. Malm, L. Tian, Psychosocial stress on neuroinflammation and cognitive dysfunctions in Alzheimer's diseasethe emerging role for microglia? Neurosci. Biobehav. Rev. 77 (2017) 148–164, https://doi.org/10.1016/j.neubiorev.2017.01.046.
[4] A. Association, Alzheimer's disease facts and figures, Alzheimer's Dement. 14 (2018) (2018) 367–429, https://doi.org/10.1016/j.jalz.2018.02.001.
[5] B. Duthey, Background paper 6.11 alzheimer disease and other dementias, update on 2004, World Heal. Organ (2013) 1–77, https://doi.org/10.1038/nprot.2013.155.
[6] U.M. Kocyigit, Y. Budak, M.B. Gürdere, F. Ertürk, B. Yencilek, P. Taslimi, İ. Gülçin, M. Ceylan, Synthesis of chalcone-imide derivatives and investigation of their anticancer and antimicrobial activities, carbonic anhydrase and acetylcholinesterase enzymes inhibition profiles, Arch. Physiol. Biochem. 124 (2018) 61–68, https://doi.org/10.1080/13813455.2017.1360914.
[7] M. Mehta, A. Adem, M. Sabbagh, New acetylcholinesterase inhibitors for alzheimer's disease, Int. J. Alzheimers Dis. (2012) (2012), https://doi.org/10.1155/2012/728983.
[8] M.B. Colovic, D.Z. Krstic, T.D. Lazarevic-Pasti, A.M. Bondzic, V.M. Vasic, Acetylcholinesterase inhibitors: pharmacology and toxicology, Curr. Neuropharmacol. 11 (2013) 315–335, https://doi.org/10.2174/1570159X11311030006.
[9] L.G. Howes, Cardiovascular effects of drugs used to treat Alzheimer's disease, Drug Saf. 37 (2014) 391–395, https://doi.org/10.1007/s40264-014-0161-z.
[10] M.M.J. Van Eijk, K.C.B. Roes, M.L.H. Honing, M.A. Kuiper, A. Karakus, M. Van Der Jagt, P.E. Spronk, W.A. Van Gool, R.C. Van Der Mast, J. Kesecioglu, A.J.C. Slooter, Effect of rivastigmine as an adjunct to usual care with haloperidol on duration of delirium and mortality in critically ill patients: a multicentre, double-blind, placebo-controlled randomised trial, Lancet 376 (2010) 1829–1837, https://doi.org/10.1016/S0140-6736(10)61855-7.
[11] A. Kumar, S. Roy, S. Tripathi, A. Sharma, Molecular docking based virtual screening of natural compounds as potential BACE1 inhibitors: 3D QSAR pharmacophore mapping and molecular dynamics analysis, J. Biomol. Struct. Dyn. 34 (2016) 239–249, https://doi.org/10.1080/07391102.2015.1022603.
[12] D.C. Ferreira Neto, M. de Souza Ferreira, E. da Conceição Petronilho, J. Alencar Lima, S. Oliveira Francisco de Azeredo, J. de Oliveira Carneiro Brum, C. Jorge do Nascimento, J.D. Figueroa Villar, A new guanylhydrazone derivative as a potential acetylcholinesterase inhibitor for Alzheimer's disease: synthesis, molecular docking, biological evaluation and kinetic studies by nuclear magnetic resonance, RSC Adv. 7 (2017) 33944–33952, https://doi.org/10.1039/C7RA04180B.
[13] S. Chigurupati, M. Selvaraj, V. Mani, K.K. Selvarajan, J.I. Mohammad, B. Kaveti, H. Bera, V.R. Palanimuthu, L.K. Teh, M.Z. Salleh, Identification of novel acetylcholinesterase inhibitors: indolopyrazoline derivatives and molecular docking studies, Bioorg. Chem. 67 (2016) 9–17, https://doi.org/10.1016/j.bioorg.2016.05.002.
[14] C. Sarikurkcu, G. Zengin, M. Oskay, S. Uysal, R. Ceylan, A. Aktumsek, Composition, antioxidant, antimicrobial and enzyme inhibition activities of two *Origanum vulgare* subspecies (subsp. Vulgare and subsp. hirtum) essential oils, Ind. Crops Prod. 70 (2015) 178–184, https://doi.org/10.1016/j.indcrop.2015.03.030.
[15] L. Scotti, M.T. Scotti, Silico studies applied to natural products with potential activity against alzheimer's disease, in: K. Roy (Ed.), Comput. Model. Drugs Against Alzheimer's Dis. Springer New York, New York, NY, 2018, pp. 513–531, , https://doi.org/10.1007/978-1-4939-7404-7.
[16] I.E. Orhan, D. Jedrejek, F.S. Senol, R.E. Salmas, S. Durdagi, I. Kowalska, L. Pecio, W. Oleszek, Molecular modeling and in vitro approaches towards cholinesterase inhibitory effect of some natural xanthohumol, naringenin, and acyl phloroglucinol derivatives, Phytomedicine. 42 (2018) 25–33, https://doi.org/10.1016/j.phymed.2018.03.009.
[17] O.A. Ojo, A.B. Ojo, B.O. Ajiboye, O. Olaiya, A. Akawa, O. Olaoye, O.O. Anifowose, O. Idowu, O. Olasehinde, T. Obafemi, J. Awe, B.E. Oyinloye, Inhibitory Effect of *Bryophyllum pinnatum* (Lam.) Oken leaf Extract and their Fractions on α-amylase, α-glucosidase and Cholinesterase Enzyme Oluwafemi, Pharmacogn. J. 10 (2018) 497–506, https://doi.org/10.5530/pj.2018.3.82.
[18] O. Politeo, F. Burcul, I. Blazevic, M. Radan, Phenylpropanoids Terpenes, Sulfur and other essential oil constituents as inhibitors of Cholinesterases, Curr. Med. Chem. 25 (2018) 13–14, https://doi.org/10.2174/0929867325666180330092607.
[19] E. Arranz, L. Jaime, M.C.L. De, G. Reglero, S. Santoyo, Supercritical fluid extraction as an alternative process to obtain essential oils with anti-inflammatory properties from marjoram and sweet basil, Ind. Crop. Prod. 67 (2015) 121–129, https://doi.org/10.1016/j.indcrop.2015.01.012.
[20] N.J. Reddy, D. Nagoor Vali, M. Rani, S.S. Rani, Evaluation of antioxidant, antibacterial and cytotoxic effects of green synthesized silver nanoparticles by Piper longum fruit, Mater. Sci. Eng. C. 34 (2014) 115–122, https://doi.org/10.1016/j.msec.2013.08.039.
[21] B. Uttara, A.V. Singh, P. Zamboni, R.T. Mahajan, Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options, Curr. Neuropharmacol. 7 (2009) 65–74, https://doi.org/10.2174/157015909787602823.
[22] E.E. Mgbeahuruike, T. Yrjönen, H. Vuorela, Y. Holm, Bioactive compounds from medicinal plants: focus on Piper species, S. Afr. J. Bot. 112 (2017) 54–69, https://doi.org/10.1016/j.sajb.2017.05.007.
[23] J.K.R. da Silva, L.C. Pinto, R.M.R. Burbano, R.C. Montenegro, E.H.A. Andrade, J.G.S. Maia, Composition and cytotoxic and antioxidant activities of the oil of *Piper aequale* Vahl, Lipids Health Dis. 15 (2016) 174, https://doi.org/10.1186/s12944-016-0347-8.
[24] G.A. Linde, N.B. Colauto, E. Alberto, Z. Gazim, Quimiotipos, extracción, composición y aplicaciones del aceite esencial de Lippia alba, Rev. Bras. Pl. Med. 18 (2016) 191–200, https://doi.org/10.1590/1983-084X/15.
[25] P.K. Mukherjee, V. Kumar, M. Mal, P.J. Houghton, In vitro acetylcholinesterase inhibitory activity of the essential oil from Acorus calamus and its main constituents, Planta Med. 73 (2007) 283–285, https://doi.org/10.1055/s-2007-967114.
[26] P. Tongnuanchan, S. Benjakul, Essential oils: extraction, bioactivities, and their uses for food preservation, J. Food Sci. 79 (2014) 1231–1249, https://doi.org/10.1111/1750-3841.12492.
[27] M.A. Elgndi, S. Filip, B. Pavlić, J. Vladić, T. Stanojković, Ž. Žižak, Z. Zeković, Antioxidative and cytotoxic activity of essential oils and extracts of Satureja montana L., *Coriandrum sativum* L. And *Ocimum basilicum* L. Obtained by supercritical fluid extraction, J. Supercrit. Fluids 128 (2017) 128–137, https://doi.org/10.1016/j.supflu.2017.05.025.
[28] O. Zenebon, N.S. Pascuet, P. Tiglea, Instituto Adolfo Lutz - métodos físico-químicos para Análise de Alimentos - 4ª edição 1ª Edição Digital, 4°, São Paulo SP-Brazil (2008), http://www.ial.sp.gov.br/resources/editorinplace/ial/2016_3_19/analisedealimentosial_2008.pdf.
[29] K.A. da F. Rodrigues, L.V. Amorim, J.M.G. de Oliveira, C.N. Dias, D.F.C. Moraes, E.H. de A. Andrade, J.G.S. Maia, S.M.P. Carneiro, F.A. de A. Carvalho, Eugenia uniflora L. Essential oil as a potential Anti- leishmania agent: effects on leishmania amazonensis and possible mechanisms of action, Evid. Complement. Alternat. Med. (2013) (2013) 279726, , https://doi.org/10.1155/2013/279726.
[30] E.W. Lemmon, M.O. McLinden, D.G. Friend, Thermophysical properties of fluid systems" in NIST chemistry webbook; NIST standard reference database No. 69, Gaithersburg MD (2017), https://doi.org/10.18434/T4D303.
[31] R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. 4th ed. Carol Stream, IL, USA: Allured Publishing Corporation; (2007), 4th ed., Allured Pub Corp; 4th edition (February 28, 2007), Carol Stream, 2007.
[32] Stein;, D. Mirokhin, T. D;, M. Mallard, G, Z. A., D. V. and Sparkmanm, The NIST mass spectral search program for the nist/epa/nih mass spectra library. Standard Reference Data Program of the National Institute of Standards and Technology. Gaithers-burg, MD, US, (2011). http://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:start.
[33] M.Y. Dar, W.A. Shah, M.A. Rather, Y. Qurishi, A. Hamid, M.A. Qurishi, Chemical composition, in vitro cytotoxic and antioxidant activities of the essential oil and major constituents of *Cymbopogon jawarancusa* (Kashmir), Food Chem. 129 (2011) 1606–1611, https://doi.org/10.1016/j.foodchem.2011.06.016.
[34] C. Sánchez-Moreno, J.a. Larrauri, F. Saura-calixto, A procedure to measure the antiradical efficienc y of polyphenols, J. Sci. Food Agric. 270 (1998) 270–276, https://doi.org/10.1002/(SICI)1097-0010(199802)76:2<270::AID-JSFA945>3.0.CO;2-9.
[35] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. 26 (1999) 1231–1237, https://doi.org/10.1016/S0891-5849(98)00315-3.
[36] N. Erkan, G. Ayranci, E. Ayranci, Antioxidant activities of rosemary (*Rosmarinus officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol, Food Chem. 110 (2008) 76–82, https://doi.org/10.1016/j.foodchem.2008.01.058.
[37] A. Marston, J. Kissling, K. Hostettmann, A rapid TLC bioautographic method for the

detection of acetylcholinesterase and butyrylcholinesterase inhibitors in plants, Phytochem. Anal. 13 (2002) 51–54, https://doi.org/10.1002/pca.623.
[38] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery, J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, D.J. Gaussian, J. Bloino, Y. Honda, K.N. Kudin, R.E. Stratmann, G.A. Voth, Gaussian 09 (2009) 2–3.
[39] A.D. Becke, Density-functional thermochemistry. III. The role of exact exchange, J. Chem. Phys. 98 (1993) 5648–5652, https://doi.org/10.1063/1.464913.
[40] R. Thomsen, M.H. Christensen, MolDock: a new technique for high accuracy molecular docking, J. Med. Chem. 49 (2006) 3315–3321.
[41] J. Cheung, E.N. Gary, K. Shiomi, T.L. Rosenberry, Structures of human acetylcholinesterase bound to dihydrotanshinone i and territrem B show peripheral site flexibility, ACS med, Chem. Lett. 4 (2013) 1091–1096, https://doi.org/10.1021/ml400304w.
[42] C.I. Bayly, P. Cieplak, W.D. Cornell, P.A. Kollman, A well-behaved electrostatic potential based method using charge restraints for deriving atomic charges: the RESP model, J. Phys. Chem. 97 (1993) 10269–10280, https://doi.org/10.1021/j100142a004.
[43] J. Wang, P. Cieplak, P.A. Kollman, How well does a restrained electrostatic potential (RESP) model perform in calculating conformational energies of organic and biological molecules? J. Comput. Chem. 21 (2000) 1049–1074, https://doi.org/10.1002/1096-987X(200009)21 12 < 1049::AID-JCC3 > 3.0.CO;2-F.
[44] J. Wang, W. Wang, P.A. Kollman, D.A. Case, Automatic atom type and bond type perception in molecular mechanical calculations, J. Mol. Graph. Model. 25 (2006) 247–260, https://doi.org/10.1016/j.jmgm.2005.12.005.
[45] J. Wang, R.M. Wolf, J.W. Caldwell, P.A. Kollman, D.A. Case, Development and testing of a general amber force field, J. Comput. Chem. 25 (2004) 1157–1174, https://doi.org/10.1002/jcc.20035.
[46] D.A. Case, T.E. Cheatham, T. Darden, H. Gohlke, R. Luo, K.M. Merz, A. Onufriev, C. Simmerling, B. Wang, R.J. Woods, The Amber biomolecular simulation programs, J. Comput. Chem. 26 (2005) 1668–1688, https://doi.org/10.1002/jcc.20290.
[47] J.N. Cruz, J.F.S. Costa, A.S. Khayat, K. Kuca, C.A.L. Barros, A.M.J.C. Neto, Molecular dynamics simulation and binding free energy studies of novel leads belonging to the benzofuran class inhibitors of Mycobacterium tuberculosis Polyketide Synthase 13, J. Biomol. Struct. Dyn. 1102 (2018) 1–12, https://doi.org/10.1080/07391102.2018.1462734.
[48] T.J. Dolinsky, J.E. Nielsen, J.A. McCammon, N.A. Baker, PDB2PQR: an automated pipeline for the setup of Poisson-Boltzmann electrostatics calculations, Nucleic Acids Res. 32 (2004) 665–667, https://doi.org/10.1093/nar/gkh381.
[49] J.A. Maier, C. Martinez, K. Kasavajhala, L. Wickstrom, K.E. Hauser, C. Simmerling, ff14SB: improving the accuracy of protein side chain and backbone parameters from ff99SB, J. Chem. Theory Comput. 11 (2015) 3696–3713, https://doi.org/10.1021/acs.jctc.5b00255.
[50] W.L. Jorgensen, J. Chandrasekhar, J.D. Madura, R.W. Impey, M.L. Klein, Comparison of simple potential functions for simulating liquid water, J. Chem. Phys. 79 (1983) 926–935, https://doi.org/10.1063/1.445869.
[51] T. Darden, D. York, L. Pedersen, Particle mesh Ewald: an N ·log(N) method for Ewald sums in large systems, J. Chem. Phys. 98 (1993) 10089–10092, https://doi.org/10.1063/1.464397.
[52] J.P. Ryckaert, G. Ciccotti, H.J.C. Berendsen, Numerical integration of the cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes, J. Comput. Phys. 23 (1977) 327–341, https://doi.org/10.1016/0021-9991(77)90098-5.
[53] J.A. Izaguirre, D.P. Catarello, J.M. Wozniak, R.D. Skeel, Langevin stabilization of molecular dynamics, J. Chem. Phys. 114 (2001) 2090–2098, https://doi.org/10.1063/1.1332996.
[54] W. Humphrey, A. Dalke, K. Schulten, VMD: visual molecular dynamics, J. Mol. Graph. 14 (1996) 33–38, https://doi.org/10.1016/0263-7855(96)00018-5.
[55] E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF Chimera - A visualization system for exploratory research and analysis, J. Comput. Chem. 25 (2004) 1605–1612, https://doi.org/10.1002/jcc.20084.
[56] T. Hou, J. Wang, Y. Li, W. Wang, Assessing the performance of the MM / PBSA and MM / GBSA methods. I. The accuracy of binding free energy calculations based on molecular dynamics simulations, J. Chem. Inf. Model. 51 (2011) 69–82, https://doi.org/10.1021/ci100275a.
[57] H. Gohlke, C. Kiel, D.A. Case, Insights into protein-protein binding by binding free energy calculation and free energy decomposition for the Ras-Raf and Ras-RalGDS complexes, J. Mol. Biol. 330 (2003) 891–913, https://doi.org/10.1016/S0022-2836(03)00610-7.
[58] J. da Silva, J. Silva, S. Nascimento, S. da Luz, E. Meireles, C. Alves, A. Ramos, J. Maia, Antifungal activity and computational study of constituents from *Piper divaricatum* essential oil against fusarium infection in black pepper, Molecules 19 (2014) 17926–17942, https://doi.org/10.3390/molecules191117926.
[59] A.F. Ribeiro, E.H.A. Andrade, F.R.G. Salimena, J.G.S. Maia, Circadian and seasonal study of the cinnamate chemotype from *Lippia origanoides* Kunth, Biochem. Syst. Ecol. 55 (2014) 249–259, https://doi.org/10.1016/j.bse.2014.03.014.
[60] K. Nakahara, N.S. Alzoreky, T. Yoshihashi, H.T.T. Nguyen, G. Trakoontivakorn, Chemical composition and antifungal activity of essential oil from *Cymbopogon nardus* (Citronella grass), Japan Agric. Res. Q. JARQ 37 (2013) 249–252, https://doi.org/10.6090/jarq.37.249.
[61] A. Zermane, O. Larkeche, A.H. Meniai, C. Crampon, E. Badens, Optimization of essential oil supercritical extraction from Algerian *Myrtus communis* L. Leaves using response surface methodology, J. Supercrit. Fluids 85 (2014) 89–94, https://doi.org/10.1016/j.supflu.2013.11.002.
[62] R.N.P. Souto, A.Y.H. Arada, E.H.A.A. Ndrade, J.G.S.M. Aia, Insecticidal activity of piper essential oils from the Amazon against the fire ant *Solenopsis saevissima* (Smith) (Hymenoptera: Formicidae), Neotrop. Entomol. 41 (2012) 510–517, https://doi.org/10.1007/s13744-012-0080-6.
[63] E. Conde, A. Moure, H. Domínguez, Supercritical CO2 extraction of fatty acids, phenolics and fucoxanthin from freeze-dried *Sargassum muticum*, J. Appl. Phycol. 27 (2015) 957–964, https://doi.org/10.1007/s10811-014-0389-0.
[64] S.I. Abdelwahab, A.A. Mariod, M.M.E. Taha, F.Q. Zaman, A.H.A. Abdelmageed, S. Khamis, Y. Sivasothy, K. Awang, Chemical composition and antioxidant properties of the essential oil of *Cinnamomum altissimum* Kosterm. (Lauraceae), Arab. J. Chem. 10 (2017) 131–135, https://doi.org/10.1016/j.arabjc.2014.02.001.
[65] J.K.R. da Silva, L.C. Pinto, R.M.R. Burbano, R.C. Montenegro, E.F. Guimarães, E.H.A. Andrade, J.G.S. Maia, Essential oils of Amazon Piper species and their cytotoxic, antifungal, antioxidant and anti-cholinesterase activities, Ind. Crops Prod. 58 (2014) 55–60, https://doi.org/10.1016/j.indcrop.2014.04.006.
[66] D. Tasdemir, A.A. Dönmez, I. Çalis, P. Rüedi, Evaluation of biological activity of Turkish plants. Rapid screening for the antimicrobial, antioxidant, and acetylcholinesterase inhibitory potential by TLC bioautographic methods, Pharm. Biol. 42 (2004) 374–383, https://doi.org/10.1080/13880200490519695.
[67] S. Dohi, M. Terasaki, M. Makino, Acetylcholinesterase inhibitory activity and chemical composition of commercial essential oils, J. Agric. Food Chem. 57 (2009) 4313–4318, https://doi.org/10.1021/jf804013j.
[68] H.J. Yeom, J. Kang, S.W. Kim, I.K. Park, Fumigant and contact toxicity of Myrtaceae plant essential oils and blends of their constituents against adults of German cockroach (*Blattella germanica*) and their acetylcholinesterase inhibitory activity, Pestic. Biochem. Physiol. 107 (2013) 200–206, https://doi.org/10.1016/j.pestbp.2013.07.003.
[69] I. Labed-Zouad, M. Ferhat, M. Öztürk, I. Abaza, S. Nadeem, A. Kabouche, Z. Kabouche, Essential oils composition, anticholinesterase and antioxidant activities of pistacia atlantica Desf, Rec. Nat. Prod. 11 (2017) 411–415.
[70] Y. Xu, J.P. Colletier, M. Weik, H. Jiang, J. Moult, I. Silman, J.L. Sussman, Flexibility of aromatic residues in the active-site gorge of acetylcholinesterase: X-ray versus molecular dynamics, Biophys. J. 95 (2008) 2500–2511, https://doi.org/10.1529/biophysj.108.129601.
[71] A. Ordentlich, D. Barak, C. Kronman, Y. Flashner, M. Leitner, Y. Segall, N. Ariel, S. Cohen, B. Velan, A. Shafferman, Dissection of the human acetylcholinesterase active center determinants of substrate specificity. Identification of residues constituting the anionic site, the hydrophobic site, and the acyl pocket, J. Biol. Chem. 268 (1993) 17083–17095.
[72] E.H. Rydberg, B. Brumshtein, H.M. Greenblatt, D.M. Wong, D. Shaya, L.D. Williams, P.R. Carlier, Y.P. Pang, I. Silman, J.L. Sussman, Complexes of Alkylene-linked tacrine dimers with Torpedo californica acetylcholinesterase: binding of bis(5)-tacrine produces a dramatic rearrangement in the active-site gorge, J. Med. Chem. 49 (2006) 5491–5500, https://doi.org/10.1021/jm060164b.
[73] S. Lu, J.W. Wu, H.-L. Liu, J. Zhao, K. Liu, C. Chuang, H. Lin, W. Tsai, Y. Ho, The discovery of potential acetylcholinesterase inhibitors: a combination of pharmacophore modeling, virtual screening, and molecular docking studies, J. Biomed. Sci. 18 (8) (2011), https://doi.org/10.1186/1423-0127-18-8.
[74] C.H.T.P. da Silva, V.L. Campo, I. Carvalho, C.A. Taft, Molecular modeling, docking and ADMET studies applied to the design of a novel hybrid for treatment of Alzheimer's disease, J. Mol. Graph. Model. 25 (2006) 169–175, https://doi.org/10.1016/j.jmgm.2005.12.002.

# 7 CAPÍTULO VI.

## 7.1 Antimicrobial, Cytotoxic activity of the *Syzygium aromaticum* essential oil, molecular docking and dynamics molecular studies of its major chemical constituent

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# Antimicrobial, Cytotoxic Activity of the *Syzygium aromaticum* Essential Oil, Molecular Docking and Dynamics Molecular Studies of Its Major Chemical Constituent

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The objective of the present work was to analyze the cytotoxic, antimicrobial activity and the action mechanism of the major component in of the *Syzygium aromaticum* essential oil obtained by supercritical $CO_2$. In this work, gingival fibroblasts were exposed to the essential oil in different concentrations for one hour: 5 $\mu$L/ml, 7.5 $\mu$L/ml and 10 $\mu$L/ml. Culture medium was used as control. Cytotoxicity analysis was performed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT®) method. The susceptibility was evaluated on three microorganisms *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus*. The statistical analyses showed significant difference in cell viability for the concentration of 10 $\mu$L/mL, as compared to the control group. As a result, the plant extract had no cytotoxicity at concentrations below 10 $\mu$L/mL in human gingival fibroblasts. The interaction mode of eugenol, the major compound and main component responsible for the biological activity of the essential oil was evaluated. The molecular docking of eugenol with important proteins of the metabolic pathway of the microorganisms *C. albicans*, *E. coli* and *S. aureus* were performed. The results demonstrated that the compound is capable of interacting with catalytic residues of the enzymes and forming an energetically favorable system with such proteins. The results of binding free energy obtained demonstrate this capacity. For the eugenol-N-myristoyltransferase (*C. albicans*) system, the value of $\Delta G_{bind}$ was −19.01 kcal/mol, for Enoyl reductase (*E. Coli)* $\Delta G_{bind}$ was equal to −11.31 kcal/mol and for SarA (*S. aureus*) $\Delta G_{bind}$ was −13.58 kcal/mol.

**Keywords:** Natural Product, Clove Oil, Biological Activity, Simulation.

## 1. INTRODUCTION

*Syzygium aromaticum,* also known as clove, is recognised for its medicinal properties [1]. Clove has anti-bacterial, antifungal, anti-inflammatory, antinociceptive, antipyretic activities and anti-cancer activities, as well as a very important anti-fungal action [2–5]. However, for oil to be a safe alternative, viability analysis should be performed.

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Viability tests have been used and accepted as a method for evaluation of biocompatibility, allowing the observation of cellular behaviour in a controlled environment. These tests are generally based on the use of vital stain, and on the direct or indirect evaluation of the metabolic activity of the cell line, mainly in fibroblasts [6–8].

Clove essential oil is a complex mixture of volatile and lipophilic substances, and has an oily consistency the major component is eugenol or 4-allyl-2-methoxyphenol [9]. In addition, $\beta$-caryophyllene and eugenol acetate are other components present at considerable ratios [10]. In general, the extraction of essential oils is carried out by hydrodistillation, however, more modern extraction techniques such as supercritical $CO_2$ have been shown to be advantageous, mainly because it is not selective and does not use high temperatures during the extraction process, this avoids degradation thermal decomposition of secondary metabolites that may be present in the essential oil. Furthermore, there is no reaction between supercritical $CO_2 +$ oil, which does not cause changes in the chemical profile [11].

Experimental studies of the antimicrobial activity are very important, as well as understanding the mechanisms of interaction among chemically active substances with proteins that can inhibit the development and consequently the proliferation of microorganisms. Hence, doping and molecular dynamics are tools that can aid the experimental studies, because they can predict the mechanisms of action of new drugs in specific active sites. In this sense, this work aimed to evaluate the cytotoxicity, susceptibility of clove essential oils and show the mechanisms of interaction between eugenol and N proteins-myristoyltransferase/*C. Albicans*, Enoyl reductase/*E. Coli* and SarA/*S. aureus*.

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## 2. MATERIALS AND METHODS

### 2.1. Supercritical $CO_2$ (SC-$CO_2$) Extraction e Análise De Compostos Voláteis

SFE was performed using a Spe-ed™ SFE system (model 7071, Applied Separations, Allentown, PA, USA) and the characterization of the volatile compounds were performed as previously described by our research group [12] under experimental conditions of 40 °C and 50 °C and pressure of 100 bar, 200 bar and 300 bar.

### 2.2. Antimicrobial Susceptibility Assays

Sensitivity test was performed using the essential oil (EO) obtained with supercritical $CO_2$ in the experimental condition of $EO_1$—40 °C/100 bar, $OE_2$—40 °C/200 bar, $EO_3$—40 °C/300 bar, $EO_4$—50 °C/100 bar, $EO_5$—50 °C/200 bar, $EO_6$—50 °C/300 bar. The essential oils were evaluated by means of the disk-diffusion method in CLSI solid medium, with modifications [13].

The microorganisms were obtained from the coleção de microrganismos de referência em Vigilância Sanitária-CRMVS, INCQS-FIOCRUZ, Rio de Janeiro, RJ. Four strains were used as test microorganisms: *C. albicans INCQS-40175*, *S. aureus INCQS-00015* and *E. coli ATCC-25911*. The cultures were maintained in their appropriate agar slants at 37 °C throughout the study.

The microorganisms were grown for 24–48 h at 30 °C in Müeller-Hinton agar (MHA) or Sabouraud agar (SDA) for bacteria and yeast, respectively. The microorganisms were then suspended in a sterile saline solution (McFarland turbidity scale 2). With the aid of a swab, the suspension containing the microorganisms was streaked onto a Müller Hinton agar plate and the discs and paper (6 mm) were added. Then the paper discs were impregnated with the pure essential oils with 30 $\mu$L (oil/plate). Plates were incubated at 37 °C for 24 h and 48 h. After this period, the inhibition halos were checked. As an antifungal drug, Nystatin was used at concentration of 50 $\mu$g/mL. All plates were sealed with plastic film to prevent evaporation of the test sample. Isolates that presented inhibition halos with diameter $<8$ mm were considered resistant $\geq 8$ mm as sensitive. All the tests were performed in duplicate and the results were obtained by the average of the halos found.

### 2.3. Cell Culture

Human gingival fibroblasts, obtained from a primary culture using the tissue explant technique, were grown in 25 $cm^2$ flasks with DMEM/F-12 medium (Sigma Chemical Co., USA) supplemented with 10% bovine foetal serum (FBS-Gibco, USA), and were maintained at 37 °C, in a humid atmosphere with 5% $CO_2$. At the confluence of the cell monolayer, subcultures were performed to obtain a sufficient number of cells to perform the cytotoxicity tests.

### 2.4. *In Vitro* Exposure to Clove Oil

For cytotoxicity analysis, 3000 cells/well were seeded in 96-well culture plates, and were maintained in a $CO_2$ incubator for cell adhesion and proliferation, for 48 h. After this period, the cells were exposed to the plant extract at different concentrations: 5 $\mu$L/ml, 7.5 $\mu$L/mL and 10 $\mu$L/mL. Culture medium was used as control. The concentrations were determined by a pilot test that evaluated the possibility of dilution of the oil. The concentration of 10 $\mu$L/mL was the maximum concentration that allowed the dilution. As solvent, TWEEN® 80 (Sigma®) was used in the 1% concentration in the final solution. The exposure time was 1h, according to Prashar et al. [14], at 37 °C and with 5% $CO_2$.

### 2.5. Cell Viability

After treatment, the cytotoxic effect was quantified using a colorimetric MTT assay, which measures mitochondrial activity in viable cells. Briefly, MTT (dilution at 5 mg/ml in culture medium) was pipetted into each well, and the plate was incubated at 37 °C. After 4 h, the medium was removed, and DMSO (200 $\mu$l) was added to each well.

The optical density of each well was measured at 595 nm using a spectrophotometer.

### 2.6. Molecular Docking

The molecular structure of eugenol was designed with the software GaussView 5.5 and optimized in the software Gaussian 09 [15], using the Density Functional Theory (DFT) with B3LYP functional and 6-31G* basis [16, 17].

The proteins used as receptors can be located in the Protein Data Bank (PDB) from the IDs: 1IYL (N-myristoyltransferase/*C. Albicans*) [18], 1C14 (Enoyl reductase/*E. Coli*) [19] and 2FNP (SarA/*S. aureus*) [20].

In order to analyze the interaction mode of eugenol with the receptors, the software Molegro Virtual Docker 5.5 (MVD) was used [21], and its parameters used to predict the interaction mode were: (a) the MolDock scoring function (GRID) with a grid resolution of 0.30 Å and radius of 6 Å encompassing the entire connection cavity; (b) The MolDock SE algorithm with maximum number of 15 runs, 1500 interactions and total population of 50 was applied with optimized H-Bonds.

### 2.7. Molecular Dynamics Simulation

Protein amino acid protonation was studied from the results obtained with the PDB2PQR server [22]. To obtain the atomic charges of the ligand, the restrained electrostatic potential (RESP) protocol was applied with HF/6-31G* [23, 24]. The parameters for the binder were constructed with the Antechamber module of the Amber 16 package [25, 26].

In all simulations, the General Amber Force Field (GAFF) [27] and ff14SB [28] were used. After the solvation of the systems in an octahedron periodic box, with water molecules described by the model TIP3P [29], counter-ions were added to neutralize the system.

Before the simulations of MD production, steps were taken to minimize energy, heat and balance the system. 1000 cycles were performed using steepest descent method and conjugate gradient algorithm and the solute was restricted with a harmonic force constant of 100 kcal/mol $\cdot$ Å$^{-2}$. In the second step the harmonic force constant was reduced to 50 kcal/mol $\cdot$ Å$^{-2}$ and additional 500 cycles were performed using steepest descent method and conjugate gradient algorithm. In the last stage of minimization, the constraints were removed and other 500 cycles were applied using the same protocol.

Then, the systems were heated from 0 to 300 K. The total time for heating was 800 ps divided into four steps. In the first three steps, a harmonic force constant of 50 kcal/mol $\cdot$ Å$^{-2}$ was applied on the solute leaving the water molecules and counter ions with freedom of movement. In the last step, the harmonic force constant was reduced to zero leaving all the components of the systems with freedom to move.

Molecular dynamics (MD) simulations of 2 ns with a temperature of 300 K and without any restriction were performed to balance the systems. For each system, 100 ns of MD simulations of production were generated. The Particle Mesh Ewald method [30] was used for the calculation of the electrostatic interactions, and the bonds involving hydrogen atoms were restricted with the SHAKE algorithm [31]. The temperature control was performed with the Langevin thermostat [32] within collision frequency of 2 ps$^{-1}$.

### 2.8. Free Energies Calculations Using MM/GBSA Approaches

For the binding affinity calculation, 1000 snapshots of the MD last 5 ns trajectory were used [33, 34]. The binding free energy was computed as the difference:

$$\Delta G_{\text{bind}} = G_{\text{complex}} - G_{\text{protein}} - G_{\text{ligand}} \quad (1)$$

$$\Delta G_{\text{bind}} = \Delta H - T\Delta S \approx \Delta E_{MM} + \Delta G_{\text{solv}} - T\Delta S \quad (2)$$

$$\Delta E_{\text{MM}} = \Delta E_{\text{internal}} + \Delta E_{\text{eletrotatic}} + \Delta E_{\text{vdW}} \quad (3)$$

$$\Delta G_{\text{solv}} = \Delta G_{\text{GB}} + \Delta G_{\text{nonpol}} \quad (4)$$

In which ($\Delta G_{\text{bind}}$) is the free energy of the protein–ligand binding, resulting from the sum of the molecular mechanics energy ($\Delta E_{\text{MM}}$), the desolvation free energy ($\Delta G_{\text{solv}}$), and the entropy ($-T\Delta S$). The energy of molecular gas phase mechanics ($\Delta E_{\text{MM}}$) can be described by the sum of the internal energy contributions ($\Delta E_{\text{internal}}$), the sum of the connection, angle and dihedral energies), electrostatic contributions ($\Delta E_{\text{eletrotatic}}$) and van der Waals terms ($\Delta E_{\text{vdW}}$). Desolvation free energy ($\Delta G_{\text{solv}}$) is the sum of the polar ($\Delta G_{\text{GB}}$) and non-polar ($\Delta G_{\text{nonpol}}$) contributions. The polar desolvation term was calculated using the implicit generalized born (*GB*) approaches.

### 2.9. Statistical Analysis

One-way analysis of variance (ANOVA) was used for comparisons among multiple groups, before which homogeneity of variance was performed. The statistical analyses were performed using GraphPad Prism 5, and the criterion for statistical significance was $p < 0.05$.

## 3. RESULTS AND DISCUSSION

### 3.1. Yield and Chemical Composition

The mass yields and chemical composition can be observed in our previously published article [12] the results show that eugenol was the main compound identified in the essential oil, ranging from 57.12%–62.88%.

### 3.2. Antimicrobial Activity

It was observed that the microorganisms are sensitive to the compounds present in the essential oils, regardless of the temperature and pressure used to obtain the essential

**Table I.** Action of *S. aromaticum* essential oils and Eugenol pattern on *C. albicans*, *E. coli* and *S. aureu* pathogens with inhibition halos in millimeters (mm).

| | *C. albicans* INCQS–40175 | *E. coli* ATCC–25911 | *S. aureus* INCQS–00015 |
|---|---|---|---|
| Essential oils and Eugenol | Diameter of the halo (mm) | Diameter of the halo (mm) | Diameter of the halo (mm) |
| OE 1 | 17 | 17 | 13 |
| OE 2 | 21 | 18 | 15 |
| OE 3 | 21 | 20 | 15 |
| OE 4 | 21 | 20 | 17 |
| OE 5 | 23 | 20 | 17 |
| OE 6 | 25 | 22 | 17 |
| Eugenol | 36 | 20 | 20 |

*Notes*: EO** Essential oil; Extraction conditions of temperature and pressure, EO 1—40 °C/100 bar, OE 2—40 °C/200 bar, EO 3—40 °C/300 bar, EO 4—50 °C/100 bar, EO 5—50 °C/200 bar, EO 6—50 °C/300 bar.

oils. Inhibition halos ranging from 17 to 25 mm for yeast and 13 to 22 mm for *E. coli* and *S. aureus* bacteria (Table I, Figs. 1–3) were observed. However, the highest inhibition halos were observed for essential oil $EO_6$ (50 °C/300bar). By analyzing Table I and Figure 4, it can be observed that the microorganisms were sensitive to the presence of Eugenol alone with inhibition halo ranging from 20 mm for *E. coli*, and *S. aureus* and 36 mm for *C. albicans*. Thus, *C. albicans* has been shown to be the most sensitive pathogen present in clove essential oils. The present work demonstrates that the compound responsible for inhibiting the growth of microorganisms may be related to the presence of Eugenol.

The studies that report the antimicrobial activity with clove essential oils obtained with $CO_2$ use are still scarce, for example, the work of [35] was one of the first reports on the evaluation of the antibacterial activity of clove essential oils obtained through supercritical $CO_2$.

Other literature has reported the antimicrobial activity of Eugenol as being a good substitute for the control of various pathogens [36–41]. Studies with eugenol demonstrate that it has antimicrobial activity at low concentrations, with minimal inhibitory concentrations (MIC) ranging from 0.1 to 1 $\mu$l/ml for microorganisms of the oral cavity such as *C. albicans, S. aureus, E. coli* and *E. fecalis* [42]. These concentrations are smaller than those tested in the cytotoxicity analysis in the current study.

### 3.3. Cell Viability

Numerical values obtained by ELISA reader analysis for three aliquots of each sample were used to determine average mitochondrial activity (cell viability). Statistical analysis showed a significant difference between the control group and the clove oil at a concentration of 10 $\mu$L/mL Figure 5. No other statistically significant differences were observed.

The essential oil of *S. aromaticum* obtained by the $CO_2$-SC method showed promising results regarding cell viability when in contact with human gingival fibroblasts. It presented favorable composition regarding the proportion of the components to which the therapeutic properties are attributed. Therefore, it is a candidate for treatment of fungal infections in the oral cavity. The eugenol was the compound that presented the highest concentration and eugenol acetate was the second most common component found in clove essential oil [12]. This compounds has anti-inflammatory actions [43]. A previous study conducted in rats investigated the treatment of hepatic lesions associated with oxidative stress [44]. Moreover, this compound has analgesic actions, which appear to be mediated by the same mechanism described above [45]. Antimicrobial activity of this compound has also been demonstrated in a study of the control of dental plaque-forming bacteria in dogs [46–49]. In terms of the biocompatibility of this

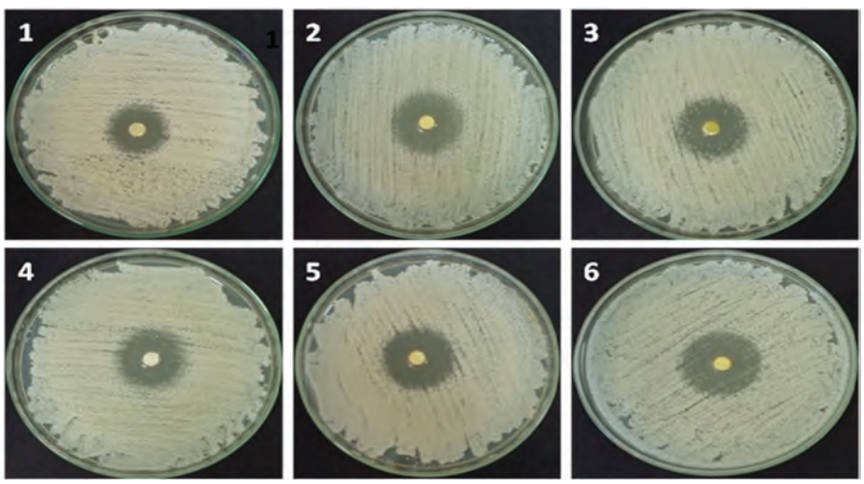


**Fig. 1.** Action of clove essential oils (*S. aromaticum*) obtained by $CO_2$ in the supercritical state under different conditions of temperature and pressure, on yeast of *C. albicans*. EO1, 100 bar/40 °C; EO2, 200 bar/40 °C; EO3, 300 bas/40 °C; EO4, 100 bar/50 °C; EO5, 200 bar/50 °C; EO6, 300 bar/50 °C.

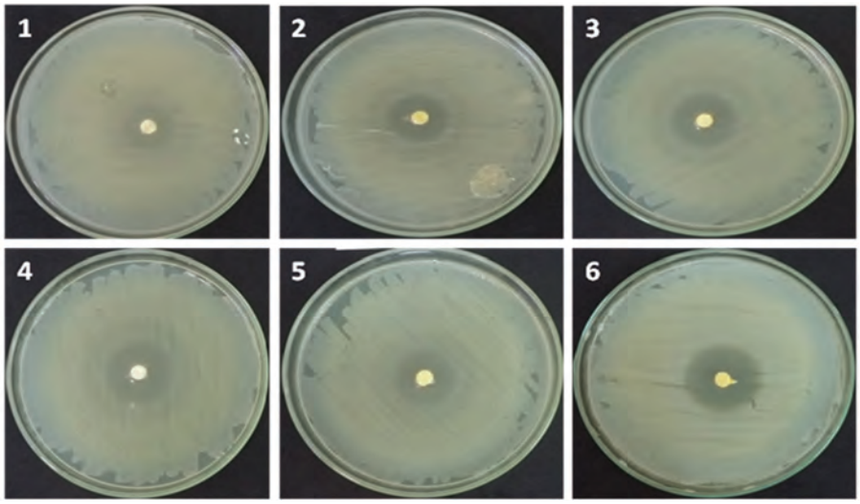


**Fig. 2.** Effect of clove essential oils (S. aromaticum) obtained by $CO_2$ in the supercritical state under different temperature and pressure conditions on cherichia coli bacteria. EO1, 100 bar/40 °C; EO2, 200 bar/40 °C; EO3, 300 bas/40 °C; EO4, 100 bar/50 °C; EO5, 200 bar/50 °C; EO6, 300 bar/50 °C.

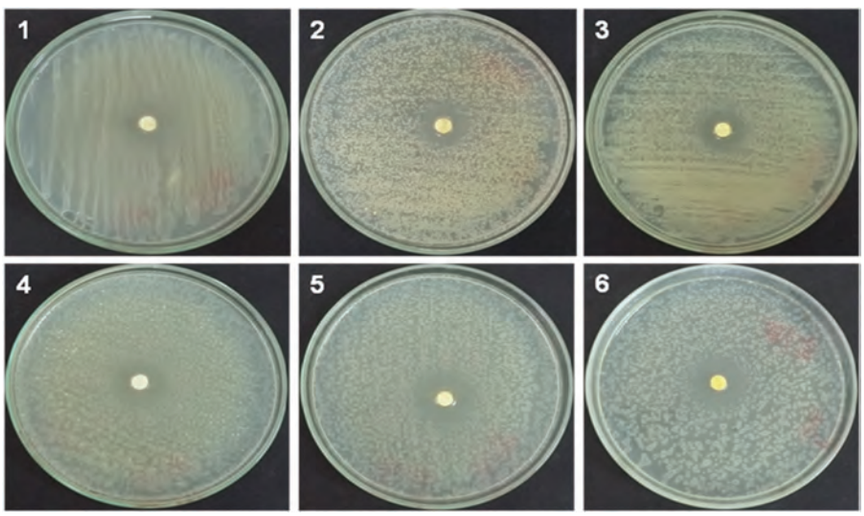


**Fig. 3.** The action of clove essential oils (*S. aromaticum*) obtained by $CO_2$ in the supercritical state under different conditions of temperature and pressure on the Staphylococcus aureus bacteria. EO1, 100 bar/40 °C; EO2, 200 bar/40 °C; EO3, 300 bas/40 °C; EO4, 100 bar/50 °C; EO5, 200 bar/50 °C; EO6, 300 bar/50 °C (Fonte: Autor).

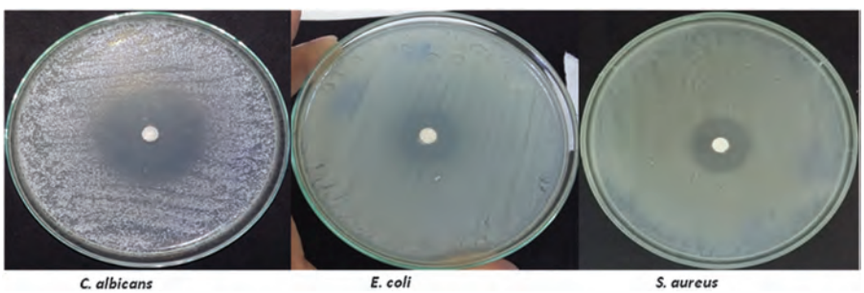


**Fig. 4.** Action of the secondary metabolite eugenol on fungal and bacterial pathogens.

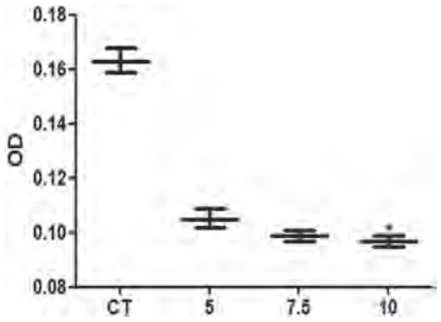


**Fig. 5.** Cell viability of gingival fibroblasts exposed to different concentrations of clove essential oil. OD, optic density. *, $p < 0.05$ compared to the control group.

compound, a toxicological study reported no significant manifestations [50, 51].

In relation to the importance of the extraction method used, the analysis of the chemical composition of the oil using the $CO_2$-SC method resulted in the absence of residues that could influence the results of antimicrobial activity analyses. This is particularly important given the very sensitive method used for evaluating cytotoxicity. Thus, this extraction process is suitable for research with plant extracts.

In this context, $CO_2$-SC has been widely used as a method for obtaining clean essential oils from various plant species that have medicinal properties [52, 53], obtained the essential extract of *Tenacetum vulgare* by using the $CO_2$-SC process, rich in phenylpropanoids and also observed antifungal activity of this extract. Given the range of properties that clove essential oil possesses, and the results of the current study, animal biocompatibility studies are the next step in the development of a safe therapeutic compound.

### 3.4. Molecular Interactions Modes Between Ligand-Receptor

In the molecular docking results, it was observed that the ligand interacted favorably with the binding site

**Table II.** Molecular docking results.

| Protein/Microorganism | MolDock | Rerank score | Interaction | $H_{bond}$ | LE1 | LE3 | Docking score |
|---|---|---|---|---|---|---|---|
| N-myristoyltransferase (*C. Albicans*) | −62.30 | −50.62 | −71.44 | −2.06 | −5.19 | −4.21 | −62.82 |
| Enoyl reductase (*E. Coli*) | −73.88 | −63.176 | −81.33 | −2.51 | −6.15 | −5.26 | −75.31 |
| SarA (*S. aureus*) | −75.56 | −45.89 | −85.46 | −2.55 | −6.59 | −3.82 | −77.88 |



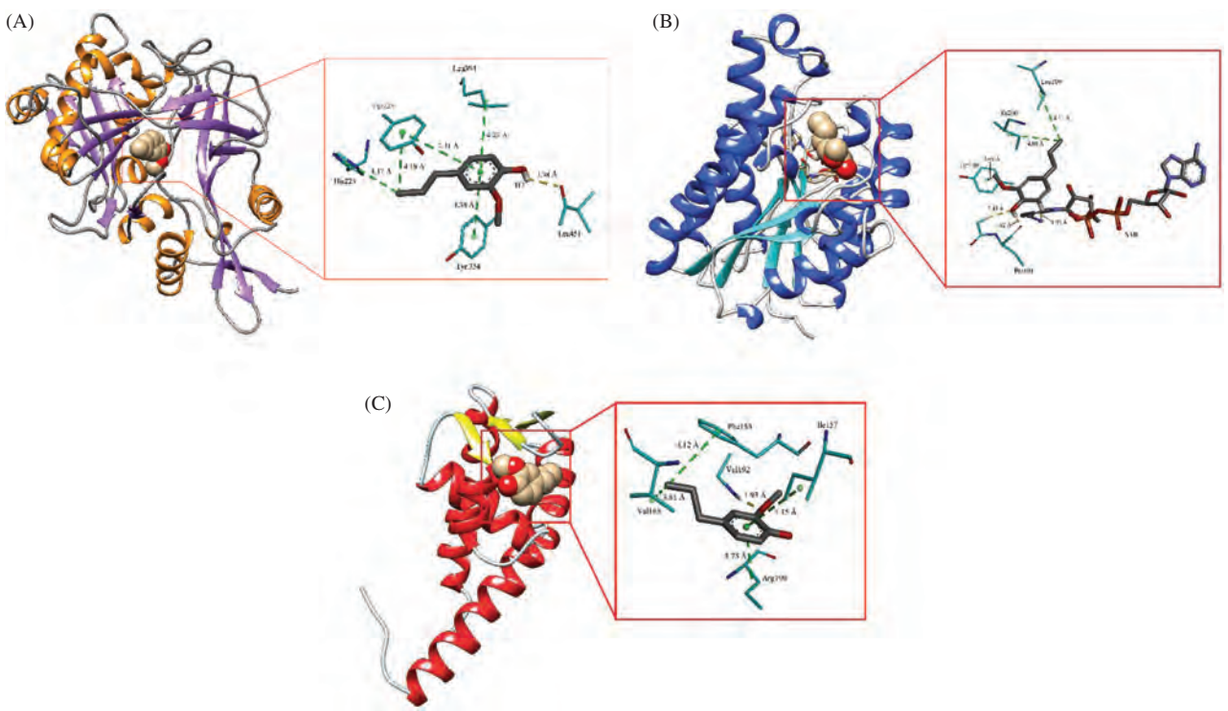


**Fig. 6.** Molecular interactions of the ligand with the residues of the active site. In green are represented the hydrophobic interactions and in blue the hydrogen bonds. (A) Intermolecular interactions between eugenol and residues of the active site of the protein N-myristoyltransferase (*C. Albicans*), (B) Intermolecular interactions between eugenol and residues of the active site of the protein Enoyl reductase (*E. Coli*) and (C) Intermolecular interactions between eugenol and residues of the active site of SarA protein (*S. aureus*).

of the proteins of the three microorganisms under analysisg.

In Table II, the energetic contributions to the interaction of eugenol with the different proteins used as molecular targets are shown. Table I includes the results of MolDock (evaluted after post-processing), Rerank Score (the ranking score) Interaction (the total interaction energy between the pose and the molecular target), $H_{bond}$ (hydrogen bond energy), LE1 (ligand efficiency 1: MolDock Score divided by heavy atoms count), LE3 (ligand efficiency 3: rerank score divided by heavy atoms count) and Docking Score (evaluated before post-processing).

The results of MolDock Scoring suggest that the interaction of eugenol with the proteins used as receptors is favorable for the formation of all systems. In addition, the interactions between eugenol and the residues belonging to the catalytic site of the proteins were evaluated. The interactions are shown in Figure 6.

In Figure 6(A) it is possible to observe the mode of attachment of eugenol to the catalytic site of the enzyme N-myristoyltransferase (*C. albicans*). The ligand interacted with the catalytic residues His255, Tyr225, Leu394, Leu451 and Tyr354. With the amino acid Leu451, a hydrogen bond with a distance of 3.38 Å was established in which eugenol was the hydrogen donor. Three interactions were performed with the benzene ring and eugenol. Two of them were hydrophobic Pi-Pi type interactions, that were established with the benzene ring of Tyr225 and Tyr354. The third interaction was of Pi-Alkyl type with Leu 394. His225 interacted with the final carbon of the eugenol carbon chain through pi-alkyl-type interactions. This same carbon interacted with Tyr225 by means of hydrophobic pi-alkyl type interactions.

The eugenol molecular docking pose shown in Figure 6(B) reveals which residues of Enoyl reductase (*E. Coli*) the ligand establishes interactions with. Eugenol made three hydrogen bonds, two with Pro191 and one with the NAD cofactor. With Tyr146 the ligand established a hydrophobic pi-alkyl type interaction with a distance of 3.89 Å. Two other alkyl-alkyl interactions were established with Leu100 and Ile200 at distances, respectively, of 4.11 and 4.08 Å.

The attachment mode of eugenol to the enzyme SarA (*S. aureus*) is shown in Figure 6(C). At this binding site,

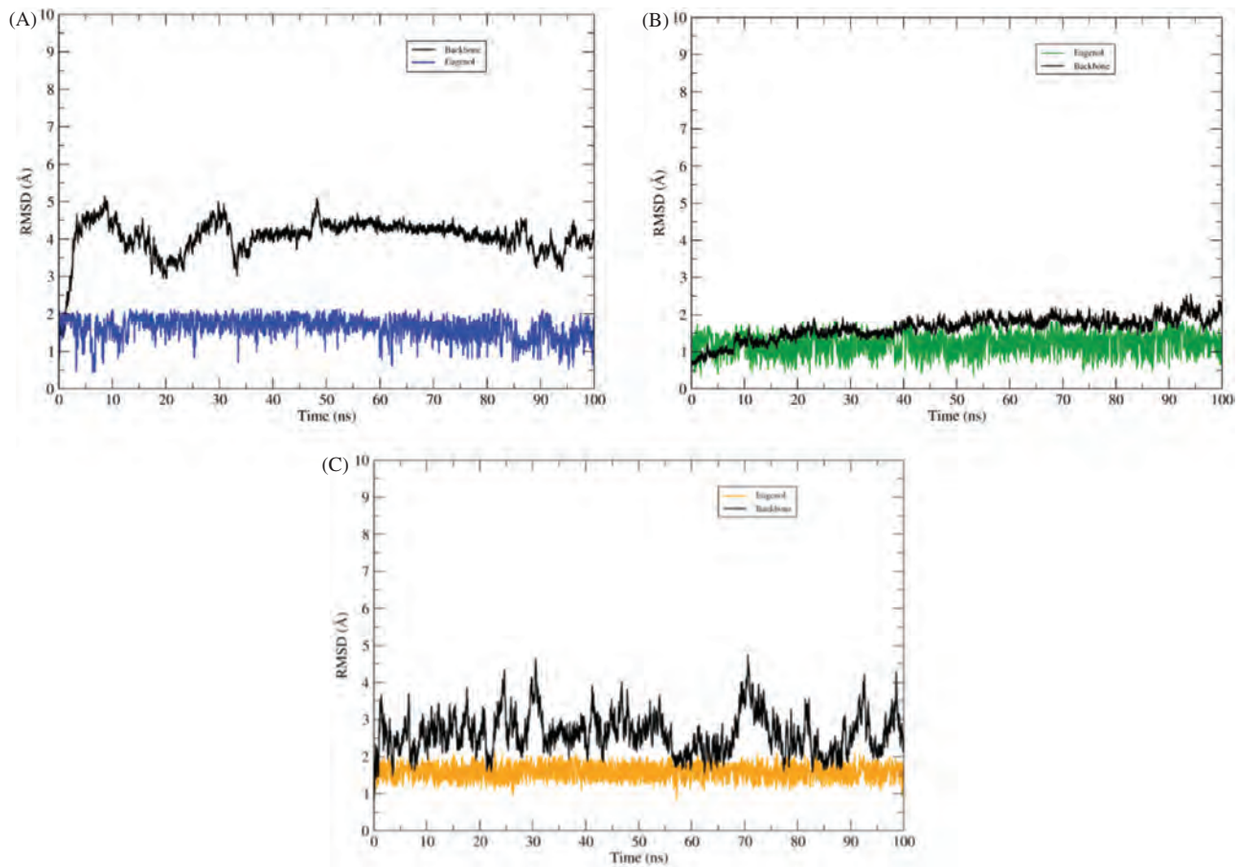


**Fig. 7.** Evolution of the RMSD chart. (A) Black represents the backbone of the N-myristoyltransferase protein of *C. albicans* microorganism and blue, the eugenol RMSD graph, (B) The RMSD of the *E. coli* microorganism protein Enoyl reductase backbone was represented in black, while eugenol was represented in green and (C) In black is represented the RMSD of the SarA enzyme backbone of *S. aureus* microorganism and in orange the RMSD of eugenol.

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**Table III.** Binding affinity values and energy components. All values are in kcal/mol.

| Targets | $\Delta E_{vdW}$ | $\Delta E_{ele}$ | $\Delta G_{GB}$ | $\Delta G_{NP}$ | $\Delta E_{non\text{-}polar}$ | $\Delta E_{polar}$ | $\Delta G_{MM\text{-}GBSA}$ |
|---|---|---|---|---|---|---|---|
| N-myristoyltransferase *(C. Albican)* | −20.42 | −4.21 | 8.70 | −3.08 | −23.50 | 4.49 | −19.01 |
| Enoyl reductase *(E. Coli)* | −15.57 | −3.15 | 9.78 | −2.37 | −17.94 | 6.63 | −11.31 |
| SarA *(S. aureus)* | −15.11 | −4.55 | 8.56 | −2.48 | −17.59 | 4.01 | −13.58 |

eugenol established a hydrogen bond with the amino acid Val192 (hydrogen donor) with a distance of 1.93 Å. Two benzene interactions of eugenol with Ile157 and Arg193 were also established, with distances of 3.75 and 4.15 Å, respectively. These two interactions are of hydrophobic nature and of pi-alkyl type. There were also hydrophobic interactions with Val168 (alkyl-alkyl) and Phe153 (pi-alkyl).

### 3.5. Stability Molecular of Systems

To evaluate the structural stability of the systems we used the root mean square deviation (RMSD). C$\alpha$ atoms were used to plot the RMSD graph of the protein backbone, and to plot the RMSD of the ligand, the heavy atoms of the molecule were used.

During the 100 ns of MD simulations the ligand remained in the binding site in all systems studied. The backbone of the protein in the different systems underwent conformational changes as can be observed in Figure 7.

The ligand was in equilibrium in all complexes, with a small conformational change as can be seen in Figure 6 and in the small mean values of RMSD. For systems: N-myristoyltransferase/*C. Albicans*, Enoyl reductase/*E. Coli* and SarA/*S. aureus*, the following mean RMSD values: 1.61 Å, 1.52 Å, and 1.49 Å, respectively were obtained

### 3.6. Free Energy Binding and Analysis of Its Components

To predict binding affinity, the MM/GBSA approach was used. In addition to the free energy values ($\Delta G_{MM\text{-}GBSA}$), the results of the contributions of van der Waals ($\Delta E_{vdW}$), polar ($\Delta G_{GB}$) and non-polar contributions ($\Delta G_{NP}$), electrostatic interactions energy ($\Delta E_{ele}$), total non-polar contribution ($\Delta E_{non\text{-}polar} = \Delta E_{vdW} + \Delta G_{NP}$) and total polar energy ($\Delta E_{polar} = \Delta E_{ele} + \Delta E_{GB}$) were computed. The results obtained are shown in Table III.

According to Table III, the binding affinity values of eugenol with N-myristoyltransferase protein was −19.01 kcal/mol, with Enoyl reductase was −11.31 kcal/mol, and with SarA was −13.58 kcal/mol. These results demonstrate that eugenol is capable of binding to these proteins and inhibiting their enzymatic activity.

In our results it is also possible to observe and evaluate the energy contributions for the calculation of the total binding affinity. The contributions of van der Waals ($\Delta E_{vdW}$) were favorable in all systems for the establishment of eugenol-receptor interactions. The nonpolar contributions ($\Delta G_{NP}$) and the energy of electrostatic interactions were also favorable for the systems formation. The total polar contribution ($\Delta E_{non\text{-}polar}$), resulting from the sum of the van der Waals ($\Delta E_{vdW}$) and nonpolar contributions ($\Delta G_{NP}$), was also very favorable for the complexes, while the total polar energy ($\Delta E_{polar}$) was shown to be unfavorable.

## 4. CONCLUSION

According to the results, the *S. aromaticum* essential oil obtained by $CO_2$-SC method, presented considerable cellular viability at concentrations below 10 $\mu$L/mL, in human gingival fibroblasts, all the six essential oil fractions tested showed antimicrobial activity, the results point to eugenol as being the main chemically active component responsible for the biological effects presented on the fungus and bacteria. Our results also demonstrate that eugenol was capable of interacting with catalytic residues of the proteins of the different microorganisms under study. During the simulation of molecular dynamics, eugenol remained interacting with the proteins and presented values of binding free energy capable of inhibiting the enzymatic activity. In complex with the enzyme N-myristoyltransferase (*C. Albicans*), $\Delta G_{bind}$ was equal to −19.01 kcal/mol, with Enoyl reductase *(E. Coli)* $\Delta G_{bind}$ was equal to −11.31 kcal/mol and with SarA (*S. aureus*), $\Delta G_{bind}$ was −13.58 kcal/mol.

### Authors' Contributions

MSO, JNC, WAC, And SGS conceived the research idea. MSO, JNC, SEMS, SAFM and TOAM conducted the experiments, GPM were assistants in experimental work. MSO, AMJC and RNCJ compiled all the data and prepared the manuscript. All authors read and approved the final manuscript.

### Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

### Ethics Approval and Consent to Participate

Not applicable.

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## References


1. Chaieb, K., Hajlaoui, H., Zmantar, T., Ben Kahla-Nakbi, A., Rouabhia, M., Mahdouani, K. and Bakhrouf, A., **2007**. *Phyther. Res.*, *21*, p.501.
2. Majeed, H., Liu, F., Hategekimana, J., Sharif, H.R., Qi, J., Ali, B., Bian, Y.Y., Ma, J., Yokoyama, W. and Zhong, F., **2016**. *Food Chemistry*, *197*, p.75.
3. Pinto, E., Vale-Silva, L., Cavaleiro, C. and Salgueiro, L., **2009**. *J. Med. Microbiol.*, *58*, p.1454.
4. Taher, Y.A., Samud, A.M., El-Taher, F.E., ben-Hussin, G., Elmezogi, J.S., Al-Mehdawi, B.F. and Salem, H.A., **2015**. *Libyan J. Med.*, *10*.
5. El-Darier, S.M., El-Ahwany, A.M.D., Elkenany, E.T. and Abdeldaim, A.A., **2018**. *Rend. Lincei*, *29*, p.131.
6. Gilmour, A.D., Woolley, A.J., Poole-Warren, L.A., Thomson, C.E. and Green, R.A., **2016**. *Biomaterials*, *91*, p.23.
7. Rashad, A., Mustafa, K., Heggset, E.B. and Syverud, K., **2017**. *Cytocompatibility of Wood-Derived Cellulose Nanofibril Hydrogels with Different Surface Chemistry*.
8. Caballero-Díaz, E. and Valcárcel Cases, M., **2016**. *TrAC—Trends Anal. Chem.*, *84*, p.160.
9. Xu, J.-G., Liu, T., Hu, Q.-P. and Cao, X.-M., **2016**. *Molecules*, *21*, p.1194.
10. de Oliveira, M.S., da Costa, W.A., Pereira, D.S., Botelho, J.R.S., de Alencar Menezes, T.O., de Aguiar Andrade, E.H., da Silva, S.H.M., da Silva Sousa Filho, A.P. and de Carvalho, R.N., **2016**. *Journal of Supercritical Fluids*, *118*, p.185.
11. Bagheri, H., Bin Abdul Manap, M.Y. and Solati, Z., **2014**. *Talanta*, *121*, p.220.
12. de Oliveira, M.S., da Costa, W.A., Pereira, D.S., Botelho, J.R.S., de Alencar Menezes, T.O., de Aguiar Andrade, E.H., da Silva, S.H.M., da Silva Sousa Filho, A.P. and de Carvalho, R.N., **2016**. *Journal of Supercritical Fluids*, *118*, p.185.
13. C. and (CLSI), L.S.I., **2004**. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Informational Supplement*, Pennsylvania.
14. Prashar, A., Locke, I.C. and Evans, C.S., **2004**. *Cell Prolif*, *37*, p.221.
15. Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Barone, V., Mennucci, B., Petersson, G.A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H.P., Izmaylov, A.F., Zheng, G., Sonnenberg, J.L., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Kitao, O., Nakai, H., Vreven, T., Montgomery, J.A., Peralta, J.E., Ogliaro, F., Bearpark, M., Heyd, J.J., Brothers, E., Kudin, N., Staroverov, V.N., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A., Burant, J.C., Iyengar, S.S., Tomasi, J., Cossi, M., Rega, N., Millam, J.M., Klene, M., Knox, J.E., Cross, J.B., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Yazyev, O., Austin, A.J., Cammi, R., Pomelli, C., Ochterski, J.W., Martin, R.L., Morokuma, K., Zakrzewski, V.G., Voth, A., Salvador, P., Dannenberg, J.J., Dapprich, S., Daniels, A.D., Foresman, J.B., Ortiz, J.V., Cioslowski, J., Fox, D.J., Gaussian, D.J., Bloino, J., Honda, Y., Kudin, K.N., Stratmann, R.E. and Voth, G.A., Gaussian 09, **2009**.
16. Becke, A.D., **1993**. *Journal of Chemical Physics*, *98*, p.5648.
17. Lee, C., Yang, W. and Parr, R.G., **1988**. *Phys. Rev. B*, *37*, p.785.
18. Sogabe, S., Masubuchi, M., Sakata, K., Fukami, T.A., Morikami, K., Shiratori, Y., Ebiike, H., Kawasaki, K., Aoki, Y., Shimma, N., D'Arcy, A., Winkler, F.K., Banner, D.W. and Ohtsuka, T., **2002**. *Chem. Biol.*, *9*, p.1119.
19. Qiu, X., Janson, C.A., Court, R.I., Smyth, M.G., Payne, D.J. and Abdel-Meguid, S.S., **1999**. *Protein Science*, *8*, p.2529.
20. Liu, Y., Manna, A.C., Pan, C.-H., Kriksunov, I.A., Thiel, D.J., Cheung, A.L. and Zhang, G., **2006**. *Proc. Natl. Acad. Sci.*, *103*, p.2392.
21. Thomsen, R. and Christensen, M.H., **2006**. *Journal of Medicinal Chemistry*, *49*, p.3315.
22. Dolinsky, T.J., Nielsen, J.E., McCammon, J.A. and Baker, N.A., **2004**. *Nucleic Acids Research*, *32*, p.665.
23. Cornell, W.D., Cieplak, P., Bayly, C.I. and Kollman, P.A., **1993**. *Journal of the American Chemical Society*, *115*, p.9620.
24. Wang, J., Cieplak, P. and Kollman, P.A., **2000**. *Journal of Computational Chemistry*, *21*, p.1049.
25. Case, D.A., Cheatham, T.E., Darden, T., Gohlke, H., Luo, R., Merz, K.M., Onufriev, A., Simmerling, C., Wang, B. and Woods, R.J., **2005**. *Journal of Computational Chemistry*, *26*, p.1668.
26. Cruz, J.N., Costa, J.F.S., Khayat, A.S., Kuca, K., Barros, C.A.L. and Neto, A.M.J.C., **2018**. Molecular dynamics simulation and binding free energy studies of novel leads belonging to the benzofuran class inhibitors of Mycobacterium tuberculosis Polyketide Synthase 13.
27. Wang, J., Wolf, R.M., Caldwell, J.W., Kollman, P.A. and Case, D.A., **2004**. *Journal of Computational Chemistry*, *25*, p.1157.
28. Maier, J.A., Martinez, C., Kasavajhala, K., Wickstrom, L., Hauser, K.E. and Simmerling, C., **2015**. *J. Chem. Theory Comput.*, *11*, p.3696.
29. Jorgensen, W.L., Chandrasekhar, J., Madura, J.D., Impey, R.W. and Klein, M.L., **1983**. *Journal of Chemical Physics*, *79*, p.926.
30. Darden, T., York, D. and Pedersen, L., **1993**. *Journal of Chemical Physics*, *98*, p.10089.
31. Ryckaert, J.P., Ciccotti, G. and Berendsen, H.J.C., **1977**. *J. Comput. Phys.*, *23*, p.327.
32. Izaguirre, J.A., Catarello, D.P., Wozniak, J.M. and Skeel, R.D., **2001**. *Journal of Chemical Physics*, *114*, p.2090.
33. Kollman, P.A., Massova, I., Reyes, C., Kuhn, B., Huo, S., Chong, L., Lee, M., Lee, T., Duan, Y., Wang, W., Donini, O., Cieplak, P., Srinivasan, J., Case, D.A. and Cheatham, T.E., **2000**. *Accounts of Chemical Research*, *33*, p.889.
34. Genheden, S. and Ryde, U., **2015**. *Expert Opin. Drug Discov.*, *10*, pp. 449.
35. Ivanovic, J., Dimitrijevic-Brankovic, S., Misic, D., Ristic, M. and Zizovic, I., **2013**. *J. Funct. Foods*, *5*, p.416.
36. Zhang, H., Dudley, E.G. and Harte, F., **2017**. *Applied and Environment Microbiology*, *83*.
37. Galotto, M. and Ulloa, P., **2010**. *Packag. Technol. Sci.*, *23*, p.253.
38. Michiels, J., Missotten, J., Fremaut, D., De Smet, S. and Dierick, N., **2007**. *Livest. Sci.*, *109*, p.157.
39. Marchese, A., Barbieri, R., Coppo, E., Orhan, I.E., Daglia, M., Nabavi, S.F., Izadi, M., Abdollahi, M., Nabavi, S.M. and Ajami, M., **2017**. *Crit. Rev. Microbiol.*, *43*, p.668.
40. Malik, K., Bashir, H., Bilal, M., Qamar, H., Mehmood, Z. and Biosci, I.J., **2016**. *Int. J. Biosci.*, *6655*, p.221.
41. Ali, S.M., Khan, A.A., Ahmed, I., Musaddiq, M., Ahmed, K.S., Polasa, H., Rao, L.V., Habibullah, C.M., Sechi, L.A. and Ahmed, N., **2005**. *Ann. Clin. Microbiol. Antimicrob.*, *4*, p.1.
42. Thosar, N., Basak, S., Bahadure, R.N. and Rajurkar, M., **2013**. *Eur. J. Dent.*, *7*.
43. de Cássia da Silveira e Sá, R., Andrade, L., dos Reis Barreto de Oliveira, R. and de Sousa, D., **2014**. *Molecules*, *19*, p.1459.
44. Al-Okbi, S.Y., Mohamed, D.A., Hamed, T.E. and Edris, A.E., **2014**. *J. Med. Food*, *17*, p.764.
45. Guenette, S.A., Beaudry, F., Marier, J.F. and Vachon, P., **2006**. *J. Vet. Pharmacol. Ther.*, *29*, p.265.
46. Hertiani, T. and Pratiwi, S., **2011**. *Maj. Farm. Indones.*, *22*, p.174.

**47.** Namba, T., Tsunezuka, M. and Hattori, M., **1982**. *Planta Medica*, *44*, p.100.
**48.** Mogoşanu, G.D., Grumezescu, A.M., Huang, K.-S., Bejenaru, L.E. and Bejenaru, C., **2015**. *Curr. Pharm. Biotechnol.*, *16*, p.94.
**49.** Benencia, F. and Courreges, M.C., **2000**. *Phyther. Res.*, *14*, p.495.
**50.** Li, J., Kong, X., Li, X., Yang, Y. and Zhang, J., **2013**. *Food and Chemical Toxicology*, *62*, p.805.
**51.** Llana-Ruiz-Cabello, M., Pichardo, S., Maisanaba, S., Puerto, M., Prieto, A.I., Gutiérrez-Praena, D., Jos, A. and Cameán, A.M., **2015**. *Food and Chemical Toxicology*, *81*, p.9.
**52.** Mo, Z.-Z., Liu, Y.-H., Li, C.-L., Xu, L.-Q., Wen, L.-L., Xian, Y.-F., Lin, Z.-X., Zhan, J.Y.-X., Chen, J.-N., Xu, F.-F. and Su, Z.-R., **2017**. *Rejuvenation Res.*, *20*, p.231.
**53.** Piras, A., Falconieri, D., Porcedda, S., Marongiu, B., Gonçalves, M.J., Cavaleiro, C. and Salgueiro, L., **2014**. *Nat. Prod. Res.*, *28*, p.1819.

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# 8 CAPÍTULO VII.

## 8.1 Chemical composition, Antimicrobial properties of *Siparuna guianensis* essential oil and a molecular docking and dynamics molecular study of its major chemical constituent

# Chemical composition, antimicrobial properties and and study of the molecular interactions of the major component present in *Siparuna guianensis* essential oil

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**Abstract**

The essential oil of *Siparuna guianensis* was obtained by hydrodistillation. The 32 identification of the chemical compounds was performed by gas chromatography coupled with 33 mass spectrometry (GC/MS). Antimicrobial activity was performed on four microorganisms: 34 *Streptococcus mutans* (ATCC 3440), *Enterococcus faecalis* (ATCC 4083), *Escherichia coli* (ATCC 25922) 35 and *Candida albicans* (ATCC-10231). The studies of doping and molecular dynamics were performed 36 with the molecule that presented the highest concentration of drug-target proteins, 1IYL (*C. albicans*), 37 1C14 (*E. coli*), 2WE5 (*E. faecalis*) and 4TQX (*S. mutans*). The main compounds identified were: 38 Elemene (7.58%), Curzerene (7.62%), Germacrene D (8.17%), β-Elemenone (12.76%) and Atractylone 39 (18.96%). Gram

positive bacteria and fungi were the most susceptible to the effects of the essential oil. The results obtained in the simulation showed that the major compound atractylone interacts with the catalytic sites of the target proteins, forming energetically favourable systems and remaining stable during the period of molecular dynamics

**Keywords:** Amazon; Natural products, Capitiú; Biomolecules; Volatile compounds.

## 1. Introduction

Fungi and bacteria can cause various pathologies in humans. Leprosy [1], tuberculosis [2], bacterial dysentery [3], gonorrhea [4], urinary tract infection, endocarditis [5, 6], onychomycosis [7], mucormycosis [8] and candidiasis [9] are examples of diseases that these microorganisms can cause. In some cases, the symbiosis between bacteria and fungi increases the virulence of bacteria, because fungi such as *C. albicans* elevates the production of exopolysaccharides, which can become an ideal shelter for *S. mutans*, thus making it difficult to control this microorganism [10]. Another important factor is the resistance that microorganisms are developing to traditional antibiotics, since this poses a threat to public health and is associated with high rates of morbidity and mortality [11]. In this sense, natural products, more specifically essential oils, can become a viable alternative for the control of fungi and bacteria [12, 13].

The plants that produce essential oils (EOs) have been an object of study for years, since their EOs present varied biological activities [14], such as cytotoxic, antimicrobial, antioxidant [15], anti-inflammatory, anti-proliferative [16, 17], antibacterial, antifungal [18–23], antiviral [24, 25], anticonvulsant [26, 27], analgesic [28], and neuroprotective properties [29]. As a result, they are increasingly attracting the attention of many industry segments [30]. Essential oils consist of a complex mixture of volatile organic substances, often involving 50, 100 or even more isolated components, and that contain chemical groups such as hydrocarbons, alcohols, aldehydes, ketones, acids and esters [31].

*Siparuna guianensis* was the first *Siparuna* species described and illustrated by Aublet [32]. This plant is present from Nicaragua to Paraguay, and in Brazil, this species is known by several names, such as *negramina*, *folha-santa*, *marinheiro*, *capitiú*, *mata-cachorro*, *catingoso*, *limão-bravo*, *cicatrizante-das-guianas*, *catingueira-de-paca* and *fedegoso*. In many countries of America, leaves of *S. guianensis* are widely used as a drink to combat stomach pains [33] and this activity may be related to the compounds present in its essential oil [34, 35]. In this context, the objective of this work was to evaluate the chemical composition, antimicrobial activity and simulate the mechanisms of interaction of the major chemical constituent present in the essential oil of *Siparuna guianensis,* using doping techniques and molecular dynamics.

## 2. Methods

### *2.1 Preparation and characterization of the Siparuna guianensis sample*

The *Siparuna guianensis* sample was obtained in the Museu Paraense Emilio Goeld (Eastern Amazon), on 09/09/2016. The geographical coordinates of the collection site were S01°27'04.3" and W048°26'38.3", with a relative humidity of 64.9% and temperature of 26.5 °C. with incorporation of an exsicata in the Herbarium of Emílio Goeldi Museum, in the city of Belém, Pará, Brazil, under the registration number *MG-150698*. Before the extraction process, the sample was dried and ground and then the moisture content was determined by infrared moisture analyser. The images of the leaves of *S. guianensis* can be observed in Figure 1.

### *2.2 Extraction procedure: Hydrodistillation*

After the drying process, the leaves of *S. guianensis* were submitted to hydrodistillation using a Clevenger-type extractor. For the extraction process, 40 g of the sample was used, for 10800 s at 100 °C. After this procedure, anhydrous sodium sulphate ($Na_2SO_4$) was added and

the essential oil was centrifuged to be moisture-free. The essential oil yield was calculated in dry basis (db).

### *2.3 Analysis of volatile compounds*

The chemical composition of the essential oils of *S. guianensis* was obtained by gas chromatography coupled to a mass spectrometer (GC/MS), according to the literature [36, 37], using a QP 2010 Shimadzu system equipped with an AOC-20i auto injector, RTX-5MS silica capillary column (30 mx 0.25 mm, film thickness 0.25 μm), at temperatures ranging from 60 °C to 250 °C with a gradient of 3 °C/min and the injector temperature of 250 ° C; helium was used as carrier gas at a flow rate of 1.2 mL/min (measured at 100 ° C), splitless injection (solution of 2 mL of oil in 1000 mL of hexane), electron impact of 70 eV and source temperature of ions of 200 °C. The compounds were identified in relation to their retention indexes, which are determined by the retention time of the compounds in a homologous series of hydrocarbons (C8-C20), comparing their mass spectra with those described in the literature [38] and in the NIST database [39].

### *2.4 Analysis of in vitro antimicrobial activity*

In the microbiological assays, standard strains of *Streptococcus mutans* (ATCC 3440), *Enterococcus faecalis* (ATCC 4083), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231) were used. All of them were purchased from the Osvaldo Cruz Foundation (FIOCRUZ), belonging to the base of standards of the Laboratory of Microbiological Quality Control of Medicines of the University Center of Pará-CESUPA.

The inoculum of each microorganism was obtained from a microbial suspension of fresh culture (maximum 24 h) in saline solution 0.85% (m/V), by comparing the inoculum

turbidity with the MacFarland scale, equivalent to a concentration of 1.5x108 UFC/mL (NCCLI M7-A7, 2006) in a turbidimeter (Grant bio, Model: DEN-1).

The culture medium used for the disk diffusion test was soybean casein agar (SCA) and brain Heart Infusion (BHI) broth containing 0.2% polysorbate 80 (m/V). 5% (v/v) of sheep blood was added for the analysis of strains of *Streptococcus mutans* (ATCC 3440) and *Enterococcus faecalis* (ATCC 4083).

### *2.5 Evaluation of the sample sensitivity by the disk diffusion method*

10 mL of Soybean Casein Agar (15x100 mm) was poured into a Petri dish. The microorganism (106 CFU/mL) was then inoculated with the aid of a sterile swab and paper discs (6 and 8 m) impregnated with 10 µL of oil. Positive and negative control were added onto the medium. The plates were incubated at 30 ± 5 °C/24 h in an aerobic environment [21, 40, 41]. The analysis was performed in triplicate. After the incubation period, the plates were revealed with triphenyltetrazolic chloride at 7 mg/mL in bacteriological agar at 1% (w/v).

The halos were measured using a pachymeter (mm) and evaluated by a descriptive analysis, compared to mean values, standard deviation and coefficient of variation;

### *2.6 Determination of minimum inhibitory concentration (MIC)*

The MIC was performed with the essential oil and was adapted from the micro dilution proposed by [40]. The test was performed on an Elisa® plate, where a 100 µL sample aliquot was diluted (1:2 v/v) in BHI broth containing 106 CFU/mL until 10 consecutive dilutions, and then positive and negatives controls were added. Plates were incubated at 30 ± 5 °C/48h. The test was performed in triplicate.

After incubation, plates were revealed with 1% (m/v) bacteriological broth containing 7 mg/mL triphenyltetrazolic chloride solution and incubated for further 30 min at 30 ± 5 °C for bacteria, and at 25 ± 5 °C for *C. albicans*. The maintenance of the red colour in the medium was interpreted as microbial growth.

## 3. Molecular docking and dynamics molecular simulations

### *3.1 Molecular Docking*

The chemical structure of atractylon, after being designed with GaussView 5.5 software, was optimized with B3LYP/6-31G* [42, 43], using Gaussian 16 [44]. To study the interaction mode of this molecule with target-proteins for drug action, the software Molegro Virtual Docker 6 was used [45]. The MolDock SE algorithm and MolDock score (GRID) function were used in all molecular docking simulations. The crystal structures of the proteins used as targets can be found in the Protein Data Bank (www.rcsb.org), from their ID: 1IYL (*C. albicans*) [46], 1C14 (*E. coli*) [47], 2WE5 (*E. faecalis*) [48], and 4TQX (*S. mutans*) [49].

### *3.2 Molecular Dynamics (MD) Simulation*

The ligand parameters were constructed with the aid of the Antechamber module, using the General Amber Force Field (GAFF) [50]. The calculations to determine the atomic charges of the ligand were performed according to the Restrained Electrostatic Potential (RESP) protocol using basis set Hartree-Fock level might with the functional 6-31G* [51, 52]. To measure the protonation status of the amino acid residues of the receptors, the results obtained from the PROPKA program were used [53, 54].

In the molecular dynamics simulations, the force field ff14SB [55] and the explicit water molecules described by the TIP3P model [56] were used. All systems were solvated in an octahedron periodic box, where a cutting radius of 12Å was applied in all directions from

154 the solute. Finally, in each system, an adequate number of counter-ions were added to neutralize
155 the charge.

156 The MD simulations were performed with the Amber 16 package [57, 58]. Sander.
157 MPI was used for the energy minimization steps, and pmemd. CUDA, for the heating,
158 equilibrium and MD simulations.

159 The energy minimization of the systems occurred in three stages. In the first step, 1500
160 cycles were performed using steepest descent method and conjugate gradient algorithm,
161 applying a harmonic force constant of 100 kcal/mol.$Å^{-2}$ on the solute. In the second step, the
162 harmonic force constant applied on the solute was 50 kcal/mol.$Å^{-2}$ and further 500 cycles were
163 performed using the steepest descent method and conjugate gradient algorithm. In the last step,
164 the restrictions were removed and 500 cycles were performed using the same protocol.

165 To raise the systems temperature from 0 to 300k, 800 ps of simulations were
166 performed. The heating was carried out in three stages. In the first stage, the solute was
167 restricted with a harmonic force constant of 50 kcal/mol.$Å^{-2}$. Thus, only the solvent and the
168 counter-ions get free to move. In the next two steps, the harmonic force constant was removed.

169 To balance the complexes, 2 ns of simulations with constant temperature and with no
170 restrictions were performed. Then, for each complex, 100 ns of MD simulation were obtained
171 with NVT ensemble.

172 The Particle Mesh Ewald method [59] was used for the calculation of electrostatic
173 interactions, and the bonds involving hydrogen atoms were restricted with the SHAKE
174 algorithm [60]. The temperature control was performed with the Langevin thermostat [61]
175 within collision frequency of 2 $ps^{-1}$.

### *3.3 Free Energy Calculations*

The binding free energy was calculated using the Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) approach [62, 63]. For the affinity energy calculation, 500 snapshots of the last 5 ns of the MD simulations trajectories were used.

The free energy was calculated according to the following equations:

$$\Delta G_{bind} = \Delta H - T\Delta S \approx \Delta E_{MM} + \Delta G_{solv} - T\Delta S \quad (1)$$

Where $\Delta G_{bind}$ is the free energy of the complex, which is the result of the sum of the molecular mechanics energy ($\Delta E_{MM}$), the desolvation free energy ($\Delta G_{solv}$), and the entropy ($-T\Delta S$).

$$\Delta E_{MM} = \Delta E_{internal} + \Delta E_{eletrotatic} + \Delta E_{vdW} \quad (2)$$

The energy of molecular gas phase mechanics ($\Delta E_{MM}$) can be described by the sum of the internal energy contributions ($\Delta E_{internal}$), the sum of the connection, angle and dihedral energies, electrostatic contributions ($\Delta E_{eletrostatic}$) and van der Waals terms ($\Delta E_{vdW}$).

$$\Delta G_{solv} = \Delta G_{GB} + \Delta G_{nonpol} \quad (3)$$

Desolvation free energy ($\Delta G_{solv}$) is the sum of the polar ($\Delta G_{GB}$) and non-polar ($\Delta G_{nonpol}$) contributions. The polar desolvation term was calculated using the implicit generalized born (GB) approaches.

## 4. Results and discussion

### *4.1 Botanical information of the sample*

Capitiú (*Siparuna guianensis*) belongs to the Siparunaceae Family. It is a shrub about three meters high, the immature fruits were greenish and the ripe ones were greenish and

purplish, with short pedunculated axillary racemes, and opposite, elliptic, and lanceolate leaves. This plant releases a characteristic odour of fish.

### *4.2 Yield and chemical composition*

The moisture content of the *S. guianensis* sample was 13.58 % and the volume of essential oil obtained in the hydrodistillation was 0.5 ml, with a yield of (1.42) % (db). Regarding the chemical profile of the essential oil of *S. guianensis*, 51 compounds were identified, the most important being trans-β-elemenone (11.78%) and atractylone (18.65) %, followed by δ-elemene (5.38) %, β-elemene (3.13) %, β- yerangene (4.14) %, γ-Elemene (7.04) %, germacrene D (7.61) %, curzerene (7.1) %, and germacrone (5.26) % (See Table 1). In Figure 2, the ion chromatogram relative to the chemical composition can be observed. Cicció and Gómez [64] analyzed the essential oil of *Siparuna thecaphora* obtained by hydrodistillation and the compounds obtained in the highest concentrations were germacrene D (32.7) %, α-pinene (16.3) %, β - pinene (13.8) % e β - caryophyllene (4.1) %. In a similar study with *Siparuna guianensis* [65], they found myrcene (28.74) % [66], β-myrcene (13.14) %, the sesquiterpenes germacrene-D (8.68) % and bicyclogermacrene (16.71) %.

In a study related to the chemical composition of *S. guianensis* essential oil from southeastern Brazil, they obtained high concentrations of capric acid (46.6) % and 2-undecane (31.7) % [67]. These compounds were not identified in other studies such as [68], who analysed the chemical composition of *S. guianensis* essential oil, collected in various cities of Northern Brazil and identified epi-α-bisabolol (25.1) % and spathulenol (15.7) % in Moju (PA); spathulenol (22) %, selin-11-en-4α-ol (19.4) %, β-eudesmol (10) % and elemol (10) % in leaves collected in Rio Branco (AC); and germacrone (23.2) %, germacrene D (10.9) %, bicyclogermacrene (8.6) %, germacrene B (8) % and atractylon (31.4) % in Belém (PA). The results found in other studies [69–71] show that the chemical composition of the essential oil

of *S. guianensis* varies according to the seasonality and site of collection. Figure 3 shows the 2D structural formulas of the major chemicals identified in the essential oil of *S. guianensis*.

### *4.3 Antimicrobial activity*

The antimicrobial activity analysed by the diffusion method can be observed in Table 2. The microorganisms presented mean inhibition halos of (11 ± 0.12) mm, (12 ± 0.57) mm, (11 ± 0.31) mm, and (12.5 ± 0.98) mm for Gram-positive *Streptococcus mutans* (ATCC 3440), Gram-positive *Enterococcus faecalis* (ATCC-4083), Gram-negative *Escherichia coli* (ATCC 25922), and *Candida albicans* (ATCC-10231), respectively. *Streptococcus mutans* (ATCC-3440) and *Candida albicans* (ATCC- 10231) were the most sensitive to the effects of essential oils, with a minimum inhibitory concentration of 125 μL/mL, whereas the bacterium *Enterococcus faecalis* (ATCC-4083) [35] demonstrated that the essential oil of *S. guianensis* exerts an inhibitory effect on fungi, and on Gram-negative and Gram-positive bacteria.

In general, Gram-positive bacteria were the most sensitive to the effects of essential oil (EO), and this may be related to the fact that Gram-positive bacteria are more susceptible to the effects of volatile components compared to the Gram-negative ones [72]. In the case of fungi, EOs can be a viable alternative in the fight against the infection caused by *Candida* [23, 73]. These biological effects can be related to the presence of chemically active compounds such as γ-elemene, curzerene, germacrene D, β-elemenone and atractylon, as there are reports in the literature that corroborate this thesis [74–76].

## 5. Interaction Mechanism

### *5.1 Molecular Binding Mode*

From our molecular docking results, it can be suggested that the ligand interacts favourably with the target proteins. In Table 3, the results of the MolDock score for each

complex formed are present. The interactions between atractylon and the catalytic site of the enzymes were analysed. The interactions that were formed can be visualized in Figure 4.

In Figure 4-A, it is possible to observe that the ligand performed several hydrophobic interactions with different residues of the catalytic site of N-myristoyltransferase (*C. albicans*). With the Tyr225 residue, two interactions were established, one of the pi-pi types and the other of the pi-alkyl type. Residues Phe339 and Tyr354 had pi-alkyl-type interactions with the ligand, whereas Leu394 established alkyl interactions. Phe117 was also able to form two interactions, both of the pi-alkyl type.

With Enoyl reductase residues (*E. coli*), atractylon established six hydrophobic interactions (Figure 4-B). Four of these interactions were of the alkyl type with the following residues: Met206, Met159, Lys163 and Ala196. In addition, two additional pi-alkyl-type interactions with Tyr156 were formed.

The interaction of the ligand with the binding pocket of the enzyme Carbamate kinase (*E. faecalis*) can be seen in Figure 4-C. With residues Val231, Cys235 and Met268, hydrophobic interactions of the alkyl type were formed. With Tyr238, two interactions were established, one of the pi-alkyl types and one of the pi-pi type. With Ala264, an interaction of the same type was formed.

All interactions formed with the residues of Sortase A (*S. mutans*) were of hydrophobic and alkyl types. These interactions were established with the following residues: Ile215, Val190, Ile191, Val188, Arg213, and Val2013.

### *5.2 Analysis of Complexes Stability*

The complexes obtained by molecular docking were used as a starting point for molecular dynamics simulations. The root means square deviation (RMSD) graphs were plotted in relation to the lowest energy structure obtained for the systems, after the execution of the

protocol of energy minimization, heating and equilibrium. To plot the RMSD of the protein's backbone, their Cα atoms were used and to plot the RMSD of the ligands, their heavy atoms were used. The correspondent graphs can be seen in Figure 5.

For the systems formed with the target proteins of *C. albicans*, *E. coli*, *E. faecalis* and *S. mutans*, the mean RMSD obtained for the ligand was 0.65 Å, 0.62 Å, 0.66 Å and 0.64 Å, respectively. Thus, it is possible to infer that during the simulations, the inhibitor remained stable at the binding site of the different targets during molecular dynamics.

The target proteins showed small conformational changes as can be observed in the RMSD plots. These changes resulted from the accommodation of the ligands at their respective binding sites.

The fluctuations observed in the RMSD for the proteins backbone may be the result of the accommodation of the ligand at the active site. The mean values for the RMSD were relatively low. These values for the backbone of the target proteins of *C. albicans, E. coli, E. faecalis* and *S. mutans* were 1.63 Å, 1.53 Å, 1.44 Å and 1.65 Å, respectively.

### *5.3 Free Energy Calculations Using MM/GBSA Approach*

For each complex, the values of affinity energy ($\Delta G_{MM\text{-}GBSA}$), in addition to the values of the energetic contributions involved in the ligand-receptor interaction were obtained. The energy contributions obtained were as follows: Van der Waals ($\Delta E_{vdW}$), polar ($\Delta G_{GB}$), non-polar ($\Delta G_{NP}$) and the electrostatic interactions energies ($\Delta E_{ele}$) (Table 4).

In all systems, the free energy values demonstrated that atractylon is capable of inhibiting enzymatic activity. The contributions of Van der Waals were the main responsible for the interaction of the ligand with the molecular targets. Moreover, the electrostatic and nonpolar contributions were favourable for the maintenance of the complexes.

## 5. Conclusions

The main compounds obtained in the essential oil of *Siparuna guianensis* were γ-elemene (7.58) %, curzerene (7.62) %, germacrene D (8.17) %, β-elemenone (12.76) % and atractylon (18.96) %. The bacterium most sensitive to the effect of the essential oil was *Streptococcus mutans* followed by the fungus *Candida albicans.* Both microorganisms had the same MIC value (125 μL / mL). In our results, it is evidenced that atractylon interacts with all catalytic sites of the proteins and may be an inhibitor. The energy contributions observed were the electrostatic interactions energies ($\Delta E_{ele}$), and of the Van der Waals ($\Delta E_{vdW}$), polar ($\Delta G_{GB}$), and nonpolar ($\Delta G_{NP}$) types. The free energy results demonstrated that the system was between receptor ligand were favourable for complex formation.

## 6. Abbreviations

EOs - Essential Oils
$Na_2SO_4$ - Anhydrous Sodium Sulphate
db - Dry Basis
GC/MS - Gas Chromatography Coupled to a Mass Spectrometer
FIOCRUZ - Osvaldo Cruz Foundation
SCA - Soybean Casein Agar
BHI - Heart Infusion
MIC - Minimum Inhibitory Concentration
C. albicans - *Candida albicans*
*E. coli* - *Escherichia coli*
*E. faecalis* - *Enterococcus faecalis*
*S. mutans* - *Streptococcus mutans*
MD - Molecular Dynamics
GAFF - General Amber Force Field
RESP - Restrained Electrostatic Potential
MM-GBSA - Molecular Mechanics-Generalized Born Surface Area
$\Delta E_{MM}$ - Molecular Mechanics Energy

$\Delta G_{solv}$ - Desolvation Free Energy

$\Delta E_{internal}$ - Internal Energy Contributions

$\Delta E_{eletrostatic}$ - Electrostatic Contributions

$\Delta E_{vdW}$ - Van Der Waals Terms

$\Delta G_{solv}$ - Desolvation Free Energy

$\Delta G_{GB}$ - Polar Contributions

$\Delta G_{nonpol}$ - Non-polar Contributions

GB - Generalized Born

RMSD - Root Means Square Deviation

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## 7. Availability of data and materials

Data can be accessed at the Museu Paraense Emílio Goeldi and at the Federal University of Para.

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## 9. Ethics approval and consent to participate

This article does not contain any studies with human or animal subjects.

## 10. Consent for publication

Not applicable.

## 11. Conflict of interest

The authors declare that they have no conflict of interest.

## References

1. O'Brien CR, Malik R, Globan M, et al (2017) Feline leprosy due to Mycobacterium lepraemurium: Further clinical and molecular characterisation of 23 previously reported cases and an additional 42 cases. J Feline Med Surg 19:737–746 . doi: 10.1177/1098612X17706469

2. Hmama Z, Peña-Díaz S, Joseph S, Av-Gay Y (2015) Immunoevasion and immunosuppression of the macrophage by Mycobacterium tuberculosis. Immunol Rev

264:220–232 . doi: 10.1111/imr.12268

3. Schofield DA, Wray DJ, Molineux IJ (2014) Isolation and development of bioluminescent reporter phages for bacterial dysentery. Eur J Clin Microbiol Infect Dis 34:395–403 . doi: 10.1007/s10096-014-2246-0

4. Fairley CK, Hocking JS, Zhang L, Chow EPF (2017) Frequent transmission of gonorrhea in men who have sex with men. Emerg Infect Dis 23:102–104 . doi: 10.3201/eid2301.161205

5. Murray BE (1990) The life and times of the Enterococcus. Clin Microbiol Rev 3:46–65 . doi: 10.1128/CMR.3.1.46

6. Fasugba O, Gardner A, Mitchell BG, Mnatzaganian G (2015) Ciprofloxacin resistance in community- and hospital-acquired Escherichia coli urinary tract infections: A systematic review and meta-analysis of observational studies. BMC Infect Dis 15: . doi: 10.1186/s12879-015-1282-4

7. Shemer A, Gupta AK, Amichai B, et al (2016) Increased Risk of Tinea Pedis and Onychomycosis Among Swimming Pool Employees in Netanya Area, Israel. Mycopathologia 181:851–856 . doi: 10.1007/s11046-016-0040-5

8. Marty FM, Ostrosky-Zeichner L, Cornely OA, et al (2016) Isavuconazole treatment for mucormycosis: A single-arm open-label trial and case-control analysis. Lancet Infect Dis 16:828–837 . doi: 10.1016/S1473-3099(16)00071-2

9. Kullberg BJ, Arendrup MC (2015) Invasive Candidiasis. N Engl J Med 373:1445–1456 . doi: 10.1056/NEJMra1315399

10. Falsetta ML, Klein MI, Colonne PM, et al (2014) Symbiotic relationship between Streptococcus mutans and Candida albicans synergizes virulence of plaque biofilms in vivo. Infect Immun 82:1968–1981 . doi: 10.1128/IAI.00087-14

11. Kaye KS, Pogue JM (2015) Infections Caused by Resistant Gram-Negative Bacteria: Epidemiology and Management. Pharmacotherapy 35:949–962 . doi: 10.1002/phar.1636

12. Łysakowska ME, Sienkiewicz M, Banaszek K, et al (2015) The sensitivity of endodontic enterococcus Spp. Strains to geranium essential oil. Molecules 20:22881–22889 . doi: 10.3390/molecules201219888

13. Marrufo T, Nazzaro F, Mancini E, et al (2013) Chemical composition and biological activity of the essential oil from leaves of Moringa oleifera Lam. cultivated in Mozambique. Molecules 18:10989–11000 . doi: 10.3390/molecules180910989

14. Mandal S, Mandal M (2015) Coriander (Coriandrum sativum L.) essential oil: Chemistry and biological activity. Asian Pac J Trop Biomed 5:421–428 . doi: 10.1016/j.apjtb.2015.04.001

15. Lai P, Rao H, Gao Y (2018) Chemical Composition , Cytotoxic , Antimicrobial and Antioxidant Activities of Essential oil from Anthriscus caucalis M . Bieb Grown in China. Rec Nat Prod 3:290–294

16. Bayala B, Bassole IHN, Gnoula C, et al (2014) Chemical composition, antioxidant, anti-inflammatory and anti-proliferative activities of essential oils of plants from Burkina

Faso. PLoS One 9:1–11 . doi: 10.1371/journal.pone.0092122

17. Arranz E, Jaime L, López de las Hazas MC, et al (2015) Supercritical fluid extraction as an alternative process to obtain essential oils with anti-inflammatory properties from marjoram and sweet basil. Ind Crops Prod 67:121–129 . doi: 10.1016/j.indcrop.2015.01.012

18. Zhang H-Y, Gao Y, Lai P-X (2017) Chemical Composition, Antioxidant, Antimicrobial and Cytotoxic Activities of Essential Oil from Premna microphylla Turczaninow. Molecules 22:381 . doi: 10.3390/molecules22030381

19. Mothana RA, Noman OM, Al-Sheddi ES, et al (2017) Chemical composition, in vitro antimicrobial, free-radical-scavenging and antioxidant activities of the essential oil of Leucas inflata Benth. Molecules 22: . doi: 10.3390/molecules22030367

20. Diao W-R, Hu Q-P, Zhang H, Xu J-G (2014) Chemical composition, antibacterial activity and mechanism of action of essential oil from seeds of fennel (Foeniculum vulgare Mill.). Food Control 35:109–116 . doi: 10.1016/j.foodcont.2013.06.056

21. Pesavento G, Calonico C, Bilia AR, et al (2015) Antibacterial activity of Oregano, Rosmarinus and Thymus essential oils against Staphylococcus aureus and Listeria monocytogenes in beef meatballs. Food Control 54:188–199 . doi: 10.1016/j.foodcont.2015.01.045

22. Da Silva Bomfim N, Nakassugi LP, Faggion Pinheiro Oliveira J, et al (2015) Antifungal activity and inhibition of fumonisin production by Rosmarinus officinalis L. essential oil in Fusarium verticillioides (Sacc.) Nirenberg. Food Chem 166:330–336 . doi: 10.1016/j.foodchem.2014.06.019

23. Freires IDA, Murata RM, Furletti VF, et al (2014) Coriandrum sativum L. (Coriander) essential oil: Antifungal activity and mode of action on Candida spp., and molecular targets affected in human whole-genome expression. PLoS One 9: . doi: 10.1371/journal.pone.0099086

24. Bektaş E, Daferera D, Sökmen M, et al (2016) In vitro antimicrobial, antioxidant, and antiviral activities of the essential oil and various extracts from thymus nummularis M. Bieb. Indian J Tradit Knowl 15:403–410

25. Pourghanbari G, Nili H, Moattari A, et al (2016) Antiviral activity of the oseltamivir and Melissa officinalis L. essential oil against avian influenza A virus (H9N2). VirusDisease 27:170–178 . doi: 10.1007/s13337-016-0321-0

26. Masoumi-Ardakani Y, Mandegary A, Esmaeilpour K, et al (2016) Chemical composition, anticonvulsant activity, and toxicity of essential oil and methanolic extract of Elettaria cardamomum. Planta Med 82:1482–1486 . doi: 10.1055/s-0042-106971

27. Randrianarivo E, Maggi F, Nicoletti M, Rasoanaivo P (2016) Evaluation of the anticonvulsant activity of the essential oil of Myrothamnus moschatus in convulsion induced by pentylenetetrazole and picrotoxin. Asian Pac J Trop Biomed 6:501–505 . doi: 10.1016/j.apjtb.2016.01.017

28. Mendes SS, Bomfim RR, Jesus HCR, et al (2010) Evaluation of the analgesic and anti-inflammatory effects of the essential oil of Lippia gracilis leaves. J Ethnopharmacol 129:391–397 . doi: 10.1016/j.jep.2010.04.005

29. Rodriguez OE, Sánchez RM, Verde MJ, et al (2014) Obtaining of the essential oil of Syzygium aromaticum, identification of eugenol and its effect on Streptococcus mutans. J Oral Res 3:218–224 . doi: 10.17126/joralres.2014.051

30. Zeng Z, Zhang S, Wang H, Piao X (2015) Essential oil and aromatic plants as feed additives in non-ruminant nutrition: A review. J. Anim. Sci. Biotechnol. 6

31. Salgado APSP, Cardoso MDG, Souza PE De, et al (2003) DE FOLHAS DE Eucalyptus SOBRE Fusarium oxysporum , FUNGITOXIC ACTIVITY EVALUATION OF ESSENTIAL LEAF OILS OF Eucalyptus ON Fusarium oxysporum , Botrytis cinerea AND Bipolaris sorokiniana. Ciênc agrotec 27:249–254

32. Valentini CMA, Rodríguez-Ortíz CE, Coelho MFB (2010) Siparuna guianensis Aublet (negramina): Uma revisão. Rev Bras Plantas Med 12:96–104 . doi: 10.1590/S1516-05722010000100014

33. Valentini CM., Rodríguez-Ortíz C., Coelho MF. (2010) Siparuna guianensis Aublet (negramina): uma revisão. Rev Bras Plantas Med 12:96–104 . doi: 10.1590/S1516-05722010000100014

34. Nelita G ccedil alves F de B, Mir eacute ia ABP, Vany F, et al (2015) Antimicrobial activity and medicinal biomass of Siparuna guianensis in Brazilian Cerrado forest, a global hotspot. J Med Plants Res 9:968–980 . doi: 10.5897/JMPR2015.5884

35. Andrade MA, Cardoso MDG, Gomes M de S, et al (2015) Biological activity of the essential oils from Cinnamodendron dinisii and Siparuna guianensis. Brazilian J Microbiol 46:189–194 . doi: 10.1590/S1517-838246120130683

36. de Oliveira MS, da Costa WA, Pereira DS, et al (2016) Chemical composition and phytotoxic activity of clove (Syzygium aromaticum) essential oil obtained with supercritical CO2. J Supercrit Fluids 118:185–193 . doi: 10.1016/j.supflu.2016.08.010

37. de Oliveira MS, da Cruz JN, Gomes Silva S, et al (2019) Phytochemical profile, antioxidant activity, inhibition of acetylcholinesterase and interaction mechanism of the major components of the Piper divaricatum essential oil obtained by supercritical CO2. J Supercrit Fluids 145:74–84 . doi: 10.1016/j.supflu.2018.12.003

38. Adams RP (2007) Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. 4th ed. Carol Stream, IL, USA: Allured Publishing Corporation; (2007)., 4th ed. Allured Pub Corp; 4th edition (February 28, 2007), Carol Stream

39. Stein;, Mirokhin D, D; T, et al (2011) The NIST mass spectral search program for the nist/epa/nih mass spectra library. Standard Reference Data Program of the National Institute of Standards and Technology. Gaithers-burg, MD, US

40. (CLSI) C and LSI (2006) (Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA

41. Knezevic P, Aleksic V, Simin N, et al (2016) Antimicrobial activity of Eucalyptus camaldulensis essential oils and their interactions with conventional antimicrobial agents against multi-drug resistant Acinetobacter baumannii. J Ethnopharmacol 178:125–136 .

doi: 10.1016/j.jep.2015.12.008

42. Becke AD (1993) Density-functional thermochemistry. III. The role of exact exchange. J Chem Phys 98:5648–5652 . doi: 10.1063/1.464913

43. Lee C, Yang W, Parr RG (1988) Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys Rev B 37:785–789 . doi: 10.1103/PhysRevB.37.785

44. Frisch MJ, Trucks GW, Schlegel HB, et al (2009) Gaussian 09. 2–3

45. Thomsen R, Christensen MH (2006) MolDock: a new technique for high accuracy molecular docking. J Med Chem 49:3315–3321

46. Sogabe S, Masubuchi M, Sakata K, et al (2002) Crystal Structures of Candida albicans N-Myristoyltransferase with Two Distinct Inhibitors. Chem Biol 9:1119–1128 . doi: 10.1016/S1074-5521(02)00240-5

47. Qiu X, Janson C a, Court RI, et al (1999) Molecular basis for triclosan activity involves a flipping loop in the active site. Protein Sci 8:2529–2532 . doi: 10.1110/ps.8.11.2529

48. Ramón-Maiques S, Marina A, Guinot A, et al (2010) Substrate binding and catalysis in carbamate kinase ascertained by crystallographic and site-directed mutagenesis studies: Movements and significance of a unique globular subdomain of this key enzyme for fermentative ATP production in bacteria. J Mol Biol 397:1261–1275 . doi: 10.1016/j.jmb.2010.02.038

49. Wallock-Richards DJ, Marles-Wright J, Clarke DJ, et al (2015) Molecular basis of Streptococcus mutans sortase A inhibition by the flavonoid natural product trans-chalcone. Chem Commun 51:10483–10485 . doi: 10.1039/c5cc01816a

50. Wang J, Wolf RM, Caldwell JW, et al (2004) Development and testing of a general Amber force field. J Comput Chem 25:1157–1174 . doi: 10.1002/jcc.20035

51. Cornell WD, Cieplak P, Bayly CI, Kollman PA (1993) Application of RESP Charges To Calculate Conformational Energies, Hydrogen Bond Energies, and Free Energies of Solvation. J Am Chem Soc 115:9620–9631 . doi: 10.1021/ja00074a030

52. Wang J, Cieplak P, Kollman PA (2000) How Well Does a Restrained Electrostatic Potential (RESP) Model Perform in Calculating Conformational Energies of Organic and Biological Molecules? J Comput Chem 21:1049–1074 . doi: 10.1002/1096-987X(200009)21:12<1049::AID-JCC3>3.0.CO;2-F

53. Dolinsky TJ, Nielsen JE, McCammon JA, Baker NA (2004) PDB2PQR: An automated pipeline for the setup of Poisson-Boltzmann electrostatics calculations. Nucleic Acids Res 32:665–667 . doi: 10.1093/nar/gkh381

54. Li H, Robertson AD, Jensen JH (2005) Very fast empirical prediction and rationalization of protein pK a values. Proteins Struct Funct Genet 61:704–721 . doi: 10.1002/prot.20660

55. Maier JA, Martinez C, Kasavajhala K, et al (2015) ff14SB: Improving the Accuracy of Protein Side Chain and Backbone Parameters from ff99SB. J Chem Theory Comput 11:3696–3713 . doi: 10.1021/acs.jctc.5b00255

56. Jorgensen WL, Chandrasekhar J, Madura JD, et al (1983) Comparison of simple potential functions for simulating liquid water. J Chem Phys 79:926–935 . doi: 10.1063/1.445869

57. Case DA, Cheatham TE, Darden T, et al (2005) The Amber biomolecular simulation programs. J Comput Chem 26:1668–1688 . doi: 10.1002/jcc.20290

58. Cruz JN, Costa JFS, Khayat AS, et al (2018) Molecular dynamics simulation and binding free energy studies of novel leads belonging to the benzofuran class inhibitors of Mycobacterium tuberculosis Polyketide Synthase 13. J. Biomol. Struct. Dyn. 1102:1–12

59. Darden T, York D, Pedersen L (1993) Particle mesh Ewald: An N ·log( N ) method for Ewald sums in large systems. J Chem Phys 98:10089–10092 . doi: 10.1063/1.464397

60. Ryckaert JP, Ciccotti G, Berendsen HJC (1977) Numerical integration of the cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. J Comput Phys 23:327–341 . doi: 10.1016/0021-9991(77)90098-5

61. Lzaguirre JA, Catarello DP, Wozniak JM, Skeel RD (2001) Langevin stabilization of molecular dynamics. J Chem Phys 114:2090–2098 . doi: 10.1063/1.1332996

62. Hou T, Wang J, Li Y, Wang W (2011) Assessing the Performance of the MM / PBSA and MM / GBSA Methods . I . The Accuracy of Binding Free Energy Calculations Based on Molecular Dynamics Simulations. J Chem Inf Model 51:69–82 . doi: 10.1021/ci100275a

63. Kollman PA, Massova I, Reyes C, et al (2000) Calculating Structures and Free Energies of Complex Molecules: Combining Molecular Mechanics and Continuum Models. Acc Chem Res 33:889–897 . doi: 10.1021/ar000033j

64. Issn O (2000) Revista de Biología Tropical. Rev Biol Trop 11:1–15

65. Nelita G ccedil alves F de B, Mir eacute ia ABP, Vany F, et al (2015) Antimicrobial activity and medicinal biomass of Siparuna guianensis in Brazilian Cerrado forest, a global hotspot. J Med Plants Res 9:968–980 . doi: 10.5897/JMPR2015.5884

66. Andrade M, das Graças Cardoso M, de Andrade J, et al (2013) Chemical Composition and Antioxidant Activity of Essential Oils from Cinnamodendron dinisii Schwacke and Siparuna guianensis Aublet. Antioxidants 2:384–397 . doi: 10.3390/antiox2040384

67. Fischer DCH, Limberger RP, Henriques AT, Moreno PRH (2005) Essential oils from fruits and Leaves of Siparuna guianensis (Aubl.) Tulasne from Southeastern Brazil. J Essent Oil Res 17:101–102 . doi: 10.1080/10412905.2005.9698844

68. Zoghbi M das GB, Andrade EHA, Santos AS, et al (1998) Essential Oils of Siparuna guianensis Aubl. J Essent Oil Res 10:543–546 . doi: 10.1080/10412905.1998.9700966

69. Machado SMF, Ribeiro VAFA, Militão JSLT, et al (1998) Seasonal variation of (E)-nerolidol in siparuna guianensis aublet and13C-NMR spectral assignments of (E)- and (Z)-nerolidol. J Essent Oil Res 10:708–710 . doi: 10.1080/10412905.1998.9701017

70. Viana FA, Andrade-Neto M, Pouliquen YBM, et al (2002) Essential oil of siparuna guianensis aublet from the amazon region of Brazil. J Essent Oil Res 14:60–62 . doi: 10.1080/10412905.2002.9699765

71. Andrade EHA, Zoghbi MGB, Machado LB (2004) Seasonal variation of germacrone and atractylone in the essential oil of Siparuna guianensis Aublet. Rev Bras Plantas Med 6:62–64

72. Smith-Palmer A, Stewart J, Fyfe L (1998) Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Lett Appl Microbiol 26:118–122 . doi: 10.1046/j.1472-765X.1998.00303.x

73. Cavaleiro C, Salgueiro L, Gonçalves MJ, et al (2015) Antifungal activity of the essential oil of Angelica major against Candida, Cryptococcus, Aspergillus and dermatophyte species. J Nat Med 69:241–248 . doi: 10.1007/s11418-014-0884-2

74. Resch M, Steigel A, Chen ZL, Bauer R (1998) 5-lipoxygenase and cyclooxygenase-1 inhibitory active compounds from Atractylodes lancea. J Nat Prod 61:347–350 . doi: 10.1021/np970430b

75. Ogunwande IA, Olawore NO, Ekundayo O, et al (2005) Studies on the essential oils composition, antibacterial and cytotoxicity of Eugenia uniflora L. Int J Aromather 15:147–152 . doi: 10.1016/j.ijat.2005.07.004

76. Govindarajan M, Rajeswary M, Senthilmurugan S, et al (2018) Curzerene, trans-β-elemenone, and γ-elemene as effective larvicides against Anopheles subpictus, Aedes albopictus, and Culex tritaeniorhynchus: toxicity on non-target aquatic predators. Environ Sci Pollut Res 25:10272–10282 . doi: 10.1007/s11356-017-8822-y

## FIGURE CAPTION

Figure 1 Leaves of *S. guianensis* before collection

Figure 2 - Ion chromatogram relative to the chemical composition *S. guianensis* essential oil.

Figure 3 - 2D Chemical structures of the main constituents identified by GC/MS in *S. guianensis* essential oil.

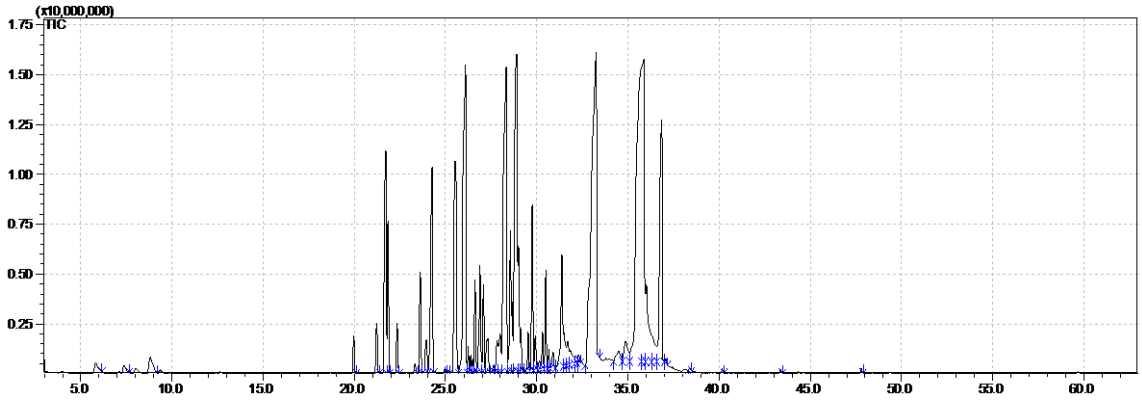
Figure 4 - Molecular interactions between ligand-receptor. (a) Molecular binding of atractylon with the protein N-myristoyltransferase of the microorganism *C. Albicans,* (b) Molecular binding of atractylon with the protein Enoyl reductase of the microorganism *E. Coli,* (c) Molecular binding of atractylon with the protein Carbamate kinase of the microorganism *E. faecalis,* and (d) Molecular binding of atractylon with the protein Sortase A of the microorganism *S. mutans*.

Figure 5 - RMSD of systems for 100 ns of MD simulations. The black colour was used to colour the backbone of all proteins, whereas various colours were used for the ligand RMSD. (a)

RMSD plot of the atractylon/N-myristoyltransferase system (*C. albicans*), (b) RMSD plot of the atractylon/Enoyl reductase system (*E. coli*), (c) RMSD plot of the atractylon/Carbamate kinase system (*E. faecalis*), and (d) RMSD plot of the atractylon/Sortase A system (*S. mutans*).

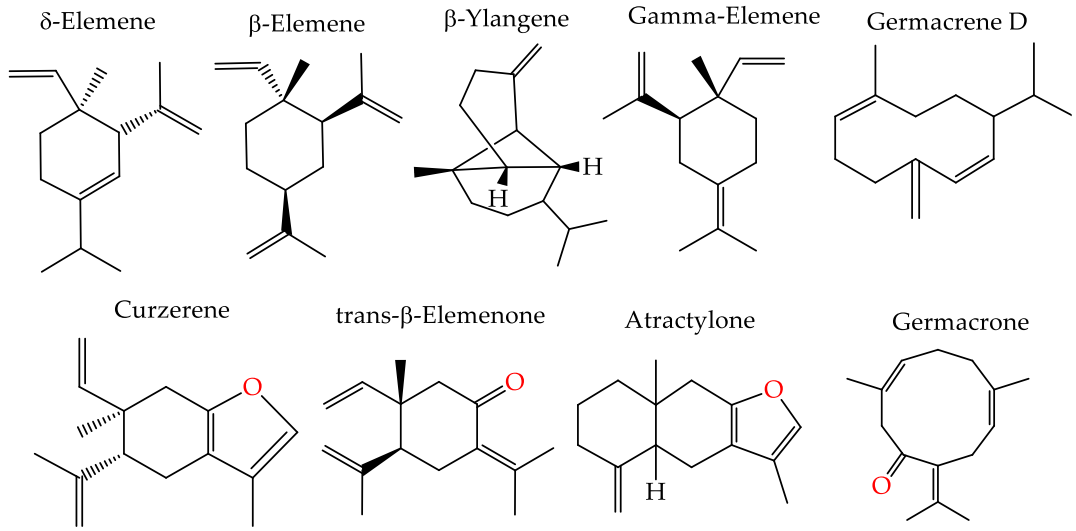
Figure 1

Figure 2



606 Figure 3

607

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609 Figure 4

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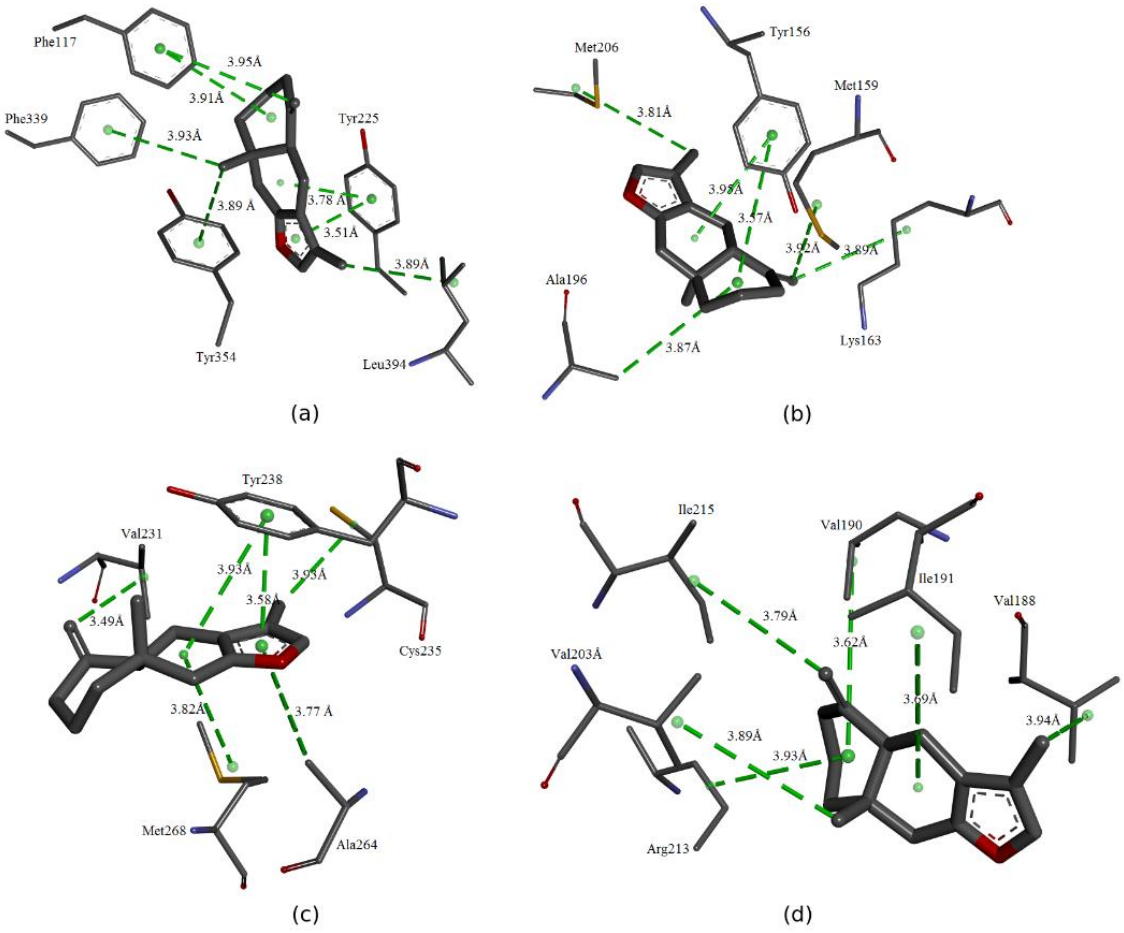


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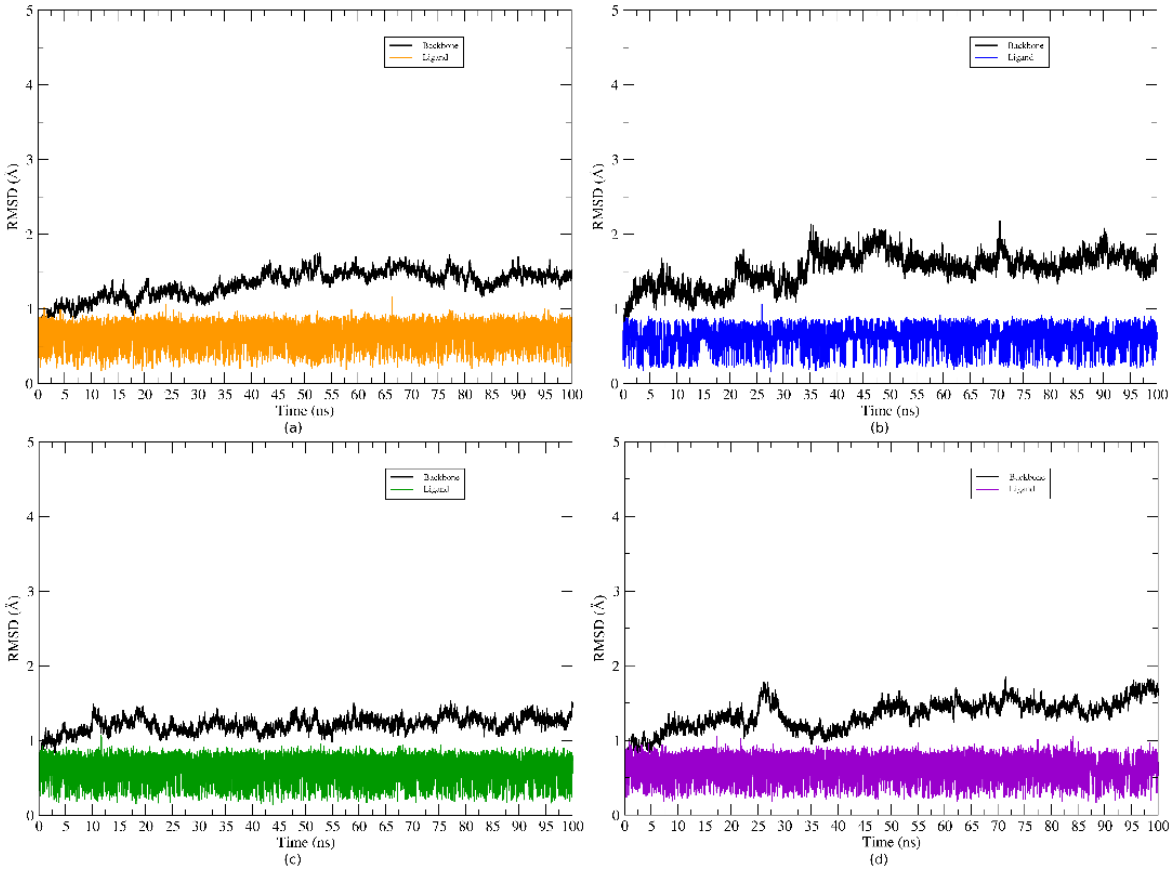
612

613 Figure 5

614




615

616 Table 1 - Chemical compounds identified in the essential oil of *S. guianensis* and their relative
617 concentrations (%).

| RI | Compound | Concentration (%) |
|---|---|---|
| 933 | α-Pinene | 0.33 |
| 978 | β-Pinene | 0.04 |
| 989 | Myrcene | 0.22 |
| 1008 | α-Phellandrene | 0.15 |
| 1030 | Sylvestrene | 0.51 |
| 1044 | (E)-β-Ocimene | 0.07 |
| 1127 | allo-Ocimene | 0.03 |
| 1292 | Undecan-2-one | 0.38 |
| **1331** | **δ-Elemene** | **5.38** |
| 1345 | α-Cubebene | 0.48 |
| 1367 | α-Ylangene | 0.12 |
| 1373 | α-Copaene | 1.1 |
| 1381 | β-Bourbonene | 0.52 |
| **1388** | **β-Elemene** | **3.13** |
| 1392 | α-Funebrene | 0.03 |
| 1404 | α-Gurjunene | 0.06 |
| 1408 | (E)-Caryophyllene | 0.03 |
| **1417** | **β-Ylangene** | **4.14** |
| **1430** | **γ-Elemene** | **7.04** |

| | | |
|---|---|---|
| 1434 | α-Guaiene | 0.23 |
| 1437 | Aromadendrene | 0.19 |
| 1439 | Guaia-6,9-diene | 0.12 |
| 1449 | cis-Muurola-3,5-diene | 1.4 |
| 1453 | α-Humulene | 0.86 |
| 1457 | Alloaromadendrene | 0.29 |
| 1459 | cis-Cadina-1(6),4-diene | 0.35 |
| 1466 | 9-epi-(E)-Caryophyllene | 0.09 |
| 1471 | γ-Gurjunene | 0.49 |
| 1475 | γ-Muurolene | 0.7 |
| **1482** | **Germacrene D** | **7.61** |
| 1488 | β-Selinene | 1.61 |
| 1490 | *trans*-Muurola-4(14),5-diene | 0.63 |
| **1496** | **Curzerene** | **7.1** |
| 1498 | α-Muurolene | 1.2 |
| 1501 | ε-Amorphene | 0.48 |
| 1506 | Germacrene A | 0.02 |
| 1511 | γ-Cadinene | 0.39 |
| 1516 | δ-Cadinene | 1.86 |
| 1521 | Zonarene | 0.48 |
| 1530 | *trans*-Cadina-1,4-diene | 0.45 |
| 1534 | Selina-4(15),7(11)-diene | 1.04 |
| 1539 | Selina-3,7(11)-diene | 0.25 |
| 1556 | Germacrene B | 1.88 |
| 1582 | Allo-hedycaryol | 0.51 |
| 1592 | *cis*-β-Elemenone | 1.43 |
| **1602** | ***trans*-β-Elemenone** | **11.78** |
| 1633 | Cubenol<1-epi-> | 0.97 |
| 1643 | Cubenol | 1.15 |
| **1661** | **Atractylone** | **18.65** |
| **1694** | **Germacrone** | **5.26** |
| 1739 | Mint sulfide | 0.05 |
| | Monoterpene hydrocarbons | 1.35 |
| | sesquiterpene hydrocarbons | 44.65 |
| | Oxygenated sesquiterpenes | 46.85 |
| | Others | 0.43 |
| | Total | 93.28 |

*RI - Retention Index in Rtx-5MS.

622 Table 2 - Antimicrobial activity of the isolated essential oil from leaves of *Siparuna guianensis*

| **Sample/ Dilution (µL/mL)** | | A | B | C | d |
|---|---|---|---|---|---|
| 1 | **500** | - | - | MIC | - |
| 2 | **250** | - | MIC | + | - |
| 3 | **125** | MIC | + | + | MIC |
| 4 | **62,5** | + | + | + | + |
| 5 | **30,625** | + | + | + | + |
| 6 | **15,3** | + | + | + | + |
| 7 | **7,6** | + | + | + | + |
| 8 | **3,8** | + | + | + | + |
| 9 | **1,9** | + | + | + | + |
| 10 | **0,95** | + | + | + | + |
| Mean halo (mm), 10 µL, N=3 | | 11±0,12 | 12±0,57 | 11±0,31 | 12,5±0,98 |
| Control | | 22,5±0,32 | 28,10±0,13 | 15,25 ± 0,58 | 19,42 ± 1,22 |

623 * (a) *Streptococcus mutans* (ATCC 3440), (b) *Enterococcus faecalis* (ATCC 4083); (c) *Escherichia coli* (ATCC 25922); (d) *Candida albicans*
624 (ATCC- 10231). mm = millimeter

625

626 Table 3 - Docking score results

| **Targets** | **MolDock score** |
|---|---|
| *C. albicans* | -71.43 |
| *E. coli* | -87.24 |
| *E. faecalis* | -80.46 |
| *S. mutans* | -65.18 |

627

628 Table 4 Energy components and values of binding affinities. All values are in kcal/mol.

| **Targets** | **$\Delta E_{vdW}$** | **$\Delta E_{ele}$** | **$\Delta G_{GB}$** | **$\Delta G_{NP}$** | **$\Delta G_{MM-GBSA}$** |
|---|---|---|---|---|---|
| *C. albicans* | -22.28 | -5.51 | 13.74 | -13.11 | -25.16 |
| *E. coli* | -25.54 | -6.88 | 15.96 | -9.87 | -26.33 |
| *E. faecalis* | -19.56 | -5.02 | 8.96 | -8.22 | -23.84 |
| *S. mutans* | -24.35 | -3.74 | 9.75 | -9.13 | -27.47 |

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# 9 APÊNDICES / OUTROS TRABALHOS

## Artigos completos publicados em periódicos

⭐ **OLIVEIRA, M. S.**; COSTA, W. A. ; BEZERRA, F. W. F. ; Araújo, M. E. ; FERREIRA, G. C. ; CARVALHO JUNIOR, R. N. . Phytochemical profile and biological activities of Momordica charantia L. (Cucurbitaceae): A review. AFRICAN JOURNAL OF BIOTECHNOLOGY JCR, v. 17, p. 829-846, 2018.

Bezerra, Fernanda Wariss Figueiredo ; DA COSTA, WANESSA ALMEIDA ; DE OLIVEIRA, MOZANIEL SANTANA ; DE AGUIAR ANDRADE, ELOISA HELENA ; DE CARVALHO, RAUL NUNES . Transesterification of Palm Pressed-Fibers (Elaeis guineensis Jacq.) Oil by Supercritical Fluid Carbon Dioxide with Entrainer Ethanol. JOURNAL OF SUPERCRITICAL FLUIDS JCR, v. 136, p. 136-143, 2018.

CRUZ, J. N. ; OLIVEIRA, M. S. ; VOGADO, J. H. ; SILVAC, S. G. ; COSTA, W. A. ; BEZERRA, F. W. F. ; COSTA, R. A. ; CARVALHO JUNIOR, R. N. ; C. NETO, A. M. J. . Molecular Insights on the Interactions of Nitrosamines from Cigarette Smoking with CYP2A13 using Molecular Docking and Molecular Dynamics Simulation. Journal of Pulmonary Medicine and Respiratory Research, v. xxx, p. xxxx-xxxx, 2018.

DA COSTA, WANESSA ALMEIDA ; FIGUEIREDO BEZERRA, FERNANDA WARISS ; DE OLIVEIRA, MOZANIEL SANTANA ; DE AGUIAR ANDRADE, ELOÍSA HELENA ; DOS SANTOS, ANA PAULA MARTINS ; BORGES CUNHA, VÂNIA MARIA ; DOS SANTOS, DANIELE CRISTINA SILVA ; DA SILVA BANNA, DÉBORA ARIANE DORNELAS ; DE CARVALHO JUNIOR, RAUL NUNES . Supercritical CO2 Extraction and Transesterification of the Residual Oil from Industrial Palm Kernel Cake with Supercritical Methanol. JOURNAL OF SUPERCRITICAL FLUIDS JCR, v. xx, p. xxxx-xxxx, 2018.

PEREIRA, D. S. ; HOLANDA NETO, J. P. ; OLIVEIRA, M. S. ; PEREIRA, N. S. ; MARACAJA, P. B. ; SOUZA FILHO, A. P. S. . PHYTOTOXIC POTENTIAL OF THE GEOPROPOLIS EXTRACTS OF THE JANDAIRA STINGLESS BEE (Melipona subnitida) IN WEEDS. Revista Caatinga JCR, v. 30, p. 876-884, 2017.

## Livros publicados/organizados ou edições

1. RODRIGUES, A. C. ; FURTADO, A. C. S. ; SOUZA FILHO, A. P. S. ; SOUZA, B. C. P. ; PEREIRA, D. S. ; ILKIU-BORGES, F. ; BEZERRA, F. W. F. ; GUILHON, G. M. S. P. ; RIPARDO FILHO, H. S. ; BITTENCOURT, H. V. H. ; RODRIGUES, I. M. C. ; HOLANDA-NETO, J. P. ; CARRERA, J. C. ; MENEZES NETO, M. A. ; OLIVEIRA, M. S. ; TREZZI, M. M. ; MOREIRA, R. N. ; CARVALHO JUNIOR, R. N. ; VIDAL, R. A. ; XAVIER JUNIOR, S. R. ; RODRIGUES, S. T. ; MARDEGAN, S. ; BARBOSA, S. M. ; COSTA, W. A. . POACEAE Barnhart. 1. ed. Belém: Marques Editora, 2017. v. 1. 272p.

**Capítulos de livros publicados**

CUNHA, V. M. B. ; SILVA, M. P. ; COSTA, W. A. ; M.S.Oliveira ; BEZERRA, F. W. F. ; MELO, A. C. ; PINTO, R. H. H. ; MACHADO, N. T. ; Araújo, M. E. ; CARVALHO JUNIOR, R. N. . Carbon Dioxide Use in High Pressure Extraction Processes. Carbon Dioxide Chemistry. xed.Rijeka: Intechopen, 2018, v. 1, p. 000-000.

DA COSTA, WANESSA ALMEIDA ; DE OLIVEIRA, MOZANIEL SANTANA; da Silva, Marcilene Paiva ; Cunha, Vania Maria Borges ; Pinto, Rafael Henrique Holanda ; Bezerra, Fernanda Wariss Figueiredo ; Junior, Raul Nunes de Carvalho . Açaí (Euterpe oleracea) and Bacaba (Oenocarpus bacaba) as Functional Food. Superfood and Functional Food - An Overview of Their Processing and Utilization. 1ed.: InTech, 2017, v. 1, p. 155-172.

**M.S.Oliveira**; COSTA, W. A. ; BEZERRA, F. W. F. ; CARVALHO JUNIOR, R. N. . COMPOSIÇÃO QUÍMICA E ATIVIDADES BIOLOGICAS DE ESPÉCIE DA FAMILÍA POACEAE. COMPOSIÇÃO QUÍMICA E ATIVIDADES BIOLOGICAS DE ESPÉCIE DA FAMILÍA POACEAE. 1ed.Belém: Marques Editora, 2017, v. 1, p. 199-219.

**Trabalhos completos publicados em anais de congressos**

BEZERRA, F. W. F. ; BEZERRA, P. N. ; OLIVEIRA, M. S. ; COSTA, W. A. ; CARVALHO JUNIOR, R. N. ; ANDRADE, E. H. A. . IDENTIFICAÇÃO E QUANTIFICAÇÃO QUÍMICA DO ÓLEO ESSENCIAL DE PIPER ADUNCUM L.. In: XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos, 2018, Belém. Anais do XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos, 2018.

COSTA, W. A. ; OLIVEIRA, M. S. ; BEZERRA, F. W. F. ; BEZERRA, P. N. ; CARVALHO JUNIOR, R. N. ; SOUZA FILHO, A. P. S. . PHYSICO-CHEMICAL AND NUTRITIONAL CHARACTERIZATION OF COWPEA BEANS (VIGNA UNGUICULATA L. WALP.) FERTILIZED WITH K2O. In: XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos, 2018, Belém. Anais do XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos, 2018.

MENEZES, E. G. O. ; COSTA, W. A. ; BEZERRA, F. W. F. ; MOZANIEL, S.O. ; BEZERRA, P. N. ; CARVALHO JUNIOR, R. N. . PREDIÇÃO DAS PROPRIEDADES FÍSICAS E TERMODINÂMICAS DO ÓLEO DE PALMISTE POR METODOLOGIA DE CONTRIBUIÇÃO DE GRUPOS. In: XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos, 2018, Belém. anais do XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos, 2018.

CRUZ, J. N. ; OLIVEIRA, M. S. ; SILVAC, S. G. ; COSTA, W. A. ; BEZERRA, F. W. F. ; BEZERRA, P. N. ; SANTOS, G. X. ; C. NETO, A. M. J. ; CARVALHO JUNIOR, R. N. . Análise das Interações entre Nitrosaminas e CYP2A13 usando Ancoragem Molecular e Simulações de Dinâmica Molecular. In: 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018, São Luís. 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018.

MENDES, M. H. S. ; MEDEIROS, I. G. ; CRUZ, J. N. ; OLIVEIRA, M. S. ; COSTA, W. A. ; MIRANDA, G. S. ; SOARES, A. M. S. ; ALBUQUERQUE, S. M. ; LIMA, A. H. L. E. ; Brasil, D. S. B. . Remoção de Poluentes Orgânicos Usando Nanofiltragem: Um Estudo por Dinâmica Molecular. In: 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018, São Luís. 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018.

MAIA, M. A. ; SILVA, L. P. S. ; SANTOS, L. S. ; RIPARDO FILHO, H. S. ; OLIVEIRA, M. S. . Atividade potencial alelopática do extrato etanólico do caule de *Acacia mangium* sobre a germinação de sementes das espécies malícia e mata-pasto. In: 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018, São Luís. 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018.

MAIA, M. A. ; RIPARDO FILHO, H. S. ; SILVA, L. P. S. ; OLIVEIRA, M. S. ; SANTOS, L. S. . Atividade potencialmente alelopática do extrato etanólico das cascas do caule de Acacia mangium. In: 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018, São Luís. 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018.

SANTOS, G. X. ; OLIVEIRA, M. S. ; BEZERRA, P. N. ; LEAO JUNIOR, J. ; FREITAS, K. ; CRUZ, J. N. ; ANDRADE, E. H. A. ; MARQUES, E. ; CARVALHO JUNIOR, R. N. ; RIBEIRO, A. . Composição química do óleo essencial da Piper divaricatum da região metropolitana de Belém/PA. In: 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018, São Luís. 58º CONGRESSO BRASILEIRO DE QUÍMICA.

SILVAC, S. G. ; COSTA, R. A. C. ; CRUZ, J. N. ; OLIVEIRA, M. S. ; NASCIMENTO, L. D. ; COSTA, W. A. ; SOUZA FILHO, A. P. S. ; CARVALHO JUNIOR, R. N. ; MAIA, J. G. S. ; ANDRADE, E. H. A. . EFEITO FITOTÓXICO DO ÓLEO ESSENCIAL DE LIPPIA thymoides Mart. & Schauer (VERBENACEAE). In: 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018, São Luís. 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018.

OLIVEIRA, M. S.; COSTA, W. A. ; BEZERRA, F. W. F. ; CRUZ, J. N. ; SILVAC, S. G. ; BEZERRA, P. N. ; CORDEIRO, R. M. ; CARVALHO JUNIOR, R. N. ; SOUZA FILHO, A. P. S. . TEOR DE COMPOSTOS FENÓLICOS TOTAIS E FLAVONOIDES TOTAIS EM GEOPRÓPOLIS E PRÓPOLIS DE ABELHAS SEM FERRÃO DA AMAZÔNIA ORIENTAL. In: 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018, São Luís. 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018.

COSTA, W. A. ; BEZERRA, F. W. F. ; DE OLIVEIRA, MOZANIEL SANTANA ; BEZERRA, P. N. ; CRUZ, J. N. ; SILVAC, S. G. ; SILVA, C. C. E. ; SILVA, A. P. S. E. ; PINTO, R. H. H. ; CARVALHO JUNIOR, R. N. . COMPOSIÇÃO DE ÁCIDOS GRAXOS DE ÓLEO DE PALMISTE OBTIDO POR EXTRAÇÃO SUPERCRÍTICA. In: 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018, São Luís. 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018.

COSTA, W. A. ; BEZERRA, F. W. F. ; OLIVEIRA, M. S. ; BEZERRA, P. N. ; CRUZ, J. N. ; SILVAC, S. G. ; SILVA, A. P. S. E. ; SILVA, C. C. E. ; FERREIRA, M. C. R. ; CARVALHO JUNIOR, R. N. . TRANSESTERIFICAÇÃO SUPERCRÍTICA DE ÓLEO DE

PALMISTE USANDO METANOL. In: 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018, São Luís. 58º CONGRESSO BRASILEIRO DE QUÍMICA, 2018.

CORDEIRO, R. M. ; BEZERRA, F. W. F. ; PINTO, R. H. H. ; DA COSTA, W. A. ; **OLIVEIRA, M. S**. . DETERMINAÇÃO DE CAROTENOIDES EM EXTRATOS DE MURICI (BYRSONIMA CRASSIFOLIA L. RICH) OBTIDOS POR EXTRAÇÃO COM FLUIDO SUPERCRÍTICO. In: XXII Congresso Brasileiro de Engenharia Química, 2018, São Paulo. Blucher Chemical Engineering Proceedings. São Paulo: Blucher, 2018. p. 2705-2709.

COSTA, W. A. ; **OLIVEIRA, M. S**. ; SILVA, A. P. S. ; SILVA FILHO, F. G. S. ; BEZERRA, F. W. F. ; MARTELLI, M. C. . DEVELOPMENT OF CERAMICS ENAMEL USING BOTTLE GLASS. In: XXII Congresso Brasileiro de Engenharia Química, 2018, São Paulo. Blucher Chemical Engineering Proceedings. São Paulo: Blucher, 2018. p. 1871-685.

COSTA, W. A. ; SILVA, C. J ; LIMA, C. R ; **OLIVEIRA, M. S**. ; GARCIA, K. C. S. P ; SANTOS, A. P. M ; SILVA, A. P. S. E ; CARVALHO JUNIOR, R. N. ; MARTELLI, M. C. ; BRASIL, D. S. B. . PROPRIEDADES FÍSICAS DA ADSORÇÃO DE BTX SOBRE CARVÃO ATIVADO. In: 15º Encontro de Profissionais da Química da Amazônia, 2017., 2017, Belém. 15º Encontro de Profissionais da Química da Amazônia, 2017.

COSTA, W. A. ; **OLIVEIRA, M. S**. ; PINTO, R. H. H. ; GARCIA, K. C. S. P ; SANTOS, A. P. M ; SILVA, C. J. ; BEZERRA, F. W. F. ; CARVALHO JUNIOR, R. N. ; SOUZA FILHO, A. P. S. . ARACTERIZAÇÃO FÍSICO-QUÍMICA E NUTRICIONAL DE FEIJÃO-DA-COLÔNIA (Vigna unguiculata L. Walp.) ADUBADO COM P2O5. In: 15º Encontro de Profissionais da Química da Amazônia, 2017, Belém. 15º Encontro de Profissionais da Química da Amazônia, 2017.

COSTA, W. A. ; SILVA, C. J ; LIMA, C. R. ; **OLIVEIRA, M. S**. ; SANTOS, A. P. M ; GARCIA, K. C. S. P ; MELO, A. C. ; CARVALHO JUNIOR, R. N. ; MARTELLI, M. C. ; BRASIL, D. S. B. . SIMULAÇÃO DA ADSORÇÃO DE TOLUENO SOBRE CARVÃO ATIVADO. In: 15º Encontro de Profissionais da Química da Amazônia, 2017, Belém. 15º Encontro de Profissionais da Química da Amazônia, 2017.

**M.S.OLIVEIRA**; Oliveira, A. H ; MENEZES, C. ; PEREIRA, D. S. ; CORDEIRO, H. K. C. ; COSTA, W. A. ; VASCONCELOS, M. A. M. ; VENTURIERI, G. C. ; SOUZA FILHO, A. P. S. . ANÁLISE QUALITATIVA DE COMPOSTOS FENÓLICOS E ÍNDICE DE OXIDAÇÃO EM AMOSTRAS DE GEOPRÓPOLIS E PRÓPOLIS DE ABELHAS SEM FERRÃO DO ESTADO DO PARÁ. In: 56° CONGRESSO BRASILEIRO DE QUÍMICA, 2016, BELÉM. 56° CONGRESSO BRASILEIRO DE QUÍMICA, 2016.

COSTA, W. A. ; SILVA FILHO, F. G. S. ; M.S.Oliveira ; CARVALHO JUNIOR, R. N. ; MARTELLI, M. C. ; BRASIL, D. S. B. . ESTUDO DA ADSORÇÃO DE p-XILENO SOBRE CARVÃO ATIVADO. In: 56° CONGRESSO BRASILEIRO DE QUÍMICA, 2016, BELÉM. 56° CONGRESSO BRASILEIRO DE QUÍMICA.

COSTA, W. A. ; BEZERRA, F. W. F. ; SANTOS, A. P. M. ; CORDEIRO, R. M. ; TUMA, J. C. ; PINTO, R. H. H. ; M.S.Oliveira ; CARVALHO JUNIOR, R. N. . CARACTERIZAÇÃO FÍSICO-QUÍMICA DA TORTA RESULTANTE DO PROCESSAMENTO DA AMÊNDOA DO DENDÊ (Elaeis guineensis Jacq.). In: 56° CONGRESSO BRASILEIRO DE QUÍMICA, 2016, BELÉM. 56° CONGRESSO BRASILEIRO DE QUÍMICA, 2016.

COSTA, W. A. ; SENA, R. S. F. ; **M.S.OLIVEIRA** ; BEZERRA, F. W. F. ; CORDEIRO, R. M. ; CARVALHO JUNIOR, R. N. ; SILVA FILHO, F. G. S. ; MARTELLI, M. C. . DESENVOLVIMENTO DE ESMALTE CERÂMICO UTILIZANDO FRITA BRANCA PARA APLICAÇÃO NA CERÂMICA DE ICOARACI. In: 56° CONGRESSO BRASILEIRO DE QUÍMICA, 2016, BELÉM. 56° CONGRESSO BRASILEIRO DE QUÍMICA, 2016.

BEZERRA, F. W. F. ; COSTA, W. A. ; CORDEIRO, R. M. ; **M.S.OLIVEIRA** ; OLIVERA, G. R. F. ; CARVALHO JUNIOR, R. N. . MODELAGEM MATEMÁTICA DA CURVA CINÉTICA DO EXTRATO DA CASCA DA UNHA-DE-GATO (UNCARIA GUIANENSIS) OBTIDO POR CO2 SUPERCRÍTICO. In: XXV Congresso Brasileiro de Ciência e Tecnologia de Alimentos/ X CIGR Section IV Technical Symposium., 2016, Gramado. XXV Congresso Brasileiro de Ciência e Tecnologia de Alimentos/ X CIGR Section IV Technical Symposium., 2016.

**M.S.OLIVEIRA**; BEZERRA, F. W. F. ; SILVA, M. V. C. ; GUIMARES SOBRINHO, A. C. ; MENDES, K. A. ; SANTOS, J. L. ; COSTA, W. A. ; CARVALHO JUNIOR, R. N. ; RODRIGUES, A. M. C. . CINÉTICA DE SECAGEM CONVECTIVA DE CARÁ BRANCO (DIOSCOREA SSP.) DA REGIÃO AMAZÔNICA. In: XXV Congresso Brasileiro de Ciência e Tecnologia de Alimentos/ X CIGR Section IV Technical Symposium., 2016, Gramado. XXV Congresso Brasileiro de Ciência e Tecnologia de Alimentos/ X CIGR Section IV Technical Symposium., 2016.

**Artigos aceitos para publicação**

1. MEDEIROS, I. G.; CRUZ, J. N. ; **OLIVEIRA, M. S**. ; COSTA, W. A. ; LIMA, L. H. A. ; BRASIL, L. S. N. S ; CARVALHO JUNIOR, R. N. ; C. NETO, A. M. J. ; BRASIL, D. S. B. . Removal of Organic Pollutants Benzene and Phenol Using Nanofiltration: A Molecular Dynamics Study. **JOURNAL OF NANOSCIENCE AND NANOTECHNOLOGY**, 2019.

2. COSTA, W. A.; BEZERRA, F. W. F.; **OLIVEIRA, M. S**. ; CUNHA, V. M. B. ; SILVA, M. P. ; CARVALHO JUNIOR, R. N. . Appliance of a High Pressure semi-Batch Reator : Supercritical Transesterification of Soybean oil using Methanol. **CIÊNCIA E TECNOLOGIA DE ALIMENTOS** (ONLINE), 2019.

3. BEZERRA, F. W. F.; BEZERRA, P. N. ; **OLIVEIRA, M. S**. ; COSTA, W. A. ; FERREIRA, G. C. ; CARVALHO JUNIOR, R. N. . Bioactive Compounds and Biological Activity of Croton Species (Euphorbiaceae): An Overview. **CURRENT BIOACTIVE COMPOUNDS**, 2019.