**UNIVERSIDADE FEDERAL DO CEARÁ**
**FACULDADE DE FARMÁCIA, ODONTOLOGIA E ENFERMAGEM**
**DEPARTAMENTO DE ANÁLISES CLÍNICAS E TOXICOLÓGICAS**
**PROGRAMA DE PÓS-GRADUAÇÃO EM DESENVOLVIMENTO E INOVAÇÃO TECNOLÓGICA EM MEDICAMENTOS**

**ROSUETI DIÓGENES DE OLIVEIRA FILHO**

# DESENVOLVIMENTO E CARACTERIZAÇÃO DE COMPLEXOS MOLECULARES DE ÓLEO ESSENCIAL DE *Alpinia zerumbet* (PERS.) BURTT & SMITH COM SULFOBUTIL-ÉTER-β-CICLODEXTRINA (CAPTISOL®)

**FORTALEZA**

**2019**

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Tese apresentada ao Programa de Pós-Graduação em Desenvolvimento e Inovação Tecnológica em Medicamentos da Universidade Federal do Ceará, como parte dos requisitos para obtenção do título de Doutor em Inovação Tecnológica em Medicamentos. Área de concentração: Inovação Tecnológica em Medicamentos.

Orientadora: Profª. Dra. Marta Maria de França Fonteles

Co-orientador: Prof. Dr. Renato de Azevedo Moreira

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A Deus.

A meus pais, Rosueti e Edneide.

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“A ciência nunca resolve um problema sem criar pelo menos outros dez”. (George Bernard Shaw)

## RESUMO

**Desenvolvimento e caracterização de complexos moleculares de óleo essencial de *Alpinia zerumbet* (Pers.) Burtt & Smith com sulfobutil-éter-β-cilodextrina (Captisol®)**. Rosueti Diógenes de Oliveira Filho. Orientadora: Profa. Dra. Marta Maria de França Fonteles. Tese de Doutorado em Desenvolvimento e Inovação Tecnológica em Medicamentos. Universidade Federal do Ceará, 2019.

O presente trabalho é composto por dois capítulos distintos. No primeiro capítulo, intitulado "Atividades biológicas e aplicações farmacológicas de ciclodextrinas complexadas com óleos essenciais e seus componentes voláteis: uma revisão sistemática", aborda-se uma revisão com estudos *in vitro* e *in vivo* que avaliaram óleos essenciais (OEs) e seus componentes voláteis (CVs) complexados com diferentes ciclodextrinas (CDs), a formação de complexos de inclusão (CIs) e sua influência em diferentes atividades biológicas e ensaios pré-clínicos. Os termos de busca 'Ciclodextrina', 'Complexo de inclusão', 'Óleos voláteis', 'Óleo essencial' e 'Componentes voláteis' foram usados para recuperar artigos nas bases de dados PUBMED, MEDLINE e SCOPUS, resultando na seleção de 38 artigos, 22 de estudos *in vitro* e 16 de estudos pré-clínicos, que foram discutidos e constatou-se que as CDs promoveram uma melhor eficácia terapêutica devido ao aumento de solubilidade, estabilidade e biodisponibilidade dos OEs e CVs. O segundo capítulo, intitulado "Preparo, caracterização e docking molecular do óleo essencial de *Alpinia zerumbet* complexado com sulfobutil-éter-β-ciclodextrina", teve como objetivo extrair e identificar os componentes do óleo essencial de *Alpinia zerumbet* (OEAz) por cromatografia gasosa acoplada à espectrometria de massas (CG-EM) e avaliar sua atividade antimicrobiana frente às cepas de microrganismos patogênicos (*klebsiella pneumoniae* ATCC 10031, *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538P, *Candida parapsilosis* ATCC 90018, *Candida albicans* ATCC 10231 e *Candida tropicallis* ATCC 750). O OEAz foi complexado com sulfobutil-éter-β-ciclodextrina (SBE-β-CD) para formação dos CIs. Os complexos foram preparados por co-precipitação (CI 1 com 24 h e CI 2 com 6 h) e por mistura física (MF), todos na razão molar estequiométrica de 1:1 (OEAz:SBE-β-CD) e avaliados por espectroscopia de infravermelho com transformada de Fourier (IVTF), análise térmogravimétrica (ATG), calorimetria exploratória diferencial (CED) e simulação de doking molecular, este último utilizando os constituintes majoritários do OEAz. Como resultados, o OEAz apresentou terpinen-4-ol (22,37%) e 1,8-cienol (19,65%) como constituintes majoritários e atividade antimicrobiana contra todas as cepas patogênicas testadas. Na análise por IVTF, ocorreram deslocamentos de bandas do modo de alongamento O-H do espectro de SBE-β-CD em relação ao CI 1 (3446 para 3421 $cm^{-1}$, 2938 para 2931 $cm^{-1}$ e 1161 para 1159 $cm^{-1}$) e desaparecimento da banda 2357 $cm^{-1}$ presente apenas no espectro de OEAz; a caracterização térmica indicou uma diminuição na perda de massa do OEAz de 95,13% para 55,7, 55,8 e 53,78 em CI 1, CI 2 e MF, respectivamente. Dados de docking molecular revelaram interação de 1,8-cienol ($\Delta E_{bind}$ = -97,20 Kcal/mol) próximo ao centro da cavidade da SBE-β-CD sugerindo formação de complexo de inclusão, enquanto o terpinen-4-ol ($\Delta E_{bind}$ = -120,46 Kcal/mol) interagiu por fora da cavidade. Portanto, o deslocamento de bandas do espetro no IVTF, o aumento da estabilidade térmica com diminuição da perda de massa nas análises termogravimétricas e a conformação no docking molecular de um dos componentes majoritários do OEAz sugerem a formação de complexos moleculares no CI 1 obtido com 24 h, sendo uma potencial alternativa para desenvolvimento biotecnológico de novas formulações para potencial aplicação terapêutica.



**Palavras-chave:** Óleo Essencial, *Alpinia zerumbet*, SBE-β-CD, Complexos de Inclusão, Docking Molecular.

## ABSTRACT

**Development and characterization of molecular complexes of *Alpinia zerumbet* (Pers.) Burtt & Smith essential oil with sulfobutylther-β-cyclodextrin (Captisol®)**. Rosueti Diógenes de Oliveira Filho. Supervisor: Professor PhD Marta Maria from França Fonteles. Doctoral thesis in Development and Technological Innovation in Drugs. Federal University of Ceara, 2019.

The present study consists of two distinct chapters. In the first chapter, entitled "Biological activities and pharmacological applications of cyclodextrins complexed with essential oils and their volatile components: a systematic review", a review with *in vitro* and *in vivo* studies that evaluated essential oils (EOs) and their volatile components (VCs) complexed with different cyclodextrins (CDs), the formation of inclusion complexes (ICs) and their influence on different biological activities and pre-clinical trials. The search terms 'Cyclodextrin', 'Inclusion complex', 'Volatile oils', 'Essential oil' and 'Volatile components' were used to retrieve articles from the PUBMED, MEDLINE and SCOPUS databases, resulting in the selection of 38 articles, 22 from *in vitro* studies and 16 from preclinical studies, which were discussed and it was found that CDs promoted better therapeutic efficacy due to increased solubility, stability and bioavailability of EOs and VCs. The second chapter, entitled "Preparation, characterization and molecular docking of *Alpinia zerumbet* essential oil complexed with sulfobutylether-β-cyclodextrin", aimed to extract and identify the components of essential oil of *Alpinia zerumbet* (EOAz) by gas chromatography coupled mass spectrometry (GC-MS) and evaluate its antimicrobial activity against strains of pathogenic microorganisms (*klebsiella pneumoniae* ATCC 10031, *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538P, *Candida parapsilosis* ATCC 90018, *Candida albicans* ATCC 10231 e *Candida tropicallis* ATCC 750). The OEAz was complexed with sulfobutylether-β-cyclodextrin (SBEβCD) to form ICs. The complexes were prepared by co-precipitation (IC 1 at 24 h and IC 2 at 6 h) and by physical mixture (PM), all in a 1:1 stoichiometric molar ratio (EOAz:SBEβCD) and evaluated by Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), differential scanning calorimetry (CSD) and molecular doking simulation, the latter using the major components of EOAz. As results, EOAz showed terpinen-4-ol (22.37%) and 1.8-cyienol (19.65%) as major constituents and antimicrobial activity against all pathogenic strains tested. In the FTIR analysis, displacements of bands of the OH stretching mode of the SBEβCD spectrum occurred in relation to IC 1 (3446 to 3421 $cm^{-1}$, 2938 to 2931 $cm^{-1}$ and 1161 to 1159 $cm^{-1}$) and disappearance of the 2357 $cm^{-1}$ band present only in the OEAz spectrum; thermal characterization indicated a decrease in EOAz mass loss from 95.13% to 55.7, 55.8 and 53.78 in IC 1, IC 2 and PM, respectively. Molecular docking data revealed an interaction of 1,8-cienol ($\Delta E_{bind}$ = -97.20 Kcal/mol) close to the center of the SBEβCD cavity suggesting formation of an inclusion complex, while terpinen-4-ol ($\Delta E_{bind}$ = -120.46 Kcal/mol) interacted outside the cavity. Therefore, the displacement of bands of the spectrum in the FTIR, the increase of the thermal stability with reduction of the mass loss in the thermogravimetric analyzes and the conformation in the molecular docking of one of the major components of the EOAz suggest the formation of molecular complexes in the IC 1 obtained with 24 h, being a potential alternative for the biotechnological development of new formulations for potential therapeutic application.



**Keywords:** Essential Oil, *Alpinia zerumbet,* SBEβCD, Inclusion Complex, Molecular Docking.

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## LISTA DE ABREVIATURAS E SIGLAS

| | |
|---|---|
| ATCC | American Type Culture Collection |
| ATG | Análise Termogravimétrica |
| B16-F10 | Tumor cell line of mouse melanoma |
| BCS | Biopharmaceutics Classification System |
| BEAS-2B | Human bronchial epithelial cells |
| BHI | Brain heart infusion |
| C1 | Complexo de inclusão EOAz: SBEβCD obtido por 24 h |
| C10 | 10 carbons |
| C2 | Complexo de inclusão EOAz: SBEβCD obtido por 6 h |
| C5 | 5 carbons |
| CDs | Cyclodextrins |
| CED | Calorimetria Exploratória Diferencial |
| CFU | Colony forming unit |
| CG-EM | Cromatografia Gasosa acoplada à Espectrometria de Massas |
| CI | Complexo de inclusão |
| CI 1 | Complexo de inclusão OEAz:SBE-β-CD obtido com 24 h |
| CI 2 | Complexo de inclusão OEAz:SBE-β-CD obtido com 6 h |
| CLSI | Clinical and Laboratory Standard Institute |
| CVs | Componentes voláteis |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl |
| DSC | Differential Scanning Calorimetry |
| EOAz | Essential oil of *Alpinia zerumbet* |
| EOAz | Essental Oil of *Alpinia zerumbet* |
| EOs | Essential oils |
| FDA | Food and Drug Administration |
| FTIR | Fourier-Transform Infrared Spectroscopy |
| FTIR | Espectroscopia de Infravermelho com Transformada de Fourier |
| GC-MS | Gas Chromatography coupled to Mass Spectrometry |
| GRAS | Generally Recognized as Safe |
| HepG2 | Tumour cell line of human hepatocellular carcinoma |
| HL-60 | Tumour cell line human promyelocytic leukaemia |
| HPβCD | Hydroxypropyl-β-cyclodextrin |

| | |
|---|---|
| HPγCD | Hydroxypropyl γ-cyclodextrin |
| i.p. | intraperitoneal |
| IC 1 | EOAz:SBEβCD inclusion complex obtained for 24 h |
| IC 2 | EOAz:SBEβCD inclusion complex obtained for 6 h |
| ICs | Inclusion Complexes |
| IVTF | Espectroscopia no Infravermelho com Transformada de Fourier |
| K562 | Tumour cell line of human chronic myelocytic leukaemia |
| MeSH | Medical Subject Headings |
| MF | Mistura física |
| MIC | Minimum inhibitory concentration |
| MLC | Minimum lethal concentration |
| MβCD | Methyl-β-cyclodextrin |
| Nd | No data |
| OEAz | Óleo essencial de *Alpinia zerumbet* |
| p.o. | Oral administration |
| PM | Physical mixture |
| SBEβCD | Sulfobutyl-ether-β-cyclodextrin |
| SD | Sprague-Dawley |
| SUS | Sistema Único de Saúde |
| TGA | Thermogravimetric analysis |
| TNF-α | Tumor necrosis factor-α |
| VCs | Volatile components |
| VFA | Volatile fatty acids |
| XFSWD | Xiang-Fu-Si-Wu Decoction |
| αCD | α-cyclodextrin |
| βCD | β-cyclodextrin |
| γCD | γ-cyclodextrin |

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## 1. INTRODUÇÃO

O uso de plantas como recurso terapêutico pode ser traçado desde a antiguidade até os dias atuais, através de hábitos observacionais do ser humano e o meio que o cerca, resultando no surgimento de um conhecimento rico em fusões culturais, religiosas e étnicas que foi utilizado para tratar a saúde das pessoas (BADKE *et al*., 2011). As plantas medicinais são consideradas como rica fonte de remédios tradicionais e dessas plantas são obtidos muitos fitofármacos. Os metabólitos secundários são geralmente responsáveis pelas características biológicas das espécies vegetais utilizadas em todo o mundo (DAR; SHAHNAWAZ; QAZI, 2017).

A família Zingiberaceae é a maior da ordem Zingiberales, constituída de 53 gêneros, sendo *Alpinia* o maior gênero da família, e mais de 1.200 espécies nativas encontradas em regiões tropicais, especialmente do sul e sudeste da Ásia (JOHN-KRESS; PRINCE; WILLIANS, 2002). Dentre elas, a *Alpinia zerumbet* (Pers.) Burtt & Smith, espécie escolhida nesse estudo, é conhecida pelos nomes populares de colônia, cuité-açu, pacová, gengibre-concha, cana-do-brejo e paco-seroso (ALBUQUERQUE; NEVES, 2005), tendo a *Alpinia speciosa* K. Schum. e *Alpinia nutans* Roscoe como nomes científicos sinônimos. É uma planta herbácea, rizomatosa, que atinge 2 ou 3 metros de altura com folhas aromáticas, longas, largas e brilhantes. As flores, que surgem no verão e outono, são róseas e brancas, agrupadas em inflorescências semi-pendentes e apresentam aroma suave e agradável (Figura 1) (SANTOS *et al.,* 2011).

**Figura 1**. Espécie vegetal *Alpinia zerumbet* (Pers.) Burtt & Smith cultivada no Horto de Plantas Medicinais Prof. Francisco José Abreu Matos representando as folhas (esquerda) e flores (direita). Fonte: O Autor (2019).

A *A. zerumbet* tem sido utilizada na medicina popular, predominantemente no tratamento de hipertensão e ansiedade (LAHLOU *et al.*, 2002), com ação antimicrobiana (VICTÓRIO *et al*., 2009; MENDES *et al*., 2015), atividade antifúngica (LEAL-CARDOSO *et al*., 2004), antioxidante (WONG; LIM; OMAR, 2009) e anti-inflamatória (SHAHINOZZAMAN *et al*., 2018).

Os óleos essenciais (OEs) são líquidos voláteis ou semi-sólidos, caracterizados por um forte odor e sintetizados como metabólitos secundários em plantas aromáticas, geralmente extraídos por hidrodestilação (BAKKALI *et al.,* 2008). A quantidade e composição desses óleos variam de acordo com a localização em diferentes órgãos da planta (flores, folhas, raízes, caules, sementes, casca e frutos inteiros) (NOVAK *et al*., 2005). As folhas contêm de 0,2-1,0 % de óleo essencial em relação à matéria seca, apresentando os monoterpenos terpine-4-ol, 1,8-cineol, α-terpineno e sabineno como componentes majoritários (LEAL-CARDOSO *et al.*, 2004; PINTO *et al.*, 2009).

Os OEs e seus constituintes são considerados os produtos naturais bioativos mais importantes derivados de plantas tradicionalmente usadas na medicina popular com amplo espectro de atividades biológicas como antibacteriana (CRUZ-GALVEZ *et al.*, 2013), antifúngica (LU; HAN; YAO, 2013), inseticida (BENELLI *et al.,* 2018) e larvicida (OSANLOOA *et al*., 2017), além de aplicações farmacológicas com efeitos anti-inflamatório (CALDEFIE-CHÉZET *et al*., 2006), analgésico (CHANDRASHEKAR; PRASANNA, 2010), antinociceptivo (LIMA *et al.,* 2009) e em doenças como diabetes (BOUKHRIS *et al.*, 2012), câncer (EDRIS, 2007), Alzheimer (PERRY *et al*., 2001) e cardiovasculares (BAKKALI *et al.*, 2008). Essas propriedades dos OEs estão diretamente correlacionadas à concentração e proporção dos constituintes, sendo dois ou três componentes bioativos principais em concentrações mais altas ou em sinergismo com outros constituintes presentes responsáveis por um papel distinto na atividade biológica (MAHMOUD; CROTEAU, 2002).

Estudos de atividade biológica do óleo essencial de *A. zerumbet* (OEAz) demonstraram um importante potencial farmacológico deste no controle da pressão arterial, atuante também na musculatura lisa do intestino como agente antiespasmódico e como anestésico local (LEAL-CARDOSO; FONTELES, 1999; LAHLOU *et al.*, 2002). Além de atividade antioxidante (ELZAAWELY; XUAN; TAWATA, 2007), antibacteriana (MENDES *et al.*, 2015), fungistática (LIMA *et al.*, 2009) e cicatrizante (SANTOS-JÚNIOR, 2013).

No entanto, a baixa solubilidade em água, alta volatilidade, meia-vida curta, susceptibilidade à degradação pela exposição ao ar, luz e calor limitam as aplicações terapêuticas dos OEs e seus componentes devido à baixa biodisponibilidade. A proteção desses compostos em formulações de sistemas de liberação de fármacos pode promover a integridade de suas bioatividades (SERAFINI, *et al*., 2012; PINHO *et al.*, 2014). Assim, a complexação de OEs em ciclodextrinas (CDs) é uma maneira de solucionar os problemas para melhorar a solubilidade e estabilidade evitando a degradação e volatilização de compostos bioativos nos OEs, além de transformá-los em pós dispersáveis em água (FERNANDES *et al.*, 2004; LIOLIOS *et al.*, 2009). As CDs são um dos agentes complexantes mais comumente usados pela indústria farmacêutica como excipientes farmacêuticos, devido ao seu baixo preço e alta produção, além de serem capazes de melhorar as propriedades biológicas, químicas, físicas e o perfil farmacológico de compostos bioativos, principalmente de plantas (PINHO *et al.*, 2014).

As CDs são oligossacarídeos macrocíclicos consistindo de 6, 7 ou 8 unidades de D-glucopiranose (nomeadamente α-, β-, ou γ-CD, respectivamente) ligadas por ligações glicosídicas α-(1,4) representados como uma estrutura de cone com superfície relativamente hidrofílica e cavidade central hidrofóbica (LI; LOH, 2008; MARQUES, 2010) (Figura 2). São moléculas quimica e fisicamente estáveis com cavidade capaz de conter moléculas hidrofóbicas em seu interior tornando-as ideais para a solubilização dos solutos em meio aquoso, devido à polaridade de sua superfície formando complexos de inclusão (CIs) que são utilizados para melhorar a estabilidade físico-química, reduzindo por sua vez as perdas por evaporação de compostos, no caso dos óleos essenciais (LIU *et al.*, 2001; LIU *et al.*, 2012).

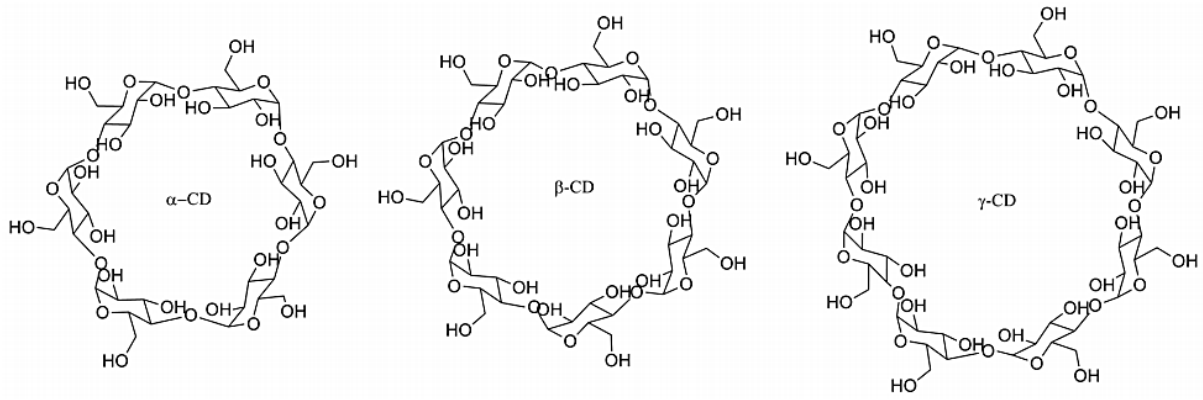


**Figure 2.** Estrutura química da α, β e γ-ciclodextrina, respectivamente. Fonte: Souza, Ferraz-Freitas e Oliveira (2016).

Além das CDs naturais (α-CD, β-CD, γ-CD), novas estruturas quimicamente modificadas como hidroxipropil-β-ciclodextrina (HP-β-CD), metil-β-ciclodextrina (M- β-CD) e sulfobutil-éter-β-ciclodextrina (SBE-β-CD) foram sintetizadas para melhorar as propriedades complexantes e solubidade em uma variedade de aplicações (BREWSTER; LOFTSSON, 2002; DEL VALLE, 2004). Algumas dessas CDs estudadas ganharam a aprovação da Food and Drug Administration (FDA) e são consideradas como sendo "geralmente reconhecidos como seguros" (GRAS) (LAKKAKULA; MACEDO, 2014).

Há diferentes métodos para obtenção dos CIs, como co-precipitação, homogeneização, mistura física, pasta de complexação, selagem, liofilização, secagem por pulverização e evaporação de solvente (MARQUES, 2010; DAS *et al.*, 2013). Estudos sobre OEs e seus componentes voláteis complexados em CDs são historicamente fundamentados em algumas revisões sobre o tema, evidenciando melhora da solubilidade como a principal característica obtida pelos CIs (MARQUES, 2010; PINHO *et al.*, 2014; LIMA *et al.*, 2016; WADHWA *et al.*, 2017).

A SBE-β-CD (Figura 3) é capaz de formar CIs com diferentes OEs, assim como seus componentes individuais (canfeno, β-cariofileno, p-cimeno, 1,8-cineol, estragol, limoneno, mirceno, α-pineno, β- pineno e γ-terpineno); enquanto, em comparação com CDs nativas, essas CDs exibem maior capacidade de complexação, eficiência na melhoria da solubilidade em água e sistema de liberação controlada, sendo um potencial agente de transporte e solubilização para OEs e seus componentes (KFOURY *et al.*, 2017).

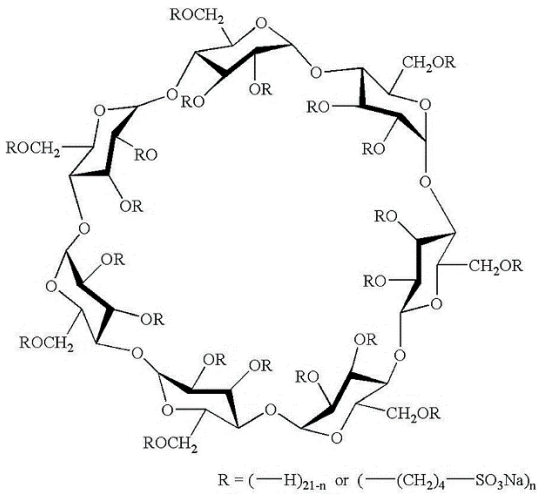


**Figure 3.** Estrutura química da sulfobutil-éter- β-ciclodextrina (SBE-β-CD). Fonte: BUDĂU et al. (2017).

Diante das evidências científicas de atividades biológicas relacionadas ao OEAz e considerando as limitações de uso terapêutico devido à baixa solubilidade, alta volatilidade e biodisponibilidade, este tabalho propõe o desenvolvimento e caracterização de CIs do OE de folhas de *A. zerumbet* complexado com SBE-β-CD como alternativa de excipiente farmacêutico na melhoria de suas propriedades físico-químicas com potencial aplicação terapêutica.

A tese está organizada, fundamentalmente, em dois capítulos. O primeiro aborda uma revisão sistemática com artigos que avaliaram as aplicações de óleos essenciais e seus componentes voláteis complexados em diferentes ciclodextrinas e o segundo consiste nos procedimentos de encapsulação do óleo essencial de *Alpinia zerumbet* em sulfobutil-éter-β-ciclodextrina, seguido das caracterizações das propriedades físico-químicas dos complexos de inclusão e docking molecular dos componentes majoritários do óleo essencial em interação com a ciclodextrina.

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# 2. OBJETIVOS

## 2.1 Objetivo Geral

Desenvolver os complexos de inclusão contendo o óleo essencial de *Alpinia zerumbet* (OEAz) com sulfobutil-éter-β-ciclodextrina (SBE-β-CD).

## 2.2 Objetivos Específicos

- Relizar revisão sistemática com artigos de aplicações de complexos de inclusão de ciclodextrinas e óleos essenciais;
- Extrair, identificar e quantificar os constituintes do óleo essencial de *Alpinia zerumbet* (OEAz) por meio de cromatografia gasosa acoplada à espectrometria de massas (CG-EM);
- Obter complexos de inclusão de OEAz com SBE-βCD (OEAz:SBE-β-CD) pelo método de co-precipitação;
- Caracterizar os complexos de inclusão OEAz:SBE-β-CD por espectrofotometria de absorção na região do infravermelho com transformada de Fourier (IVTF);
- Caracterizar a estabilidade dos complexos de inclusão OEAz:SBE-β-CD por análise termogravimétrica (TGA) e calorimetria exploratória diferencial (CED);
- Determinar a concentração inibitória mínima (CIM) e concentração letal mínima (CLM) do OEAz e SBE-β-CD;
- Avaliar possíveis interações componentes majoritários do OEAz com SBE-β-CD por meio de simulação de docking molecular.

# CAPÍTULO 1. Atividades biológicas e aplicações farmacológicas de ciclodextrinas complexadas com óleos essenciais e seus componentes voláteis: uma revisão sistemática

**CAPÍTULO 1**

## TITLE PAGE

This chapter refers to the article published on the journal **Current Pharmaceutical Design**.

# Biological Activities and Pharmacological Applications of Cyclodextrins Complexed with Essential Oils and Their Volatile Components: A Systematic Review

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**Abstract:** ***Background:*** Essential oils (EOs) and their volatile components (VCs) have varied biological and pharmacological activities, but the low solubility and bioavailability hamper their applications, so that inclusion in cyclodextrins (CDs) is likely to improve their physicochemical properties and pharmacological effects. ***Objective:*** The authors conducted a systematic review to evaluate the biological activities and pharmacological applications of essential oils and their volatile components complexed with cyclodextrins. ***Methods:*** The search terms 'Cyxlodextrin', 'Inclusion Complex', 'Volatile oils', 'Essential oil' and 'Volatile components' were used to retrieve articles from the PUBMED, MEDLINE and SCOPUS databases. ***Results:*** A total of 38 articles were identified. In *in vitro* and preclinical studies, a greater efficacy of EOs and their VCs complexed with different CD types was found when compared to free forms in the various biological activities and animal models of the pharmacological tests evaluated. ***Conclusion:*** This review of selected studies showed that the use of CDs promotes greater solubility, bioavailability and efficacy of EOs and their VCs indicating an interesting alternative for the biotechnological development of new therapeutic formulations.

**Keywords:** Cyclodextrin, volatile oils, essential oils, inclusion complex, biological activities, pharmacological application, systematic review.

## 1. INTRODUCTION

Essential oils (EOs) are volatile or semi-solid liquids characterized by strong odor and synthesized as secondary metabolites in aromatic plants, usually extracted by hydrodistillation [1]. The amount and composition of these oils varies according to the location in different plant organs (flowers, leaves, roots, stems, seeds, bark and whole fruits) [2]. EOs are highly complex mixtures of volatile components (VCs) that can be classified according to the chemical structure of the hydrocarbon as terpenes (mono-, sesqui- and diterpenes), its oxygenated derivatives, terpenoids (phenolic compounds, aliphatic aldehydes, ketones, amines, lactones, alcohols and esters) and aromatic compounds, all present in very different concentrations [3-5]. Terpenes are derived from combinations of 5-carbon (C5) units called isoprenes. Among these, monoterpenes (C10) are the most common molecules in the composition of essential oils (e.g. myrene, terpinenes, p-cymene, sabinene, geraniol, linalool, citronellol, menthol, camphor, 1,8-cineole, thymol and carvacrol) [6,7]. Sesquiterpenes (C15) have similar structure and function as monoterpenes (e.g. β-bisabolene, cadinenes, β-caryophyllene, logifolene, farnesenes, bisabol, cedrol, farnesol and germacrona) [8]. Aromatic compounds, in turn, are derived from phenylpropane and occur less frequently than terpenes (e.g. cinnamaldehyde, chavicol, eugenol, anethole and estragol) [9, 10].

EOs and their constituents are considered the most important bioactive natural products derived from plants [11,12] which have traditionally been used in folk medicine with a broad spectrum of biological activities such as antioxidant [13], antibacterial [14], antifungal [15], insecticide [16] and larvicide [17], in addition to pharmacological applications with anti-inflammatory [18], antidepressive [19], analgesic [20], antinociceptive [21] effects and in the management of health problems such as diabetes [22], cancer [23], Alzheimer [24] and cardiovascular diseases [1]. These properties of the EOs are directly correlated to the concentration and proportion of their constituents, where two or three major bioactive components are present in higher concentrations [25] or in synergism with other constituents, being responsible for a distinct role in the biological activity of EOs [3]. However, it is likely that the activity of the major components is modulated by other smaller molecules [26]. In relation to the main components, these reflect the biological characteristics of the EOs, in which the effectiveness of their activities depends on their concentration in the isolated form or comprised in EOs [27]. Volatile compounds due to low solubility and high permeability, mainly for passive transport through plasma membranes, belong to the class II drugs of the biopharmaceutical classification system (BCS) [28].

However, the low solubility in water, high volatility, short shelf life, susceptibility to degradation by exposure to air, light and heat limit the therapeutic applications of EOs and their VCs due to the low bioavailability. Protection of these compounds in formulations of drug delivery systems can promote the integrity of their bioactivities [29,30]. Thus, the complexation of EOs and their VCs in inclusion complexes with cyclodextrins (CDs) is a way of solving the problems by improving the solubility and stability and avoiding the degradation and volatilization of bioactive compounds in the EOs, besides transforming them into water-dispersible powders [31,32,33]. CDs are one of the complexing agents most commonly used by the pharmaceutical industry as pharmaceutical excipients due to their low price and high production, as well as their potential to improve the biological, chemical, physical and pharmacological profile of bioactive compounds, mainly of plants [30,34].

Cyclodextrins are non-toxic macrocyclic oligosaccharides consisting of 6, 7 or 8 D-glucopyranose units (notably α, β, or γCD, respectively) attached by α-(1,4) glycosidic bonds represented as a truncated cone with a relatively hydrophilic surface and a hydrophobic central cavity with approximately 4.7-5.3, 6.0-6.5 and 7.5-8.3 Å of diameter, respectively [35,36]. They are chemically and physically stable molecules with a cavity capable of containing hydrophobic molecules in their inner portion. These properties make them ideal for solubilization of solutes in aqueous media due to the polarity of their surface, forming inclusion complexes (ICs) which, in turn, are used to improve the physicochemical stability, reducing losses by evaporation [37,38]. Besides natural CDs (αCD, βCD, γCD), new chemically modified structures such as hydroxypropyl-β-cyclodextrin (HPβCD), methyl-β-cyclodextrin (MβCD) and sulfobutylether-β-cyclodextrin (SBEβCD) have been synthesized to improve complexing and solubility properties in a variety of applications [39,40]. Some of these studied CDs were approved by the Food and Drug Administration (FDA) and were believed to be "generally recognized as safe" (GRAS) [41]. There are different methods for obtaining ICs, such as co-precipitation, homogenization, physical mixture, complexing paste, sealing, lyophilization, spray drying and solvent evaporation [42,36]. Studies on EOs and their VCs complexed with CDs are historically based on some revisions on the theme [30,34,36,43], with the improvement of solubility being the main characteristic obtained by the inclusion complexes. However, no specific systematic review on the biological activities and pharmacological applications has been done. Thus, our article represents a timely contribution to the scientific community.

In this context, the present study aimed to evaluate, through a systematic review, studies that investigated the biological activities and pharmacological applications of EOs and their VCs complexed with CDs, analyzing whether complexation improves the physicochemical properties and the pharmacological profile of these compounds.

## 2. METHODS

### 2.1 Systematic literature search

This systematic review was carried out in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA statement) with modifications.

### 2.2 Selection procedures

#### *2.2.1 Search strategy*

We conducted a survey between February and April 2018 in specialized databases (PUBMED, MEDLINE and SCOPUS) including all articles published between 2008 and the end of April 2018 in the following blocks of search terms: 'Cyclodextrin' OR 'Inclusion Complex' AND 'Volatile Oils' OR 'Essential Oil' OR 'Volatile Components' which include terms related to cyclodextrins and essential oils located in the Pubmed Medical Subject Headings (MeSH) database or as free text words adapted to other databases. We also researched the references of the articles identified and included in the systematic review to further substantiate and discuss our work based on relevant articles.

Our objective was to provide an overview of the research of biological and pharmacological properties of EOs and their VCs complexed on CDs by organizing all the selected studies in tables.

#### *2.2.2 Inclusion and exclusion criteria*

The selection of articles was performed according to the search terms found in the titles and abstracts, followed by screening and evaluation of complete documents in order to identify studies that met the inclusion and exclusion criteria. The studies were included only if they met the following criteria: (a) being an original publication in a peer-reviewed journal; (b) evaluating the complexation of essential oils and/or their volatile components with cyclodextrins; (c) preparation and characterization of the inclusion complex (IC) and (d)

studies on biological activities *in vitro* and pharmacological properties *in vivo*. We reviewed only articles written in English and published in the last ten years. Exclusion criteria were articles written in a language other than English, review articles, duplicates, any other studies with compounds complexed with CDs other than EOs and their VCs, and articles that were not evaluated and applied in the pharmaceutical field.

### 2.3 Registered variables

#### *2.3.1 Data extraction and analysis*

Data were extracted manually and separated into a standardized form in the Microsoft Office Excel 2010 software (Microsoft Corporation, Redmond, Washington, USA), in which descriptive and quantitative analyses were performed. The results are reported in different tables to aid the reader in establishing an independent view on the subject in order to provide an understandable interpretation of the main findings and conclusions drawn. We obtained three summary tables of all the studies included in the systematic review, a table of *in vitro* studies evaluating the biological activity of inclusion complexes (Table 1), one illustrating the pharmacological effects of ICs in preclinical studies (Table 2) and another with a summary of aspects improved by complexation (Table 3). The variables extracted from each article and included in the review were: authors and year of publication, identification of complexed EOs and VCs, cyclodextrin type, encapsulation technique and biological activity evaluated. In the case of *in vivo* studies, the dose and route of administration, type of animal and the N (per group) were also recorded, and the protocol used in *in vitro* evaluations in preclinical studies.

## 3. RESULTS

This review investigated studies of EOs and their VCs complexed with CDs that evaluated their *in vitro* biological activities and *in vivo* pharmacological applications based on preclinical studies. In the primary survey, we found a total of 758 potentially relevant articles in the entries listed in the databases until April 2018, using the search terms shown above. A secondary survey selected 358 articles that met the inclusion and exclusion criteria: 85 from PUBMED, 77 from MEDLINE and 196 from SCOPUS. From this total, 120 were indexed in two or more databases and were therefore considered only once. Two hundred articles were excluded from final sample consisted because were review studies, studies that investigated the application of ICs in the area of food technology and textile industry, or were published in a language other than English.

After filtration and screening, 38 studies published between 2008 and April 2018 met the inclusion criteria and were included in the review. Twenty-two articles on the preparation and evaluation of the biological activity of inclusion complexes were included as *in vitro* studies, while sixteen articles investigated the pharmacological profiles of inclusion complexes in animal models and were included as preclinical studies. The flowchart in Figure 1 illustrates the research selection procedure and the number of articles in each phase. The general characteristics of the *in vitro* and preclinical studies identified and included in this systematic review are described in Tables 1 and 2, respectively.

The *in vitro* studies selected in this systematic review evaluated different biological activities of several EOs and isolated VCs complexed with different types of CDs. The articles compared the use of these compounds in free and complexed forms. The twenty-two studies were divided according to the complexed substance; a total of 14 articles used EOs and 8 used isolated VCs. Among these, two studies with EOs and one with VCs evaluated the antibacterial activity; three studies with EOs and two with VCs evaluated antifungal activity; two studies with EOs evaluated larvicidal activity; and one with VCs evaluated acaricidal activity; two studies with EOs and two with VCs evaluated antioxidant activity; an study with EOs evaluated the activity of methane and ruminal hydrogen production *in vitro*; a study with three different VCs evaluated the bioxidation activity for conversion of these compounds by cytochrome P450 based *Escherichia coli;* and another study with four different VCs evaluated the inflammatory activity in lung and liver cells. Among these articles, some studies evaluated more than one activity in which four of these studies with EOs evaluated the antibacterial and antioxidant activity and a study with EO evaluated the antioxidant and antifungal activity.

The selected preclinical studies evaluated different pharmacological effects in animal models with several EOs and isolated VCs, all complexed with βCD. The articles compared the effect of these compounds in free and complexed forms. The sixteen studies were divided according to the complexed substance, where a total of 9 articles used EOs and 7 articles used isolated VCs. Among these, two studies with EOs and one with VCs evaluated antihyperalgesic activity; a study with EOs evaluated anti-inflammatory activity; a study with EOs evaluated antitumor activity; two studies with EOs evaluated orofacial antinociceptive activity; two studies with EOs and one with VCs evaluated the pharmacokinetic profile; a study with EOs evaluated analgesic activity; a study with VCs evaluated the gastroprotective activity; and another evaluated anticonvulsive; three studies with VCs evaluated antinociceptive activity, and among them, two also evaluated antihyperalgesic activity.

Most of the studies reported an improvement in other characteristics and properties of the EOs and their VCs. The *In vitro* studies demonstrated that the use of CDs was able to improve solubility, stability and controlled release, resulting in increased antibacterial, antifungal and antioxidant activity of these compounds. In preclinical studies, there was an increase in solubility, oral bioavailability, absorption and stability, resulting in an improvement in the antihyperalgesic, antinociceptive and gastroprotective activity, with reduction in the dose of the compounds. Details of the characteristics improved by the use of CDs in *in vitro* and preclinical studies are presented in Table 3.

## 4. DISCUSSION

The present systematic review showed that EOs and their VCs complexed with CDs improved their biological activities and pharmacological properties when compared to their non-complexed form. *In vitro* studies tested different EOs (*Hyptis martiusii* [44], *Melaleuca alternifólia* quimiotipo terpinen-4-ol [45], *Thymus catharinae* quimiotipo carvacrol [46], *Lippia graveolens* [47], *Psidium guajava* L. [48], *Eugenia caryophyllata* L. [49], *Achillea millefolium* L. [50], *Anemopsis californica* [51], *Piper nigrum* L. [52], *Citrus sinensis* L. [53], *Satureja montana* [54,56], *Origanum vulgare*, *Rosmarinus officinalis* [55], Eucalyptus, peppermint, thyme, wasabi, cineole and menthol [57] and isolated VCs (Eugenol [58,61,65], limonene [59], 2-nonanona [60], carvacrol [61,64], cinnamaldehyde [61], tymol [61,62,64], α-longipinene, isolongifolene and α-humulene [63], trans-anethole, estragol e isoeugenol [65]), complexed with different CDs (αCD, βCD, HPβCD, HPγCD and MβCD) (table 1). The preclinical studies evaluated several EOs (*Lippia grata* [66,73], *Ocimum basilicum* [67,71], *Annona vepretorum* [68], *Cymbopogon winterianus* [69], Xiang-Fu-Si-Wu Decoction [70, 74], and *Hyptis pectinata* [72]) and isolated VCs (Linalol [75,78,81], Citronellal [76], carvacrol [77], β-caryophyllene [79] and geraniol [80]) complexed with βCD. These studies used a variety of protocols in animal models and there was a better pharmacological effect on the complexed than on the non-complexed compounds (table 2). These studies demonstrated the effectiveness of CDs in a variety of biological and pharmacological activities (Table 3).

### 4.1 *In vitro* studies

#### *4.1.1 EOs complexed with CDs*

EOs have several relevant biological activities, including antibacterial, antifungal and antioxidant [1], but the low solubility and bioavailability compromise their use. In view of their properties, several EOs have already been complexed with CDs to improve stability and

efficacy, because the molecular inclusion technique protects the EOs from degradation and increases their solubility and bioavailability [43].

Andrade *et al.* [44] evaluated the antibacterial and antibiotic-modulatory activity of the essential oil of *Hyptis martiusii* complexed with βCD, demonstrating that the complex and the βCD alone did not have any activity, whereas the essential oil alone demonstrated an antibacterial effect against *Staphylococcus aureus* and synergism against *Pseudomonas aeruginosa* when associated with norfloxacin, and against *Escherichia coli* when associated with gentamicin. Yim *et al.* [45] tested the larvicidal activity of *Melaleuca alternifolia* oil in emulsion and complexed into two forms of βCD (slow and faster releases) against tick larvae (*Rhipicephalus australis*) and showed that the repellency provided by the faster release formulation exceeded that of slow release in the study, indicating the potential in modifying formulations to increase the load and change the release characteristics of the essential oil aiming at optimal effect of repellent activity. Delogu *et al.* [46] evaluated the essential oil of *Thymus catharinae* Camarda (carvacrol chemotype) complexed with βCD and showed that the inclusion complex had antimicrobial activity with a very slow death kinetics against *E. coli* and to a lesser degree against *Candida albicans*, being efficiently modulated when complexed with a biomatrix, which improved its effectiveness. Arana-Sánchez *et al.* [47] tested the antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus*, and the antioxidant activity of three *Lippia graveolens* essential oils with different ratios of carvacrol/thymol/p-cymene (38:3:32, 23:2:42, 7:19:35) complexed with βCD and showed that the complexation preserved the antibacterial activity in all cases and increased the antioxidant activity from four to eight-fold, acting as a protection for the main compounds and improving the quality of the oils. Rakmai *et al.* [48] evaluated the antibacterial and antioxidant activity of the essential oil of *Psidium guajava* L. complexed with hydroxypropyl-βCD, demonstrating that the complexation increased the antibacterial activity of the oil against *S. aureus* and *E. coli* by four-fold and two-fold, respectively, and the capacity of free radicals from the encapsulated oil was 26-38% more stable than the free oil, indicating the protection of the active components of the oil from the effects of sunlight incidence. Babaoglu *et al.* [49] demonstrated that the essential oil of *Eugenia caryophyllata* L. complexed with hydroxypropyl-β-CD had its antioxidant activity increased, with an elimination of free radicals superior to that obtained with the free oil, and the *in vitro* release profile of the inclusion complexes indicated a slow and sustained release. Rakmai *et al.* [50] tested the antibacterial and antioxidant activity of the essential oil of *Achillea millefolium* L. complexed

with hydroxypropyl-β-CD; they demonstrated that antibacterial efficacy was greatly improved against *S. aureus* and *E. coli* with MIC value of 62.5 μg/mL after complexation, and that the inclusion complex can protect active compounds of the essential oil and preserve the antioxidant activity after sun exposure. Perez-Perez *et al.* [51] evaluated the antioxidant activity and the prolonged release profile of the essential oil of *Anemopsis californica* complexed with βCD demonstrating that only the elemycin and methyleugenol compounds were efficiently complexed and that the inclusion complex promoted the retention of the antioxidant activity, and that the prolonged release profile was influenced by increasing relative humidity. Rakmai *et al.* [52] tested the antioxidant and antibacterial activity of the essential oil of *Piper nigrum* L. complexed with hydroxypropyl-βCD and showed that the inclusion complexes increased oil stability but obtained a slightly lower antioxidant activity during the reaction with DPPH radicals. Meanwhile, the complexed oil had the antibacterial activity against *S. aureus* and *E. coli* improved by four-fold. Galvão *et al.* [53] evaluated the larvicidal activity of *Citrus sinensis* L. essential oil complexed with βCD against *Aedes aegypti* larvae and showed that the oil was effectively complexed and the activity of this complex induced 93% of mortality at 50 ppm, close to the pure oil. This product becomes an interesting alternative with a biodegradable, non-toxic and economically viable characteristic for the control of *A. aegypti* larvae.

Haloci *et al.* [54] and Haloci *et al.* [56] tested the *Satureja montana* essential oil complexed with βCD, the first study on both antioxidant and antifungal activity. The first [54] showed that the complexed essential oil had a high percentage of growth inhibition of dermatophyte colonies with controlled release of the complex and high antioxidant activity. The second [56] showed that the complexation improved the antifungal properties of the essential oil and its stability after storage. Papajani *et al.* [55] tested the antifungal activity of essential oils of *Origanum vulgare* and *Rosmarinus officinalis* complexed with βCD and showed that the complexation increased the antifungal properties of the oils, with higher activity of *O. vulgare* due to the high concentration of carvacrol, suggesting the use of these two essential oils incorporated in dermatological formulations as antifungal agent and industrial applications. Tatsuoka *et al.* [57] evaluated the essential oils of eucalyptus, peppermint, wasabi and thyme and two major constituents (menthol and cineol) complexed with αCD and βCD on the ruminal production of methane and hydrogen, volatile fatty acids (VFA) and number of protozoa *in vitro* and showed that methane production was significantly reduced with eucalyptus-αCD, wasabi-α-CD and -βCD, and increased with cineol-αCD and -

βCD, while other complexes showed no changes. The total VFA remained unchanged in almost all experiments, except for cineol-βCD, eucalypt-αCD and -βCD, which promoted a slight increase according to the concentration of complexes; in relation to the total number of protozoa, the eucalyptus-βCD, wasabi-βCD and both cineol-αCD and -βCD had no effect, but eucalyptus-αCD caused a reduction in this parameter. It was observed that the addition of fumaric acid or malic acid with wasabi and eucalypt-αCD complexes reduced both methane and hydrogen production.

*4.1.2 VCs complexed with CDs*

A variety of volatile compounds derived from plants, specifically isolated from EOs, have presented different biological activities [82]. The inclusion of these compounds in CDs for the improvement of their properties has been described in the literature.

Celebioglu *et al.* [58] and Celebioglu *et al.* [62] evaluated eugenol and thymol (essential oil components), respectively, complexed with three modified cyclodextrins (hydroxypropyl-βCD, hydroxypropyl-γCD and methyl-βCD) in the formation of self-sustaining functional nanofiber networks produced by electrospray and the samples showed higher thermal stability, increased water solubility, improved compound elimination property and higher antioxidant activity compared to pure eugenol and thymol. Aytac *et al.* [59] tested the limonene component complexed with hydroxypropyl-βCD, hydroxypropyl-γCD and methyl-βCD in the formation of self-sustaining nanofiber networks produced by electrospray and demonstrated that the complex with methyl-β-CD released much more limonene at 37, 50 and 75 ºC than the hydroxypropyl-βCD, hydroxypropyl-γCD complexes. However, with respect to the whole, nanofibers exhibited a rapid dissolution character, enhanced thermal stability and extended shelf life along with antibacterial activity against *E. coli* and *S. aureus*. Abarca *et al.* [60] evaluated the antifungal activity of 2-nonanone (2-NN) compound complexed with βCD and showed a reduction in the radial growth of the filamentous fungus *Botrytis cinerea*, indicating a fungistatic behavior of the inclusion complexes of the active compound. Bernardos *et al.* [61] tested the antifungal activity of four different volatile components such as carvacrol, cinnamaldehyde, eugenol and thymol complexed with βCD and incorporated into silica mesoporous mobile composition of matter No. 41 (MCM-41) against *Aspergillus niger* and showed that even after 30 days, carvacrol and thymol encapsulated in mesoporous frameworks were able to maintain antifungal activity, unlike compounds complexed with βCD, among which none exhibited antifungal properties to such

an extent. Ly *et al.* [63] evaluated the oxidation of the α-longipinene, isolongifolene and α-humulene sesquiterpenes complexed with hydroxypropyl-β-cyclodextrin and an alternative ferredoxin reductase in the CYP264B1-based recombinant *E. coli* whole cell system and showed that the use of cyclodextrin and alternative redox standards promoted improvement in the system for efficient conversion of the compounds by the biocatalyst cell corresponding to efficiencies of 82.1, 51.8 and 71.5% for the conversion of α-longipinene, isolongifolene and α-humulene, respectively, to 200 mL scale. LeBlanc *et al.* [64] also tested thymol and carvacrol complexed with βCD regarding the acaricidal activity against Varroa mite (*Varroa destructor*), tested by feeding honey bees (*Apis mellifera*), and showed that after the first and second weeks of feeding, thymol levels and carvacrol were elevated in bees'tissues without any imposed toxicity in an effort to prevent Varroa mites from feeding on the bees' hemolymph. Kfoury *et al.* [65] evaluated the effect of four components of essential oil, trans-anetol, estragol, eugenol and isoeugenol complexed with hydroxypropyl-βCD in reducing inflammation induced by particulate matter with aerodynamic diameter below 2.5 μm in human bronchial epithelial cell lines (BEAS-2B) and human liver carcinoma (HepG2). They showed that the addition of free or complexed essential oil components to cells exposed to particles reduced the IL-6 cytokine level by up to 96%, and of IL-8 cytokine by up to 87%. Thus, the components neutralized the inflammatory effects of the particles and the cyclodextrins preserved their properties.

## 4.2 Preclinical studies

### *4.2.1 EOs complexed with CDs*

Several EOs have been evaluated in preclinical studies for their pharmacological properties, including analgesic and anti-inflammatory activity [83] but their low solubility hinders clinical applications. Several studies in animal models have evaluated the pharmacological profile of EOs complexed with CDs to increase their bioavailability, dose reduction and therapeutic effect [36].

Siqueira-Lima *et al.* [66] and Siqueira-Lima *et al.* [73] evaluated the essential oil of *Lippia grata* complexed with βCD in the antihyperalgesic activity in the test of chronic musculoskeletal pain and orofacial antinociceptive activity in mice, respectively. The first [66] showed that the treatment with the complex at a dose of 24 mg/kg was able to reduce primary and secondary hyperalgesia without producing a reduction of muscle strength in the hind and front legs, being the activity of the complex involved with opioid and serotonergic

receptors. The second [73] showed that the complex significantly reduced orofacial antinociceptive activity in formalin, capsaicin and glutamate-induced tests and did not cause changes in the motor coordination of the mice in the rota-rod test.

Rodrigues *et al.* [67] and Nascimento *et al.* [71] tested the essential oil of *Ocimum basilicum* complexed with βCD in acute anti-inflammatory activity in paw edema, vascular permeability and peritonitis models, and chronic granuloma model, and tested its antihyperalgesic activity in fibromyalgia models in mice, respectively. Rodrigues *et al.* [67] showed that the complex prevented the formation of paw edema by decreasing vascular permeability *in vivo*, inhibited the recruitment of leukocytes into the peritoneal cavity and inhibited the formation of granuloma. Nascimento *et al.* [71] demonstrated that all doses of complexes tested produced a significant reduction in mechanical hyperalgesia. Bonfim *et al.* [68] evaluated the antitumor activity of *Annona vepretorum* essential oil complexed with βCD in C57BL/6 mice inoculated with B16-F10 melanoma and showed that the inhibition of tumor growth was potentiated by the complexation of the essential oil with the CD. Santos *et al.* [69] tested the orofacial antinociceptive activity of *Cymbopogon winterianus* essential oil complexed with βCD and showed that nociceptive orofacial behavior was significantly reduced, the complexes did not cause changes in motor coordination in the rota-rod test, and reduced the essential oil dose considerably when compared to commonly used doses.

Xi *et al.* [70] and Pan *et al.* [74] evaluated the essential oil of Xiang-Fu-Si-Wu Decoction, a famous Chinese formulation composed of seven herbs (*Angelica sinensis*, *Ligusticum chuanxiong*, *Cyperus rotundus*, *Aucklandia lappa*, *Rehmannia glutinosa*, *Paeonia lactiflora* and *Corydalis yanhusuo*) complexed with βCD in a pharmacokinetic and oral bioavailability study in rats and showed that the inclusion complexes exhibited higher maximum plasma concentration ($C_{max}$), prolonged half-life ($T_{1/2}$), greater area under the time-concentration curve ($AUC_{0–24\ h}$) and promoted an increase in oral absorption and bioavailability when compared to the non-complexed form. Menezes *et al.* [72] tested the analgesic activity of the essential oil of *Hyptis pectinata* complexed with βCD in the formalin-induced pain model in mice and demonstrated that the complex promoted the pharmacological profile of the essential oil suggesting the applicability of the formulation in the treatment of painful conditions.

*4.2.2 VCs complexed with CDs*

The main volatile constituents of EOs present a range of possible pharmacological uses, being interesting candidates for the development of new drugs [34]. Several approaches have been undertaken to improve the therapeutic properties of VCs, including the use of βCDs which are widely applied in inclusion complexes to improve the bioavailability, solubility and therapeutic effect of such constituents [84].

Silva *et al.* [75], Quintans-Júnior *et al.* [78] and Nascimento *et al.* [81] evaluated the linalool monoterpene complexed with βCD in different animal models. Silva *et al.* [75] tested the gastroprotective activity in acute and chronic gastric ulcer models in rodents and demonstrated that the complexes had a significantly improved gastroprotective effect compared to non-complexed linalool due to increased solubility and stability. The oral administration of the complexes in doses did not cause changes to the point of causing signs of toxicity in animals, such as death or macroscopic changes in the liver, stomach, heart, lungs, spleen and kidneys. This indicates that the complexation of monoterpene with βCD improves the cure response. Quintans-Júnior *et al.* [78] demonstrated that complexed linalool produced an antinociceptive effect superior to that of its free form, with a strong activity in all the mice models induced by chemicals and heat, such as the test of contortion reflex of acetic acid, formalin and heating plate, as well as in the test of carrageenan-induced peritonitis with reduction in total leukocyte migration and TNF-α levels. In turn, Nascimento *et al.* [81] demonstrated that the complexes promoted antihyperalgesic activity and antinociceptive effect with a significant reduction of mechanical hyperalgesia in a non-inflammatory chronic muscular pain model, remaining for 24 h due to increased stability and bioavailability.

Santos *et al.* [76] tested the antihyperalgesic activity of the citronellal component, a monoterpene present in the essential oil of *Cymbopogon* species, complexed with βCD in a model of chronic muscular pain in mice, showing that the complexes reduced mechanical hyperalgesia in all treatment days without altering muscle strength, with a greater effect than citronellal in the free form. Guimarães *et al.* [77] evaluated the carvacrol component, a monoterpene present in oregano essential oil, complexed with βCD in a nociception model induced by tumor cells (Sarcoma 180) and hyperalgesia in rodents, demonstrating that the complex administered in mice with tumor in the hind paw was able of reducing hyperalgesia for 24 h, unlike non-complexed carvacrol which promoted effects only for 9 hours. Liu *et al.* [79] evaluated the beta-caryophyllene sesquiterpene complexed with β-CD in a

pharmacokinetic and oral bioavailability study after a single oral dose of 50 mg/kg in rats, demonstrating that the complex exhibited short $T_{max}$, greater $C_{max}$ and $AUC_{0–12h}$ 2.6-fold greater than the non-complexed sesquiterpene, thus significantly increasing the oral bioavailability of the drug. Lins *et al.* [80] tested the geraniol compound complexed with β-CD in an anticonvulsive activity model and showed that complexes at doses of 100 and 200 mg/kg significantly increased the latency to the first seizure induced by pentylenetetrazole and reduced the percentage of animals that convulsed; therefore, the activity was potentiated by βCD complexation.

## 5. CONCLUSION

This systematic review found *in vitro* and preclinical studies that tested a variety of EOs and VCs complexed with different types of CDs. *In vitro* studies showed greater efficacy in the properties of EOs and their components when complexed with CDs in a variety of biological activities. Preclinical studies gave evidence that inclusion complexes with CDs are able to improve the pharmacological profile in various animal nociception, anti-inflammatory, antihyperalgesic and antitumor models with improved efficacy, reducing therapeutic doses and side effects. In general, these studies demonstrated a greater solubility, stability and bioavailability of the OEs and their components. Thus, the complexation of drugs with CDs may be an alternative method for the biotechnological development of novel formulations with improved therapeutic efficacy and, therefore, a perspective of clinical applicability.

## List of Abbreviation

| | | |
|---|---|---|
| B16-F10 | = | Tumor cell line of mouse melanoma |
| BCS | = | Biopharmaceutics Classification System |
| BEAS-2B | = | Human bronchial epithelial cells |
| C5 | = | 5 carbons |
| C10 | = | 10 carbons |
| CDs | = | Cyclodextrins |
| DPPH | = | 2,2-diphenyl-1-picrylhydrazyl |
| EOs | = | Essential oils |
| FDA | = | Food and Drug Administration |
| GRAS | = | Generally Recognized as Safe |
| HepG2 | = | Tumour cell line of human hepatocellular carcinoma |
| HL-60 | = | Tumour cell line human promyelocytic leukaemia |
| HPβCD | = | Hydroxypropyl-β-cyclodextrin |
| HPγCD | = | Hydroxypropyl γ-cyclodextrin |
| ICs | = | Inclusion Complexes |
| i.p. | = | intraperitoneal |
| K562 | = | Tumour cell line of human chronic myelocytic leukaemia |

MeSH = Medical Subject Headings
MIC = Minimum inhibitory concentration
MβCD = Methyl-β-cyclodextrin
Nd = No data
p.o. = oral administration
SD = Sprague-Dawley
TNF-α = Tumor necrosis factor-α
VCs = Volatile components
VFA = Volatile fatty acids
XFSWD = Xiang-Fu-Si-Wu Decoction
αCD = α-cyclodextrin
βCD = β-cyclodextrin
γCD = γ-cyclodextrin

**Conflict of interest**

All authors confirm the content of this article has no conflict of interest.

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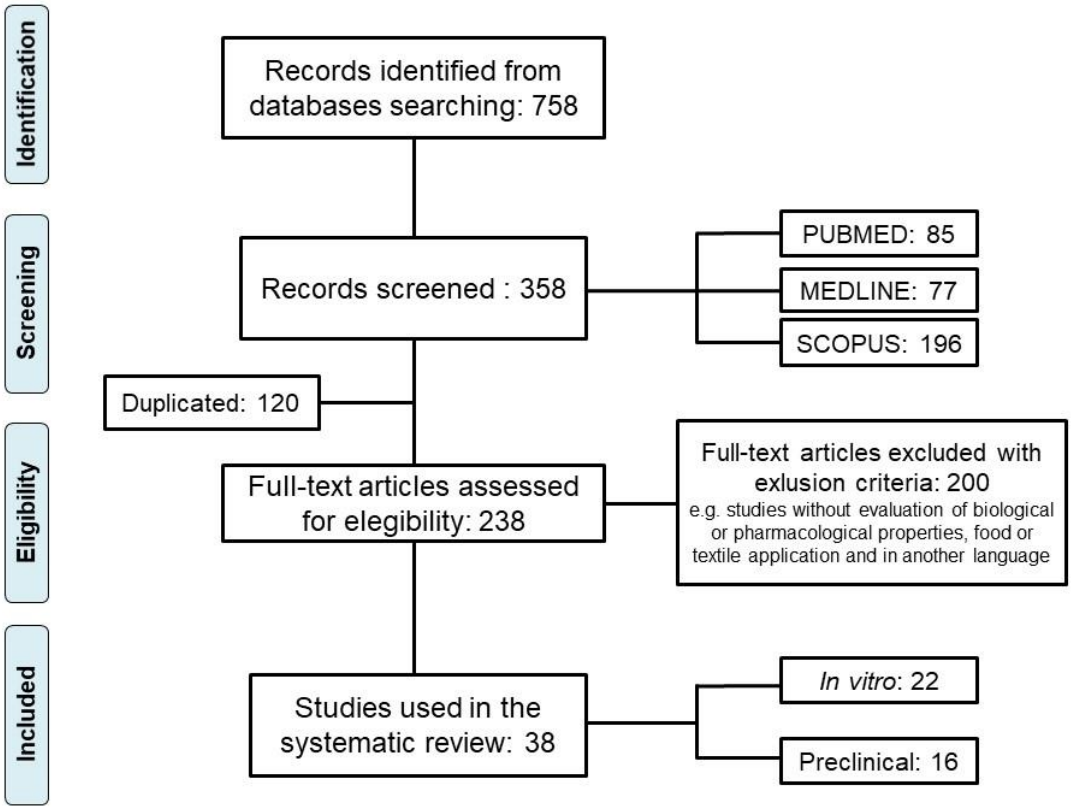
## Appendix A - Figure

**Figure 1.** Flowchart (selection strategy) of the articles included in the systematic review.



## Appendix B - Tables

**Table 1.** Details of the main aspects of the *in vitro* studies included in the systematic review.

| Authors (year) | Substance | CD type | Dose, Concentration or Quantity | Complexation technique | Biological activity |
|---|---|---|---|---|---|
| ***Essential oils*** | | | | | |
| Andrade *et al.* (2017) [44] | *Hyptis martiusii* | βCD | 1-1024 µg/mL | Physical mixture, paste complexation and slurry complexation | Antibacterial and modulatory-antibiotic |
| Yim *et al.* (2016) [45] | *Melaleuca alternifolia* (terpinen-4-ol chemotype) | βCD | 300 mg | Precipitation | Larvicide and repellent effects |
| Delogu *et al.*(2015) [46] | *Thymus catharinae* Camarda (carvacrol chemotype) | βCD | 42.8, 82.6, 85.5 and 165.2 mg | Precipitation | Antimicrobial |
| Arana-Sánchez *et al.*(2010) [47] | *Lippia graveolens* | βCD | 0.05, 0.10 and 0.20% | Spray-drying | Antibacterial and antioxidant |
| Rakmai *et al.* (2018) [48] | *Psidium guajava* L. | HPβCD | 2 - 2000 µg/mL | Freeze-drying | Antibacterial and antioxidant |
| Babaoglu *et al.* (2017) [49] | *Eugenia caryophyllata* L. | HPβCD | 410 µg/mL and 25 mg | kneading | Antioxidant and release study |
| Rakmai *et al.* (2017) [50] | *Achillea millefolium* L. | HPβCD | 2 - 2000 µg/mL | Freeze-drying | Antibacterial and antioxidant |
| Perez-Perez *et al.* (2017) [51] | *Anemopsis californica* | βCD | Nd | Precipitation | Antioxidant and release study |
| Rakmai *et al.* (2017) [52] | *Piper nigrum L.* and β-caryophyllene (major component) | HPβCD | 2 - 2000 µg/mL | Freeze-drying | Antioxidant and antibacterial |
| Galvão *et al.* (2015) [53] | *Citrus sinensis* (L.) | βCD | 5–70 ppm | Kneading, co-precipitation and physical mixture | Larvicide |
| Haloci *et al.* (2014) [54] | *Satureja montana* | βCD | 20 and 100 µg/ml | Co-precipitation | Antioxidant and antifungal |
| Papajani *et al.*(2015) [55] | *Origanum vulgare* and *Rosmarinus officinalis* | βCD | 20 and 100 µg/ml | Co-precipitation | Antifungal |
| Haloci *et al.* (2014) [56] | *Satureja montana* | βCD | 20 and 100 µg/ml | Co-precipitation | Antifungal |
| Tatsuoka *et al.* (2008) [57] | Eucalyptus, peppermint, thyme, wasabi, cineol and menthol | αCD and βCD | 1–40 mg | Precipitation | Ruminal methane and hydrogen production, volatile fatty acid (VFA) and protozoa |
| ***Volatile components*** | | | | | |
| Celebioglu *et al.* (2018) [58] | Eugenol | HPβCD, HPγCD and MβCD | 3-1600 µg/mL | Electrospinning | Antioxidant |
| Aytac *et al.* (2016) [59] | Limonene | HPβCD, MβCD and HPγCD | 20 mg | Electrospinning | Antibacterial |
| Abarca *et al.* (2016) [60] | 2-nonanone (2-NN) | βCD | 0.5 g | Co-precipitation | Antifungal |
| Bernardos *et al.*(2015) [61] | Carvacrol, Cinnamaldehyde, Eugenol and Thymol | βCD | 5 mg | Co-precipitation | Antifungal |
| Celebioglu *et al.* (2018) [62] | Thymol | HPβCD, HPγCD and | 50–4000 µg/mL | Electrospinning | Antioxidant |

| | | | | | |
|---|---|---|---|---|---|
| | | MβCD | | | |
| Ly *et al.* (2017) [63] | α-Longipinene, Isolongifolene and α-Humulene | HPβCD | 1.0% | Precipitation | Biooxidation by P450-based *E. coli* whole-cell |
| LeBlanc *et al.* (2008) [64] | Thymol and Carvacrol | βCD | 0.01, 0.1 and 1.0 % | Precipitation | Acaricide |
| Kfoury *et al.* (2016) [65] | Trans-anethole, Estragole, Eugenol and Isoeugenol, | HPβCD | 120 µM | Freeze-drying and physical mixture | Effect on pulmonary and hepatic cells inflammation induced by air pollution particulate matter |

Abbreviations – CD: Cyclodextrin; HPβCD: Hydroxypropyl β-cyclodextrin; HPγCD: Hydroxypropyl-γ-cyclodextrin; MβCD: Methyl-β-cyclodextrin; αCD: α-cyclodextrin; βCD: β-cyclodextrin.

**Table 2.** Details of the main aspects of the preclinical studies included in the systematic review.

| **Authors (year)** | **Substance** | **CD type** | **Complexation technique** | **Dose (route)** | **Animal (N per group)** | **Pharmacological application (protocols used)** | ***In vitro* evaluation** |
|---|---|---|---|---|---|---|---|
| ***Essential oils*** | | | | | | | |
| Siqueira-Lima *et al.* (2017) [66] | *Lippia grata* | βCD | Slurry complexation | 6, 12 and 24 mg/kg; (p.o.) | Male Swiss mice (8) | Chronic musculoskeletal pain model, Mechanical sensitivity of the muscle and paw | Antioxidant assays, Immunofluorescence for Fos protein |
| Rodrigues *et al.* (2017) [67] | *Ocimum basilicum* | βCD | Physical mixture, kneading and co-evaporation | 5 and 10 mg/kg (p.o.) | Female and Male Swiss mice (6) | Anti-inflammatory activities in acute (paw edema, vascular permeability and Peritonitis) and chronic (granuloma formation) inflammation | - |
| Bonfim *et al.* (2016) [68] | *Annona vepretorum* | βCD | Co-precipitation | 50 mg/kg (i.p.) | Pathogen-free C57BL/6 males mice (8 or 9) | Antitumour activity in C57BL/6 mice inoculated with B16-F10 melanoma | Cytotoxic activity assay in tumor cell lines B16-F10, HepG2, K562 and HL-60 |
| Santos *et al.* (2015) [69] | *Cymbopogon winterianus* | βCD | Physical mixture, kneading and co-evaporation | 50, 100 and 200 mg/kg (p.o.) | Male Swiss mice (6) | Orofacial antinociceptive activity | Immunofluorescence for Fos protein |
| Xi *et al.* (2015) [70] | XFSWD | βCD | Co-precipitation and physical mixture | 4 g/kg (p.o.) | Female SD rats (6) | Pharmacokinetic study and oral bioavailability | - |
| Nascimento *et al.* (2015) | *Ocimum basilicum* | βCD | Physical mixture, past | 25, 50 and | Male Swiss | Antihyperalgesic Effect in Animal | Immunofluorescence for Fos protein |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| [71] | | | complexation and slurry complexation | 100 mg/kg (p.o.) | mice (8) | Models for Fibromyalgia | |
| Menezes *et al.* (2015) [72] | *Hyptis pectinata* | βCD | physical mixture, paste complexation and slurry complexation | 40 mg/kg, (p.o.) | Female Swiss mice (7) | Analgesic Effect | - |
| Siqueira-Lima *et al.* (2014) [73] | *Lippia grata* | βCD | Slurry complexation | 6, 12 and 24 mg/kg (p.o.) | Male Swiss mice | Orofacial Nociception in Mice | Immunofluorescence for Fos protein |
| Pan *et al.* (2017) [74] | XFSWD | βCD | Co-precipitation | 37.8 mg/g/d (p.o.) | Female SD rats (8) | Pharmacokinetic study | - |
| ***Volatile components*** | | | | | | | |
| Silva *et al.* (2016) [75] | (−)-Linalool | βCD | Physical mixture, past complexation and slurry complexation | 5, 10, 20 and 40 mg/kg (p.o.) | Female Swiss mice and Wistar rats (5) | Gastroprotective activities in acute and chronic gastric ulcers in rodents | Antioxidant activity |
| Santos *et al.* (2016) [76] | Citronellal | βCD | Physical mixture | 50 mg/kg (p.o.) | Male Swiss mice (7) | Anti-hyperalgesic activity in chronic muscle pain model | Immunofluorescence for Fos protein |
| Guimarães *et al.* (2015) [77] | Carvacrol | βCD | Physical mixture and slurry complexation | 12.5, 25 and 50 mg/kg (p.o.) | Male Swiss mice (Nd) | Nociception induced by tumor cells (Sarcoma 180) and hyperalgesia in rodents | - |
| Quintans-Júnior *et al.* (2013) [78] | (−)-Linalool | βCD | Physical mixture, past complexation and slurry complexation | 20 and 40 mg/kg (p.o.) | Male Swiss Mice (6-8) | Antinociceptive effect | Determination of TNF-α levels in the peritoneal fluid |
| Liu *et al.* (2013) [79] | β-caryophyllene | βCD | Co-precipitation | 50 mg/kg (p.o.) | Male and female SD rats (6) | Pharmacokinetic study and oral bioavailability | Dissolution study |
| Lins *et al.* (2014) [80] | Geraniol | βCD | Slurry complexation | 50, 100 and 200 mg/kg (i.p.) | Male Swiss mice (8-12) | Anticonvulsant effect | - |
| Nascimento *et al.* (2014) [81] | Linalool | βCD | Physical mixture, past complexation and slurry complexation | 25 mg/kg (p.o.) | Male Swiss Mice (Nd) | Antinociceptive and anti-hyperalgesic activity | Immunofluorescence for Fos protein |

Abbreviations – B16-F10: Tumour cell line of mouse melanoma; CD: Cyclodextrin; HepG2: Tumour cell line of human hepatocellular carcinoma; HL-60: Tumour cell line human promyelocytic leukaemia; i.p.: intraperitoneal; K562: Tumour cell line of human chronic myelocytic leukaemia; Nd: No data; p.o.: oral administration; SD: Sprague-Dawley; XFSWD: Xiang-Fu-Si-Wu Decoction formula composed of seven crude Chinese herbs, including *Angelica sinensis*, *Ligusticum chuanxiong*, *Cyperus rotundus*, *Aucklandia lappa*, *Rehmannia glutinosa*, *Paeonia lactiflora* and *Corydalis yanhusuo*;. βCD: β-cyclodextrin.

**Table 3.** Summary of the results of aspects improved by complexation describe in *in vitro* and preclinical studies included in the systematic review.

| Authors | Complexed substance | Aspects improved |
|---|---|---|
| ***In vitro*** | | |
| *Essential oil* | | |
| Yim *et al.* [45] | *Melaleuca alternifolia* (terpinen-4-ol chemotype) | IC extended the time of repellency |
| Delogu *et al.* [46] | *Thymus catharinae* Camarda (carvacrol Chemotype) | Antimicrobial activity of the EO was efficiently modulated and had its effectiveness improved |
| Arana-Sánchez *et al.*[47] | *Lippia graveolens* | Antioxidant activity was improved by four-to eightfold |
| Rakmai *et al.* [48] | *Psidium guajava* L. | Antibacterial activity was improved by 2 and 4-fold after encapsulation and the antioxidant activity of the IC was more stable |
| Babaoglu *et al.* [49] | *Eugenia caryophyllata* L | Encapsulation increased the total phenolic content, antioxidant activity, solubility and controlled release |
| Rakmai *et al.* [50] | *Achillea millefolium* L | The antibacterial efficacy was much improved after encapsulation |
| Rakmai *et al.* [52] | *Piper nigrum* L. and β-caryophyllene (major component) | ICs increase their stability and antibacterial activity was improved by 4-fold |
| Galvão *et al.* [53] | *Citrus sinensis* L | IC induced high larval mortality |
| Haloci *et al.* [54] | *Satureja montana* | ICs showed high % of growth inhibition, antioxidant activity and the longer contact of the oil is due to its controlled released |
| Papajani *et al.* [55] | *Origanum vulgare* and *Rosmarinus officinalis* | Improved stability of both EOs |
| Haloci *et al.* [56] | *Satureja montana* | Antifungal activity and stability of the EO was improved |
| Tatsuoka *et al.* [57] | Eucalyptus, peppermint, thyme and wasabi essential oils; cineol and menthol as constituints | ICs decreased the pH of the fluid, and eucalyptus-αCD or wasabi-CD with fumaric acid reduced the rumen fluid |
| *Volatile components* | | |
| Celebioglu *et al.* [58] | Eugenol | The fast-dissolving character, water solubility, high temperature stability and antioxidant activity were enhanced |
| Aytac *et al.* [59] | Limonene | IC nanofibrous webs exhibited high antibacterial activity, thermal stability and increased water solubility |
| Abarca *et al.* [60] | 2-nonanone (2-NN) | ICs improved fungistatic behavior. |
| Celebioglu *et al.* [62] | Thymol | Water solubility, high temperature stability and antioxidant activity was enhanced |
| Ly *et al.* [63] | α-Longipinene, Isolongifolene and α-Humulene | This system was successfully applied for the efficient conversion of the components |
| LeBlanc *et al.* [64] | Thymol and Carvacrol | ICs improved the effective parasitic control |

| **Preclinical** | | |
|---|---|---|
| *Essential oils* | | |
| Siqueira-Lima *et al.* [66] | *Lippia grata* | ICs produced an antihyperalgesic effect, reducing primary and secondary hyperalgesia |
| Rodrigues *et al.* [67] | *Ocimum basilicum* | ICs prevented paw edema formation by decreasing vascular permeability, inhibiting leukocyte recruitment and inhibiting granuloma formation |
| Bonfim *et al.* [68] | *Annona vepretorum* | ICs enhanced tumor growth inhibition |
| Santos *et al.* [69] | *Cymbopogon winterianus* | ICs considerably reduced the dose of EO |
| Xi *et al.* [70] | Xiang-Fu-Si-Wu Decoction | ICs increased oral bioavailability |
| Menezes *et al.* [72] | *Hyptis pectinata* | ICs improved the pharmacological profile of the EO |
| Siqueira-Lima *et al.* [73] | *Lippia grata* | ICs were significantly capable of reducing the nociceptive face-rubbing behavior |
| Pan *et al.* [74] | Xiang-Fu-Si-Wu Decoction | ICs improved the absorption and bioavailability of all compositions |
| *Volatile components* | | |
| Silva *et al.* [75] | (−)-Linalool | ICs improved the gastroprotective effect and increased the solubility and stability |
| Santos *et al.* [76] | Citronellal | ICs improved the antihyperalgesic effect |
| Guimarães *et al.* [77] | Carvacrol | ICs increased stability, solubility and improved modulation on painful responses |
| Quintans-Júnior *et al.* [78] | (−)-Linalool | ICs improved the antinociceptive effect |
| Liu *et al.* [79] | β-caryophyllene | ICs improved oral bioavailability |
| Lins *et al.* [80] | Geraniol | The anticonvulsant activity was potentialized by ICs |
| Nascimento *et al.* [81] | Linalool | ICs improved analgesic profile |

Abbreviations – CD: Cyclodextrin; EO: Essentia oil; IC: Inclusion complex; αCD: α-cyclodextrin.

# CAPÍTULO 2. Preparo, caracterização e docking molecular do óleo essencial de *Alpinia zerumbet* complexado com sulfobutiléter-β-ciclodextrina

**CAPÍTULO 2**

## TITLE PAGE

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# Preparation, characterization and molecular docking of *Alpinia zerumbet* essential oil complexed with sulfobutylether-β-cyclodextrin

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## ABSTRACT

***Backgriund:*** Essential oil of *Alpinia zerumbet* (EOAz) is a natural product that displays a variety of biological activities and demonstrates an important pharmacological potential but its low solubility in water, low bioavailability and high volatility limit its therapeutic applications. Sulfobutylether-β-cyclodextrin (SBEβCD) is a chemically modified cyclodextrin belongs to a group of pharmaceutical excipients having a hydrophobic central cavity, the structure of which enables stable inclusion complexes (ICs) to be formed, increase solubility of substances in water. ***Porpuse:*** We aimed to evaluate physicochemical properties and possible interactions of EOAz complexed with SBEβCD. ***Methods:*** The EOAz was obtained by hydrodistillation, characterized by GC-MS and evaluated the antimicrobial activity against strains of pathogenic microorganisms. The EOAz was complexed with SBEβCD. The samples were prepared by co-precipitation (IC 1 and IC 2) and physical mixture (PM) in the molar ratio of 1:1 (EOAz:SBEβCD) and evaluated by FTIR spectroscopy, thermal analysis (TGA and DSC) and molecular docking. ***Results:*** EOAz presented terpinen-4-ol (22.37%), 1,8-cienole (19.65%) and γ-terpinene (14.77%) as major constituents and antimicrobial activity against all strains tested. In FTIR analysis there were shifts in bands of the O-H stretching mode of the SBEβCD spectrum in relation to IC 1 (3446 to 3421 $cm^{-1}$, 2938 to 2931 $cm^{-1}$ and 1161 to 1159 $cm^{-1}$) and the disappearance of the 2357 $cm^{-1}$ band present only in the OEAz spectrum; Thermal characterization indicates a decrease in mass loss of the EOAz of 95.13 % to 55.7, 55.8 and 53.78 in IC 1, IC 2 and PM, respectively. Molecular docking data revealed an interaction of 1,8-cienole ($\Delta E_{bind}$ = – 97.20 Kcal/mol) close to the center of the SBEβCD cavity suggesting formation of inclusion complex, while terpinen-4-ol ($\Delta E_{bind}$ = – 120.46 Kcal/mol) interacted outside the cavity. ***Conclusion:*** The displacement of bands of the spectrum in FTIR, the increase in thermal stability with decreased mass loss of EOAz and the conformation in the molecular docking of the interaction of 1,8-cineole suggest the formation of molecular complexes in CI 1 obtained with 24 h. Thus, the encapsulated of EOAz and their antimicrobial potential may contribute as a promising agent with potential biotechnological applications.


**Keywords:** Essential Oil, *Alpinia zerumbet*, Cyclodextrin, SBEβCD, Inclusion Complex.

## Introduction

Natural products, such as medicinal plants and secondary metabolite seem to offer an interesting alternative for the development of new drugs, due to their invaluable role as a source of new molecules that can act by innovative mechanisms of action (Li; Vederas, 2009). Essential oils (EOs) and their individual components are generally recognized as flavouring and fragrance agents in cosmetics and food industries (Marques, 2010). They can also be used in pharmaceutical and medical applications for their antioxidant, antimicrobial and anti-inflammatory activities or to neutralize undesirable taste of bitter drugs (Kfoury et al., 2016). EOs and their components are well accepted by consumers due to their natural origin and nutraceutical potential.

Among the plant species that produce essential oil, we have the *Alpinia zerumbet* (Pers.) Burtt. & Smith belonging to the Zingiberacea family. A study of the biological activity of essential oil of *A. zerumbet* (EOAz) has demonstrated an important pharmacological potential in blood pressure control, also acting on the smooth muscle of the intestine as an antispasmodic agent and as a local anesthetic (Santos et al., 2011). In addition to antioxidant, antibacterial and healing activity (Elzaawely et al., 2007; Mendes et al., 2015). However, a major issue is the low solubility and stability as well as the high volatility of EOs and their components that limit their application in the different fields (Turek; Stintzing, 2013).

Moreover, they do evaporate suggesting a need for encapsulation. A method of enhancing EOs solubility is their molecular encapsulation by cyclodextrins (CDs) (Marques, 2010). CDs are cyclic oligosaccharides derived from enzymatic degradation of starch. They have a truncated shape with a hydrophilic surface and a hydrophobic cavity that allows them to encapsulate guests and form inclusion complexes in solution or in solid state (Thompson, 1997). The most common native CDs are α-, β- and γ-CDs and are made up of six, seven and eight glucosyl units, respectively. CDs have been used to improve water solubility and also to reduce the number of doses and toxic effects (Oliveira et al., 2009).

Incorporating EOs as inclusion complexes in cosmetic, food or pharmaceutical formulations present several advantages. CDs might enhance the solubility of EOs and their components thus higher concentrations could be used (Loftsson et al., 2005; Kfoury et al., 2014), produce easy measurable dosage forms, facilitate their dispersion and protect them from interactions with other excipients (Salústio et al., 2011). CDs could also retain and allow a controlled release for EOs (Yang; Xiao, 2015; Kfoury et al., 2016) offer them thermal,

oxidative, light and chemical stability (Decock , 2008) and increase their oral bioavailability (Marques, 2010).

Among them, Captisol®, the trade name of SBEβCD (sulfobutylether-β-cyclodextrin) is a chemically modified β-cyclodextrin (βCD) with an average degree substitution of seven. Its solubility is significantly higher than the βCD (Lockwood; Malley; Mosher, 2003) and has been widely used as pharmaceutical excipients (Zhang et al., 2016). Moreover, its four-carbon butyl chain coupled with the repulsion of the end group's negative charge provides an extended hydrophobic cavity and an extremely hydrophilic exterior surface, thus forming an inclusion complex more stable, compared with other modified CDs (Yang et al., 2013; Deng et al., 2016). The SBEβCD showed characteristics as an efficient carrier and solubilizing agent for different essential oils (*Artemisia dracunculus, Citrus reticulata Blanco, Citrus aurantifolia, Melaleuca alternifolia, Melaleuca quinquenervia* and *Rosmarinus officinalis cineoliferum*) and their components (camphene, β-caryophyllene, p-cymene, 1,8-cineole, estragole, limonene, myrcene, α-pinene, β-pinene and γ-terpinene) in the formation of inclusion complexes (Kfoury et al., 2017).

To the best of our knowledge no previous study attempted to investigate the inclusion complexes of Captisol® (sulfobutylether-β-cyclodextrin, SBEβCD) and EOAz and their components, while compared to native CDs, these CDs exhibit greater water solubility and a more desirable safety profile (Kfoury et al., 2017). The aim of this study was to evaluate the ability of SBEβCD to forming inclusion complexe with the EOAz, characterize physicochemically by FTIR, TGA and DSC analysis, and also evaluate the possible interactions of the major constituents of EOAz with SBEβCD by molecular docking simulation.

## Materials and methods

### *Plant material*

Leaves of *Alpinia zerumbet* were collected in the Garden of Medicinal Plants Prof. Francisco José Abreu Matos at Federal University of Ceará (UFC), Fortaleza-Ceará, Brazil (3°44'48"S, 38°34'29"W), always in the morning. A voucher was deposited under registration number 56966 at Herbarium Prisco Bezerra of the UFC.

***Extraction of the essential oil of Alpinia zerumbet (EOAz) by hydrodistillation***

EOAz was obtained by hydrodistillation in a Cleavenger-type dispenser at the Natural Products Laboratory of the UFC using 1000 g of leaves. The freshly collected leaves were cut and placed in a glass flask along with distilled water and boiled for 2 h. After this period, the EOAz was separated from the water/oil mixture of the metered dose and dehydrated with anhydrous sodium sulfate and transferred to a clean flask with the aid of a Pasteur pipette. Then, the EOAz collected was weighed to calculate the % yield (w/w) and stored at -20°C.

***Physicochemical characterization and quantification of EOAz constituents by gas chromatography coupled to mass spectrometry (GC-MS)***

EOAz was analyzed at Technological Development Park (PADETEC) in UFC by Gas Chromatography coupled to Mass Spectrometry (GC-MS), model CG 17A and EM QP5050A of Shimadzu, equipped with OV-5 capillary column (30m x 0.25 mm x 0.25 mm) under the experimental conditions: electron impact ionization at 70 eV; injector in split mode (1:6); drag as helium gas and flow 1 ml/min; increasing temperature of 4 °C/min from 40 to 180 °C and 20 °C/min from 180 to 280 °C and maintained at this temperature for 10 minutes; temperature of the injector 250 °C and that of the detector 280 °C. The identification of the constituents was performed by comparing the adjusted mass spectrum and retention index using data from the literature retention rates.

***Preparation of the EOAz:SBEβCD inclusion complexes by co-precipitation***

The SBEβCD (CAPTISOL®) was kindly provided by Prof. Dr. Angelo Roncali and the preparation of the inclusion complexes (ICs) between the EOAz and SBEβCD was performed at the Laboratory of Drug Development at University of Fortaleza (UNIFOR) by co-precipitation method. All samples were prepared in a 1:1 stoichiometric molar ratio of SBEβCD and EOAz. The SBEβCD (300 mg) was previously solubilized in 15 ml of ethanol:water (1:2) solution heated at 55 °C for 30 min (150 rpm), 200 µl aliquot of EOAz was solubilized in ethanol PA (1300 µl) and added to the solution of SBEβCD and stirred at 150 rpm for 24 h (IC 1) and 6 h (IC 2) at 25 °C. The suspension was then removed from the stirring and cooled to 4 °C for 12 h and the precipitate was submitted to freeze-drying. To prepare the physical mixture (PM), an equimolar mixture (1:1) of EOAz and SBEβCD was weighted and mixed for 15 min. All the samples were stored in a glass desiccator at room temperature until submission for characterization analyses.

***Physicochemical characterization of the EOAz:SBEβCD inclusion complexes***

*Fourier-Transform Infrared Spectroscopy (FTIR) absorption*

The analysis was performed using a Fourier Transform Infrared Spectrometer (FTIR) (Spectrum Two, Perkin Elmer). Prior to the analysis, potassium bromide (KBr) tablets were prepared as a hydraulic press diluent containing the samples of EOAz, SBEβCD, IC 1, IC 2 and PM. The spectra were collected by accumulation of 64 scans between 4000 and 400 $cm^{-1}$ with a resolution of 4 $cm^{-1}$.

*Thermal Analysis*

The samples of EOAz, SBEβCD, IC 1, IC 2 and PM were subjected to thermogravimetric analysis (TGA) and differential scanning calorimetric (DSC) and the curves were obtained simultaneously using the thermal analysis system equipament (Jupiter STA 449, Netzsch). The DSC curves starting below room temperature were recorded with a Netzsch Maia 200 F3 calorimeter. The samples (10 mg) were placed insealed aluminum crucibles with pierced lids under $N_2$ atmosphere and a heating rate of 10 °C.$min^{-1}$ in the temperature range between 25 and 500 °C.

***Molecular docking assay***

The interaction between SBEβCD with 1,8-cineol and terpinen-4-ol (major components of the EOAz) were analyzed using molecular docking simulation, which consists of the use of a computational software with an algorithm for coupling two molecules, seeking to form a stable complex. The three-dimensional structure of the SBEβCD, 1,8-cineol and terpinen-4-ol are available in PubChem (133065445, 2758 and 11230, respectively). The docking was performed using the software HEX 8.0.0 (Macindoe et al., 2010), which performs the fittings automatically, based on the interaction energy of the components. The SBEβCD molecule was kept as a fixed truncated-cone structure and the 1,8-cineol and terpinen-4-ol were allowed free motion. The results were clustered to identify similar conformations and analyzed using the software PyMol v1.4.7, which allows a detailed investigation of the inclusion complexes formed (Delano, 2002). The host-guest inclusion complexes were selected based on the lowest binding energy. The parameters used inside the software interface for the fitting process were: Correlation type: shape only; Calculation Device – GPU (graphic process units); FFT Mode – 3D fast life; Grid Dimension – 0.6; Receptor range – 180; Ligand Range – 180º; Twist range – 360º and Distance range – 40.

***Antimicrobial assay***

*Determination of Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC)*

The determination of MIC was performed using the microdilution technique in microplates according to the Norm M7-A6 (CLSI, 2009). Suspensions of microbial strains (*klebsiella pneumoniae* ATCC 10031, *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538P, *Candida parapsilosis* ATCC 90018, *Candida albicans* ATCC 10231 and *Candida tropicallis* ATCC 750) were incubated overnight in brain heart infusion (BHI) broth for bacteria and broth sabouraud to yeasts at 37 °C until reaching the exponential growth stage. After this period, the cultures had their cell density adjusted to obtain a turbidity corresponding to 0.5 tube of the McFarland scale ($1x10^8$ CFU/mL). This suspension was diluted 100-fold in sterile BHI and sabouraud medium ($1x10^6$ CFU/mL). 80 μL of the microbial suspension, 100 μL of BHI broth and 20 μL of OEAZ (10.0 mg/ml - 0.0048 mg/ml) and SBEβCD (5.0 mg/ml - 0.0024 mg/ml). For the negative control, culture medium, the sterile diluent (sterile Tween 80 aqueous solution) and inoculum of the microorganism were used. The microplates were incubated at 37 °C for 24 h and after that time the reading was carried out at 620 nm. MIC was considered the lowest concentration of the sample capable of completely inhibiting microbial growth by naked eye inspection (absence of visible turbidity). The assays were performed in triplicate and the results expressed in mg/mL. The MCL determination was performed by counting the colonies using the drop plate technique. Aseptically, inoculums obtained from the wells of microplates used for determination of the MIC that did not show visible cloudiness were seeded on the surface of plate-count agar. The microplates were then incubated at 37 °C and after 24 h the counts of the colonies grown on the agar surface were counted. The lowest concentration of the sample able to determine the death of 99.9% of the microbial cells contained in the initial inoculum was considered MCL. The assays were performed in triplicate and the results expressed in mg/mL.

***Statistical analysis***

The results were analyzed with the statistical program GraphPad Prism® version 6.00 for Microsoft Windows®, where the Student's t test and Analysis of variance (ANOVA with Tukey post test) will be used. Data were expressed as mean ± standard error of the mean (M ± SEM) and were considered statistically significant when $p < 0.05$. The GraphPad Prism 6 program was used and the results were represented by graphs and tables.

## Results and Discussion

### *Extraction and identification of composition of the essential oil of Alpinia zerumbet (EOAz)*

EOAz extracted showed a strong characteristic odor, yellow color, density of 0.82 g/cm$^3$ and yield of 0.29 % (w/w) in relation to the mass of fresh leaves collected. The yield obtained was low, which is characteristic in the isolation of essential oils compared to the data cited in the literature (Santos *et al.*, 2011). The yield value found is among the values described by Mendes et al. (2015) and Victório et al. (2009), 0.17 and 0.44 %, respectively.

The GC-MS analysis identified and quantified 18 constituents present in the EOAz (Figure 1). The chemical composition of the EOAz was identified in its totaly (100%) with the following monoterpenes as major constituents: terpinen-4-ol (22.37 %), 1,8-cineole (19.65 %), γ-terpinen (14.77 %), sabinen (11.43 %) and p-cimene (5.58 %). These five components correspond to approximately 74% (73.80 %) of the total chemical composition of the oil. These and other components at lower concentrations are listed in Table 1. In other studies with EOAz, the results showed considerable differences between the concentrations of the compounds. Elzaawely et al. (2007) reported values of 6.18 and 16.63 %, Mendes et al. (2015) described values of 32.9 and 21.4 %, and Murakami et al. (2009) found concentrations of 21.1 and 29.1 to the main cnstituents terpinen-4-ol and 1,8-cienol, respectively.

The chemical composition, concentration of active constituents and yield of essential oils can vary according to the geographical parameters (soil, humidity, temperature), growth phases and parts used of the plants (leaves, flowers and seeds), genetic factors and the extraction method of essential oils (Rajabi et al., 2014).

### *Characterization of the EOAz:SBEβCD inclusion complexes*

*Fourier-Transform Infrared Spectroscopy (FTIR) absorption*

The FTIR technique is helpful in detecting the interaction between cyclodextrin and the guest molecules in a solid phase (Singh et al., 2010). The inclusion complex can be affirmed by the variation of shape, intensity and position of the peaks (Guo, 2018). FTIR spectra of EOAz, SBEβCD, IC 1, IC 2 and PM are shown in Graphic 1.

The EOAz spectrum showed several characteristic absorption peaks. The band at 3465 cm$^{-1}$ represents the vibration of stretching of the -OH groups of the essential oil substances and another band at 2922 cm$^{-1}$ is associated with the antisymmetric stretching vibration of the

-$CH_2$ groups. The C-H bonds of the hydrocarbon skeleton were observed at 2956 $cm^{-1}$. The monoterpene compounds of the essential oils have significant absorbance in the regions of 3,000 $cm^{-1}$, 2800 to 1650 $cm^{-1}$, and between 900 and 850 cm -1, as observed in the bands at 2956, 2357, 1650, 875 and 811 $cm^{-1}$.

For the SBEβCD spectrum, the prominent peaks shown were at 3446 $cm^{-1}$ corresponding to the O-H hydroxyl groups stretching vibration of βCD, similar to the results observed by Menezes et al. (2014). The band at 2938 $cm^{-1}$ is related to C–H stretching vibrations, bands in the region of 1450-1375 $cm^{-1}$ vibrations indicate stretching of $CH_3$ bonds of variable intensity, as observed at band 1417 $cm^{-1}$ and bands at 1160 to 1043 $cm^{-1}$ represents C–O-C stretching vibrations.

Nevertheless, some significant differences between SBEβCD and IC 1 were noted in the FTIR spectra. The band of O−H stretching mode of the SBEβCD was shifted from 3446 to 3421 $cm^{-1}$, 2938 to 2931 $cm^{-1}$, 2884 to 2879 $cm^{-1}$ and 1160 to 1159 $cm^{-1}$ and is related to changes in the hydration structure of cyclodextrin molecules, suggesting the possibility of constituents of EOAz was included in the SBEβCD cavity, which enabled it to form intermolecular hydrophobic interaction with SBEβCD and which was conducive to the formation of a more stable inclusion complex system. The band at 2357 $cm^{-1}$ present only in the OAEz spectrum, characteristic of monoterpene compounds, has also disappeared in the IC 1 spectrum and appears in the IC 2 spectrum changed to the band at 2386 $cm^{-1}$, suggesting that these bonds are possibly involved in the formation of these complexes, since the insertion of a host molecule within the cavity of the CD causes a conformational restriction, reducing the free movement of the encapsulated molecules, thereby reducing the signal strength (Aguiar et al., 2014).

The vibrations that appear in the SBEβCD spectrum also appear in the PM with similar profile (bands at 3430, 2928, 2873, 1165 and 1035 $cm^{-1}$). However, this pattern changes when compared to the complexed product in the IC 1 spectrum. The lower intensity of the PM spectra indicates a difference when the molecules are not complexed. The possible formation of inclusion complexes was only observed by physicochemical analysis in IC 1.

*Thermal Analysis*

Thermal analysis were conducted in order to evaluate the water/moisture content as well as the dissociation process of the host-guest supramolecular system to confirm the

formation of the inclusion complexes and their thermal stability (Fourmentin; Crini; Lichtfouse, 2018).

The curves obtained by TGA and DSC for EOAz, SBEβCD, IC 1, IC 2 and PM are shown in Graphic 2. The DSC curve of the EOAz presented one endothermic event followed by decomposition. The event occurred in the following temperature range: 124-146 °C ($T_{peak}$ = 134.34 °C) corresponding to its volatilization and decomposition at 230 °C. The TGA curve corroborate with this result, showing one step of mass loss of 95.13 % up to 229 °C. For SBEβCD sample, the DSC curve presented one endothermic event followed by decomposition. The event occurred in the following temperature range: 100-264 °C ($T_{peak}$ = 213.22 °C) corresponding to the dehydration in the cavity of SBEβCD and 267-307 °C ($T_{peak}$ = 289.03 °C) corresponding to its decomposition. The TGA curve showed three steps of weight loss whereas the first indicates 12.1 % up to 135 °C (water dissociation and evaporation), followed by 22.7 % up to 347 °C (thermal evaporation of EOAz), and 33.4 % up to 473 °C (thermal degradation), resulting in an overall mass loss of 68.2 %.

The TGA and DSC curve for IC 1 and IC 2 showed similar behaviors of weight loss at similar temperatures. The samples presented two endothermic events followed by decomposition. In the IC 1, the event occurred in the following temperature range: 89-271 °C (release of water molecules as well as to release of some volatile compounds from essential oil) and 271-300 °C ($T_{peak}$ = 282.89 °C) corresponding to its decomposition. The TGA curve showed three steps of weight loss whereas the first indicates 10.5 % up to 147 °C (water release), followed by 25.5 % up to 334 °C (thermal evaporation of EOAz), and 19.7 % up to 470 °C is the thermal degradation of SBEβCD, resulting in an overall mass loss of 55.7 %. For the IC 2 sample, the event occurred in the following temperature range: 89-269 °C (water release) and 269-311 °C ($T_{peak}$ = 286.22 °C) corresponding to its decomposition. The TGA curve showed three steps of mass loss whereas the first indicates 8.5 % up to 124 °C, followed by 26.1 % up to 331 °C, and 21.2 % up to 466 °C is the thermal degradation of SBEβCD, resulting in an overall mass loss of 55.8 %. The DSC curve of the PM obtained the similar profile of endothermic event as SBEβCD. The event occurred in the following temperature range: 100-268 °C ($T_{peak}$ = 241.48 °C) corresponding to the dehydration and 266-301 °C ($T_{peak}$ = 279.42 °C) corresponding to its decomposition. The TGA curve showed three steps of mass loss whereas the first indicates 12.97 % up to 153 °C, followed by 21.5 % up to 349 °C (thermal evaporation of EOAz), and 19.31 % up to 459 °C, resulting in an overall

mass loss of 53.78 %. The overlap of DSC and TGA curves of all samples are shown in Graphic 3 and 4, respectively.

In the inclusion complexes, the complexation process some of water molecules are replaced by hydrophobic compounds from essential oils. The disappearance of thermal transitions such as melting point or glass transition of guest molecules in the presence of CDs is well known, which is used to confirm the formation of inclusion complexes between CDs and guest molecules (Celebioglu et al., 2018). The characteristic event of CD dehydration on the complex's DSC curve may shift due to the replacement of water molecules in the cavity by guest molecules, which results in altered energy status (LI et al., 2005). In addition, when compared to pure EOAz, the thermal evaporation and mass loss of the essential oil has shifted to much higher temperature in IC 1 and IC 2 samples, which is due to the interaction between EOAz and SBEβCD.

***Molecular docking assay***

At present, molecular docking based on molecular mechanics is been widely applied to characterize the three-dimensional structure of inclusion complexes with cyclodextrins by considering the complex binding energy (Sapte; Pore, 2016).

Terpinen-4-ol and 1,8-cineole, the main components identified in the EOAz of this study, were used as model compounds. The docking calculations revealed, out of 50,000 possibilities of conformations, the 10 most energetic in the combination of 1,8-cineole and terpinen-4-ol with SBEβCD. Regarding affinity and specificity, the most energetic clusters promote overlaps at the same site for the guest molecules and CD. We observed that the docking of the 10 conformers generated by 1,8-cineole with the SBEβCD ocorred forming six clusters in close to the cavity center and by terpinen-4-ol forming eight cluster with the binder complex shape outside the receptor cavity, located near the narrow-side of the host molecule. The stick modes obtained by molecular docking for 1,8-cineole and terpinne-4-ol complexed with SBEβCD are shown in Figure 2.

The binding energy ($\Delta E_{bind}$) acted as the effective interaction energy in the composite, which is summarized in Table 3. The lowest energy binding was – 97.20 Kcal/mol for 1.8-cineole possibly forming an inclusion complex stable close to the cavity of the SBEβCD and for the terpinen-4-ol presentend the lowest energy binding was -120.46 Kcal/mol forming an interaction outside the SBEβCD cavity. Generally, the more negative the binding energy is,

the stronger the interaction is between host and guest molecules (Zhang et al., 2015). Regarding the interaction of molecules with SBEβCD, Figure 3 shows the spatial proximity of protons of 1,8-cineole and terpinen-4-ol close and protons of the SBEβCD cavity, presents the distance of intermolecular interaction that were observed between 1.1 and 2.1 Å for 1,8-cineol stabilized by several hydrophobic contacts and between 1.1 and 1.9 Å for terpinen-4-ol. The overlap of interaction of 1,8-cineole and terpinen-4-ol in the SBEβCD structure are shown in Figure 4.

Physicochemical properties of the guest molecule such as the size, charge, and polarity can influence the ability and stability of inclusion complex formation (Lawtrakul; Inthajak; Toochinda, 2014). There was a direct correlation with the host-guest hydrophobic interaction: the wider the contact surface, the stronger the hydrophobic bonds (Faucci et al., 2002). On account of molecular size, the 1,8-cineole is smaller, thus it was better able to fit into the SBEβCD cavity. Hence, it has a wider contact interface with SBEβCD as opposed to terpinen-4-ol.

Lawtrakul, Inthajak and Toochinda (2014) described the molecular calculations on βCD inclusion complexes with five essential oil compounds from *Ocimum basilicum* (sweet basil) which included linalool, eugenol, methyl eugenol, estragole, and eucalyptol (1,8-cineole). As results, only 1,8-cineole shows a single possibility conformation while other guest molecules provide more than one possible conformation due to the molecular structure of 1,8-cineole, with no available rotatable bond in the molecule. According to the steric hindrance, 1,8-cineole can enter βCD cavity at the wider rim and can form only inclusion complex conformation. The dimethyl group of 1,8-cineole molecule provides the possibility of inclusion complex by hydrophobic interaction.

***Antimicrobial activity***

The antimicrobial activity of EOAz were evaluated against Gram-negative and Gram-positve bacterial and yeast strains at different concentrations. The EOAz showed antimicrobial activity against all strains tested with MICs and MLCs ranging from 2.5 to 5.0 mg/mL. The EOAz showed the same MIC value of 2.5 mg/mL to all strains and MLCs values of 2.5 mg/mL to *K. pneumoniae, E. coli* and *C. albicans* and 5.0 mg/mL to *S. auereus, C. parapsilosis* and *C. tropicallis.* The MIC and MLC values of the EOAz are listed in Table 2. The SBEβCD showed no antimicrobial activity against all strains tested. Optical density readings at 620 nm of the microplates confirmed the MIC values determined in the visual

inspection of the growth. In all graphics the values are expressed by the mean ± SEM of three assays, in triplicate each, using as control the culture medium + microorganism (Graphic 5).

The antimicrobial activity of the EOAz was attributed to the high monoterpene concentration, mainly terpinen-4-ol and 1,8-cineole, which are compounds showing antimicrobial activity and synergism with the other constituents (Rodrigues et al., 2009; Victório et al., 2009). It is generally admitted that the major compounds determine the biological properties of an essential oil (Kerdudo et al., 2017). Essential oils from *A. zerumbet* present a great variety of compounds involved in the antimicrobial activity. Terpinen-4-ol and 1,8-cineol have been reported to inhibit several microorganisms (Matasyoh et al., 2006; Mendes et al., 2015). Similar results with Gram-positive and Gram-negative bacteria tested with EOAz revealed the same major constituents (terpinen-4-ol and 1,8 cineole) (Indrayan; Tyagi; Agrawal, 2010). Other studies have reported the inhibitory ability of 1,8-cineole against Gram-positive and Gram-negative bacteria (Hendry et al., 2009; Marzoug et al., 2011).

The effectiveness of antimicrobial activity of essential oils differs from one type to another as well as against different target bacteria depending on their structure (Gram-positive and Gram-negative bacteria. (Swamy; Akhtar; Sinniah, 2016). In general, Gram-negative bacteria were more resistant to the essential oil components duo the presence of a hydrophilic outer layer, which can block the penetration of hydrophobic components through the target cell membrane (Dorman; Deans, 2000). The mechanism of action of terpene compounds from essential oils is related to their effects on the cellular membrane, altering its function, causing swelling and increasing permeability (Rodrigues et al., 2011). Essential oils tested against yeast strains are capable of causing damage to the cell envelope and therefore interfere with the development of yeast pathogenetic structures and cell division (Nakamura et al., 2004; Oliveira et al., 2016).

**Conclusion**

EOAz leaves have a rich essential oil content, which was analysed by CG-MS and presented 1,8-cineole and terpinen-4-ol as major constituents, also showed antimicrobial acitivity against gram-negative and gram-positive bacterial and yeast strains tested. The complexation of EOAz with SBEβCD was obtained by the co-precipitation and the results of FTIR and thermal analysis (TGA and DSC) revealed the improvement in the thermal stability of EOAz when interacterd with SBEBCD compared to free EOAz in the IC 1 sample,

obtained with 24 hours, suggesting the formation of inclusion complex. Interactions close to the cavity and outside of the SBEβCD were observed in the molecular docking data to 1,8-cineole possibly forming an inclusion complex and terpinen-4-ol, interecting outside. Thus, these characteristics may contribute to the processing and storage of the essential oil and its bioviablity as well as the possibly EOAz:SBEβCD inclusion complex is a promising agent with potential biotechnological applications.

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**Conflict of interest**

Authors declare no conflict of interest

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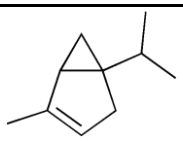
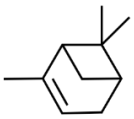
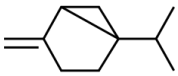
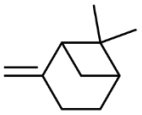
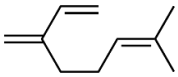
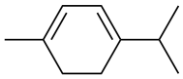
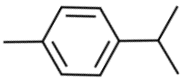
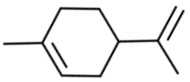
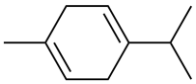
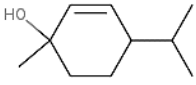
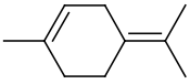
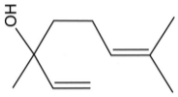
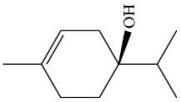
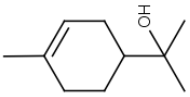
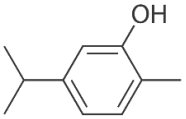
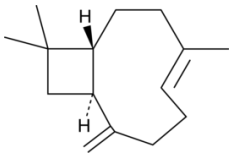
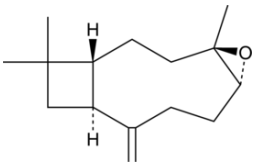
**Table legends**

Table 1. Chemical composition of leaf essential oil of *Alpinia zerumbet* (EOAz).

| Peak | Constituents | Structure | RT (min) | Area (%) |
|---|---|---|---|---|
| 1 | α-Thujene |  | 11.985 | 3.94 |
| 2 | α-Pinene |  | 12.394 | 1.53 |
| 3 | Sabinene |  | 14.580 | 11.43 |
| 4 | β-Pinene |  | 14.807 | 2.73 |
| 5 | β-Mircene |  | 15.423 | 1.40 |
| 6 | α-Terpinen |  | 17.029 | 3.22 |
| 7 | p-Cimene |  | 17.509 | 5.58 |
| 8 | Limonene |  | 17.770 | 2.27 |
| 9 | 1,8-Cineole |  | 17.975 | 19.65 |
| 10 | γ-Terpinen |  | 19.584 | 14.77 |
| 11 | p-Menth-2-en-1-ol |  | 20.072 | 0.82 |
| 12 | Terpinolene |  | 21.356 | 1.77 |
| 13 | Linalol |  | 21.927 | 2.24 |

| | | | | |
|---|---|---|---|---|
| 14 | Terpinen-4-ol |  | 26.915 | 22.37 |
| 15 | α-Terpineol |  | 27.609 | 1.25 |
| 16 | Carvacrol |  | 33.438 | 1.49 |
| 17 | β-Cariophyllene |  | 40.944 | 1.85 |
| 18 | Cariophyllene Oxyde |  | 47.933 | 1.70 |
| | | | | 100.00 |

RT = Retention Time.

**Table 2**. Minimum inhibitory concentration (MIC) and minimal lethal concentration (MLC) of the essential oil of *Alpinia zerumbet* (EOAz) against all strains tested.

| **Microbial strains** | **MIC (mg/mL)** | **MLC (mg/mL)** |
|---|---|---|
| *klebsiella pneumoniae* ATCC 10031 | 2.5 | 2.5 |
| *Escherichia coli* ATCC 10536 | 2.5 | 2.5 |
| *Staphylococcus aureus* ATCC 6538P | 2.5 | 5.0 |
| *Candida parapsilosis* ATCC 90018 | 2.5 | 5.0 |
| *Candida albicans* ATCC 10231 | 2.5 | 2.5 |
| *Candida tropicallis* ATCC 750 | 2.5 | 5.0 |

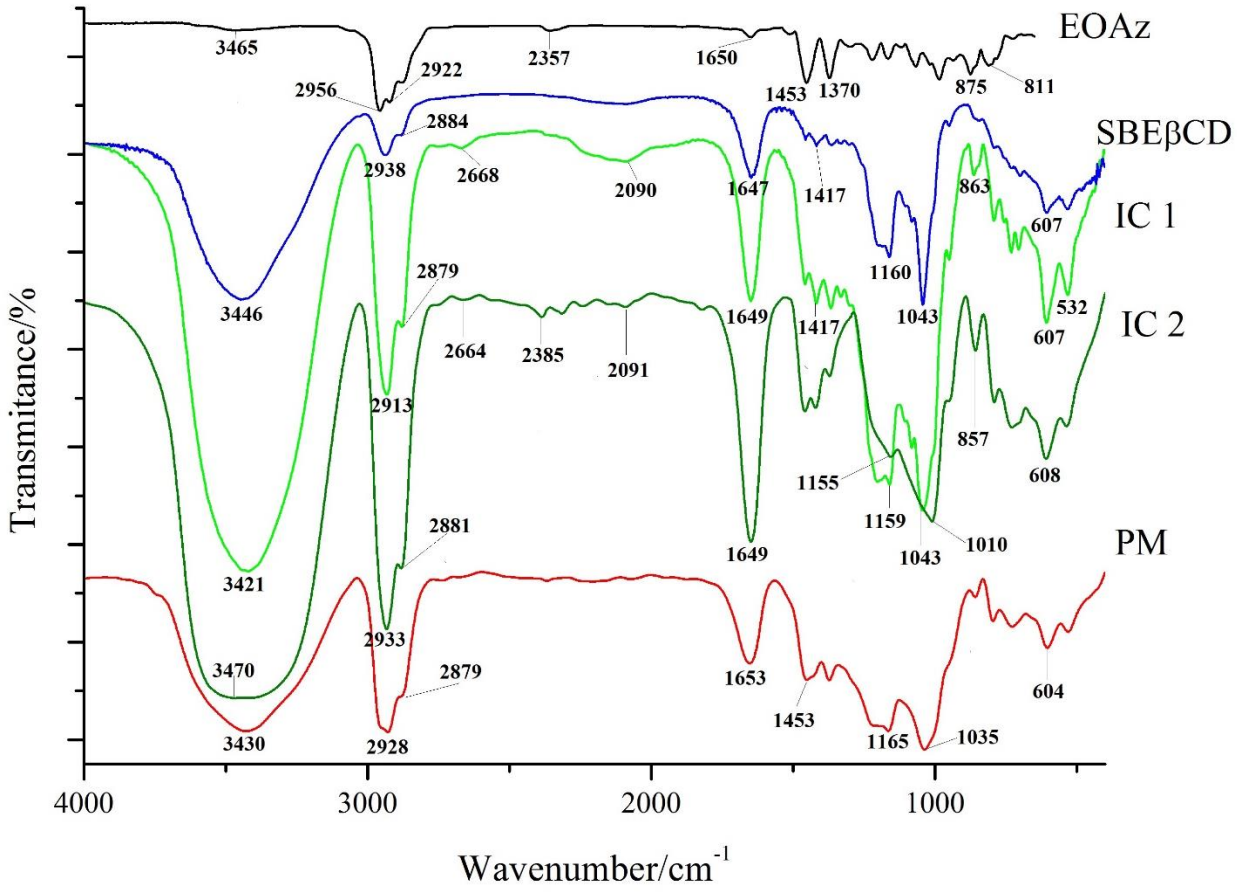
ATCC = American Type Culture Collection

**Table 3.** The binding energy between 1,8-Cineole and Terpinen-4-ol against SBEβCD ($E_{total}$ – Kcal/mol).

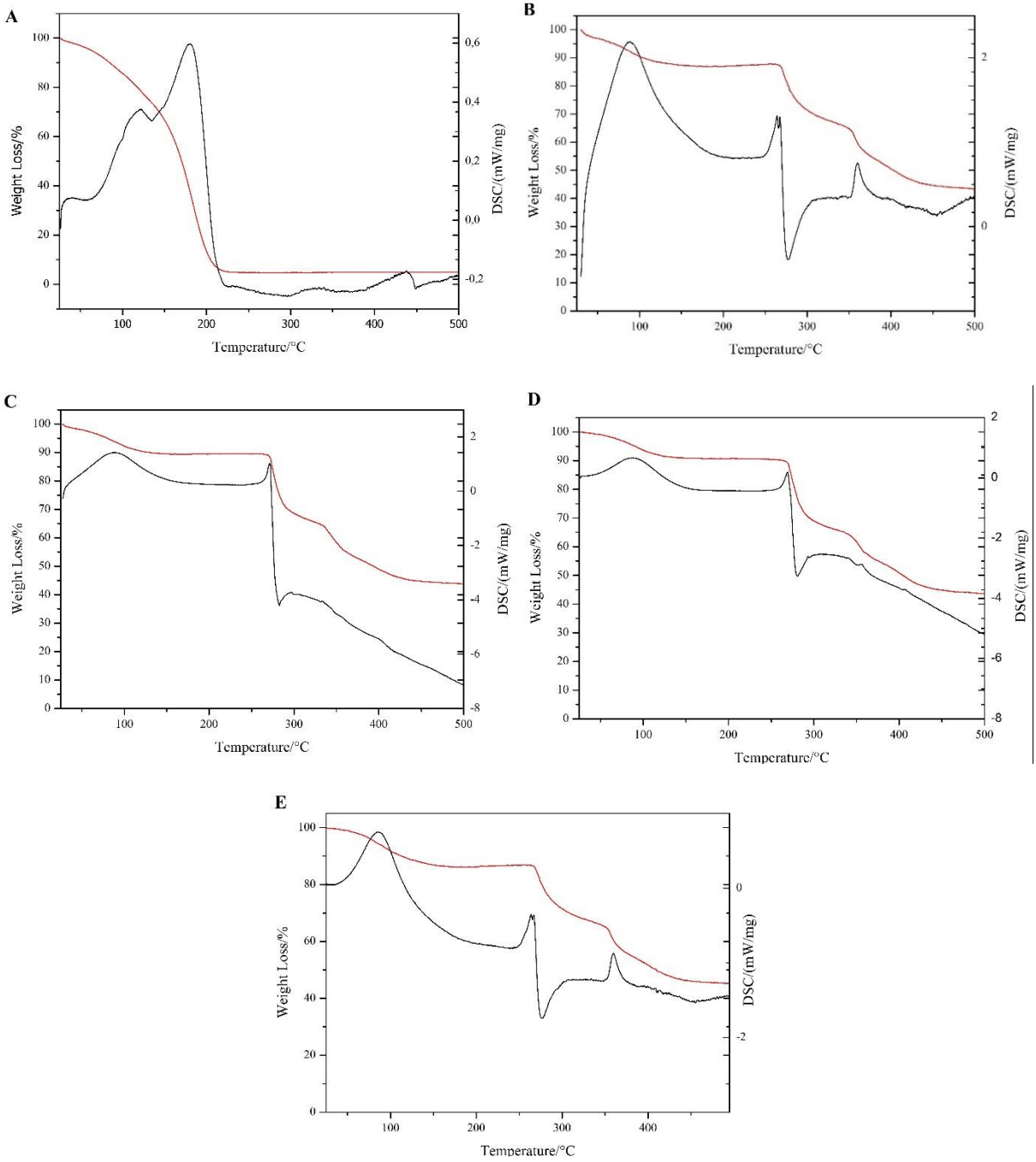
| **Clusters** | **1,8-Cineole x SBEβCD $E_{total}$ (Kcal/mol)** | **Terpinen-4-ol x SBEβCD $E_{total}$ (Kcal/mol)** |
|---|---|---|
| 01 | -97.20 | -120.46 |
| 02 | -96.82 | -119.22 |
| 03 | -93.93 | -116.52 |
| 04 | -93.27 | -113.36 |
| 05 | -93.01 | -113.05 |
| 06 | -92.54 | -112.39 |
| 07 | -91.27 | -112.10 |
| 08 | -91.07 | -111.46 |
| 09 | -90.91 | -111.19 |
| 10 | -90.43 | -110.64 |

**Grahpic legends**

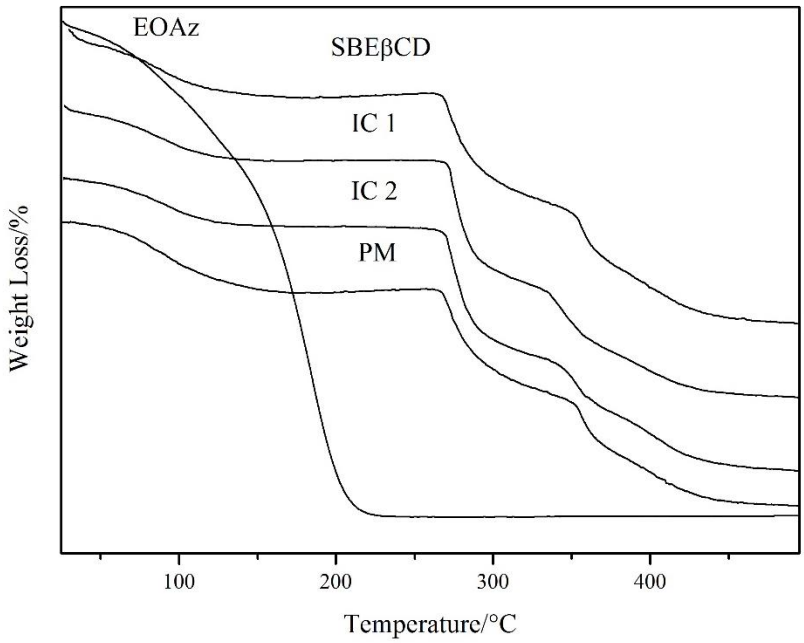
**Graphic 1.** FTIR spectra of essential oil of *Alpinia zerumbet* (EOAz) (black line), sulfobutylether-β-cyclodextrin (SBEβCD) (blue line), EOAz:SBEβCD inclusion complex obtained for 24 h (IC 1) (light green line), EOAz:SBEβCD inclusion complex obtained for 6 h (IC 2) (dark green line) and physical mixture (PM) (red line).



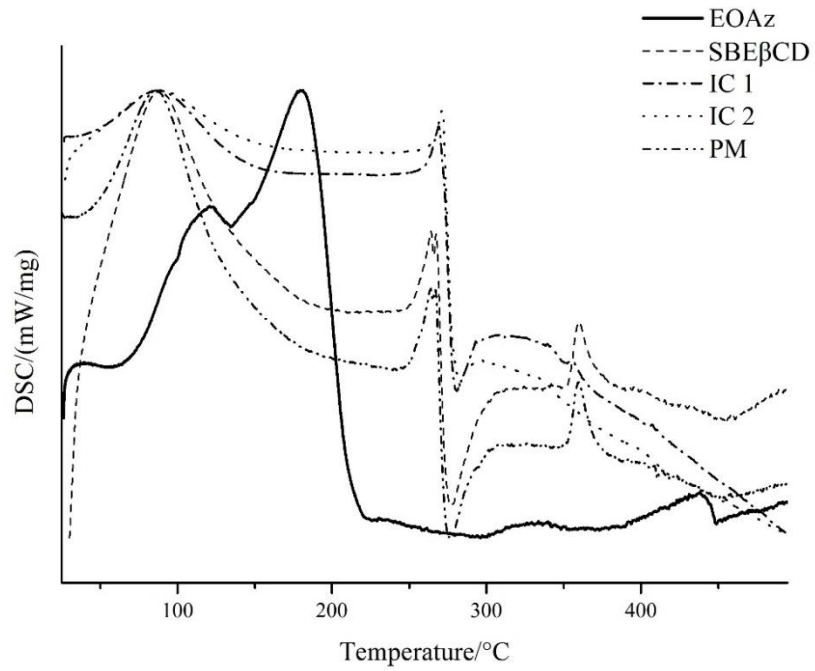
**Graphic 2.** TGA (red line) and DSC (black line) curves of essential oil of *Alpinia zerumbet* (EOAz) (A), sulfobutylether-β-cyclodextrin (SBEβCD) (B), EOAz:SBEβCD inclusion complex obtained for 24 h (IC 1) (C), EOAz:SBEβCD inclusion complex obtained for 6 h (IC 2) (D) and physical mixture (PM) (E) in dynamic nitrogen atmosphere and heating of 10 °C.min$^{-1}$ in the temperature range from 25 to 500 °C.



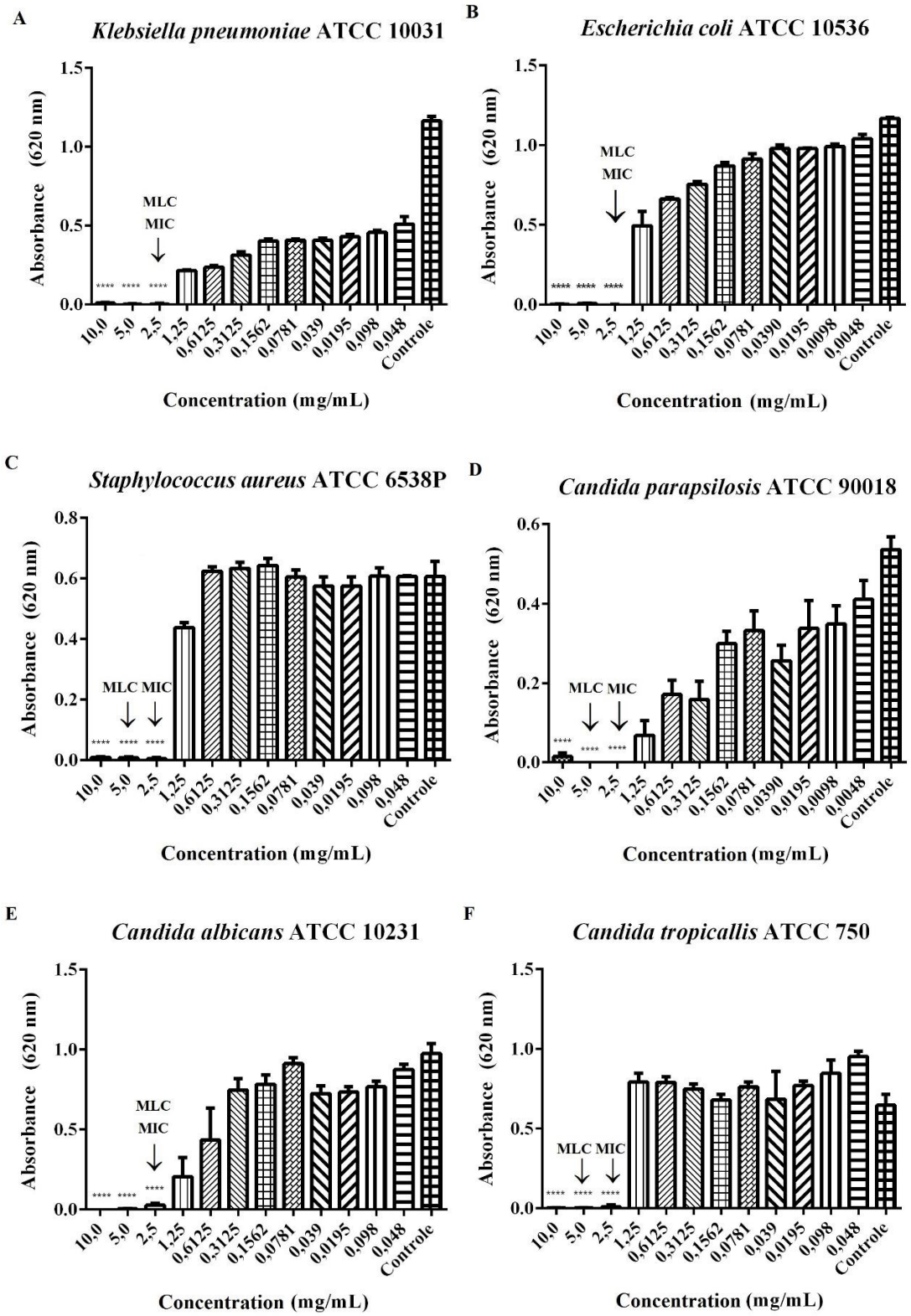
**Graphic 3.** Overlap of TGA curves of essential oil of *Alpinia zerumbet* (EOAz), sulfobutylether-β-cyclodextrin (SBEβCD), EOAz:SBEβCD inclusion complex obtained for 24 h (IC 1), EOAz:SBEβCD inclusion complex obtained for 6 h (IC 2) and physical mixture (PM).




**Graphic 4.** Overlap of DSC curves of essential oil of *Alpinia zerumbet* (EOAz), sulfobutylether-β-cyclodextrin (SBEβCD), EOAz:SBEβCD inclusion complex obtained for 24 h (IC 1), EOAz:SBEβCD inclusion complex obtained for 6 h (IC 2) and physical mixture (PM).



**Graphic 5.** Antimicrobial activity of essential oil of *Alpinia zerumbet* (EOAz) against (A) *K. pneumoniae* ATCC 10031, (B) *Escherichia coli ATCC* 10536, (C) *Staphylococcus aureus* ATCC 6538P, (D) *Candida parapsilosis* ATCC 90018, (E) *Candida albicans* ATCC 10231) and (F) *Candida tropicalis* ATCC 750. Control = culture medium + microorganism.



## Figure legends

**Figure 1**. Total chromatogram of the essential oil of *Alpinia zerumbet* (EOAz) constituents by gas chromatography coupled to mass spectrometry (GC-MS).

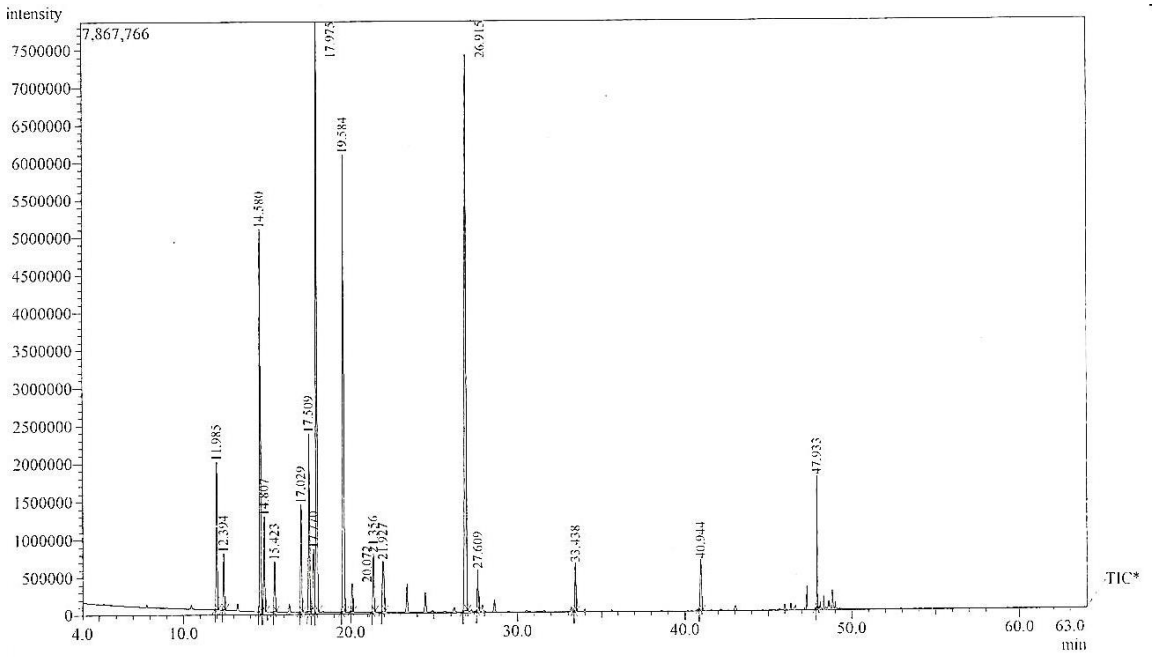



**Figure 2.** SBEβCD, terpinen-4-ol and 1,8-cineole are presented as stick model in the docking molecular simulation. The three-dimensional structure of the SBEβCD (purple stick) is responsible for the inclusion of overlapping molecules from the ten most energetic clusters. (A) 1,8-cineole formed six clusters (yellow, green and blue sticks) close to the center of the cavity and (B) terpinen-4-ol formed eight cluster (yellow, light blue and pink sticks) with the binder complex shape outside the cavity, located near the narrow-side of the host molecule.

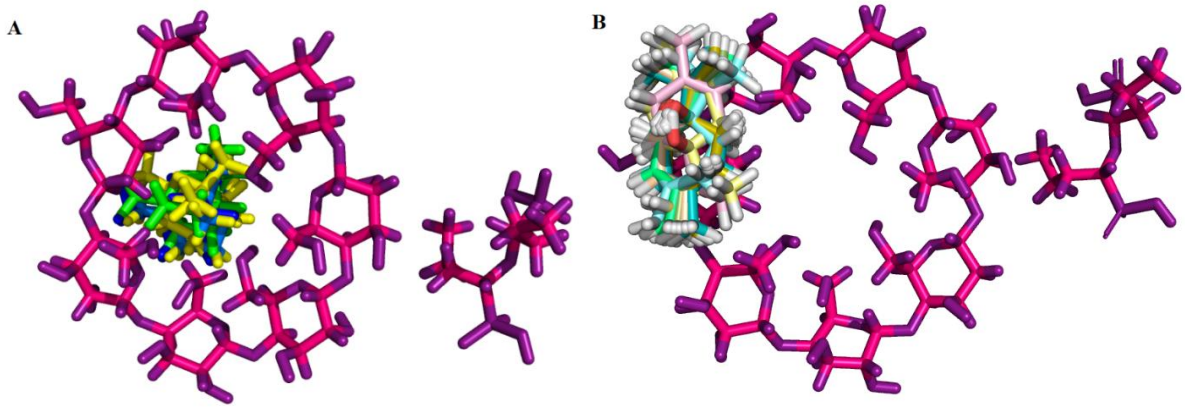


**Figure 3.** SBEβCD, terpinen-4-ol and 1,8-cineole are presented as stick model in the docking molecular simulation. Theorical spatial proximities between the protons of (A) 1,8-cineole (yellow and blue sticks) and (B) terpinen-4-ol (yellow and green sticks) and protons of the cavity of SBEβCD (purple sticks) obtained throught molecular docking in a vacuum with the energy-minimized mode.

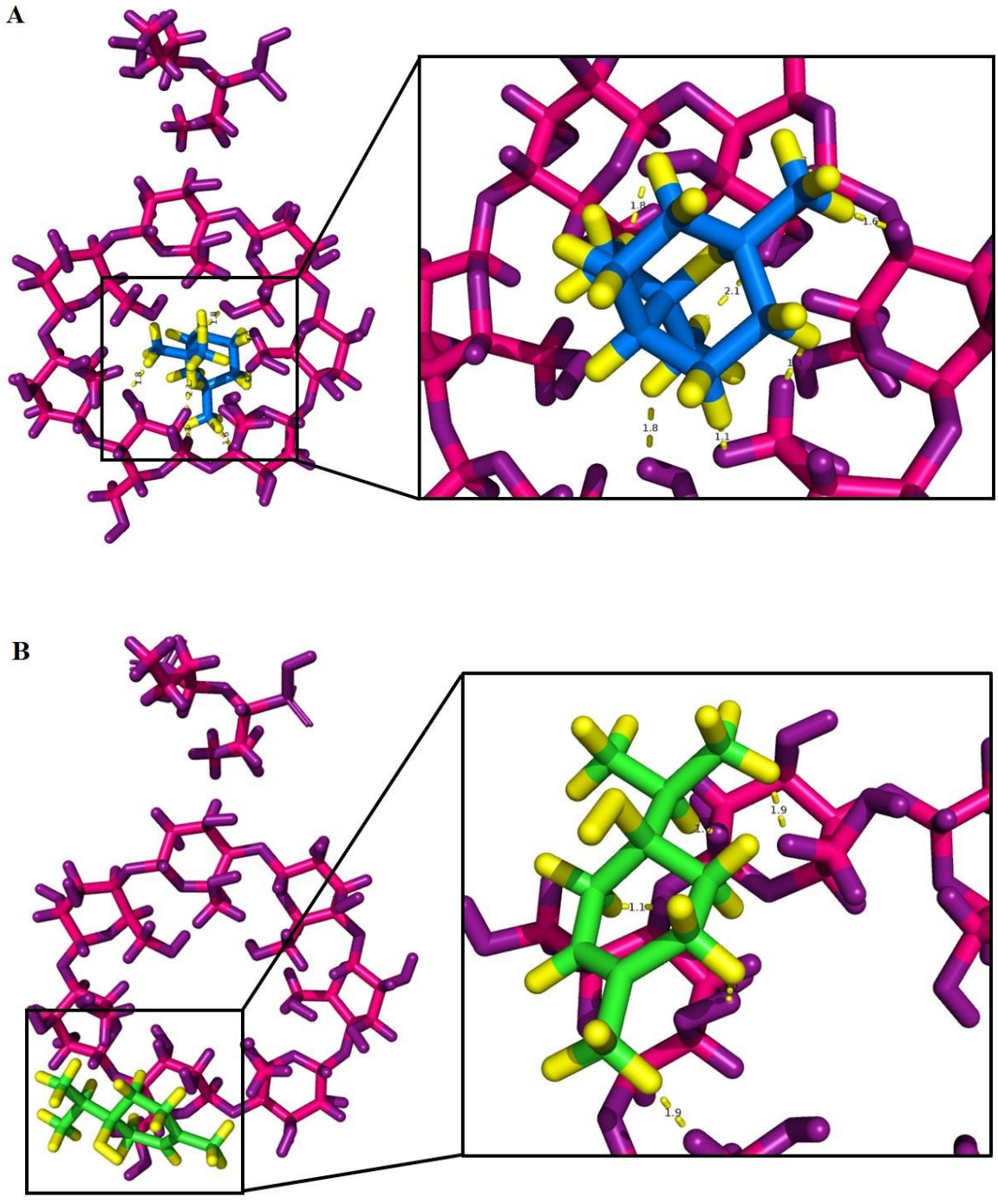
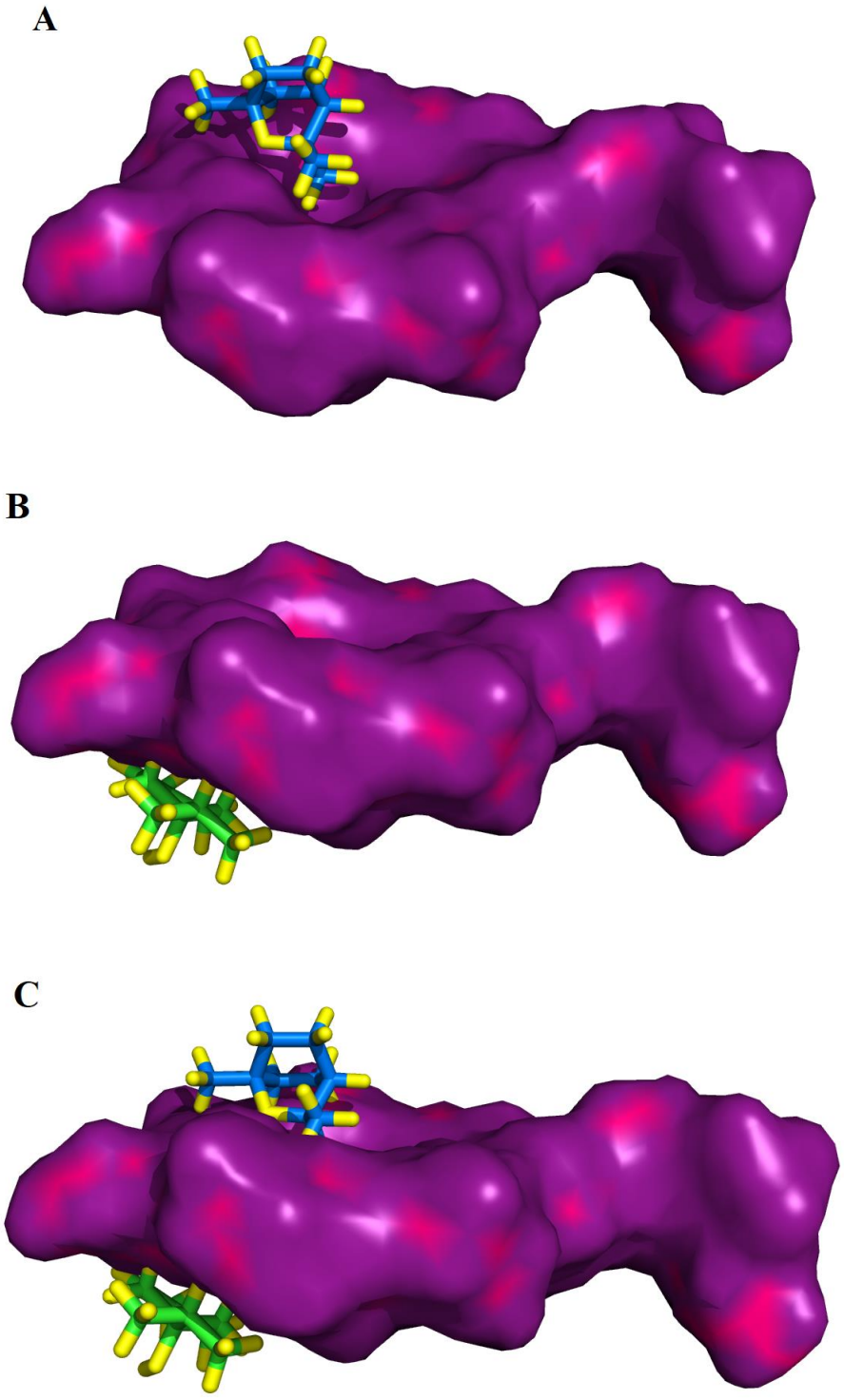


**Figure 4.** Terpinen-4-ol and 1,8-cineole are presented as stick model and the SBEβCD is molecular surface representation of electron density omission graphic (omit map). Molecular models of interaction of (A) 1,8-cineole (yellow and blue sticks), (B) terpinen-4-ol (yellow and green sticks) and the (C) overlap of both molecules between the SBEβCD (purple molecular surface) structure.



## 2. DISCUSSÃO

A revisão sistemática é um tipo de estudo que tenta reunir todos os artigos que realizaram ensaios que se encaixam em critérios preestabelecidos a fim de responder uma questão específica. O método sistemático é utilizado de maneira explícita de modo a minimizar erros e apresentar resultados confiáveis, para guiar a tomada de decisões em futuras pesquisas (GALVÃO; PEREIRA, 2014). A revisão sistemática realizada no capítulo 1 investigou atividades biológicas (*in vitro*) e aplicações farmacológicas (*in vivo,* pré-clínico) de óleos essenciais (OEs) e seus componentes voláteis (CVs) complexados com ciclodextrinas (CDs), em que o maior objetivo foi avaliar se a formação de complexos de inclusão (CI) promoviam a melhor eficácia desses compostos nas aplicações testadas, quando comparado à sua aplicação em formas não complexadas.

Os estudos *in vitro* selecionados apresentaram diversos tipos de atividades avaliadas, dentre elas antibacteriana, antifúngica, larvicida, acaricida e antioxidante, com diferentes OEs e CVs isolados complexados em uma grande varidade de CDs (α-CD, β-CD, HP-β-CD, HP-γ-CD e M-β-CD). Estudos como de Arana-Sànchez et al. (2010) demosntrou que a atividade antioxidante do óleo essencial de *Lippia graveolens* foi melhorada de quatro a oito vez quando complexado em β-CD, isso se evidencia pelo aumento da biodisponibilidade desses compostos com característica hidrofóbica (MARQUES, 2010). Rakmai et al. (2017) demonstoru que o óleo essencial de *Piper nigrum* L. e seu componente majoritário β-cariofileno complexados com HP-β-CD tiveram aumento de sua estabilidade e a atividade antibacteriana aumentou em quatro vezes.

Os estudo *in vivo* selecionados, tratando-se de ensaios pré-clínicos, avaliaram diferentes efeitos farmacológicos em modelos animais, dente eles atividade antihiperalgésica, anti-inflamatória, antitumoral, antinociceptiva, analgésica e gastroprotetora, com diferentes OEs e CVs isolados, todos complexados apenas com β-CD. Dentre esses estudos, Bonfim et al. (2016) mostrou que o óleo essencial de *Annona vepretorum* complexado obteve um aumento na inibição dos tumores indizidos em camundongos machos. Assim como Siqueira-Lima et al. (2017) evidenciou que o óleo essencial de *Lippia grata* complexado produziu efeito anti-hiperalgésico, reduzindo a hiperalgesia primária e secundária em camundongos machos. Os estudos desmonstraram a efetividades do uso de CDs em uma variedade de atividades biológicas e farmacológicas testadas com OEs e seus CVs com intuinto de melhorar a eficácia terapêutica.

A elaboração, apresentação e discussão dos artigos selecionados na revisão sistemática promove um material importante no embasamento científico comprovado do uso de CDs para complexação de OEs e CVs. A melhoria da solubilidade tem sido a principal característica obtida pela formação dos complexos de inclusão, o que proporciona outros fatores como biodisponibilidade e eficácia terapêutica (PINHO *et al*., 2014; LIMA *et al*., 2016).

Com base no capítulo 2, a utilização de plantas com fins medicinais para tratamento, cura e prevenção de doenças, é uma das mais antigas formas de prática medicinal da humanidade. O Brasil é o país com maior biodiversidade do planeta, apresentando grande potencial para geração de pesquisas, desenvolvimento e inovações de produtos oriundos de plantas medicinais, com grande diversidade de princípios ativos, essências e formulações para a obtenção de medicamentos, despertando interesse econômico, principalmente do mercado farmacêutico (BRANDÃO *et al.*, 2006). Os estudos multidisciplinares com plantas medicinais são essenciais em decorrência das possibilidades terapêuticas dos princípios ativos das plantas, sendo a fitoterapia uma área alvo de farmacêuticos e biotecnologistas (VICTÓRIO *et al*., 2011).

Nesse âmbito, o mercado farmacêutico nacional e internacional tem valorizado a pesquisa e o desenvolvimento de medicamentos oriundos de plantas, devido o fato dessa produção apresentar uma melhor relação custo-benefício quando comparada aos produtos sintéticos, ação biológica com baixa toxicidade e efeitos colaterais, custo de produção e preço de venda mais acessíveis (CORRÊIA; SCHEFFER, 2014). Em 2006, foi criada a Política Nacional de Plantas Medicinais e Fitoterápicos nos serviçoes de saúde do SUS (Sistema Único de Saúde) representando além da incorporação de mais uma terapêutica às possibilidades de tratamento à disposição dos profissionais de saúde, o resgate de uma prática milenar, onde se utiliza o conhecimento científico e o conhecimento popular e seus diferentes entendimentos sobre o tratamento de enfermidades (FIGUEREDO; GURGEL; GURGEL-JUNIOR, 2014)

A presença de óleos essenciais (OEs) é uma das principais causas das propriedades farmacêuticas das plantas, formados como metabólitos secundários. São compostos voláteis, naturais e complexos caracterizados por um forte odor, consistem em grande parte em grupos moleculares como os álcoois simples, aldeídos, cetonas, fenóis, ésteres, ácidos orgânicos, lactonas, entre outros, sendo a grande maioria constituída de derivados de terpenóides, principalmente de monoterpenos e sesquiterpenos (SIMÕES *et al*., 2010; PROPERZI *et al*.,

2013). Possuem papel de destaque nas pesquisas em produtos naturais por possuírem o seu potencial farmacológico comprovado em estudos científicos, serem de fácil extração e economicamente viáveis (MARQUES, 2010).

As variações de composição assim como as respectivas concentrações e rendimento, para uma mesma espécie, variam com as diferenças climáticas, localização, estações do ano e métodos de extração (BAKKALI *et al*., 2008; REHMAN *et al*., 2016). Geralmente, os componentes principais determinam as propriedades biológicas do OE, contudo, estudos farmacológicos tem mostrado que a intensidade de seus efeitos depende da concentração dos componentes majoritários modulados sinergicamente por outros componentes de menores concentrações (MENDES et al., 2010).

Como possibilidade fitoterápica, têm-se a *Alpinia zerumbet*, planta facilmente encontrada no nordeste e popularmente conhecida como "colônia". O óleo essencial de *Alpinia zerumbet* (OEAz) vem sendo apresentado como uma fonte rica em monoterpenos como 1,8-cineol e terpinen-4-ol (NASCIMENTO *et al*., 2005; PINHO *et al.*, 2005; PINTO *et al.*, 2009). Há uma ampla variedade de compostos com atividade antimicrobiana no OEAz (VICTÓRIO *et al*., 2009), o que indica seu grande potencial para desenvolvimento de novas drogas. O uso de óleos essenciais como agentes antimicrobianos oferece um baixo risco de desenvolvimento de resistência microbiana, pois sendo misturas de diferentes compostos, sua atividade pode estar relacionada a diferentes mecanismos de ação o que dificulta a adaptação dos microrganismos (DAFERERA; ZIOGAS; POLISSIOU, 2003).

A concentração inibitória mínima (CIM) e concentração bactericida mínima (CBM) foram empregadas para a avaliação da propriedade antimicrobiana do OEAz com diferentes tipos de bactérias (Gram-positivas e Gram-negativas) e leveduras. O OEAz apresentou atividade inibitória para os todos microrganismos patogênicos testados e, por isso, é um composto que tem potencial de uso terapêutico. Esses resultados corroboram com outros autores (VICTÓRIO *et al*., 2009; MENDES *et al*., 2015). A atividade antimicrobiana dos OEs está relacionada com a sua hidrofobicidade, característica que favorece a interação com os lipídeos das membranas celulares e com as mitocôndrias das células microbianas. Essas interações geralmente alteram a permeabilidade das células bacterianas causando distúrbios nas estruturas e resultando em vazamento de íons, moléculas e conteúdo celular, conduzindo os microrganismos à morte ou à inibição do seu crescimento (BURT, 2004). Com base nestas

constatações científicas, as células bacterianas Gram-negativas são mais resistentes por possuírem uma parede celular hidrofílica (KIM *et al*., 1995).

Os OEs apresentam alta volatilidade, baixa solubilidade em água e instabilidade química em resposta à luz, calor, umidade e ar, o que limita sua aplicabilidade terapêutica (ADORJAN; BUCHBAUER, 2010). Para melhorar essas propriedades fisico-químicas, algumas estratégias tecnológicas como a complexação em sistemas de liberação de fármacos com ciclodextrinas (CDs) ou o uso de nanocarreadores, têm sido empregadas (LIMA *et al*., 2016; QUINTANS-JÚNIOR *et al*., 2018). Diversos autores têm demostrado que a βCD forma complexos de inclusão com os OEs, e seus derivados isolados, melhorando a biodisponibilidade, efeito e estabilidade (QUINTANS-JÚNIOR *et al*., 2018; SIQUEIRA-LIMA *et al*., 2017).

As CDs apresentam uma cavidade apolar e em solução aquosa são capazes de interagir com diversas classes de moléculas, formando complexos estabilizados por ligações não covalentes, denominados de complexos de inclusão, caracterizados pela penetração parcial ou total de moléculas na cavidade das ciclodextrinas. Esta propriedade tem sido principalmente explorada no que diz respeito à encapsulação de fármacos e na química supramolecular, podendo vincular fármacos hidrofóbicos dentro de sua cavidade lipofílica e, assim, reduzir sua volatilidade e toxicidade, aumentar a estabilidade e a solubilidade em água, controlar a biodisponibilidade e mascarar características organolépticas (LAZA-KNOERR; GREF; COUVREUR, 2010).

A principal interação que proporciona a formação dos complexos de inclusão consiste na substituição de moléculas de água, presentes nas estruturas das CDs, por moléculas hóspedes de menor entalpia, gerando um processo energicamente favorável por desencadear uma alteração de entalpia, aumento da entropia e redução da energia total do sistema, corroborando para o aumento da estabilidade do complexo (LAZA-KNOERR; GREF; COUVREUR, 2010). Outras interações, como as forças de Wan der Waals, ligações de hidrogênio, interações eletrostáticas e hidrofóbicas, também auxiliam para a formação e estabilização dos complexos (YAO *et al*., 2014).

A formação dos complexos de inclusão (CIs) pode ser avaliada com auxílio da técnica de espectroscopia na região do infravermelho com transformada de Fourier (FTIR), empregada para confirmar a presença dos grupos químicos funcionais específicos dos

substratos de componentes de OEs, dos grupos funcionais específicos da CD utilizada como molécula encapsulante, bem como as possíveis modificações químicas ou físicas ocorridas devido ao processo de complexação (QUINTANS-JÚNIOR et al., 2018). Para o complexo de inclusão, são observadas características importantes nos espectros de FTIR que corroboram a complexação do OE com a CD, em que das modificações significativas pode ser observada na banda associada com os modos vibracionais dos grupos O-H (WANG *et al*., 2011).

A banda associada com os modos de vibração do O-H do OEAz é apresentada em 3465 $cm^{-1}$, enquanto que no espectro obtido com SBE-β-CD (sulfobutil-éter-β-ciclodextrina) a banda mostrou-se em 3446 $cm^{-1}$, enquanto que para o complexo CI 1 (OEAz:SBE-β-CD obtido por 24 horas) os mesmos modos de vibração são apresentados em 3421 $cm^{-1}$. Esse deslocamento vibracional sugere relação com as alterações da estrutura de hidratação das moléculas de CD. É possível considerar que a interação física entre as moléculas de OEAz e as moléculas de SBE-β-CD modificam as ligações entre as moléculas da SBE-β-CD e as moléculas de água em sua cavidade ou com os grupos químicos funcionais O-H (WANG *et al*., 2011).

A análise termogravimétrica (TGA) é uma técnica fundamentada na medida da massa de um composto enquanto ocorre a variação da sua temperatura por um período de tempo. Os termogramas fornecem informações importantes a respeito da estabilidade térmica e de transições térmicas do material em análise (SKOOG *et al*., 1991). Segundo Wang et al. (2011), de acordo com os termogramas obtidos para as amostras o processo de perda de massa pode ser dividido em dois estágios para as ciclodextrinas, em que o primeiro ocorre no intervalo de temperatura de 25 a 82°C e corresponde à perda de água adsorvida na estrutura da CD e o segundo estágio inicia próximo a 300°C e corresponde a sua decomposição térmica.

A SBE-β-CD apresenta-se estável em temperaturas de 100°C até próximo a 300°C, enquanto as amostras CI 1 e CI 2, com possíveis complexos de inclusão formados, apresentam perda de massa contínua nesse mesmo intervalo de temperatura. Essa perda de massa pode estar associada com a decomposição térmica ou volatilização das moléculas do OEAz que não estão complexadas. A massa residual do complexo é menor que a massa residual da decomposição da ciclodextrina pura, devido ao fato de que, considerando o o possível complexo, além da perda de massa da decomposição da ciclodextrina, ocorre a perda de massa de OEAz encapsulado (PRABU *et al*., 2015).

A fim de se avaliar possíveis interações de componentes presentes no OEAz, realizou-se uma simulação de docking molecular para avaliar a conformação de tais componentes em maior concentração e com probabilidade na formação do complexos de inclusão. Utilizou-se as moléculas 1,8-cineol e terpinen-4-ol, componentes majoritários identificados no OEAz do estudo, e a SBE-β-CD como estrutura complexante. O encaixe dos 10 conformações geradas pela melócula de 1,8-cineol com o SBE-β-CD ocorreu formando seis aglomerados próximo ao centro da cavidade, apresentando energia de ligação $\Delta E_{bind}$ = -97,20 Kcal/mol, enquanto o terpinen-4-ol formou oito aglomerados com interagindo por fora da cavidade receptora da CD com energia de ligação $\Delta E_{bind}$ = 120,46 Kcal/mol.

As propriedades físico-químicas da molécula complexada, como tamanho, carga e polaridade podem influenciar a capacidade e a estabilidade da formação do complexo de inclusão (LAWTRAKUL; INTHAJAK; TOOCHINDA, 2014). Devido o tamanho molecular de 1,8-cineol, que é menor, e portanto, capaz de se encaixar na cavidade do SBE-β-CD, há uma correlação direta com a interação hidrofóbica hospedeiro-hóspede à medida que quanto maior a superfície de contato, mais fortes as ligações hidrofóbicas (FAUCCI *et al*., 2002).

Sengundo Lawtrakul, Inthajak e Toochinda (2014), descreveram os cálculos moleculares dos complexos de inclusão de β-CD com cinco compostos de óleo essencial de Ocimum basilicum (manjericão), dentre eles o 1,8-cineol, que apresentou uma única possibilidade de conformação, devido sua estrutura molecular sem ligação rotativa disponível na molécula. De acordo com o impedimento estérico, o 1,8-cineol pode entrar na cavidade β-CD na borda mais larga e pode formar apenas uma conformação complexa de inclusão. O grupo dimetil da molécula 1,8-cineol fornece a possibilidade de complexo de inclusão por interação hidrofóbica.

A SBE-β-CD é uma β-CD modificada pela ação do grupo sulfobutil-éter à sua superfície hidrofílica, logo, o diâmetro da cavidade é mesmo entre as β-CD naturais (KFOURY *et al.*, 2017). Tais características estruturais e fisico-químicas do 1,8-cineol, sugerem a formação de um possível complexo com interação próximo à cavidade hidrofóbica da SBE-β-CD, com base em cálculos da simulação de docking molecular.

## CONSIDERAÇÕES FINAIS

A proposta inicial desse trabalho tinha como objetivo o desenvolvimento de complexos de inclusão com o óleo essencial de *Alpinia zerumbet* (OEAz) com sulfobutil-éter-β-ciclodextrina (SBE-β-CD), mas pela necessidade de conhecimento em produção, caracterização e aplicação desses complexos, a elaboração de uma revisão sistemática promoveu uma busca fundamentada e direcionada em ciclodextrinas e óleos essenciais, tornando-se uma contribuição oportuna para a comunidade científica.

A revisão sistemática realizada encontrou estudos *in vitro* e pré-clínicos (*in vivo*) que testaram uma grande variedade de óleos essenciais (OES) e seus componentes voláteis complexadas em diferentes tipos de ciclodextrinas (CDs). Estudos *in vitro* mostraram maior eficácia nas propriedades de EOs e seus componentes quando complexados em CDs em uma variedade de atividades biológicas, devido aumento de solubilidade, estabilidade e biodisponibilidade. Os estudos pré-clínicos mostram que os complexos de inclusão foram capazes de melhorar o perfil farmacológico em vários modelos animais de nocicepção, anti-inflamatório, anti-hiperalgésico e antitumoral com melhor eficácia, reduzindo as doses terapêuticas e os efeitos colaterais.

A partir do óleo essencial de *Alpinia zerumebt* (OEAz) extraído e da análise por GC-MS foi possível determinar a composição química, onde também apresentou atividade antimicrobiana *in vitro* contra leveduras, bactéricas Gram-negativas e Gram-positivas de importância clínicas. A complexação do OEAz com SBE-β-CD com o intuito de potencializar as propriedades fisico-químicas do OE foi avaliada e sugerida a possível formação de complexos de inclusão pelas técnicas de caracterização como FTIR, TGA e DSC. As amostras de complexos CI 1 e CI 2 obtidas possibilitaram uma maior estabilidade térmica quando comparado ao OEAz de forma não complexada.

A abordagem molecular adotada pelo uso de docking molecular apresentou a perspectiva de possível interação de dois dos componentes majoritários 1,8-cineol e terpinen-4-ol do OEAz com a SBE-β-CD. Apenas, a molécula de 1,8-cinoel foi possível de encaixe próximo à cavidade hidrofóbica da SBE-β-CD.

O uso de CDs é um método alternativo para o desenvolvimento biotecnológico de novas formulações farmacêuticas com melhora de eficácia terapêutica e, portanto, com perspectiva de aplicabilidade clínica. O desenvolvimento de complexos de inclusão

representam potenciais inovações biotecnológicas, bem como evidenciam a importância da novos estudos para permitir a ampliação de mais compostos terapêuticos e maior eficácia farmacológica dos tratamentos existentes.

Diante do exposto, a realização de mais técnicas de caracterização são imprescindíveis para o estudo de ciclodextrinas e formação de complexos de inclusão como estudo de Solubilidade de fases, Liberação *in vitro*, Estabilidade, Difração de raios X e Microscopia eletrônica de varredura. Estudos adicionais utilizando os complexos de inclusão obtidos para avaliação de atividade antimicrobiana com objetivo de otimizar a ação do óleo em comparação ao mesmo em sua forma não complexada. Assim como avaliar a complexação de componentes isolados do OEAz, principalmente majoritários, com a SBE-β-CD e também sua atividade antimicrobiana.

Com relação ao docking molecular, é importante realizar testes com os demais componentes presentes no OEAz, sejam em maior (majoritário) ou menor concentração de forma a compreender a possível interação supramolecular dos compomentes e da SBE-β-CD.

Com a obtenção e mais dados complementares de caracterização dos complexos de inclusão, cabe, ainda, a avaliação de propriedades farmacológicas evidenciadas do OEAz como anti-hipertensiva, antitumoral e analgésica em modelos animais. O uso de CDs é um método alternativo para o desenvolvimento biotecnológico de novas formulações farmacêuticas com melhora de eficácia terapêutica e, portanto, com perspectiva de aplicabilidade clínica. O desenvolvimento de complexos de inclusão representam potenciais inovações biotecnológicas, bem como evidenciam a importância da novos estudos para permitir a ampliação de mais compostos terapêuticos e maior eficácia farmacológica dos tratamentos existentes.

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# ANEXOS

## ANEXO I - COMPROVANTE DE PUBLICAÇÃO - ARTIGO DO CAPÍTULO 1

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**REVIEW ARTICLE**

# Biological Activities and Pharmacological Applications of Cyclodextrins Complexed with Essential Oils and Their Volatile Components: A Systematic Review

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**Abstract:** ***Background:*** Essential oils (EOs) and their volatile components (VCs) have varied biological and pharmacological activities, but low solubility and bioavailability hamper their applications, so that inclusion in cyclodextrins (CDs) is likely to improve their physicochemical properties and pharmacological effects.

***Objective:*** The authors conducted a systematic review to evaluate the biological activities and pharmacological applications of essential oils and their volatile components complexed with cyclodextrins.

***Methods:*** The search terms 'Cyxlodextrin', 'Inclusion Complex', 'Volatile oils', 'Essential oil' and 'Volatile components' were used to retrieve articles from the PUBMED, MEDLINE and SCOPUS databases.

***Results:*** A total of 38 articles were identified. A greater efficacy of EOs and their VCs complexed with different CDs types was found in *in vitro* and preclinical studies when compared to free forms in the various biological activities and animal models of the evaluated pharmacological tests.

***Conclusion:*** This review of selected studies showed that the use of CDs promotes greater solubility, bioavailability and efficacy of EOs and their VCs, thus indicating an interesting alternative for the biotechnological development of new therapeutic formulations.

**Keywords:** Cyclodextrin, volatile oils, essential oils, inclusion complex, biological activities, pharmacological application, systematic review.

### 1. INTRODUCTION

Essential oils (EOs) are volatile or semi-solid liquids characterized by strong odor and synthesized as secondary metabolites in aromatic plants, usually extracted by hydrodistillation [1]. The amount and composition of these oils varies according to the location in different plant organs (flowers, leaves, roots, stems, seeds, bark and whole fruits) [2]. EOs are highly complex mixtures of volatile components (VCs) that can be classified according to the chemical structure of the hydrocarbon as terpenes (mono-, sesqui- and diterpenes), its oxygenated derivatives, terpenoids (phenolic compounds, aliphatic aldehydes, ketones, amines, lactones, alcohols and esters) and aromatic compounds, all present in very different concentrations [3-5]. Terpenes are derived from combinations of 5-carbon (C5) units called isoprenes. Among these, monoterpenes (C10) are the most common molecules in the composition of essential oils (e.g. myrcene, terpinenes, p-cymene, sabinene, geraniol, linalool, citronellol, menthol, camphor, 1,8-cineole, thymol and carvacrol) [6, 7]. Sesquiterpenes (C15) have similar structure and function as monoterpenes (e.g. β-bisabolene, cadinenes, β-caryophyllene, logifolene, farnesenes, bisabol, cedrol, farnesol and germacrona) [8]. In turn, aromatic compounds are derived from phenylpropane and occur less frequently than terpenes (e.g., cinnamaldehyde, chavicol, eugenol, anethole and estragol) [9, 10].

EOs and their constituents are considered the most important bioactive natural products derived from plants [11, 12] which have traditionally been used in folk medicine with a broad spectrum of biological activities such as antioxidant [13], antibacterial [14], antifungal [15], insecticide [16] and larvicide [17], in addition to pharmacological applications with anti-inflammatory [18], antidepressive [19], analgesic [20], and antinociceptive [21] effects, and also in the management of health problems such as diabetes [22], cancer [23] and Alzheimer's diseases [24]. These properties of EOs are directly correlated to the concentration and proportion of their constituents, where two or three major bioactive components are present in higher concentrations or in synergism with other constituents, being responsible for a distinct role in the biological activity of EOs [25]. However, it is likely that the activity of the major components is modulated by other smaller molecules [26]. In relation to the main components, these reflect the biological characteristics of the EOs, in which the effectiveness of their activities depends on their concentration in the isolated form or comprised in EOs [27]. Volatile compounds belong to the class II drugs of the biopharmaceutical classification system (BCS) due to their low solubility and high permeability, mainly for passive transport through plasma membranes [28].

However, the low solubility in water, high volatility, short shelf life, susceptibility to degradation by exposure to air, light and heat limit the therapeutic applications of EOs and their VCs due to the low bioavailability. Protection of these compounds in formulations of drug delivery systems can promote the integrity of their bioactivities [29, 30]. Thus, the complexation of EOs and their VCs in inclusion complexes (ICs) with cyclodextrins (CDs) is a way of solving the problems by improving the solubility and stability and avoiding the degradation and volatilization of bioactive compounds in the EOs, in addition to transforming them into water-dispersible powders [31-33]. CDs are one of the most commonly used complexing agents by the pharmaceutical industry as pharmaceutical excipients due to their low price and high production, as well as

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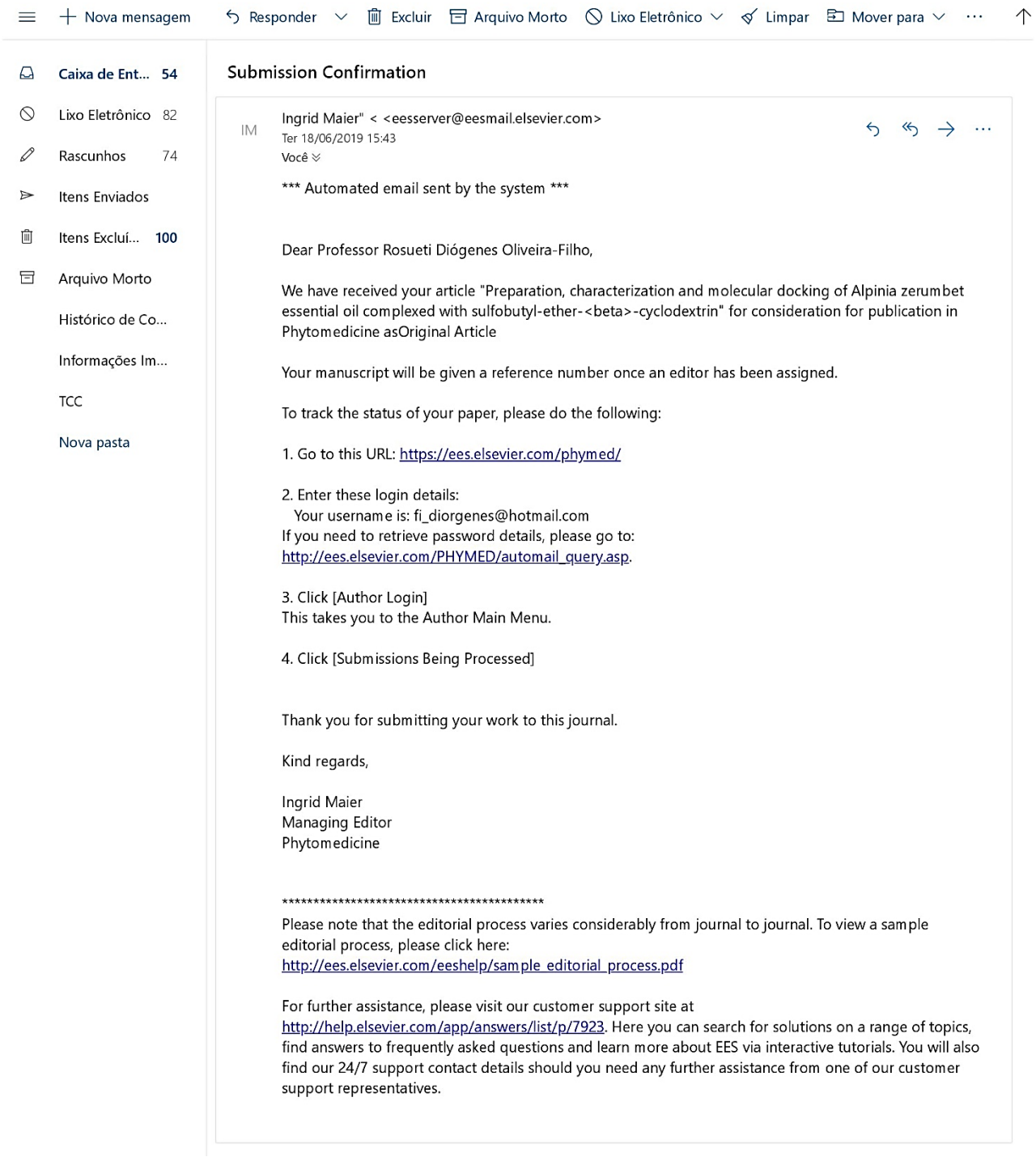
# ANEXO II - COMPROVANTE DE SUBMISSÃO - ARTIGO DO CAPÍTULO 2



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