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NATÁLIA KAORI AIDA

ATIVIDADE ANTIMICROBIANA DO ÓLEO ESSENCIAL DE *Origanum vulgare* E SEU COMPOSTO ISOLADO SOBRE CÉLULAS DE *Streptococcus* spp., *Candida* spp. e *Staphylococcus aureus* MRSA.

ANTIMICROBIAL ACTIVITY OF *Origanum vulgare* ESSENTIAL OIL (OREGANO) AND ITS COMPOUND ISOLATED AGAINST CELLS OF *Streptococcus* spp., *Candida* spp. and *Staphylococcus aureus* MRSA.

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Trabalho de Conclusão de Curso apresentado à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Cirurgião Dentista.

Udergraduate final work presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Dental Surgeon

Orientador: Prof. Dr. José Francisco Hofling

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DO TRABALHO DE CONCLUSÃO DE CURSO APRESENTADO PELA ALUNA NATÁLIA KAORI AIDA E ORIENTADA PELO PROF. DR. JOSÉ FRANCISCO HOFLING.

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RESUMO

A literatura demonstra que o óleo essencial de Origanum vulgare e seu composto isolado carvacrol apresentam efeitos antimicrobianos como atividade antibacteriana e antifúngica. O objetivo deste trabalho foi avaliar a atividade antifúngica do óleo essencial e do composto contra cepas padrão de Candida spp., Streptococcus spp. e Staphylococcus aureus Meticilina resistente pelo método de microdiluição em caldo M27-A3 (CLSI, 2008), na determinação da CIM e CFM. O antifúngico comercial Fluconazol e a Clorexidina foram utilizados como parâmetros de susceptibilidade. O óleo essencial de Origanum vulgare e o composto isolado Carvacrol mostraram-se biologicamente ativos de maneira dose dependente contra as espécies testadas em sua forma planctônica.

Palavras-chave: Origanum. Plantas medicinais. *Candida. Staphylococcus aureus. Streptococcus*

ABSTRACT

The literature has shown that the Origanum vulgare essential oil and its isolated compound carvacrol have antimicrobial effects as antibacterial and antifungal activity. The objective of this work was to evaluate the antifungal activity of essential oil and compound against standard strains of Candida spp., Streptococcus spp. and Staphylococcus aureus Methicillin resistant by the M27-A3 broth microdilution method (CLSI, 2008) in the determination of MIC and CFM. Commercial antifungal Fluconazole and Chlorhexidine were used as susceptibility parameters. *Origanum vulgare* essential oil and isolated compound Carvacrol showed up biologically active in a dose dependent manner against the species tested in their planktonic form.

Key words: Origanum. Plants, Medicinal. *Candida. Staphylococcus aureus. Streptococcus*

SUMÁRIO

1 INTRODUÇÃO

Desde os tempos remotos, a natureza é fonte de meios de sobrevivência para o homem. Dela ele utilizava as plantas, como alimento e remédio. A utilização de plantas medicinais tem feito parte da evolução humana e foram os primeiros recursos terapêuticos utilizados pelos antigos povos. A aplicação destas, ora curavam, ora causavam a morte ou produziam efeitos colaterais. O conhecimento sobre elas e suas propriedades, vinham na por meio da observação dos animais ou por meio do conhecimento empírico, e eram passadas oralmente de geração em geração. Os primeiros relatos do uso de plantas com finalidade terapêutica foram escritos em cuneiforme. Esses relatos datam de 2.600 a.C. e são originários da Mesopotâmia. Outras descrições escritas são encontradas na obra Pen Ts'ao ("A grande fitoterapia"), de 2.800 a.C. do fundador da medicina chinesa Shen-Nong. E também nos antigos papiros do Egito, de 2.000 a.C., mostram que um grande número de médicos utilizavam plantas como remédio e consideravam as doenças como resultado de causas naturais, e não como resultado de magia ou poderes de espíritos maléficos. Outros relatos do uso de plantas medicinais em antigas civilizações mostram que os egípcios, assírios e hebreus cultivavam, desde 2.300 a.C., diversas ervas e traziam tantas outras de suas expedições. Na Índia por volta de 1.500 a.C., foi criado um dos sistemas medicinais mais antigos da humanidade, a Ayurveda. Na antiga Grécia, Hipócrates (460 – 377 a. C.), o pai da medicina, foi responsável por grande parte da sabedoria sobre plantas, reunindo em sua obra Corpus Hipocratium, uma síntese dos conhecimentos médicos de seu tempo, indicando para cada enfermidade, um remédio vegetal e um tratamento adequado. No Ocidente, os relatos da utilização da fitoterapia datam do século III a.C. que o botânico Teofrasto listou cerca de 455 plantas medicinais com detalhes de como preparar e usar cada produto. Na Era Cristã, o médico grego militar Pedanius Dioscórides (40 – 90 d.C.) catalogou e ilustrou cerca de 600 plantas utilizadas para fins medicinais, descrevendo o emprego terapêutico da maioria. De 129 – 216 d.C., o médico filósofo grego Claudius Galeno, o pai da farmácia, desenvolveu misturas complexas, advindas de antigas misturas egípcias e gregas, conhecidas como "misturas galênicas". Na Idade Moderna, Paracelso, um famoso médico, físico, alquimista e astrólogo suíço, buscava novos medicamentos tendo como fonte os produtos naturais e lançou as bases da medicina natural. Na Idade Contemporânea, o progresso científico na área da química permitiu analisar e separar os princípios ativos das plantas promovendo grande avanço na fitoterapia. Na Alemanha, Hahnemann desenvolveu o conceito de homeopatia. Em 1860, a cocaína foi extraída das folhas de coca tornando-se possível muitas cirurgias.

No Brasil, a utilização de plantas nos tratamentos de doenças apresenta contribuição das culturas africana, indígena e europeia. Os escravos africanos trouxeram consigo plantas que eram utilizadas em rituais religiosos e apresentavam propriedades farmacológicas descobertas empiricamente. Os índios gozavam da imensa biodiversidade brasileira e seus usos foram aprimorados a cada geração.

Com o advento de tecnologias para elaboração de fármacos sintéticos, no fim do século XIX, a medicina tradicional que tinha como base terapêutica medicamentosa as plantas medicinais e seus derivados foi deixada de lado e substituída pelo uso dos medicamentos industrializados.

Contudo, nas últimas décadas, os estudos sobre plantas medicinais têm ganhado cada vez mais relevância e vem se tornando tendência mundial. Seu consumo como tratamento das doenças com base nas tradições familiares são bastante observados em zonas rurais ou em regiões de baixo desenvolvimento econômico e tem se tornado prática comum na medicina popular. Por ser considerada uma fonte barata, por possuir propriedades culturais e devido aos efeitos colaterais do uso crônico ou indiscriminado dos medicamentos industrializados, ao difícil acesso de algumas populações à assistência médica, novas pesquisas com plantas medicinais vêm sendo realizadas. A fim de encontrar biocompostos ativos para a formulação de novas drogas.

O *Origanum vulgare* tem sido usado desde a antiguidade como cura tradicional para tratamento de infecções (Karaman et al., 2017). Estudos revelam que o óleo essencial de *O. vulgare*, rico em Carvacrol, possui atividade anti-inflamatória, antioxidante, anti-espática, antimicrobiana e antifúngica (Król et al., 2019). Microrganismos resistentes têm surgido ao longo dos anos e têm sido considerados uma ameaça mundial. A resistência microbiana tem trazido grandes problemas no tratamento de doenças infecciosas, sendo necessário o desenvolvimento de novos agentes antimicrobianos eficazes (Ayaz et al.,2019). A ocorrência de cepas do gênero *Candida* tem se mostrado resistente ao tratamento com antifúngicos comerciais, pertencentes à família azoles e polienos, sendo motivo de preocupação dos profissionais de saúde. As infecções causadas por *Candida* afetam principalmente pacientes imunodeprimidos (Barbosa et al., 2019). Diante desse cenário, as plantas têm sido consideradas um agente antimicrobiano promissor para o desenvolvimento de novos fármacos, atuando na prevenção e tratamentos de doenças (Ayaz et al.,2019). Portanto, este trabalho teve como objetivo estudar a atividade antimicrobiana do óleo essencial de *Origanum vulgare* e seu composto isolado Carvacrol contra microrganismos do gênero *Candida, Estreptococos* e *Estafilococos*

2 ARTIGO: *Origanum vulgare* (OREGANO) AND ITS CARVACROL BIOCOMPONENT AS AN ALTERNATIVE OF ANTIMICROBIAL AGENT

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Abstract- The use of plants as an alternative to medicinal treatments is an old practice. The increased resistance of microorganisms to conventional antimicrobials has made studies with medicinal plants increasingly relevant, and ethnobotanical and ethnopharmacological knowledge is considered essential for the development of new drugs. The essential oil of Origanum vulgare and its isolated compound Carvacrol have antimicrobial effects demonstrated in the literature as antibacterial and antifungal activity. Therefore, the present study evaluated the antibacterial and antifungal activity of O. vulgare and Carvacrol using the broth microdilution method (CLSI, 2008), determining MIC (Minimum Inhibitory Concentration) and MFC and MBC (Minimum Fungicidal Concentration and Concentration Minimum Bactericide). Used as standard comparative the antimicrobials Fluconazoleand Chlorhexidine. The essential oil of Origanum vulgare and the isolated compound Carvacrol are biologically active against the genders Candida and Streptococcus and in the strain of Staphylococcus aureus methicillin resistant, exhibiting promising antimicrobial properties. The antimicrobial activity can be seen highlighted with a combination of traditional commercial antimicrobials.

Keywords: origanum vulgare; carvacrol; candida spp., streptococcus spp., staphylococcusaureus MRSA.

I. Introduction

The use of plants as an alternative to medicinal treatments is an ancient practice, which provide primary health care to 80% of the world's population. It has also been an important source for new drug discoveries (Wangchuk & Tobgay, 2015). World Health Organization has estimated that around 80% of the population of developing countries use traditional herbal medicines as a source of treatment for diseases as a cheap and alternative source, other factors such as the lack of modern health facilities, Cultural priorities and choices also contribute to the use of medicinal plants as a therapeutic alternative (Aziz et al., 2018). Studies on medicinal plants have became increasingly relevant, ethnobotanical and ethnopharmacological knowledge is considered essential for the development of n ew drugs. Researches studies the effectiveness and use of traditional plants as alternative medicine and adjuvants in a treatment (Amjad et al., 2017). In this context, Origanum vulgare is a plantused since ancient times as a traditional cure for treating infections (Karaman et al., 2017). Studies show that O. vulgare essential oil is rich in Carvacrol, has anti-inflammatory, antioxidant, antispasmodic, antimicrobial, and antifungal activity (Król et al., 2019). Also, resistant microorganisms have emerged over the years and have been considered a global health threat. Microbial resistance has brought problems in the treatment of infectious diseases, and the development of new antimicrobial agents is required (Ayaz et al., 2019). The occurrence that strains of the genus Candida have been resistant to treatment with commercial antifungals, belonging to the azoles and polyenes family, has been a reason for concern by health professionals. Candida infections mainly affect immunosuppressed patients (Barbosa et al., 2019). Given this scenario, the plants are promising antimicrobial agent for the development of new drugs, acting in disease prevention and treatment (Ayaz et al., 2019). Therefore, this work aimed to study the antimicrobial activity of Origanum vulgare essential oil and its isolated compound Carvacrol against microorganisms of genus Candida, Streptococci and Staphylococci.

II. Material and Methods

a) Essential oil The essential oil was purchased commercially from Quinarí Fragrâncias e Cosméticos LTDA and the isolated compound from Sigma Aldrich. Essential Oil - Lot: 09818DIV Isolated Compound - Lot: S40656V

b) Minimum Inhibitory Concentration Assessment (MIC) To determine the minimum inhibitory concentration (MIC), we tested samples of the essential oil and isolated compound against Streptococcus spp., Candida spp. and Staphylococcus aureus MRSA by broth microdilution technique following the M27-A3 protocol (CLSI, 2008).

c) Candida spp. Inoculum adjustment It was prepared with saline in spectrophotometer an absorbance of 530nm in the range of 0.08 to 0.1 equivalent to 5.0 x 106 CFU/mL. Then, the inoculum was standardized, diluting to 2.5x 103, according to CLSI, 2008. In asterile microplate was deposited with 100 µl of RPMI, 100 µl of the essential oil or 100 µl of the isolated compound at the initial concentration, followed by serial microdilution and then added with 100 µl of the adjusted inoculum. Groups of test: CG: positive control group; OL: Essential Oil Treatment Group; IC: Treatment group with isolated compound; AC: Treatment group with the commercial antifungal Fluconazole at an initial concentration of 64ug/mL (CLSI, 2002). Incubated the plates for 48h at 37°C. Defined the MIC as the lowest concentration of compound that did not exhibit visible growth of the microorganism.

d) Inoculum adjustment of the Streptococcus spp. and Staphylococcus aureus MRSA

After growth in BHI liquid culture medium, the inoculum was adjusted by spectrophotometer with a wavelength of 625nm in the range 0.08 to 0.1absorbance, equivalent to 1.0 x 108 CFU/mL. In a sterile microplate were deposited 100 µl of BHI, 100 µl of the essential oil or 100 µl of the isolated compound at the initial concentration, followed by serial microdilution, and then added of 100µl of the adjusted inoculum. Groups of test: CG: positive control group; OL: Essential Oil Treatment Group; IC: Treatment group with isolated compound; AC: Treatment group with the commercial antifungal chlorhexidine at the initial concentration of 64ug/mL (CLSI, 2008). Incubation of the plates was made for 24h at 37°C with 10% CO2 for Streptococcus strains and aerobically for Staphylococcus aureus MRSA. Defined the MIC as the lowest concentration of compound that did not exhibit visible growth of the microorganism.

e) Determination of Minimum Fungicidal Concentration (CFM) and Minimum Bactericidal Concentration (CBM)

In a petri dish containing Sabouraud Dextrose Agar (SDA) for yeast and Brain Heart Infusion Agar (BHI) for bacteria tested the determination of minimum fungicidal concentration (MFC) and minimum bactericidal concentration (MBC). Homogeinized the wells containing target concentrations and transferred the aliquot to the Petri dish with the solid culture medium. After incubation at 37ºC for 24h, established the lowest fungicidal/bactericidal concentration. Determined the MFC/MBC as the lowest concentration of essential oils and isolated compounds that did not allow the growth of any colony of the microorganism on the solid medium after the incubation period. Through visual reading, the inhibition of growth or death provided by the tested substances was confirmed (GULLO et al., 2012).

III. Results

a) Evaluation of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal / Bactericidal Concentration (CFM / CBM)

Tested the Origanum vulgare essential oil and its isolated compound Carvacrol against reference strains of the genus Candida, Streptococcus, and Staphylococcus aureus methicillin-resistant strain to determine its inhibitory effect by broth microdilution technique. Both substances showed inhibitory activity on microbial cells (Table 1).For reference strains of the genus Candida, inhibitory concentrations were between 0.125 mg/mL and 0.5 mg/mL for O. vulgare essential oil and 0.125 mg/mL and 0.0625 mg/mL for Carvacrol isolated compound. The antifungal Fluconazole was also tested against strains to determine the minimum inhibitory concentration by broth microdilution technique. Fluconazole has shown inhibitory activity between 1 and 32 µg/ml (Table 1). For bacterial strains of the genus Streptococcus, the essential oil of O. vulgare behaved similarly, varying the MIC between concentrations of 0.5 mg/mL and 0.250 mg/mL, as well as its isolated compound Carvacrol. The essential oil and Carvacrol inhibited the strain Staphylococcus aureus at concentrations of 0.5 and 0.250 mg/mL respectively. Chlorhexidine antimicrobial was also tested against strains to determine the minimum inhibitory concentration by broth microdilution technique. Chlorhexidine demonstrated inhibitory activity between 3.75 to 7 µg/ml (Table 1).After the determined the MIC values, used an aliquot of the susceptibility test to determine the minimum fungicidal/bactericidal concentration (MFC/MBC) of the strains. For Candida spp. Strains, O. vulgare essential oil, showed fungicidal activity against Candida spp. strains, varying its effect on concentrations between 0.5 mg/mL and 0.250 mg/mL; while Carvacrol showed 0.250 mg/mL fungicidal activity for all Candida strains tested (Table 1). Streptococcus bacterial strains showed a bactericidal concentration ranging from 0.5 mg/mL to 0.250 mg/mL for the essential oil and ranged from 0.5 mg/mL to 0.125 mg/mL for the isolated compound. S. aureus(MRSA) showed a bactericidal concentration of 0.5 mg/mL for both.

Table 1: Visual reading results of MIC and CFM/CBM of the strains tested.

Reference strain	*O. vulgare*		Carvacrol		Fluconazole / Chlorhexidine
	MIC	MFC	MIC	MFC	
	mg/mL	mg/mL	mg/mL	mg/mL	µg/mL
C. albicans	0.25	0.5	0.125	0.25	1
C. dubliniensis	0.125	0.5	0.0625	0.25	1
C. glabrata	0.125	0.5	0.0625	0.25	1
C. parapsilosis	0.125	0.25	0.0625	0.25	2
C. krusei	0.25	0.5	0.125	0.25	32
C. guilliermondii	0.125	0.25	0.0625	0.25	1
S. mutans	0.5	0.5	0.25	0.5	3.75
S. mitis	0.25	0.25	0.125	0.125	3.75
S. oralis	0.5	0.5	0.25	0.25	15
S. gordonii	0.25	0.25	0.125	0.125	7.5
S. salivarius	0.25	0.5	0.25	0.25	3.75
S. sanguinis	0.25	0.5	0.25	0.25	7.5
S aureus MRSA	0.5	0.5	0.25	0.5	

IV. Discussion

Excessive and indiscriminate use of antimicrobials is a major determinant of some emerging infections, selection of resistant pathogens, and the continued development of antimicrobial resistance globally. The increasing emergence of multi-drug resistant organisms and the limited development of new agents available to combat them have caused an imminent crisis with alarming implications (Sartelli et al., 2016).In view of the increasing numbers of cases of conventional drug-resistant microorganisms, researchers are looking for alternatives to biocompounds that have antimicrobial properties against microorganisms. Studies with plants as promising agents in the search for new compounds.

In this study, the obtained data showed antimicrobial activity of the tested essential oil, as well as its isolated compound against planktonic cells of Candida spp., oral Streptococcus species, and S. aureus methicillin resistant strain. The essential oil inhibited antimicrobial growth between concentrations of 0.5 to 0.125mg/mL against all strains tested. At the same time, the isolated compound showed antimicrobial activity between concentrations of 0.250 to 0.0625mg/mL against all strains tested. The minimum fungicidal/bactericidal concentration (MFC/MBC) of the essential oil in the strains tested was between 0.5 mg / ml and 0.250 mg / ml. The isolated compound showed MFC/MBC between concentrations of0.5 mg/mL to 0.125 mg/mL (Table 1).

These data, initially reveal the antimicrobial action of this essential oil, as well as its isolated compound, corroborating with the literature, pointing out its antimicrobial activity. In a study by Bharti et al. (2013). O. vulgare essential oil also demonstrated antimicrobial activity in synergism with ciprofloxacin against clinical isolates of Salmonella typhi, considerably decreasing the inhibitory concentration of conventional antimicrobial. According to the study by Bhat et al. (2018), antifungal activity demonstrated an inhibition zone of 30 mm for O. vulgare compared with 22 mm for nystatin against the three Candida species tested, Candida glabrata, Candida tropicalis, and Candida albicans. Cleff et al. (2010) also observed the antifungal activity of O. vulgare against C. albicans, C. parapsilosis, C. krusei, C. lusitaniae, and C. dubliniensis strains, and in this same study, the action of the essential oil against isolates was also tested. Clinical results of C. albicans showed dose-dependent antifungal activity for the strains tested. The mechanism of Carvacrol action was investigated by Wang et al. (2016) showing that exposure to Carvacrol at low concentrations induced a marked increase in unbranched fatty acid content and at higher levels substantially altered the integrity and morphology of S. aureus cell membrane.

Nobrega et al. (2016) evaluated the minimum inhibitory concentration and the minimum fungicidal concentration of Carvacrol, ranging from 25 to 81 µg/mL MIC and 25 to 102 µg/mL CFM. According to Duarte et al. (2005), the MIC value is parameter of the classification of the acceptance level of plant materials, up to 0.5 mg/mL being considered strong, from 0.55 to 1.5 mg/mL, moderate and above. 1.5 mg/mL as weak. In this sense, the results obtained with O. vulgare essential oil and isolated compound showed MICs considered strong for all strains tested.

The data obtained in this study added to the data in the recent literature, suggest that the essential oil of O. vulgare and also its isolated compound Carvacrol show antibacterial and antifungal potential.

These data open possibilities for many other studies, such as the performance of these oils and biocomponents in mature biofilms and multispecies biofilms, evaluating cell viability and possible morphological changes, added to cytotoxicity tests, action on cancer cells, in an attempt to add more information about this plant and its use as an alternative agent in the treatment of infections and acting as coadjuvants.

V. Conclusion

• Both the essential oil of O. vulgare, and its isolated compound Carvacrol, can inhibit the growth of the tested microorganisms in low concentrations;

• The isolated compound is more effective when compared to the essential oil, inhibiting the microorganisms in a lower concentration;

• About the microorganisms, essential oil and Carvacrol are more effective against Candida spp. when compared to bacterial strains;

• Both the EO and its main biocomponent Carvacrol show fungicidal/bactericidal activity against the tested strains

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3 CONCLUSÃO

O estudo mostrou que o óleo essencial (OE) de Origanum vulgare e o seu composto isolado Carvacrol são biologicamente ativos apresentando atividade fungicida/bactericida de maneira dose dependente contra as espécies testadas em sua forma planctônica.

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O relatório está bem redigido e bem organizado. A bolsista realizou as metodologias propostas e cumpriu o cronograma de trabalho. Os resultados foram apresentados de forma adequada do ponto de vista acadêmico e discutidos de maneira clara. A aluna manteve bom desempenho acadêmico durante a execução do projeto de IC.

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