GC-MS ANALYSIS AND IN VITRO ANTIMICROBIAL ACTIVITY OF Ocotea catharinesis MEZ essential oil (LAURACEAE)

https://doi.org/10.56238/sevened2025.018-046

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ABSTRACT

The use of plants to treat and prevent diseases is an ancestral practice of humanity. In recent years, the resistance of microorganisms to antibiotics has generated a growing interest in the research of therapeutic alternatives, including the chemical compounds present in essential oils. The aim of the present study was to evaluate the antibacterial and antifungal effect of Ocotea catharinesis essential oil (EOOc) and to identify its main compounds. The extraction of the essential oil was done by hydrodistillation for 4.5 hours, using 50g of dried leaves. To identify the chemical components, gas chromatography with mass spectrometry was used. For the microbiological tests, the strains tested included Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and Candida tropicalis. The Minimum Inhibitory Concentration (MIC) was determined by the microdilution method. The essential oil was tested as a modulator of antibiotic resistance, using gentamicin, penicillin and norfloxacin as references for antibacterials and fluconazole for fungi, with or without the presence of the oil. Analysis of the oil revealed two main compounds: alpha pinene (13.9%) and limonene (13.4%). Alpha pinene, a terpenoid, can help reduce oxidative stress by acting as an antioxidant. The cEOOc showed resistance against E. coli and P. aeruginosa bacteria, with values equal to or greater than 1024 µg/mL, showing that gram-negative bacteria are more resistant. With the antibiotic penicillin, the EOOc showed similar results, also because of receptor proteins. Tests with Norfloxacin and Gentamicin showed values of 512 µg/mL, with no synergistic potential with antibiotics. It was observed that against the strains of Candida tropicalis, the EOOc presented a modulating effect with a lower concentration than the drug tested. The results indicate that the essential oil of Ocotea catharinesis Mez may have an antifungal effect against Candida tropicalis strains.

Keywords: Essential oil. Chemical Analysis. Antimicrobial.

INTRODUCTION

The use of plant species for the prevention, treatment or cure of various diseases is a practice that dates back to the beginning of human civilization (Elharas; Ouhssine, 2025; Carmignan et al., 2020).

The resistance that microorganisms have shown in recent decades to pharmaceutical products and the research studies of the biological and chemical potential from the presence of chemical compounds in essential oils make the search for new therapeutic alternatives an important tool in the development of new antimicrobial drugs (Brixner et al., 2022; Hou et al. 2022; Rodrigues et al., 2018).

A large part of the world's population uses these plants as an alternative source for the treatment and cure of various diseases, as they are more accessible, both in terms of financial requirement and practicality, compared to industrially obtained medicines (Domingo-Fernández et al., 2023). In this way, the search for plants for therapeutic use has been accentuated and passed on from generation to generation (Lima Mota et al., 2023).

Among the compounds provided by plant species are essential oils, consisting of monoterpenes, sesquiterpenes, and phenylpropanoids that foster chemical and structural properties from their volatility, thus demonstrating great potential for antifungal and antibacterial activities, (Angane et al., 2022).

Ocotea catharinesis Mez, also known as Black Cinnamon is a species of woody tree found in the south of the Atlantic Forest in Brazil, which has excellent quality wood (Dos Santos Alves et al., 2024). Second Medeiros (2022)), based on its characteristics, it fits into the Lauraceae family, being a group that provides great phytogenic advances.

The species *Ocotea catharinesis Mez* has several compounds with antimicrobial characteristics, in addition to being used as aromatics in the food sector (Diast al., 2022). Thus, the objective of this study was to evaluate the antibacterial and antifungal effect of *Ocotea catharinesis* essential oil (EOOc) as well as to identify its main compounds.

MATERIALS AND METHODS

COLLECTING BOTANICAL MATERIAL

The species *Ocotea catharinensis* was collected for essential oil extraction in Campos Gerais, Paraná State, according to the following coordinates: S 25º 20.948' and W 049º 47.148'. The exsicata was transported and deposited in the Herbarium of the Integrated Spiritist Colleges (HFIE) which received the following number: 8,559 (Lawrence, 1951; IBGE, 1992).

EXTRACTION OF THE ESSENTIAL OIL

The extraction of the essential oil was carried out by hydrodistillation for 4.5 hours in a graduated Clevenger device using 50g of dried leaves in 1L of distilled water, with 3 replications (Wasicky, 1963). To dry the leaves, an electric dryer model FANEM - Mod. 320 SE with air circulation at 40º C for 24 hours. After extraction, the sample was collected with a precision pipette and stored in a freezer where it remained until the moment of analysis.

DETERMINATION OF THE CHEMICAL COMPOSITION OF ESSENTIAL OILS

The identification of the chemical constituents was performed by gas chromatography coupled to mass spectrometry (GC/MS). The essential oil was diluted in dichloromethane in the proportion of 1 % and 1.0 µL of the solution was injected, with a flow division of 1:20 in an Agilent 6890 chromatograph (Palo Alto, CA) coupled to an Agilent 5973N selective mass detector. The injector was kept at 250 °C. The separation of the constituents was obtained in HP-5MS capillary column (5%-phenyl-95%-dimethylpolysiloxane, 30 m x 0.25 mm x 0.25 µm) and using helium as carrier gas (1.0 mL min-1). The oven temperature was programmed from 60 to 240°C at a rate of 3°C min-1. The mass detector was operated in the electron ionization mode (70 eV), at a rate of 3.15 s-1 scans and a mass range of 40 to 450 u. The transfer line was maintained at 260º C, the ion source at 230º C and the analyzer (quadrupole) at 150º C.

For quantification, the diluted sample was injected into an Agilent 7890A chromatograph equipped with a flame ionization detector (DIC), operated at 280ºC. The percentage composition was obtained by the electronic integration of the DIC signal by dividing the area of each component by the total area (area%).

The identification of the chemical constituents was obtained by comparing their mass spectra with those of the spectrotheques (Wiley, 1994; Nist, 2016) and by their linear retention indexes, calculated from the injection of a homologous series of hydrocarbons (C7-C26) and compared with data from the literature (Adams, 2007).

MICROBIAL TESTS

Culture media and strains

The following strains were used: standard strains *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* INCQS 40006 and *Candida Tropicalis* INCQS 40042. These strains were inoculated in Sabouraud Dextrose Agar (SDA - KASVI), for fungal strains, and in Brain

Heart Infusion (BHI), for bacterial strains, being incubated at 37 C for 24 h. The inoculum concentration was standardized according to McFarland, comparing the inoculum turbidity with the standard 0.5 on the scale. The prepared inocula were used in the Inhibitory Concentration Tests (MIC) and in the test performed to verify the potential of the extracts in the modulation of antibiotics by contact.

Drugs, reagents and preparation of solutions

Dimethyl sulfoxide (DMSO - Merck, Darmstadt, Germany) was used to dilute the oil. Norfloxacin, Gentamicin, Penicillin and Fluconazole (Capsule - FLUCOMED) was diluted in water and used as a reference drug in the tests. The matrix solutions of the products, Ocotea catharinesis essential oil mez, was prepared by weighing 0.3 g of each product and then diluting them in 1 mL of DMSO. To obtain the desired concentration for the tests, the product underwent a new dilution in sterile distilled water, so that the concentration of DMSO in the product affected the tested cells.

Determination of the minimum inhibitory concentration (MIC)

This test was performed on 96-well plates using juice microdilution, a method according to document M7-A10. Briefly, serial dilutions were performed yielding concentrations of natural products ranging from 4 to 512 µg/mL for the bacterial strains and from 4 µg/mL to 4096 µg/mL for the fungal strains. The latter well was used as a growth control microorganism. Dilutions of the product (using saline instead of inoculum) and medium sterility controls were also achieved. All bacterial tests were performed in triplicate and fungal tests in quadrupled. The plates were incubated at 37 °C for 24 h. Antibacterial activity was detected using a colorimetric method by adding 25 µL of aqueous solution of resazurin (0.01%) to each at the end of the incubation period. For antifungal activity, the reading was performed by the ELISA spectrophotometer (Termoplate ®) at 630 nm. MIC was defined as the lowest concentration of natural products capable of inhibiting the growth of microorganisms.

Modulatory activity by direct contact

The essential oil was tested as a modulator for antibiotic resistance from the obtaining of MICS, using as reference the aminoglycoside gentamicin and the beta-lactam penicillin and norfloxacin against bacterial strains, and fluconazole, against fungal strains, in the presence or absence of the natural product using the microdilution test. Subinhibitory concentrations (MIC/8 for bacteria and MIC /16 for fungi) in 10% of specific culture medium

were used. According to the methodology used by Coutinho et al, 2008, the microbial inocula were distributed in microdilution plates followed by the addition of different antimicrobial concentrations, ranging from 4 to 4096 µg/mL for fluconazole and 0.5 to 5000 µg/mL for the others. The bacterial tests were performed in triplicate and the fungal tests in quadrupled, and the plates were incubated at 37°C for 24 h. The readings were performed as previously described, where with the antifungal results the cell viability curve was obtained.

Statistical Analysis

The tests were expressed as the geometric mean. The MIC values were obtained by nonlinear regression for the purpose of interpolating values from standard curves (using the Graphpad Prism software, v. 7.0).

RESULTS AND DISCUSSIONS

The chemical analysis of the oil showed the presence of two major compounds, alpha pinene with 13.9% of the composition and limonene with 13.4% (Table 1). Studies carried out with alpha pinene, which point to it as an identified terpenoid, have shown its ability to reduce factors responsible for causing oxidative stress, a result of the formation of free radicals, being seen as a potential antioxidizer (Zamyad et al., 2019).

Table 1.Identification of Ocotea catharinensis *essential oil compounds* using gas chromatography coupled to mass spectrometry (GC-MS).

Compounds	Retention time (min)	%
alpha-pinene	937	13.9
Sabinene	976	4.2
Beta-Pinene	980	10.0
Mircene	992	3.0
alpha-felandrene	1006	1.1
alpha-terpinene	1019	0.8
P-Cimene	1027	1.3
limonene + beta-felandrene	1032	13.4
(*Z*)-beta-ocimene	1040	2.5
(*E*)-beta-ocimene	1051	1.5
gamma-terpinene	1062	1.7
terpinen-4-ol	1179	2.7
(*E*)-caryophyllene	1417	2.9
alpha-humulene	1452	1.2
germacrene D	1479	6.1
Viridiflorene + Bicyclogermacrene	1493	7.9
Cadinene-Delta	1522	2.1
spatulenol	1575	1.4
Caryophyllene oxide + globulol	1580	2.5
Viridiflorol	1588	2.0
khusimona	1598	2.3
alpha-cadinol	1652	2.8
kaurenoic acid	2025	7.8
Total	–	95.1

Limonene is a compound found in several essential oils of citrus fruits, presenting low toxicity and pharmacological potential, but it has low solubility in water and high volatility, which may compromise its therapeutic use (Kazyoba & Viljoen, 2008).

Alpha pinene, alone, has already shown bacterial activity against gram-positive strains, but has not been shown to be effective against gram-negative strains It is important to emphasize that there is a search by the food industry for new antimicrobials, as some bacteria have the power to produce enterotoxins, a virulence factor capable of causing food poisoning (Cervantes-García; García-González; Salazar-Schettino, 2014).

In another study, alpha pinene had inhibitory power against strains of *Staphylococcus aureus*, proving to be a potent antibacterial (Villeda, 2014), a result that was not observed in tests with the essential oil *Ocotea catharinesis Mez* which presented a concentration equal to or greater than 1024 µg/mL, possibly due to the fact that limonene and alpha pinene, the main compounds of the oil, are antagonistic.

Against the bacteria *Escherichia coli and Pseudomonas,* the EOOc obtained a value equal to or greater than 1024 µg/mL (Table 2), indicating that gram-negative microorganisms are more resistant than gram-positive bacteria, due to their different resistance mechanisms, including their modifying enzymes and binding proteins (Tafur; Towers; Villegas, 2011).

Table 2. Modulating effect of *Ocotea catharinensis* essential oil in combination with aminoglycoside, beta-lactam and fluorokilone antibiotics.

Antibiotics	***Escherichia coli* ATCC 10536**		***Staphylococcus aureus* ATCC 25923**		***Pseudomonas aeruginosa* ATCC 27853**	
	Control	+ EOOc	Control	+ EOOc	Control	+EOOC
Gentamicin	256	512	128	512	512	512
Penicillin	512	≥1024	≥1024	≥1024	512	≥1024
Norfloxacin	**512**	**512**	**512**	**512**	**512**	**512**

Antibiotic modulation against bacteria using strains of *S. aureus, E. coli and Pseudomonas,* when performed with the antibiotic penicillin, it was shown that it presented a value equal to or greater than 1024 µg/mL, probably due to some bacteria having penicillin receptor proteins. Similarly, when tested with the antibiotics Norfloxacin and Gentamicin, the EOOc presented values equal to 512 µg/mL, where it was seen that the oil did not have synergistic potential with the antibiotics (Table 2).

The union of natural products with allopathic medicines usually has modulatory activity (Abreu et al., 2016), however in recent decades bacteria have been developing

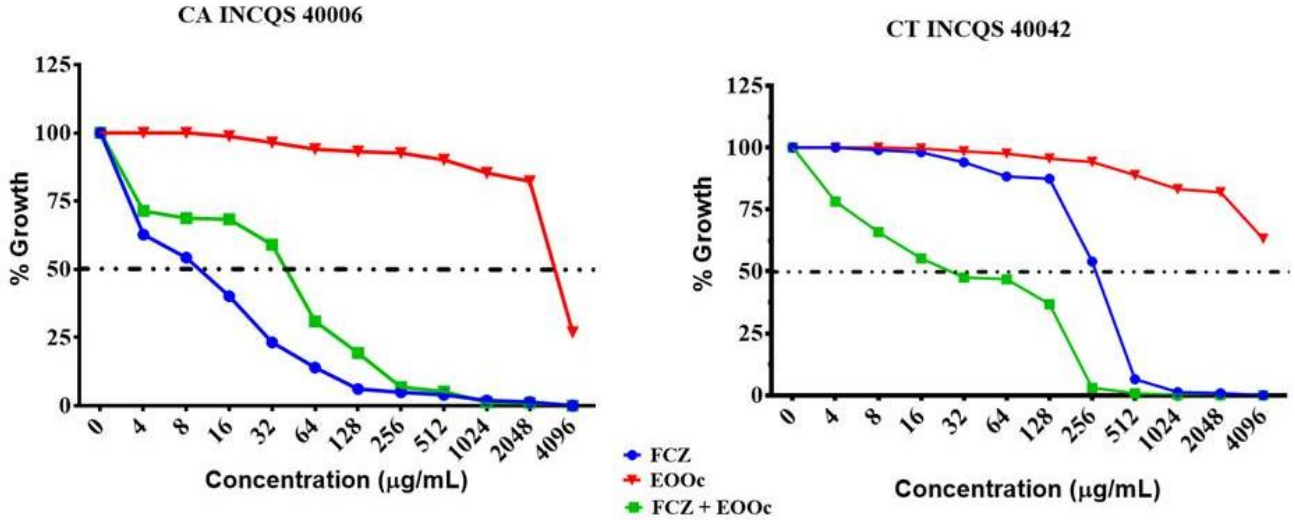
several defense mechanisms, such as the transferable resistance method, the production of enzymes Inactivators reduction or absence of protein channels responsible for the transport of possible drugs, alteration in receptors, among others (Del Fio et al., 2000).

Fungal infections, caused by gender *Candida* sp, are increasingly present, especially in hospitalized patients, although the *Candia albicans* is the most responsible for most pathologies, species such as *Candida parapsilosis, Candida tropicalis* and *Candida glabrata*, have become common (Fernandes, 2020).

Several studies show resistance of candida species to fluconazole, the main antifungal used against infections caused by these strains. Thus, substances of natural origin, especially essential oils, have been widely studied in recent years in relation to their antifungal activity (Scallop; Santos, 2017).

When tested against fungal strains of *Candida albicans and* Candida *tropicalis* , *O. Catharinesis*, in isolation, was not effective (Figure 1).

Figure 1. Antifungal effect of *Ocotea Catharinensis essential oil* alone or in combination with fluconazole. CA: *Candida albicans*; CT: Candida tropicalis; INCQS: National Institute for Quality Control in Health; FCZ: Fluconazole; EOOc: Essential Oil of *O. Catharinensis*



The modulatory potential of the product when associated with the antimycotic drug, fluconazole, against the Candida strains was shown to have an antagonistic effect. However, against the strains of C*andida tropicalis* it was possible to notice modulatory activity with lower concentrations than the drug tested. This synergistic effect can be analyzed according to the viability curve of the fungal strains (CI50) (Table 3).

Table 3. Values of the 50% inhibitory concentration (IC50) of *Ocotea catharinensis essential oil against* Candida albicans *and* Candida tropicalis *strains*.

	IC50 µg/mL	
Product	**CA INCQS 40006**	**CT INCQS 40042**
Fluconazole (FCZ)	10.65 ± 0.029	284.38 ± 0.022
OEOc	≥ 4096 ± 0.038	≥ 4096 ± 0.011
OEOc + FCZ	44.71 ± 0.018	29.42 ± 0.042*

* IC50 values with a statistically significant difference ($p < 0.01$) when compared to the commercial antifungal fluconazole (FCZ).

Essential oils are aromatic compounds obtained from plants, giving them complex potentials, making it difficult to associate antifungal activity with their possible bioactive compound (Naeem et al., 2018). Studies show that this type of activity may be related to the presence of limonene, a low-toxicity compound with antifungal potential (Yu et al., 2022). In this sense, the participation of some of these secondary metabolites can interfere in several metabolic routes or in different enzymatic reactions, which would explain their easy interaction with the antibiotic (Júnior & Pastore, 2007). In addition, they can also act directly on the plasma membrane of the pathogen, causing morphological and structural changes (Ahmad et al, 2010), act on electrolyte enzymes causing cell leakage (Darvishi et al, 2013), among other essential methods for the survival of the fungus.

Thus, antibiotic therapy combined with natural agents can bring benefits, such as reduced toxicity, side effects, minimum effective dose, and reduced treatment costs, through its blocking of cellular communication mechanisms, biofilm formation, or even in the production of mycotoxins (Nazzaro et al., 2017).

CONCLUSION

The results show the potential of the essential oil of *Ocotea catharinesis Mez* as an antifungal modulating effect against Candida *tropicalis strains,* whose activity may be related to one of its major compounds, limonene, which shows to be an antifungal potential when isolated. Such data insure that the essential oil possesses antimicrobial bioactive metabolites.

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