Capítulo 100

Gc-Ms Analysis And In Vitro Antibacterial Activity Of The Essential Oil Of Cinnamodendron dinisii Schwacke (Canellaceae) Against Multirresistant Strains

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ABSTRACT
Medicinal plants are used as cheaper alternatives for the treatment of certain pathologies. Due to the great Brazilian biodiversity, many plants do not have in-depth studies such as Cinnamodendron dinisii Schwacke (pepper or pau-pra-tudo), which is native to the south and southeast. The aim of this study was to evaluate the antibacterial activity of Cinnamodendron dinisii Schwacke essential oil (OECd) against multiresistant strains: Escherichia coli 06 (EC 06) and Staphylococcus aureus (SA 10). Ampicillin, Norfloxacin and Gentamicin were diluted in distilled water and used for the modulatory test. The tests followed the methodology of microdilution in broth to obtain the Minimum Inhibitory Concentration (MIC). The MIC/8 of the products was used in the resistance reversal test. In terms of direct activity, OECd showed the best result against S .aureus with a MIC of 512µg/mL. For bacterial resistance reversal tests, OECd showed better synergistic interactions with the antibiotics ampicillin and gentamicin, both against E.coli strains. The results demonstrate that OECd has antibacterial action when tested alone or in combination with antibiotics against multidrug-resistant strains.

Keywords: Cinnamodendron dinisii, essential oil, antibacterial activity

1 INTRODUCTION

The use of plant species with the aim of preventing, treating or curing various diseases is a practice that dates back to the beginning of human civilization (PEREIRA et al., 2019).

The resistance that microorganisms have shown in recent decades to pharmaceutical products and 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 (RODRIGUES et al., 2018; JANTAPAN et al. 2017; DHIFI et al. 2016).

A large part of the world's population uses these plants as an alternative source for the treatment and also for the cure of various diseases, since they are more accessible, both in financial terms and in practicality, in relation to medicines obtained industrially (RAWAT et al. al., 2017). In this way, the search for plants for therapeutic use has been accentuated, and also passed on from generations to generations (SEM & SAMANTA, 2015).

Among the compounds provided by plant species are essential oils, consisting of monoterpenes, sesquiterpenes and phenylpropanoids, which promote chemical and structural properties based on their volatility, thus demonstrating great potential for antifungal and antibacterial activities (MACHADO; JUNIOR, 2011).

Popularly known as “pau-pra-tudo” or “pimenteira”, the species Cinnamodendron dinisii Schwacke belongs to the Canellaceae family. It is found in the Southeast and South of Brazil, being used to treat several diseases, justifying its name (BARTH; BARBOSA, 1976; SOUZA; LORENZI, 2012). Some studies suggest that this species has insecticidal, antifungal larvicidal activity (VEDOVATTO et al., 2015).

The objective of this present work was to obtain the chemical profile and evaluate the antibacterial activity of the essential oil of Cinnamodendron dinisii Schwacke (Canellaceae) against strains multiresistant to conventionally used antibiotics.

2 MATERIALS AND METHODS

2.1 BOTANICAL MATERIAL COLLECTION

The Cinnamodendron dinisii species was collected for essential oil extraction in Campos Gerais, Paraná State, according to the following coordinates: S 25º 20.958' and W 049º 47.131'. The specimen was deposited in the Herbarium of Faculdades Integradas Espírita (HFIE) which received the number 9.009 (LAWRENCE, 1951; IBGE, 1992)

2.2 EXTRACTION AND DETERMINATION OF THE CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF Cinnamodendron dinisii

Essential oil extraction was performed by hydrodistillation for 4.5 hours in a Clevenger-type graduated apparatus using 50g of dry leaves in 1L of distilled water, with 3 repetitions (Wasicky, 1963).

To dry the leaves, an electric dryer model FANEM - Mod. 320 SE with air circulation at 40ºC for 24 hours. After extraction, samples were collected with a precision pipette and placed in a freezer where they remained until analysis.

The essential oil were analyzed using a Shimadzu GC–MS QP2010 series, provided by Shimadzu Scientific Instruments Inc. (Columbia, MD, USA), with fused silica capillary column SH-Rtx-5 (30 m × 0.25 mm I.D.; 0.25 m film thickness) and the following temperature program: 80–180 ºC at 4 ºC/min, then to 246 ºC at 6.6 ºC/min, closing with 10 min at 280 ºC, at 3.4 ºC/min, totaling analysis time of 30 min. It was used as the carrier gas, flow rate of 1.5 mL/min, split mode (1:100), and injection port was set at 220 ºC. The quadrupole MS operating parameters: interface temperature (280 ºC) and ion source (200 ºC); electron impact ionization at 70 eV; scan mass range of 40–350 m/z with sampling rate of 1.0 scan/s. Injection volume: 1 µL of 500 ppm solution prepared with dichloromethane. The constituents were identified by computational search using digital libraries of mass spectral data (NIST 08) and by comparing their authentic mass spectra and reported in the literature (ADAMS, 2017).

2.3 CULTURE MEDIA AND STRAINS

The following strains were used: multidrug-resistant bacterial strains isolated from hospitalized patients and numerically designated according to their specific serotype in S. aureus 10 (SA 10) and E. coli 06 (EC 06). All strains were obtained from cultures cultivated in the Laboratory of Microbiology and Molecular Biology (LMBM) of the Regional University of Cariri (URCA). The source for obtaining multidrug-resistant serotypes and the classes of antibiotics associated with this resistance are described in Table 1 below.

Table 1. Microorganism resistance profile to the tested antibiotics

Bacterium	Collect	Resistance profile
Staphylococcus aureus 10	Swab retal	β-lactâmicos, macrolídeos e quinolonas
Escherichia coli 06	Cultura de urina	β-lactâmicos

2.4 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

It was carried out in 96-well plates using the microdilution of the test solution, according to document M7-A10. Briefly, serial dilutions were performed producing natural product concentrations ranging from 4 to 512 µg/mL, for the bacterial strains. The last well was used as a microbial growth control. Product dilutions (using saline instead of inoculum) and sterility medium controls were also prepared to check for interference. All bacterial tests were performed in triplicate. Plates were incubated at 37 °C for 24 h. Antibacterial activity was detected using a colorimetric method by adding 25 µL of resazurin aqueous solution (0.01%) to each well at the end of the incubation period. The MIC was defined as the lowest concentration of natural products capable of inhibiting the growth of microorganisms (COUTINHO et al., 2008)

2.5 MODULATORY ACTIVITY

This technique consists of the same procedure as the previous assay, however, with the following modifications: R eppendorfs are filled with the BHI solution, the inoculum and the OECd, in a volume corresponding to a subinhibitory concentration (MIC/8) of the previous step, with plaque microdilution being performed with clinically used antibiotics. Control plates were prepared from eppendorfs® tubes containing only 10% BHI medium and bacterial inoculum. Ampicillin, norfloxacin and gentamicin, all at a concentration of 1024 μg/mL, were the antibiotics used in the modulation assays. At this point, the plates were used in numerical order, with concentrations ranging from 512 μg/mL in the first well to 0.5 μg/mL in the penultimate well. In an attempt to infer a possible mechanism of action for essential oils, a standard Efflux Pump Inhibitor (EPI), Chlorpromazine (CPZ), was added at subinhibitory concentrations to the test of the antibiotic's modulatory activity. Lower MICs, with statistical significance, in the presence of the standard inhibitor compared to MICs of antibiotics alone suggest the presence of an efflux pump for a given antibiotic for a given bacterium (COUTINHO et al., 2008; ALMEIDA et al., 2020)

3 STATISTICAL ANALYSIS

Central data and standard deviations were obtained according to the methodology by Freitas et al. (2021), on microbiological analysis in microdilution plates. Data were analyzed using the statistical program GraphPad Prisma 6.01 through a one-way ANOVA test. Then, a post hoc Bonferroni test was performed (where $p < 0.05$ was considered significant and $p > 0.05$ not significant).

4 RESULTS AND DISCUSSION

In the chemical analysis, the oil showed two major compounds, namely spathulenol (48.07%), a terpene, and α-Pinene (10.22%) (Table 2). Studies carried out with terpenoid compounds have shown that these substances are capable of reducing factors responsible for causing oxidative stress, resulting from the formation of free radicals, being seen as a potential antioxidant (ZAMYAD et al., 2019).

Table 2. Chemical composition of Cinnamodendron dinisii essential oil.

Compound	IR*	%
α-Felandreno	3,876	0,80
α-Pineno	3,986	10,22
Verbeneno	4,182	0,64
β-Pineno	4,466	5,36
o-Cimeno	4,914	1,63
Limoneno	5,025	5,96
Linalol	5,666	0,83
6-Camfenol	5,918	1,07

L-trans-Pinocarveol	6,115	2,24
4-Terpineol	6,454	1,99
Mirtenal	6,548	2,60
(R)-Carvona	6,920	1,60
α-Copaeno	8,254	0,31
β- Maalieno	8,343	1,29
trans-Cariofileno	8,666	1,36
α-Cariofileno	8,983	1,33
β-Guaieno	9,625	6,36
Oxido de cariofileno	9,989	1,79
(-)-Espatulenol	10,271	48,07
(+)-Aromadendrene	11,027	1,84
β-Eudesmol	11,314	2,48
Total		**99,77**

IR* - Adams Literature Retention Index (2017)

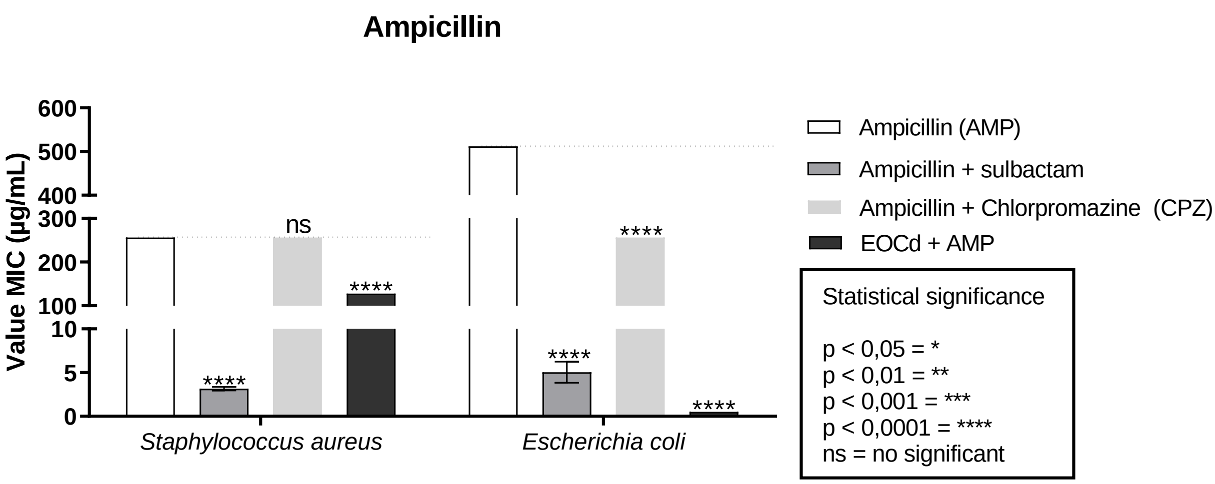
The essential oil of Cinnamodendron dinisii Schwacke presents in its composition the majority of oxygenated sesquiterpenes, however the chemical composition is highly influenced by the environment, age of plant development, altitude and forms of extraction, for example (ANDRADE et al., 2013) .

Essential oils are made up of different compounds, giving them complex potential, facilitating the association of antimicrobial activity with its possible bioactive compound. In this sense, the participation of some of the 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 pathogen's plasma membrane, causing morphological and structural changes (AHMAD et al, 2010), act on electrolytic enzymes causing cellular extravasation (DARVISHI et al, 2013) among other essential methods for survival. of the microorganism.

Three antibiotics (norfloxacin, gentamicin and ampicillin (a β-lactam)) were tested for the reversal of bacterial resistance. The mechanism of action of these antibiotics occurs in the cell wall. The multiresistant strains, S. aureus 10 and E. coli 06, were used to potentiate the antibacterial activity. An efflux pump inhibitor was added as a modulation control in order to establish a probable mechanism of action of OECd through modulation of bacterial resistance.

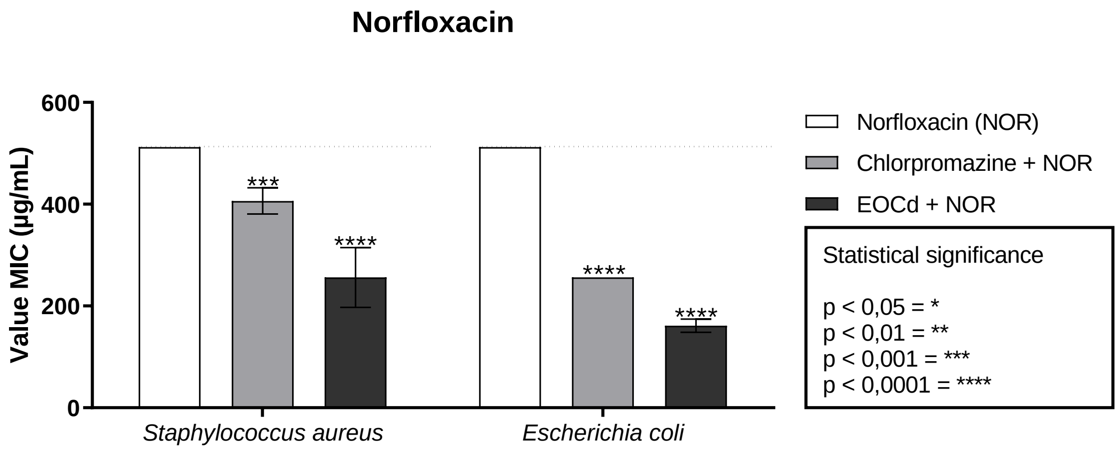
A mechanism present in the cells of all living beings are the efflux pumps (EB) where, for example, in bacteria it acts in intercellular communication, pathogenicity and in the expulsion of antibiotics, thus becoming a target of inhibition (BLANCO et al. 2018). The modulation results expressed in graphs (Figures 1-3) show the effect of OECd against Gram-positive and Gram-negative strains from modulation with different conventional antibiotics.

Figure 1. Synergistic modifying activity of Cinnamodendron dinisii essential oil (dOEC) with the antibiotic ampicillin.



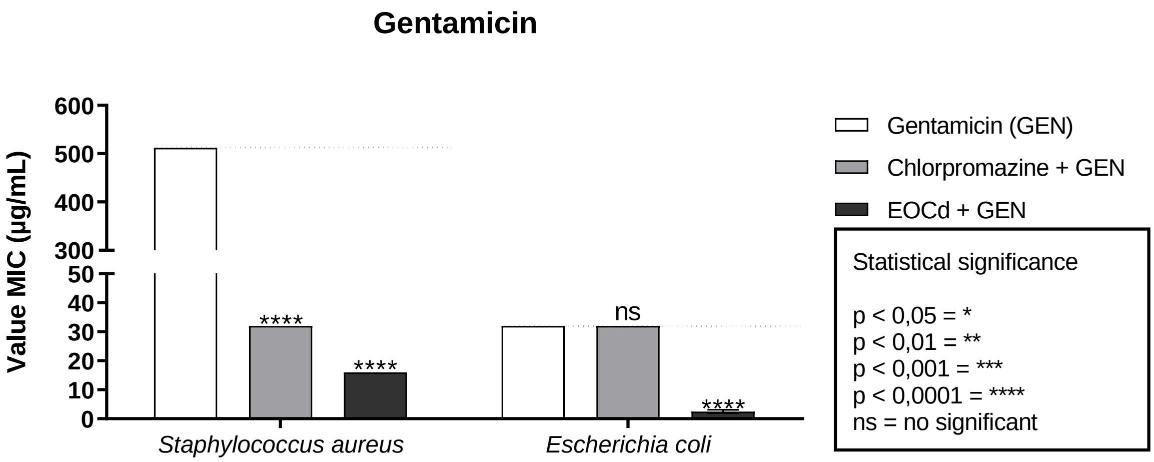
Source: Authorship, 2023.

Figure 2. Synergistic modifying activity of Cinnamodendron dinisii essential oil (dOEC) with the antibiotic norfloxacin.



Source: Authorship, 2023.

Figure 3. Synergistic modifying activity of Cinnamodendron dinisii essential oil (dOEC) with the antibiotic gentamicin.



Source: Authorship, 2023.

The strains used were sensitive to chlorpromazine (CPMZ) in one or both antibiotics tested. This result indicates the presence of efflux pumps acting in the expulsion of norfloxacin in the S. aureus and

E.coli strain, and in the expulsion of gentamicin in the S. aureus strain. Chlorpromazine is an efflux pump inhibitor that acts by inhibiting calcium uptake, which is essential to supply the energy necessary for pump activation (BLANCO et al., 2018). In this sense, the suggested action of OECd from the synergism presented with the antibiotic occurs in the inhibition of the efflux pump mechanism sensitive to chlorpromazine. On the other hand, the E.coli strain did not show sensitivity when tested with CPMZ against the antibiotic gentamicin, which may indicate the presence of other mechanisms responsible for its resistance to that antibiotic, or even an efflux pump that makes it insensitive to CPMZ.

Essential oils are liposoluble substances and, therefore, present the possibility of altering the permeability of the cell membrane, increasing the susceptibility of penetration of various substances, facilitating or hindering the entry of the antibiotic. The proteins that participate in the efflux process are transmembrane and are sensitive to alterations. Thus, possible membrane damage occurs by inhibiting the pump from the action of OECd (KIM et al., 2019; TINTINO et al., 2020)

In this sense, antibiotic therapy combined with natural agents can bring benefits, such as reduced toxicity, side effects, minimum effective dose and reduced treatment costs (NAZZARO et al., 2017).

4 CONCLUSION

The essential oil of C. dinisii influenced the reversibility of bacterial resistance of S.aureus and E. coli with regard to the mechanisms presented by the efflux pumps, in addition to presenting a synergistic effect with the antibiotics tested when efflux pumps were sensitive to chlorpromazine. Thus, OECd is shown to be an antimicrobial modulator against multidrug-resistant bacterial strains. Such data support that the essential oil has bioactive metabolites with antibacterial potential.

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