



Chemical composition, antimicrobial, modulator and antioxidant activity of essential oil of *Dysphania ambrosioides* (L.) Mosyakin & Clemants



José Weverton Almeida Bezerra^{a,*}, Adrielle Rodrigues Costa^b, Maria Audilene de Freitas^c, Felicidade Caroline Rodrigues^a, Mikael Amaro de Souza^b, Ana Raquel Pereira da Silva^e, Antonia Thassya Lucas dos Santos^d, Karina Vieiralves Linhares^e, Henrique Douglas Melo Coutinho^f, Jailson Renato de Lima Silva^f, Maria Flaviana Bezerra Moraes-Braga^d

^a Postgraduate Program in Plant Biology - PPGBV, UFPE, Recife, PE, Brazil

^b Postgraduate Program in Molecular Bioprospecting - PPBM, URCA, Crato, CE, Brazil

^c Laboratory of Medical Mycology Sylvio Campos, UFPE, Recife, PE, Brazil

^d Laboratory of Applied Mycology of Cariri - LMAC, URCA, Crato, CE, Brazil

^e Herbarium Caririense Dárdano de Andrade Lima - HCDAL, Crato, Ce, Brazil

^f Laboratory of Microbiology and Molecular Biology - LMBM, URCA, Crato, CE, Brazil

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ABSTRACT

The oil presented the α -Terpinene as the major compound with 54.09% presence. Antibacterial activity demonstrated significant MIC against *Staphylococcus aureus* (256 $\mu\text{g/mL}$) and moderate against *Pseudomonas aeruginosa* (512 $\mu\text{g/mL}$). The modulating effect of antibiotics was significant against *P. aeruginosa* potentiating the effect of all the antibiotics tested. The IC_{50} observed for CT LM 23 was clinically relevant (19.3 $\mu\text{g/mL}$), similar to that obtained for CA INCQS 40006 (25.2 $\mu\text{g/mL}$). The combined effect with fluconazole also showed significant results, 0.1 and 22.7 $\mu\text{g/mL}$, for CT LM 23 and CA INCQS 40006, respectively. For CA LM 77 the IC_{50} was 101.9 $\mu\text{g/mL}$ and for CT INCQS 40042 a value of 53.3 $\mu\text{g/mL}$. Regarding the modulation, both were considered of clinical relevance, 3.3 and 6.4 $\mu\text{g/mL}$. OEDA has low antioxidant activity (> 1024 $\mu\text{g/mL}$). Therefore, the popular use against infections was corroborated by this work.

1. Introduction

One of the main public health problems is the indiscriminate use of antibiotics that has caused a growing development of defense mechanisms in microorganisms, this happens mainly with bacteria, but occurs in both fungi and protozoa. Therefore, the processing of new drugs can not keep pace with the evolution and proliferation of microorganisms [1]. In addition to the microbial resistance factor, populations from developing and mainly underdeveloped countries do not have access to medicines in a way that has high mortality rates due to bacterial and fungal infections [2].

These infections are caused by several bacterial strains, however some are of great clinical interest, such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* [3]. The first strain is a Gram-positive strain that causes infections in the skin and the

subcutaneous tissue, besides being present in infected surgeries and that may represent foci for the development of systemic infections [1]. Despite the antibiotic treatment, the strains are able to acquire resistance to practically all the antibiotics [4].

As for the other two bacteria, both are Gram-negative so that *E. coli* despite being a commensal species in the vertebrate intestine, it can lead to infections that lead to the death of 2 million people per year, the species is linked to clinical pictures of hemorrhagic colitis, dysentery, cystitis, nephritis and uremia-hemolytic [5,6]. Whereas *P. aeruginosa* causes infections in the urinary tract, respiratory and its contamination occurs, mainly because of being opportunistic. In addition, strains of *P. aeruginosa* are resistant to a range of antibiotics [7].

In addition to bacteria, some commensal fungi have the capacity to infect immunocompromised individuals, who are called opportunists [8]. Among these, *Candida* strains have caused high mortality rates

* Corresponding author at: Bioscience Center, Department of Botany, Street Professor Moraes Rego, s/n. Cidade Universitária, Recife, Pernambuco, CEP - 50.670-901 Brazil.

E-mail address: weverton.almeida@urca.br (J.W. Almeida Bezerra).

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even after treatment, varying from 40 to 60% of mortality [9]. The search for drugs in the fight against fungal infections has been a challenge since, like humans, fungi are eukaryotic organisms, so that there is a cellular similarity, consequently many drugs end up causing cytotoxicity both in the parasite and in the host [10].

As an alternative to the treatment of these infections caused by microorganisms, the deprived populations use medicinal plants because they are easy to access and at low cost, besides being culturally accepted. Among these plants include *Dysphania ambrosioides* (L.) Mosyakin & Clemants, for use against general infections [11]. This species, belonging to the family Amaranthaceae and is popularly known in Brazil as "mastruz" and "santa-maria", and in addition to the use against infections, its infusions, decoctions and macerations are used in the treatment of colds, inflammation, wound healing, and bone fracture [12]. It has been demonstrated *in vitro* that the essential oil of the species exhibits antibacterial activity against cariogenic bacteria as *Streptococcus sobrinus* (ATCC 33478) and *Enterococcus faecalis* (ATCC 4082) [13].

A parallel beneficial effect of the use of medicinal plants in the combat against infectious and parasitic diseases, is the antioxidant action of the compounds present in the natural product [14,15]. This antioxidant action consists in the reduction or even elimination of free radicals from biological organisms, which are produced by endogenous and exogenous reactions, such as exposure to ultraviolet rays and the use of cigarettes. These radicals have the ability to sequester electrons from various cell sites, including the plasma membrane and DNA itself, so the multiplication of these radicals is detrimental to biological systems. Thus, the antioxidant acts by donating electrons so that it is stabilized and does not cause a cascade reaction [16,17].

Thus, due to the resistance of pathogenic microorganisms to antibiotics, studies are necessary to evaluate the antimicrobial effect of natural products. Therefore, as *D. ambrosioides* is used in folk medicine for the treatment of infectious and parasitic diseases, this study aimed to evaluate the antibacterial and antifungal effect of its essential oil and the ability to modulate the effect of commercial drugs against bacteria. In addition, its *in vitro* efficacy in reduction free radicals has been investigated.

2. Materials and methods

2.1. Plant material

The leaves of *D. ambrosioides* were collected in the Medicinal Plants Garden of the Regional University of Cariri - URCA, Crato, CE, Brazil, in March 2015 at 09:00 ± 00:30 h under coordinates 07°14'19.2" S and 39°24'52.8" longitude of Greenwich. One specimen was pressed, identified by the and deposited in the Herbarium Anchieta - PACA-AGP under voucher #116226.

2.2. Extraction of the essential oil of *D. ambrosioides* (EODA)

The leaves were punched into pieces to increase the contact surface with the extraction solvent, and then added in a 5 L glass flask containing 2 L of distilled water which were boiled steadily for two hours [18]. After extraction, the oil with a yield of 0.1393% was stored in

amber glass under refrigeration at -4 °C until the conduction of the antimicrobial and antioxidant experiments.

2.3. Chemical composition of essential oil

2.3.1. Gas Chromatography (GC-FID)

The gas chromatography (GC) analyses was performed with Agilent Technologies 6890 N GC-FID system, equipped with DB-5 capillary column (30 m x 0.32 mm; 0.50 mm) and connected to an FID detector. The thermal programmer was 60 °C (1 min) to 180 °C at 3 °C/min; injector temperature 220 °C; detector temperature 220 °C; split ratio 1:10; carrier gas Helium; flow rate: 1.0 mL/min. The volume injected 1 µL diluted in chloroform (1:10). Two replicates of samples were processed in the same way. Component relative concentrations were calculated based on GC peak areas without using correction factors [14].

2.3.2. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analyses were performed on a Agilent Technologies AutoSystem XL GC–MS system operating in the EI mode at 70 eV, equipped with a split/splitless injector (220 °C). The transfer line temperature was 220 °C. Helium was used as carrier gas (1.0 mL/min) and the capillary columns used were an HP 5MS (30 m x 0.35 mm; film thickness 0.50 mm) and an HP Innowax (30 m x 0.32 mm i.d., film thickness 0.50 mm). The temperature programmer was the same as that used for the GC analyses. The injected volume was 1 µL of the essential oil diluted in chloroform (1:10).

2.3.3. Identification of the components

Identification of the constituents was performed on the basis of retention index (RI), determined with reference of the homologous series of n-alkanes, C7-C30, under identical experimental conditions, comparing with the mass spectra library search (NIST and Wiley), and with the mass spectra literature date [19]. The relative amounts of individual components were calculated based on the CG peak area (FID response).

2.4. Antibacterial activity

2.4.1. Bacterial strains, culture media and drugs

Standard bacterial strains were used to determine Minimum Inhibitory Concentration (MIC) being *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25853 and *Staphylococcus aureus* ATCC 25923. While bacterial clinical isolates of *Escherichia coli* 06, *Pseudomonas aeruginosa* 03 and *Staphylococcus aureus* 10, with a multidrug resistance profile, according to Table 1.

As for the culture medium for the antibacterial assays, Brain Heart Infusion (BHI) was prepared according to the measures recommended by the manufacturer. While the drugs used to evaluate the modulatory capacity of EODA were gentamicin of the class of aminoglycosides, Norfloxacin, belonging to the classes of fluoroquinolones and Imipenem of the class of carbapenems.

2.4.2. Minimal inhibitory concentration (MIC)

The MIC is the minimum concentration responsible for totally inhibiting bacterial growth. Thus, following the methodology of Gomes et al. [20], a 1000 µL solution containing 100 µL of inoculum and

Table 1

Isolated clinical bacterial strains used for MIC and modulation tests with their antibiotic resistance and origin profile. Source: Laboratory of Microbiology and Molecular Biology - LMBM - Regional University of Cariri - URCA.

Bacteria	Origin	Resistance profile
<i>Escherichia coli</i> 06	Urine culture	Cephalothin, cephalixin, cefadroxil, ceftriaxone, cefepime, ampicillin-sulbactam
<i>Pseudomonas aeruginosa</i> 03	Uroculture	Amikacin, imipenem, ciprofloxacin, levofloxacin, piperacillin-tazobactam, ceftazidime, merpenem, cefepime
<i>Staphylococcus aureus</i> 10	Rectal swab culture	Cefadroxil, cephalixin, cephalothin, oxacillin, penicillin, ampicillin, amoxicillin, moxifloxacin, ciprofloxacin, levofloxacin, ampicillin-sulbactam, amoxicilin/ac. Clavulanic, erythromycin, clarithromycin, azithromycin, clindamycin

900 μL of the 10% BHI liquid culture medium was prepared in eppendorf tubes. Subsequently, this solution was distributed into 96 well plates filled in the numerical sense by the addition of 100 μL in each well. Subsequently, microdilutions were run in series with 100 μL of EODA at concentrations ranging from 1024 $\mu\text{g}/\text{mL}$ to 1 $\mu\text{g}/\text{mL}$, so the plates were incubated for 24 h at 37 °C. To read the MIC, 20 μL of a solution of resazurin was added to each well in order for oxidoreductive reactions to occur in the wells where there was still bacterial growth. After 1 h the color change of the wells was observed, where the modification of the blue to red color corresponds to the microbial growth and the blue stay the absence of growth.

2.4.3. Modulating effect of antibiotics

Some natural products do not present antibacterial activity at concentrations of clinical interest, however, some of them are able to modulate the effect of usual antibiotics. In order to evaluate the modulatory capacity of EODA, the methodology proposed by Coutinho et al. [21] was used, in which, after MIC tests with resistant bacteria, the results were used to determine the subinhibitory concentrations (MIC/8) to be used with the antibiotics at concentrations ranging from 1024 $\mu\text{g}/\text{mL}$ to 1 $\mu\text{g}/\text{mL}$. Thus, for the tests, 1162 μL of 10% BHI were used, with 150 μL of the inoculum of each strain and the essential oil with volume corresponding to a subinhibitory concentration, whereas the control group were prepared with only 1350 μL of BHI (10%) and 150 μL of bacterial suspension. Subsequently a serial microdilution was performed using the antibiotic, being performed with 100 μL of each drug until the penultimate well. The plates were incubated (24 h at 37 °C) and read through the addition of 20 μL of resazurin.

2.5. Antifungal activity

2.5.1. Fungal strains and culture media

The fungal strains *Candida albicans* CA INCQS 40006 and *Candida tropicalis* CT INCQS 40042 were donated by the National Institute of Quality Control in Health (INCQS) and *C. tropicalis* LM 23 and *C. albicans* LM 77 were donated by the Laboratory of Mycology of the Federal University of Paraíba (UFPB). The strains were inoculated into Sabouraud Dextrose Agar (SDA, KASVI) and incubated for 24 h at 37 °C. Subsequently, small aliquots of the yeast were transferred into test tubes, each containing 3 mL of sterile saline (0.9%). The inoculum concentration was standardized by comparison with the McFarland 0.5 scale [22]. Sabouraud Dextrose Broth (CSD, HIMEDIA), doubly concentrated, was used in the microdilution assay.

2.5.2. Drugs, reagents and solution preparation

Dimethylsulfoxide (DMSO, Merck, Germany) was used to initially dilute the essential oil, while the antifungal fluconazole (Capsule; Prati donaduzzi, Brazil) was diluted with water and used as reference medicine. The product solution was prepared by weighing 0.15 g of the oil and diluting it in 1 mL of DMSO. To obtain the desired concentration for the test, the oil was further diluted in sterile distilled water (1024 $\mu\text{g}/\text{mL}$) so that the DMSO concentration did not exert any activity on the cells tested [23].

2.5.3. Determination of IC₅₀ and cell viability curve

In this test, we were adopted in the broth microdilution method in 96-well plates according to the methodology used by Santos et al. [24]. In each well of the plate was added 100 μL of CSD containing 10% inoculum of fungal strains examined in this study and in the first well was started with 100 μL serial microdilution essential oil *D. ambrosioides* (1024 $\mu\text{g}/\text{mL}$) or fluconazole in same concentrations followed by dilution reaching the penultimate well at a concentration of 1 $\mu\text{g}/\text{mL}$. In the case of the last well, it did not contain essential oil or drug and served as growth control. Controls were also prepared for the diluent product (with 0.9% sodium chloride solution instead of the inoculum) and the sterile medium. Subsequently the plates were

incubated at 37 °C for 24 h, and subsequently read on an ELISA spectrophotometer (Thermoplate) with a wavelength of 630 nm. The results obtained in the ELISA reading were used to construct the cell viability curve and to determine the IC₅₀ of the EODA. This IC₅₀ is the concentration of the product or drug in $\mu\text{g}/\text{mL}$ which is capable of inhibiting 50% of *Candida* yeast growth.

2.5.4. Determination of Minimum Fungicide Concentration (MFC)

MFC is the concentration in $\mu\text{g}/\text{mL}$ of the natural product or drug that is capable of causing the death of that yeast colony. In this way the tip of a rod was inserted into each well of the previously tested plaque (except for the sterility control). After mixing the medium in each well, the stem was taken to a Petri dish containing SDA with the aid of a guide plate affixed to the bottom of the plate for colony growth checks. After 24 h of incubation, the plates were analyzed observing the formation of colonies by *Candida* yeasts. The concentration in which there was no fungal colony growth was considered as the MFC of the tested product [24].

2.5.5. Evaluation of the modulating effect of fluconazole

After obtaining the results of the MFC, the modulation tests were carried out to evaluate if the oil in sub-inhibitory concentrations (MFC/8) was able to modify positively or negatively the effect of fluconazole. For this, the oil was used in a subinhibitory concentration, according to the methodology used by Coutinho et al. [21] with amendments. In 96-well plates was added 100 μL of medium + oil + inoculum and were microdiluídos fluconazole 100 μL at a concentration of 2.048 $\mu\text{g}/\text{mL}$. The mixture was added to the first well of each column to undergo serial dilutions at concentrations ranging from 1024 to 1 $\mu\text{g}/\text{mL}$. The last well was intended to control yeast growth. Dilution controls were also made for the natural product (where saline replaced the inoculum) and sterility control. The IC₅₀ of fluconazole was also determined for comparative purposes, while a dilution control was also required. Plates were incubated at 37 °C for 24 h. The reading was performed on an ELISA spectrophotometric apparatus (Thermoplate®).

2.6. Antioxidant activity

For the antioxidant assay, the DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical method was used according to Choi et al. [25] with modifications. A solution of 0.3 mM DPPH was initially prepared with ethanol, and then in 96-well plates, 100 μL of the DPPH solution was added along with 50 μL of ethanol and 50 μL of the solution of the natural product with final concentrations ranging from 32 to 1024 $\mu\text{g}/\text{mL}$. The plate was incubated for 30 min at room temperature and absorbance reading was performed at a wavelength of 517 nm in plate reader SpectraMax, USA. As a positive control, ascorbic acid was used and the antioxidant effect was calculated as a percentage, as well as its IC₅₀.

2.7. Statistical analysis

The results were analyzed in the GraphPad Prism program, version 6, in which data were analyzed using Anova One-way and followed by post hoc Bonferroni test and were considered significant when $p < 0.05$.

3. Results

3.1. Chemical composition of essential oil

According to Table 2 the chromatographic results of the oil identified 98.15% of its constitution and indicates that it presents 16 constituents. Among these, the monoterpene α -Terpinene was the major compound with 54.09% of the constitution. The second most terpene present in the oil was Ascaridol with 15.13%.

Table 2
Composition of *Dysphania ambrosioides* essential oil.

Compounds	RI ^a	RI ^b	oil %
<i>E-p</i> -Mentha-2,8-dien-1-ol	987	985	0.02
α -Terpinene	1018	1018	54.09
β -myrcene	994	991	0.36
<i>p</i> -cymene	1025	1029	4.87
Limonene	1033	1031	1.82
γ -terpinene	1062	1061	0.91
Citronellal	1151	1153	0.24
Naphthalene	1170	1178	0.12
α -Terpineol	1084	1189	2.05
Ascaridol	1235	1237	15.13
Carvacrol	1297	1298	4.57
Isoascaridole	1309	1304	2.32
Ascaridole Epoxide	1315	1311	9.77
Thymol Acetate	1354	1355	0.51
Limonene Oxide	1581	1586	1.26
Caryophyllene Acetate	1703	1700	0.11
Total identified (%)			98.15

Relative proportions of the essential oil constituents were expressed as percentages.

^a Retention indices experimental (based on homologous series of *n*-alkane C₇-C₃₀).

^b Retention indices from literature (Adams, 1995).

3.2. Antibacterial activity

3.2.1. Minimum Inhibitory Concentration (MIC) of essential oil of *D. ambrosioides*

The EODA showed antibacterial activity for *S. aureus* ATCC 25923 standard strains, with a MIC of 256 μ g/mL, but did not present antibacterial activity in concentrations of clinical relevance for strains of *P. aeruginosa* ATCC 9027 and *E. coli* ATCC 25922, since its MIC was \geq 1024 μ g/mL. While for multiresistant strains the oil had moderate antibacterial activity for *P. aeruginosa* 03 strains with MIC of 512 μ g/mL and no activity at low concentrations for *E. coli* 06 and *S. aureus* 10 with MIC of both \geq 1024 μ g/mL (Table 3).

3.2.2. Effect of modulating antibiotics

According to Fig. 1, the essential oil at subinhibitory concentrations was able to significantly modify the effect of antibiotics. For *P. aeruginosa* 03 multiresistant strains the oil modulated the effect of gentamicin, imipenem and norfloxacin so that it decreased the concentration of antibiotic required to inhibit bacterial growth. However, when the oil along with the antibiotics gentamicin and imipenem were evaluated against the strains of *E. coli* 06 there was an antagonistic effect, so that a higher concentration of the antibiotic was required to inhibit the growth of *E. coli*. While the oil modulated norfloxacin positively for the bacterium. Finally, when evaluated in the strains of *S. aureus* 10, the oil positively modulated the effect of imipenem, and antagonistic the effect of gentamicin and norfloxacin.

Table 3
Minimum Inhibitory Concentration (μ g/mL) of *Dysphania ambrosioides* essential oil against standard bacterial (ATCC) and multiresistant strains. Standard bacterial strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25853 and *Staphylococcus aureus* ATCC 25923; bacterial clinical isolates *Escherichia coli* 06, *Pseudomonas aeruginosa* 03 and *Staphylococcus aureus* 10.

Strains	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>
Strains standards (ATCC)	\geq 1024	\geq 1024	256
Multi-Resistant Strains	512	\geq 1024	\geq 1024

3.3. Antifungal activity

In the trials evaluating the cellular viability of *C. albicans* when exposed to EODA, it is possible to notice that for the CA LM 77 strains, the natural product had better antifungal activity than the standard drug, fluconazole, since its IC₅₀ was lower (19.3 μ g/mL) than the antifungal (22.7 μ g/mL) (Fig. 2). However, for CA INCQS 40006 strains, the oil had an IC₅₀ of 25.2 μ g / mL that was higher than fluconazole (22.2 μ g/mL), it is worth mentioning that these values are of great clinical relevance (Fig. 3). Regarding the modulating action, for the CA LM 77 strains, the oil was able to synergistically modulate the effect of fluconazole so that it presented an IC₅₀ of 0.1 μ g/mL whereas the drug alone had an IC₅₀ of 22.7 μ g/mL (Fig. 2). Similarly, for CA INCQS 40006 strains, the oil was able to modulate the effect of fluconazole by lowering the IC₅₀ from 22.2 μ g/mL to 12.4 μ g/mL (Fig. 3).

Regarding the cellular viability of *C. tropicalis*, the oil showed antifungal activity with an IC₅₀ of 101.9 μ g/mL for CT LM 23 strains, whereas the reference drug had an IC₅₀ of 15.8 μ g/mL. For the CT INCQS 40042 strains the oil had a higher antifungal potential than fluconazole, because the natural product had an IC₅₀ of 53.3 μ g/mL while fluconazole had a value of 385.1 μ g/mL, higher than the oil (Fig. 5). As for the modulating action, for CT LM 23 and CT INCQS 40042 the oil modulated fluconazole with an IC₅₀ of 3.3 μ g/mL and 6.4 μ g/mL respectively (Figs. 4 and 5).

3.4. Antioxidant activity

It is possible to observe in Fig. 6 that EODA presented moderate antioxidant activity since its IC₅₀, ie its ability to eliminate 50% of free radicals, was higher than 1024 μ g/mL. So that at this concentration, inhibition of the DPPH free radical was 33.2 \pm 5.9%. For the positive control ascorbic acid, this in the concentration of 8.1 μ g/mL was able to inhibit 50% of free radicals.

4. Discussion

The use of *D. ambrosioides* in ethnobotany for infectious diseases has scientific support, as is demonstrated in our study, since the oil of the species presents antimicrobial activities in concentrations of clinical and pharmacological relevance, and that even the species not being used for combat to neurodegenerative diseases, we show that the oil presents a moderate antioxidant activity.

In the present work, *D. ambrosioides* oil has the presence of α -Terpinene (C₁₀H₁₆) with presence > 40%, however, its percentage varies from work to work [26–28]. This variation is due to several factors, whether abiotic or biotic, such as differences in organism cultivation, collection periods, climatic stress, plant origin, and especially genetic factors [29,30]. An alternative to avoid a wide variation in the essential oil composition is that it should be extracted under the same conditions, resulting in a more constant composition [14].

The EODA presented antibacterial activity for *S. aureus* ATCC 25923, while there was no activity for the other ATCC strains, one of the justifications is that *S. aureus* is a gram-positive bacterium, and these have simpler cell walls when compared with gram-negative, so that the oil action was effective. The EODA in the study by Soares et al. [13] also showed antibacterial action on the strains of a gram-positive cariogenic bacterium *Streptococcus sobrinus* (ATCC 33478) with a MIC of 1000 μ g/mL, however in our study the action for *S. aureus* had a better MIC.

As for the mechanism of action, Sikkema et al. [31] states that natural products with antibacterial actions can disintegrate their cytoplasmic membranes, as well as destabilization of proton motive force (PMF), electron flow, active transport and coagulation of the cellular content. In the case of antibiotic modulating activity against multiresistant strains of *S. aureus* 10, this is due to the hydrophobic properties of monoterpenes and sesquiterpenes of the essential oil, in which

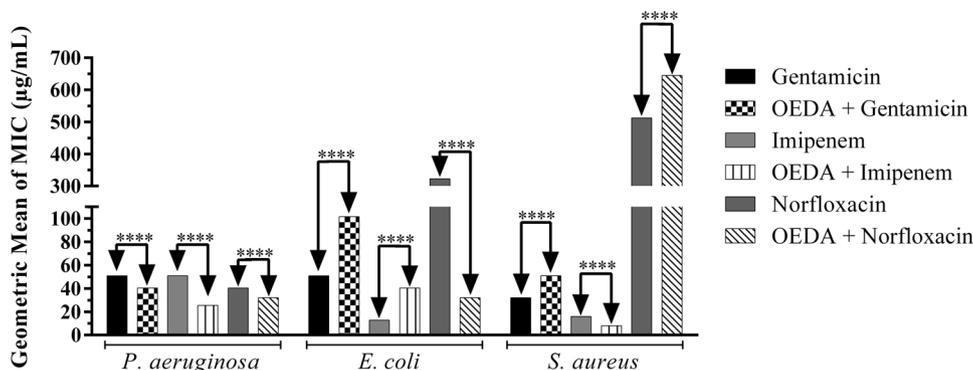


Fig. 1. Geometric Mean of Minimal Inhibitory Concentration (MIC) in µg/mL of *Dysphania ambrosioides* essential oil (EODA) against different multiresistant bacterial strains. ****: $p < 0.0001$.

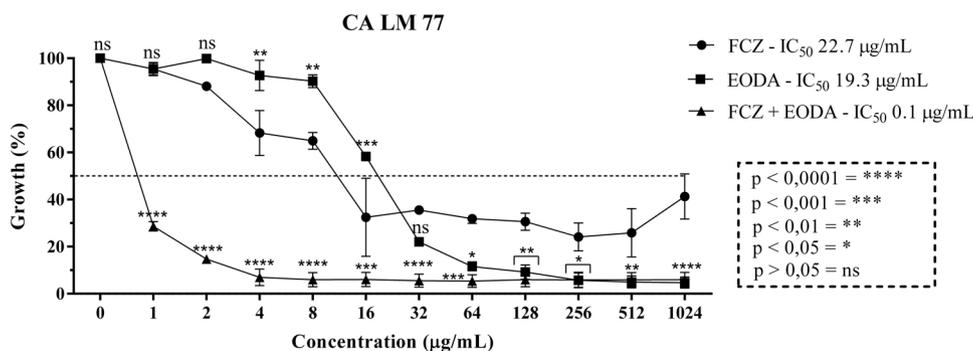


Fig. 2. Cell viability curve of essential oil of *Dysphania ambrosioides* (EODA) and Fluconazole (FCZ) in µg/mL against *Candida albicans* LM 77 (CA) and IC₅₀ (Concentration responsible for 50% inhibition of colony growth).

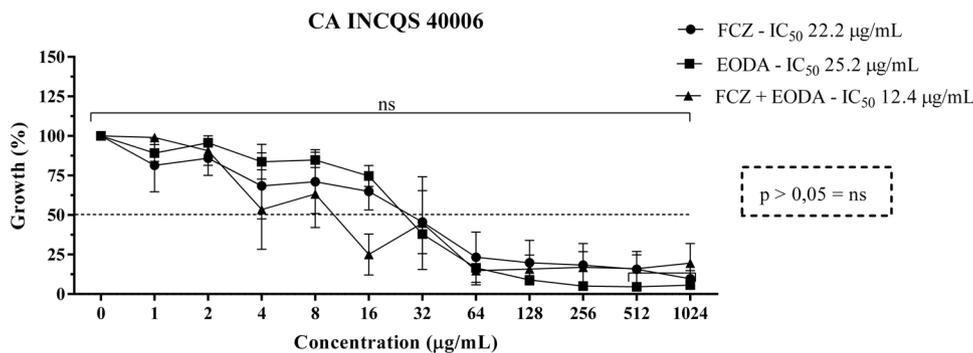


Fig. 3. Cell viability curve of essential oil of *Dysphania ambrosioides* (EODA) and Fluconazole (FCZ) in µg/mL against *Candida albicans* INCQS 40006 (CA) and IC₅₀ (Concentration responsible for 50% inhibition of colony growth).

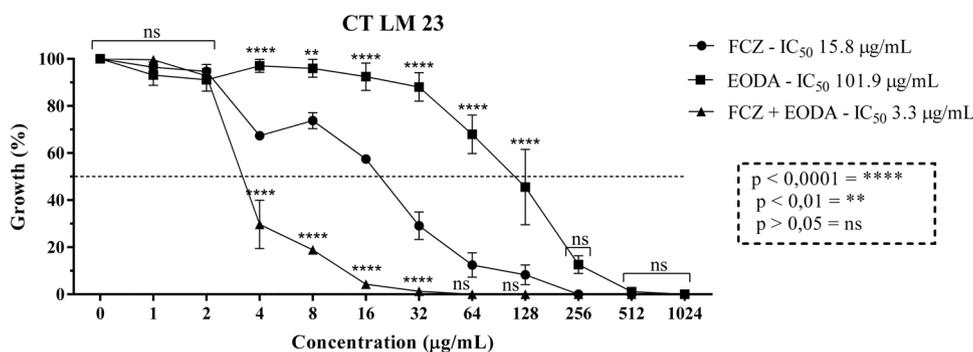


Fig. 4. Cell viability curve of essential oil of *Dysphania ambrosioides* (EODA) and Fluconazole (FCZ) in µg/mL against *Candida tropicalis* LM 23 (CT) and IC₅₀ (Concentration responsible for 50% inhibition of colony growth).

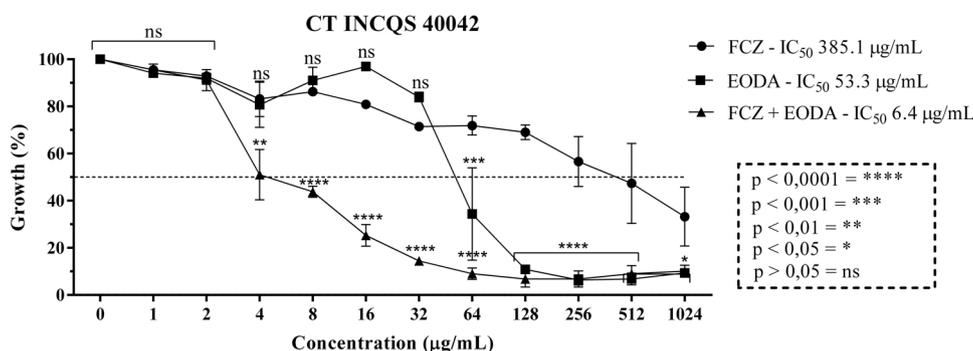


Fig. 5. Cell viability curve of essential oil of *Dysphania ambrosioides* (EODA) and Fluconazole (FCZ) in µg/mL against *Candida tropicalis* INCQS 40042 (CT) and IC₅₀ (Concentration responsible for 50% inhibition of colony growth).

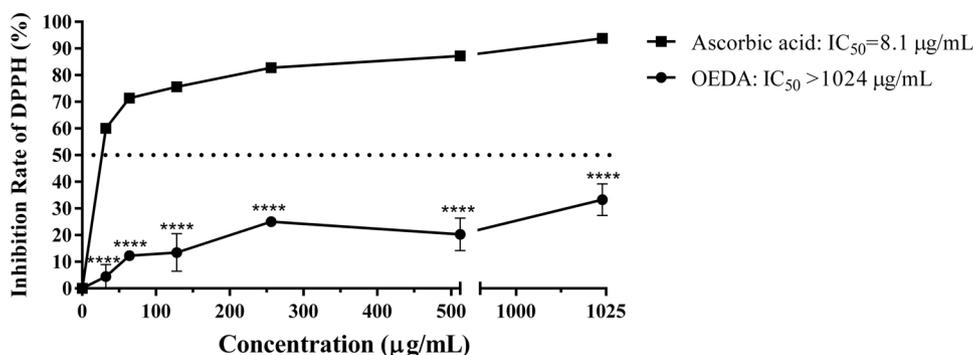


Fig. 6. Antioxidant effect of the essential oil of *Dysphania ambrosioides* (EODA) and ascorbic acid against free radical DPPH. ****: $p < 0.0001$.

such characteristics favor changes in the cell membrane in order to make them more permeable and consequently the entry of the antibiotic [32].

Oliveira-Tintino et al. [1] evaluating the action of the essential oil of *D. ambrosioides* on strains of *S. aureus* 1199B that had a pump of efflux NorA, showed that the oil alone was not able to inhibit bacterial growth. This is directly linked to the efflux pump, which may have expelled from the intracellular medium the possible phytochemical constituents that have passed through the wall and cell membrane. Jesus et al. [33] showed that fractions of ethyl acetate *D. ambrosioides* leaves showed antibacterial activity against *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 340, *Enterococcus faecalis* ATCC 29212, *Paenibacillus apiarius* and *Paenibacillus thiaminolyticus*, however, MIC varied from 4,290 µg/mL to 34,370 µg/mL, these concentrations being of no pharmacological relevance.

Despite the low activity against bacteria, EODA presented high antifungal potential against *Candida* strains, so our data corroborate with the results of Chekem et al. [26], in which in their *in vitro* experiments the oil had antifungal activity against the yeasts of various *Candida* species. In the same study, the authors evaluated the effect of *in vivo* oil on female mice that were induced vaginal candidiasis with strains of *Candida albicans* and found that after 12 days of treatment with the natural producer, all rodents were cured.

Other studies also evaluated the effect of essential oils in the search for antimicrobials and modulators, such as the case of Santos et al. [24] evaluated the effect of *Eugenia uniflora* L. (Myrtaceae) oil on *C. albicans* yeasts INCQS 40006 and *C. tropicalis* INCQS 40042, with the oil having IC₅₀ of 1,892.47 µg/mL and 4,511.82 µg/mL respectively. Although *E. uniflora* oil does not present antifungal activity in concentrations of clinical relevance, the oil was able to modulate the effect of Fluconazole when evaluated against the strains of *C. tropicalis* INCQS 40042.

In addition to the antimicrobial potential observed, the oil has the ability to inhibit free radicals. Ajaib et al. [34] also evaluating the antioxidant effect of *D. ambrosioides* showed that the aqueous extract of the vegetable presents high antioxidant potential. It is worth

mentioning that in aqueous extracts its composition is different from essential oils, because while in these the predominance is of volatile terpenes, aqueous extracts present phenolic compounds that are natural sources of antioxidant substances [35].

Despite the activities shown in this study, the oils may present toxicity to the individuals who are using them, and in the case of EODA, this natural product presents cytotoxicity [36,37]. Therefore, it is necessary to investigate the isolated constituents of EODA aiming at both antimicrobial activities and their toxicity, so that it can be used.

5. Conclusion

The essential oil of *D. ambrosioides* presents antibacterial and modulating activities in concentrations of clinical relevance, so that its use in pharmacopoeia by traditional communities has scientific support. In addition, the oil has a moderate antioxidant action.

Conflicts of interest

None.

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