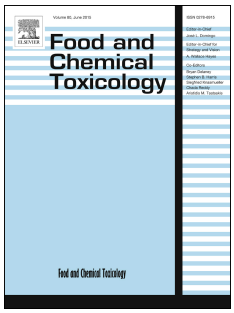


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# GC-MS-FID characterization and Antibacterial activity of the *Mikania cordifolia* essential oil and limonene against MDR strains

Ana Carolina Justino de Araújo[a], Priscilla Ramos Freitas[a], Cristina Rodrigues dos Santos Barbosa[a], Débora Feitosa Muniz[a], Janaína Esmeraldo Rocha[a], Ana Cristina Albuquerque da Silva[a], Cícera Datiane de Morais Oliveira-Tintino[b], Jaime Ribeiro-Filho[c], Luiz Everson da Silva[d], Camila Confortin[d], Wanderlei do Amaral[e], Cícero Deschamps[f], José Maria Barbosa-Filho[g], Natanael Teles Ramos de Lima[g], Saulo Relison Tintino[a], Henrique Douglas Melo Coutinho[a*]

a- Department of Biological Chemistry, Regional University of Cariri - URCA, Crato-CE, Brazil; b- Department of Antibiotics, Federal University of Pernambuco - UFPE, Recife-PE, Brazil; c- Gonçalo Moniz Institute, Oswaldo Cruz Foundation - IGM-FIOCRUZ/BA, Salvador-BA, Brazil; d- Post Graduation course of Sustainable Territorial Development, Federal University of Paraná – UFPR, Matinhos-PR, Brazil; e- Department of Chemical Technology, Federal University of Paraná – UFPR, Curitiba-PR, Brazil; f - Post Graduation course of Agronomy, Federal University of Paraná – UFPR, Curitiba-PR, Brazil; g – Department of Pharmacy, Federal University of Paraiba - UFPB, João Pessoa - PB, Brazil.

* Corresponding author:
Henrique Douglas Melo Coutinho
Universidade Regional do Cariri, Urca, Brasil
Rua Cel. Antonio Luis 1161, Pimenta, 63105-000
Fone: +55(88)31021212; Fax +55(88) 31021291
E-mail: hdmcoutinho@gmail.com



## ABSTRACT

The present study evaluated the effect of the essential oil of *Mikania cordifolia* (EOMc) and its major constituent limonene alone or associated with antibacterial drugs against Multidrug Resistant Bacteria (MDR). To evaluate the antibacterial activity, the minimum inhibitory concentrations (MIC) of the oil and limonene against *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* were determined. The antibiotic-modulating activity was assessed using subinhibitory concentrations (MIC /8) of these substances in combination with conventional antibacterial drugs. Although no relevant antibacterial activity of the natural products was detected, both substances modulated the action of antibiotics against resistant bacteria. The EOMc demonstrated the best modulating effect against *P. aeruginosa*, presenting synergistic effects when associated with gentamicin and norfloxacin. In addition, the oil reduced the MIC of norfloxacin against *E. coli* as well as reduced the MIC of gentamicin against *S. aureus*. On the other hand, the best effect of limonene was obtained against *S. aureus*. Thus, it is concluded that the essential oil *Mikania cordifolia* and the isolated compound limonene do not have clinically significant antibacterial effect, but modulate the action of antibiotics against MDR bacteria.

Keywords: *Mikania cordifolia*. Limonene. Bacterial resistance. Antibiotics. Modulation.

## 1. Introduction

The products of the secondary metabolism products of plants act as part of their defense system and, as such, have antimicrobial properties that can be harnessed for the development of novel drugs (Gurib-Fakim et al., 2006). Essential oils, including monoterpenes, sesquiterpenes and phenylpropanoids are among the main bioactive secondary metabolites of plants. They are naturally found in plants with strong odor as complex mixtures of volatile compounds. (Franz, 2011). Regarding the biological activities of these substances, earlier studies have shown that they have antimicrobial properties

against bacteria, fungi, protozoa and viruses (Ahmad et al., 2011; Perez et al., 2012; Sadekuzzaman et al., 2015).

The genus *Mikania* (L. f.) Willd (Asteraceae) is composed of more than 400 species, 171 of which occur in Brazil (Dalla et al., 2010). *Mikania cordifolia*, popularly known as "guaco", is used in folk medicine as anti-inflammatory, antiasthmatic, antiparasitic and analgesic (Agostini-Costa et al., 2016). These ethnopharmacological data are corroborated by experimental studies proving that this species has anti-inflammatory (Peluso et al., 1995), antiprotozoal (Laurella et al., 2012) and insecticide (Arais et al., 1995) activities. Even with all these activities, studies with *Mikania glomerata* using liver of hypertensive and normotensive rats prove its low toxicity (Sguarezi et al., 2017).

Bacterial infections are among the major problems in public health today. In this context, *Staphylococcus aureus*, coagulase-negative staphylococci, *Klebsiella spp*, *Escherichia coli* and *Enterobacter* spp are considered the main causative agents of hospital infections (Lima et al., 2015). Historically, bacterial infections represent some of the leading causes of diseases in mankind. However, the development of antibiotics caused a real revolution in this scenario, since non-treatable bacterial infections were practically eradicated (Silva, Hertel, 2014).

As a result, the indiscriminate use of antibiotics over the years has created a selective pressure environment that has stimulated the emergence of resistant microorganisms, such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (De Brito, Cordeiro, 2012).

Bacterial resistance is considered as a major public health problem because several bacterial strains have become unsusceptible to the currently available antibiotics (Da Costa, Junior, 2017). The main mechanisms involved in this phenomenon include: enzymatic modification of the antibiotic; reduction of cellular permeability or presence of

antibiotic efflux pumps (avoiding the intracellular accumulation of the antibiotic); structural modification of target molecules making antibiotic binding impossible (Medina, Machado, Machado, 2015).

*Pseudomonas aeruginosa* is a Gram-negative bacillus that causes opportunistic infections, mainly in immunodeficient patients (Lee, Zhang, 2015). It has a wide range of virulence and cell signaling mechanisms that favor its escape from the immune system of the host (Balasubramanian et al., 2013; Reinhart, Sherrouse, 2016), in addition to antibacterial resistance mechanims (e.g. enzymes and efflux systems) against the main classes of antibiotics (Rincón et al., 2014).

Despite the importance of *Mikania cordifolia* in the context of folk medicine, the antibacterial effects of the essential oil of this species remain to be investigated. Therefore, the objective of the present study is to evaluate the antibacterial activity and the antibiotic-modulating effect of the *Mikania cordifolia* essential oil.

## 2. Methodology

### *2.1. Plant Material*

The essential oil was extracted from terminal branches and / or inflorescences collected in the Private Reserve of Natural Patrimony (RPPN), a segment of the Atlantic Forest in the State of Paraná, Southern Brazil, It is located at 25º20.884'S and 049° 47.258' W with altitudes ranging from 985 to 1.145 m. with annual average temperatures around 17 °C, frequent frosts, and an average annual rainfall of 1,200 mm/y. The collection was performed in 28/02/16, dried specimens were herborized and deposited in the Herbarium of *Faculdades Integradas Espirita* (HFIE) under the number HFIE 8.325.

*2.2. Essential oil extraction and analysis*

The essential was extracted by hydrodistillation in Clevenger type apparatus, using 50 g of dry material in 1 L of distilled water. The chemical constituents of the essential oil were identified by gas chromatography coupled to mass spectrometry (GC / MS). The mass spectra were compared to those of the library (Linstron, Mallard, 2013) and the linear retention indices were calculated from the injection of a homologous series of hydrocarbons (C7 - C26) and compared with data in the literature (Adams, 2007). Only peaks with relative area greater than 1% in CG/MS were considered for quantification and identification. The mass detector was operated in electron ionization mode (70 eV) at a rate of 3.15 $min^{-1}$ scan and mass range of 40 to 450 u. The transfer line was maintained at 260 °C, the ion source at 230° C and the quadrupole analyzer at 150 °C. Diluted samples were injected into an Agilent 7890A chromatograph equipped with a flame ionization detector (FID), operated at 280 °C for quantification. The same column and analytical conditions described above were employed, except for the carrier gas, which was hydrogen used at a flow rate of 1.5 mL $min^{-1}$. The percentage composition was obtained by electronic integration of the FID signal by dividing the area of each component by the total area (area %). The constituents were identified by comparing their mass spectra with those of the Wiley library and NIST and also with their linear retention indices which were calculated from injection of a homologous series of hydrocarbons (C7 - C26) and compared with literature data. Limonene was purchased from Sigma Aldrich.

*2.3. Bacterial strains*

The resistance profile of *Staphylococcus aureus* 10, *Pseudomonas aeruginosa* 24 e *Escherichia coli* 06 is described in the work of Bezerra et al., (2017) that used the same strains.

*2.4. Preparation of the essential oil and Limonene*

Prior to the experiments, 10 mg of each substance were weighed and placed in individualized tubes and diluted in 0.5 ml of DMSO. This solution was transferred to a larger tube and added with 9.265 mL of sterile distilled water resulting in a 1024 μg / mL solutions of the oil or limonene, which were used in the tests.

**Preparation of inocula and antibiotics**

Bacterial cultures were seeded in Petri dishes containing Heart Agar Infusion (HIA) and maintained for growth in the oven at 37 ° C for 24 h. After this period, a trawl of each microbial culture was diluted in test tubes containing sterile saline solution in triplicate. After this procedure, the turbidity of the solution was compared to the control of 0.5 of the McFarland scale. The antibiotics used in the tests were norfloxacin, penicillin and gentamicin at an initial concentration of 1024 μg / mL.

**Determination of the Minimum Inhibitory Concentration (MIC)**

In the MIC determination assay, 900μL of 10% liquid BHI medium and 100μL of the inoculum (corresponding to 10% of the total solution) were added to a tube (NCCLS, 2003). A 100μL aliquot of this solution was transferred to each well of a 96-well plate and then serial dilution was performed by adding 100μL of the essential oil or limonene in

concentrations ranging from 512 to 8μg / mL. The plates were incubated at 35 ± 2 ° C for 24 h and after that period the MIC of the substances was determined. To this end, resazurin sodium (20 μg) was added in each well. Interpretation of the results was made by ocular analysis of the color change of resazurin after 1 h of reaction (Coutinho et al., 2008; Javadpour et al., 1996). In the positive controls, no treatments were added and in the negative controls bacterial inocula were not used. The tests were performed in triplicate

**Evaluation of the antibiotic-modulating activity**

The antibiotic-modulating activity of the oil and its manjor constituent limonene was analyzed by the method proposed by Coutinho et al. (2008). In Eppendorf tubes, 1,350 μL of a solution containing the treatments at a subinhibitory concentration (MIC / 8) and 10% BHI was prepared. To this solution, 150 μL of the bacterial suspension was added, resulting in a final volume of 1.5mL. As a control, eppendorf tubes were prepared with 1.5mL of a solution containing 1,350μL of BHI (10%) and 150μL of bacterial suspension. The plate was filled numerically by adding 100μL of the final solution into each well. Subsequently, serial microdilutions were performed by using 100μL of each antibiotic at concentrations ranging from 512 – 0,5μg /μL. The plates were incubated at 37 ° C for 24 h and the readings were performed as described above.

*2.5. Statistical Analysis*

The results were expressed as mean ± standard deviation and differences were evaluated through analysis of variance (ANOVA) followed by *Bonferroni's* post-test using

the GraphPad Prism software. The differences with a $p < 0.05$ were considered as significant.

## 3. Results and discussion

### *3.1. Chemical profile of the Mikania cordifolia essential oil*

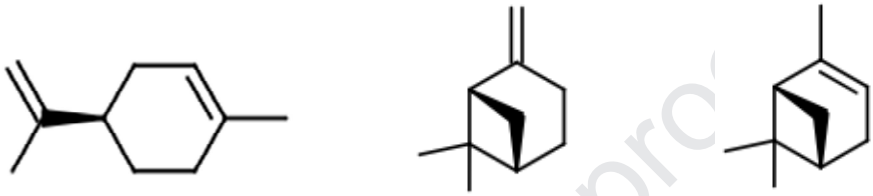
The essential oil of Mikania cordifolia showed a yield of 0.26%, which is expected for species of the genus, such as Mikania glomerata, whose essential oil presented yields of 0.12% to 0.76% according to chemotype (Bolina et al., 2009). The present study is a pioneer in the study of the essential oil of the species Mikania cordifolia (L.f.) Willd. In this context, a phytochemical analysis revealed the presence of 13 compounds (Table 1), including Limonene (19.2%) β-pinene (17.8%) and α-pinene (16%) as major compounds (Fig. 1 and Fig. 2).

**Table 1:** Chemical compounds identified in the essential oil of *Mikania cordifolia* by GC-MS-FID

| RI calculadted | Composition | % |
|---|---|---|
| 937 | α-pinene | 16,0 |
| 976 | Sabinene | 2,5 |
| 979 | β-pinene | 17,8 |
| 991 | Myrcene | 13,3 |
| 1031 | Limonene | 19,2 |
| 1050 | (*E*)-β-ocimene | 3,0 |
| 1390 | β-elemene | 3,2 |
| 1451 | α-humulene | 2,8 |
| 1478 | γ-muurolene | 3,3 |

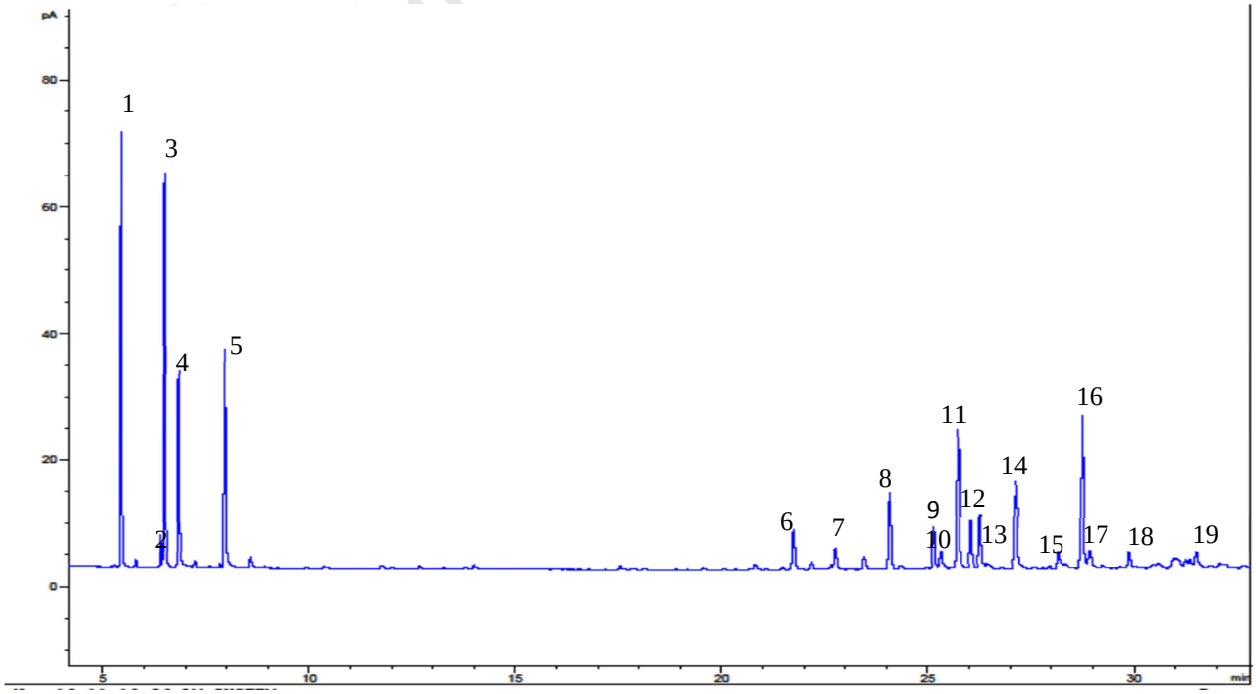
| | | |
|---|---|---|
| 1493 | bicyclogermacrene | 8,8 |
| 1501 | germacrene A | 2,6 |
| 1531 | (*E*)-γ-bisabolene | 5,4 |
| 1575 | Spathulenol | 1,9 |

**Figure 1:** Chemical structure of the major compounds found in the EOMc



Limonene β-pinene α-pinene

**Figure 2:** GC-MS Chromatogram of the Essential Oil of *Mikania cordifolia* (EOMc)




1:α-pinene; 2:sabinene; 3:β-pinene; 4: myrcene; 5: limonene; 6: (*E*)-β-ocimene; 7:β-elemene; 8:(*E*)-caryophyllene; 9: α-humulene; 10: γ-muurolene; 11: *ar*- curcumene; 12:bicyclogermacrene; 13: germacrene A; 14: β-bisabolene; 15: *(E)*-γ-bisabolene; 16: elemicin; 17:spatulenol; 18: caryophyllene oxide; 19:humulene epoxide II.

The phytochemical analysis of the EOMc indicates a similarity between this oil and the essential oil of *M. glomerata*, since there are five common constituents in the oils of these two species, including D-limonene, which was found as the major compound and in similar concentrations (19.2 and 19.5%) in both species, corroborating with the data from the current study (Silva-Junior et al., 2015). However, *Mikania glomerata* oil has coumarin, a compound derived from o-coumaric acid, which was not identified in *M. cordifolia* oil. This metabolite is synthesized more frequently in end shoots and young leaves and can be induced by successive collections (Czelusniak et al., 2012). In addition, this compound was identified as one of the major constituents of a *Mikania* species from the interior of São Paulo (Taleb-Contini et al. 2006). These differences, however, may be due to factors such as: collection time, component of the plant analyzed, light intensity and precipitation. In fact, these factors directly influence the synthesis of secondary metabolites of any plant (Czelusniak et al., 2012).

Some species of the genus Mikania were evaluated to verify their toxicological activity as in the study by Gasparetto et al. (2010) who evaluated Mikania sp administered as syrup, infusions and extracts in rats to evaluate their liver and spermatogenic toxicity evidencing its low toxicity and in some cases was considered non-toxic. A study evaluated the possible toxicity of a drug developed from six phytotherapeutic plants including Mikania glomerata. Blood tests showed no signs of toxicity. Adverse effects were reported in both the placebo and medication groups (Viana et al., 2018).

*3.2. Antibacterial Activity of the Mikania cordifolia essential oil and Limonene*

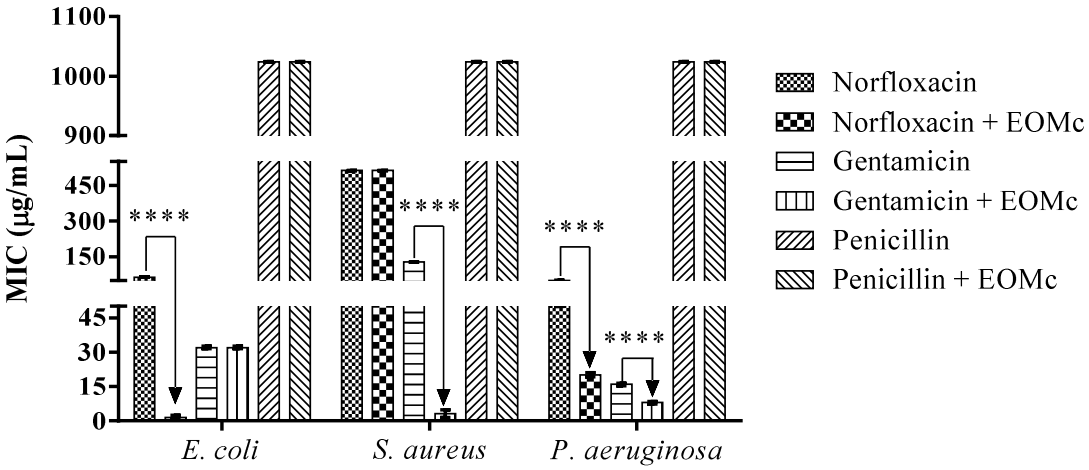
The antibacterial activity of the *Mikania cordifolia* essential oil was tested against three multiresistant strains: *Staphylococcus aureus* 10, *Pseudomonas aeruginosa* 24 and

*Escherichia coli* 06. However, the products did not inhibit bacterial growth at any of the concentrations tested, indicating that they do not have antibacterial activity when tested alone. These results are important especially in the context of traditional medicine, since many species of the genus *Mikania* are used empirically for the treatment of diseases of the respiratory tract. In addition, although studies indicate that plants of this genus have antibacterial, anti-inflammatory and antiparasitic activities (Oliveira et al., 2007; Rufatto et al., 2012), the biological effects of *M. cordifolia* need to be better investigated

*3.3. Antibiotic-modulating effect of the Mikania cordifolia essential oil*

As shown in Figure 2, penicillin did not inhibit bacterial growth even at the highest concentration tested (1024μg / mL). In addition, the association with subinhibitory concentrations of the oil did not alter the MIC of this antibiotic, indicating that the EOMc does not modulate the activity of penicillin against resistant bacteria.

**Figure 3:** Antibiotic-modulating effect of the *Mikania cordifolia* essential oil (EOMc) against multiresistant bacteria. **** statistically significant value, p<0.0001



On the other hand, the association between norfloxacin and the *M. cordifolia* essential oil presented synergism against *P. aeruginosa* and *E. coli*, with consequent reduction of the MICs of this antibiotic. This indicates that the EOMc reversed the resistance profile of the bacterium to the drug and, therefore, acts as an antibiotic modulator. However, the oil did not affect the action of this antibiotic on *S. aureus*, indicating that the modulating effect varies depending on the bacterial strain. In addition, this effect may vary according to the plant material used (Li et al., 2013).

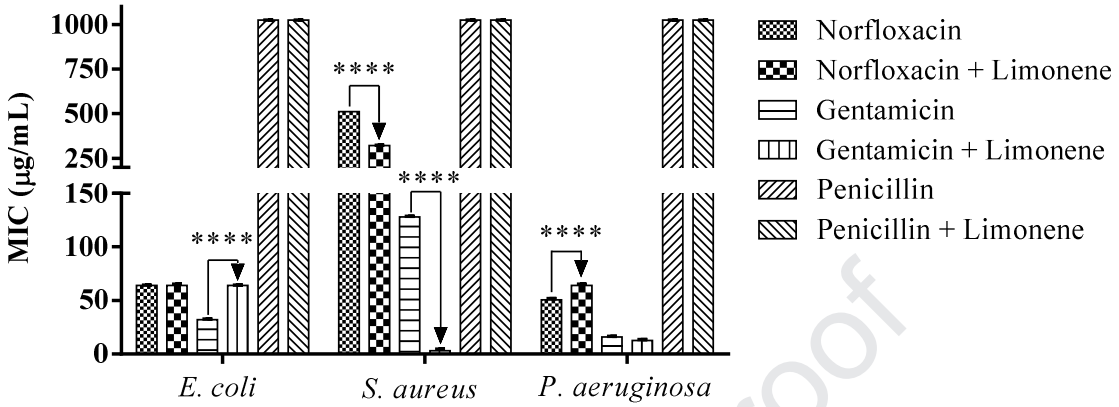
The association of the oil with the aminoglycoside gentamicin significantly reduced the MIC of the antibiotic against *S. aureus* and *P. aeruginosa*, but not against *E. coli*. It is worth mentioning that the results with the Gram-positive bacteria suggest that there was a reversal of resistance, since the MIC of gentamicin MIC decreased from 128 to 3 μg / mL. In general, the antibiotic-modulating effect of the *Mikania cordifolia* essential oil was more evident against *P. aeruginosa* compared to the other strains tested. However, studies suggest that essential oils are more effective against Gram-positive bacteria, because the lipopolysaccharides (LPS) found in the cell membranes of Gram-negative bacteria hinder the intracellular absorption and dissemination of oils (Barbosa et al., 2015).



*3.4. Antibiotic-modulating effect of limonene*

Limonene is a cyclic monoterpene found in several plant species and used mainly in the fragrances industry (Ali et al., 2011).

**Figure 4:** Antibiotic-modulating effect of limonene against multiresistant bacteria. **** statistically significant value, p<0.0001




Like the EOMc, limonene was unable to modulate penicillin activity against resistant bacterial strains. The main mechanism of resistance to Penicillin is the synthesis of β-lactamases. These enzymes hydrolyze the β-lactam ring, which has an antibacterial effect due to inhibition of cell wall synthesis (Zeng, Lin, 2013). Thus, even if the substances tested increase the permeability of the antibiotic, β-lactamases destroy the drug before it can bind to its target.

The association of norfloxacin with limonene did not affect the MIC of the antibiotic against *E. coli*, but potentiated and antagonized the effect of the antibiotic against S. aureus and P. aeruginosa, respectively. Finally, by analyzing the association of limonene with gentamicin against *P. aeruginosa*, it is possible to observe a small reduction in the MIC of the antibiotic, but the differences were not statistically significant. The same association potentiated and antagonized the effect of the antibiotic against *S. aureus* and *E. coli*, respectively. These data demonstrate that the modulating effect of limonene in combination with different antibiotics is dependent on the intrinsic resistance characteristics of each strain (Figure 3).

Essential oils containing limonene as a major component have proven antibacterial activity against resistant strains of *S. aureus* and some strains of *Salmonella enterica* and *Listeria monocytogenes*, as shown in the study by Settanni et al. (2012). In general, the cell wall of Gram-positive bacteria is simpler than that of Gram-negative bacteria, which makes them more susceptible to the action of essential oils. Thus, due to its lipophilic characteristics, it is possible that limonene crosses the cell wall, altering bacterial cell membrane permeability (Maia et al., 2014; Obidi et al., 2013).

## 4. Conclusion

The data of the present study indicate that the essential oil of *Mikania cordifolia* does not have significant antibacterial activity, but it is able to modulate the action of antibiotics, reversing some patterns of bacterial resistance. On the other hand, the antibiotic-modulating action of limonene seems to be strongly influenced by the intrinsic resistance characteristics of each strain, although promising results have been obtained against *S. aureus*.

These data suggest that the modulating activity of *M. cordifolia* is dependent on the isolated or synergistic action of other components present in the oil. However, further studies are needed to characterize the antibacterial and antibiotic-modulating properties of different constituents of the essential oil obtained from this species.

## References

Adams, R.P., 2007. Identification of essential oil components by gas chromatography/mass spectrometry, Carol Stream: Allured Publishing Corporation.

Agostini-Costa, T.S., Gomes, I.S., Fonseca, M.C.M., Alonso, A.M., Pereira, R.C.A., Montanari-Junior I, Silva, J.P., Pereira, A.M.S., Vieira, R., Vaz, A.P.A., 2016. Effect of accessions and environment conditions on coumarin, o-coumaric and kaurenoic acids levels of *Mikania laevigata*. Plant med. 82, 1431–1437.

Ahmad, A., Khan, A., Kumar, P., Bhatt, R.P., Manzoor, N., 2011. Antifungal activity of *Coriaria nepalensis* essential oil by disrupting ergosterol biosynthesis and membrane integrity against *Candida*. Yeast. 28, 611–617.

Ali, M.S., Islam, M.S., Rahman, M.M., Islam, M.R., Sayeed, M.A., et al., 2011. Antibacterial and cytotoxic activity of ethanol extract of Mikania cordata (Burm. F.) BL Robinson leaves. Journal of basic and clinical pharmacy. 2, 103.

Arias, A.R., Ferro, E., Inchausti, A., Ascurra, M., Acosta, N., Rodriguez, E., Fournet, A., 1995. Mutagenicity, insecticidal and trypanomicidal activity of some Paraguayan Asteraceae. J Ethnopharmacol. 45, 35-41.

Balasubramanian, D., Schneper, L., Kumari, H., Mathee, K., 2013. A dynamic and intricate regulatory network determines *Pseudomonas aeruginosa* virulence. Nucleic Acids Res. 41, 1–20.

Barbosa, L.N., Silva-Probst, I., Andrade, B.F.M.T., Alves, F.C.B., Albano, M., et al., 2015. In vitro antibacterial and chemical properties of essential oils including native plants from Brazil against pathogenic and resistant bacteria. Journal of oleo science. 64, 289-298.

Bezerra, C.F. et al. 2017 A vanilina modula seletivamente a ação dos antibióticos contra bactérias resistentes. Patogênese microbiana, 113, 265-268.

Bolina, R.C., Garcia, E.F., Duarte, M.G.R., 2009. Estudo comparativo da composição química das espécies vegetais Mikania glomerata Sprengel e Mikania laevigata Schultz Bip. ex Baker. Rev Bras Farmacogn, 19, 294-298.

Coutinho, H.D.M., Costa, J.G., Lima, E.O., Falcão-Silva, V.S., Siqueira-Júnior, J.P., 2008. Enhancement of the antibiotic activity against a multiresistant *Escherichia coli* by *Mentha arvensis* L. and chlorpromazine *Chemother.* 54, 328–330.

Czelusniak, K.E., Brocco, A., Pereira, D.F., Freitas, G.B.L., 2012. Farmacobotânica, fitoquímica e farmacologia do Guaco: revisão considerando Mikania glomerata Sprengel e Mikania laevigata Schulyz Bip. ex Baker. Revista Brasileira de Plantas Medicinais. 14, 400-409.

Dalla, N.G., Pastori, T., Laughinghouse, H.D., Canto-Dorow, S.T., Tedesco, S.B., 2010. Antiproliferative and genotoxic effects of *Mikania glomerata* (Asteraceae). Biocell. 34.

De Brito, M.A., Cordeiro, B.C., 2012. Necessidade de novos antibióticos. J Bras Patol Med Lab. 48, 247-249.

Da Costa, A. L. P., Junior, A. C. S. S. 2017. Resistência bacteriana aos antibióticos e Saúde Pública: uma breve revisão de literatura. Estação Científica (UNIFAP), 7, 45-57.

Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Diagnostic microbiology. 12th ed. St. Louis, MO: Mosby Elsevier.

Franz, C.M., 2011. Essential oil research: past, present and future. Flavour Fragr J. 25, 112-113.

Gasparetto, J.C., Campos, F.R., Budel, J.M., Pontarolo, R. 2010. Mikania glomerata Spreng. e M. laevigata Sch. Bip. ex Baker, Asteraceae: estudos agronômicos, genéticos, anatômicos, químicos, farmacológicos, toxicológicos e seu uso em programas de fitoterapia no Brasil. Revista Brasileira de Farmacognosia, 20, 627-640.

Gurib-Fakim, A., 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol Aspects Med. 27,1–93.

Hawkey, P.M., 1998. The origins and molecular basis of antibiotic resistance. BMJ. 317, 657–660.

Javadpour, M.M., Juban, M.M., Lo, W.S., Bishop, S.M., Alberty, J.B., Mann, C.M., Markhan, J.L., 1996. A new method for determine the minimum inhibitory concentration of essential oils. J Appl Microbiol. 84, 538–544.

Laurella, L.C., Frank, F.M., Sarquiz, A., Alonso, M.R., Giberti, G., Cavallaro, L., Catalán, C.A., Cazorla, S.I., Malchiodi, E., Martino, V.S., Sulsen, V.P., 2012. In Vitro Evaluation of Antiprotozoal and Antiviral Activities of Extracts fromArgentinean *Mikania* Species. Sci World J. 58, 639-646.

Lee, J., Zhang, L. 2015. Rede de detecção de quorum de hierarquia em Pseudomonas aeruginosa. Proteína e célula, 6, 26-41.

Lima, M. F. P. et al. 2015. Staphylococcus aureus e as infecções hospitalares–Revisão de Literatura. Revista Uningá Review, 21.

Li, Y., Li, J., Li, Y., Wang, X.X., Cao, A.C., 2013. Antimicrobial Constituents of the Leaves of Mikania micrantha HB K. Plos one. 76725.

Linstron, P.J., Mallard, W.G., 2013. NIST Chemistry Webbook, Eds. http://webbook.nist.gov (accessed in September 2013).

Lyczak, J.B., Cannon, C.L., Pier, G.B. Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. Microbes Infect. 2,1051–106.

Maia, A.J., Schwan-Estada, K.R.F., Faria, C.M.D.R., Oliveira, J.S.B., Jardinetti, V.A., et al., 2014. Óleo essencial de alecrim no controle de doenças e na indução de resistência em videira. Pesquisa Agropecuária Brasileira. 49, 330-339.

Medina, D.A.M., Machado, M.E.D, Machado, A.J.E. 2015. Resistencia a antibióticos, una crisis global. Revista Médica de Risaralda, 21, 74-74.

NCCLS. 2003. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. USA.

Obidi, O.F., Adelowotan, A.O., Ayoola, G.A., Johnson, O.O., Hassan, M.O., et al., 2013. Antimicrobial activity of orange oil on selected pathogens. The International Journal of Biotechnology. 2, 113-122.

Oliveira, P.A., Gregorio, L.E., Oliveira, D.C.R., 2007. Comparative analysis of sesquiterpene lactones from Mikania cordifolia collected from three diferente-locations. Chemistry of Natural Compounds. 43, 140-142.

Peluso, G., Feo, V., Simone, F., Bresciano, E., Vuotto, M.L., 1995. Studies on the inhibitory effects of caffeoylquinic acids on monocyte migration and superoxide íon production. J Nat Prod. 58, 639-649.

Perez, S.G., Lopez, M.A.R., Miranda, E.S., Fresan-Orozco, M.C., Perez-Ramos, J., 2012. Antiprotozoa activity of some essential oils. J Med Plants Res. 6, 2901–2908.

Reinhart, A.A., Sherrouse, A.G.O., 2016. Regulation of *Pseudomonas aeruginosa* Virulence by Distinct Iron Sources. *Genes (Basel).* 7.

Rincón, S. et al. 2014. Resistencia a antibióticos de última línea en cocos Gram positivos: la era posterior a la vancomicina. Biomedica: revista del Instituto Nacional de Salud, 34, 188- 191.

Rufatto, L.C., Gower, A., Schwambach, J., Moura, S., 2012. Genus Mikania: chemical composition and phytotherapeutical activity. Revista Brasileira de Farmacognosia. 22, 1384-1403.

Sadekuzzaman, M., Yang, S., Mizan, M.F.R., Ha, S.D., 2015. Current and recent advanced strategies for combating biofilms. Comp Rev Food Sci Food Saf. 14, 491–509.

Settanni, L., Alazzolo, E., Guarrasi, V., Aleo, A., Mammina, C., et al., 2012. Inhibition of foodborne pathogen bacteria by essential oils extracted from citrus fruits cultivated in Sicily. Food Control. 6, 326-330.

Silva, A., Hertel, V. L. 2014. Perfil epidemiológico de crianças hospitalizadas em uso de antibióticos. Revista eletrônica de enfermagem do Vale do Paraíba, 1.

Silva-Junior, A.A., Ritter, M.R., Zambonim, F.M., Deschamps, F.C., Tcacenco, F.A., et al., 2015. Um novo ecótipo de Mikania glomerata Spreng. (Asteraceae) rico em óleo essencial no sul do Brasil. Revista Fitos. 9, 19-28.

Sguarezi, J.G.D., Gonçalves, V.F., Rocha, T., Murakami, D.Y., Uzuelle, M.A., et al., 2017. Fitoterápicos na Rede Pública de Saúde (SUS) no Brasil: Um estudo toxicológico de Mikania glomerata em fetos de ratas Wistar.10, 460-468.

Taleb-Contini, S.H., Santos, P.A., Veneziani, R., Pereira, A.M.S., França, S.C., et al., 2006. Differences in secondary metabolites from leaf extracts of Mikania glomerata Sprengel obtained by micropropagation and cuttings. Revista Brasileira de Farmacognosia. 16, 596-598.

Viana, I.O.L., de Moraes, M.E.A., de Moraes Filho, M.O., Bezerra, F.A. F., et al., 2018. Avaliação da toxicologia clínica do xarope Melagrião® em voluntários sadios. Revista Biociências, 23, 15-35.

Zeng, X., Lin, J., 2013. Beta-lactamase induction and cell wall metabolism in Gram-negative bacteria. Frontiers in microbiology. 4, 128-132.

Zowalaty, M.E., Thani, A.A., Webster, T.J., Zowalaty, A.E., Schweizer, H.P., Nasrallah, G.K., Marei, H.E., Ashour, H.M., 2015. Future Microbiol. 10, 1683-706.

Ana Carolina Justino de Araújo - METHODOLOGY

Priscilla Ramos Freitas- METHODOLOGY

Cristina Rodrigues dos Santos Barbosa- METHODOLOGY

Débora Feitosa Muniz- METHODOLOGY

Janaína Esmeraldo Rocha - VALIDATION

Ana Cristina Albuquerque da Silva- VALIDATION

Cícera Datiane de Morais Oliveira-Tintino - VALIDATION

Jaime Ribeiro-Filho - RESOURCES

Luiz Everson da Silva- SUPERVISING

Camila Confortin- METHODOLOGY

Wanderlei do Amaral- METHODOLOGY

Cícero Deschamps- METHODOLOGY

José Maria Barbosa-Filho - RESOURCES

Natanael Teles Ramos de Lima - METHODOLOGY

Saulo Relison Tintino- SUPERVISING

Henrique Douglas Melo Coutinho- SUPERVISING

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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