











Antimicrobial activity of plant extracts and compounds over resistant Gram-negative isolates from domestic animals¹

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ABSTRACT.- Santos JP, Santos NS, Sant'ana AS, Silva JJ, Andreo N, Santos MWC, Almeida JRGS, Costa MM. **Antimicrobial activity of plant extracts and compounds over resistant Gram-negative isolates from domestic animals.** *Pesquisa Veterinária Brasileira* 45:e07570, 2025. Laboratório de Microbiologia e Imunologia Animal, Universidade Federal do Vale do São Francisco, Campus Ciências Agrárias, Rodovia BR-407 Km 12, Lote 543, Projeto de Irrigação Nilo Coelho, PE 56300-000. Brazil. E-mail: mmatiuzzicosta@gmail.com

Gram-negative bacteria present significant challenges due to their antimicrobial resistance. Members of the Enterobacterales order, such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*, pose the greatest risk to public health because of their numerous mechanisms of antimicrobial resistance. Therefore, this study aimed to evaluate the antimicrobial activity of crude ethanolic extracts (CEE) of *Hymenaea martiana* Hayne (jatobá), *Commiphora leptophloeos* (Mart.) Gillett (imburana), *Ocimum campechianum* Mill. (alfavaca) and *Morus nigra* L. (amora miura), as well as the hexane and ethyl acetate fractions of the last one, against clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* obtained from domestic animals. Furthermore, three commercially available compounds – gallic acid (GA), *p*-Coumaric acid (*p*-CA), benzoic acid (BA) – and one conducting polymer (synthesized for the test), polypyrrole (Ppy), were also evaluated for their antimicrobial potential against these isolates. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts and compounds were determined. All *E. coli* and *K. pneumoniae* isolates were resistant to the tested extracts. The crude ethanolic extracts showed inhibitory activity against *P. mirabilis* isolates at concentrations of 3,125 µg/mL for *H. martiana* and 12,500 µg/mL for *O. campechianum*. However, *C. leptophloeos*, *M. nigra*, and their fractions (hexane and ethyl acetate), as well as GA, did not exhibit bactericidal activity against any of the pathogens. Benzoic acid demonstrated both inhibitory and bactericidal activity against all isolates, with pronounced bactericidal activity against two *K. pneumoniae* isolates (MBC of 2,000 µg/mL). *p*-Coumaric acid showed inhibitory activity against all isolates and bactericidal activity (MBC of 5,000 µg/mL) against 66.66% (4/6) of the isolates, proving effective against *E. coli* and *K. pneumoniae*. Polypyrrole exhibited the most potent antibacterial activity, inhibiting 100% of the isolates and showing bactericidal activity against 83.33% (5/6) of the clinical isolates tested. These results suggest that the tested extracts and compounds, particularly polypyrrole, may offer a viable alternative against multidrug-resistant microorganisms.

INDEX TERMS: Antimicrobial activity, domestic animals, phenolic acids, plant extracts.

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INTRODUCTION

The Enterobacteriaceae family is a heterogeneous group with a wide natural distribution, causing significant impacts on human and veterinary health (Janda & Abbott 2021). This bacterial family consists of more than 170 species, including about 53 genera, of which 26 are associated with acquired bacterial infections in hospital or community environments (Rodríguez-Baño et al. 2018). About 80% of infections caused by Gram-negative bacteria in hospital environments are caused by enterobacteria (García-Bustos et al. 2022). Furthermore, Enterobacteriaceae have strains and/or pathogenic sorovars associated with various clinical syndromes, dietary and zoonotic infections, including gastroenteritis, urinary tract infections, peritonitis, hospital bacterial pneumonia (HABP), pneumonia related to mechanical ventilation (PAV), meningitis, bacteremia, sepsis of several foci, pyogenic liver abscesses, among others (Paczosa & Meccas 2016, Janda & Abbott 2021, Oliveira & Reygaert 2023).

The dissemination of multidrug-resistant (MDR) and extensively drug-resistant (XDR) isolates of the Enterobacteriaceae family is one of the greatest challenges faced by One Health due to the high rates of morbidity and mortality they cause (Gupta & Datta 2019). The Gram-negative bacteria that stand out in the Enterobacteriaceae family due to high resistance to antimicrobials are *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* (Santos et al. 2020, Garcia-Bustos et al. 2022, Oliveira & Reygaert 2023). Therefore, the rapid dissemination of antimicrobial resistance has been identified as a priority research area, and mitigation strategies at different fronts are being planned and explored further (Sharma et al. 2018).

In this sense, the antimicrobial activity of medicinal plants offers a new hope in combating the dangerous threats posed by antimicrobial resistance. A large diversity of bioactive compounds from plants has proved to have therapeutic potential as antimicrobial resistance modifiers. In this sense, there is an urgent need to identify and isolate new bioactive compounds that have yet to be adequately explored (Vaou et al. 2021). In this scenario, possible antimicrobial alternatives emerge, such as the ethanolic extracts from different plant species, e.g., *Hymenaea martiana* Hayne (jatobá), *Commiphora leptophloeos* (Mart.) Gillett (imburana), *Ocimum campechianum* Mill. (alfavaca) and *Morus nigra* L. (amora miura), as well as bioactive compounds from plants, like *p*-Coumaric, gallic and benzoic acids. In addition to the plant alternatives already mentioned, conductive polymers such as polypyrrole have also been studied and stand out due to their numerous applications and bactericidal potential (Varesano et al. 2013, Silva Júnior et al. 2016).

From this perspective, the aim of this research was to evaluate the antimicrobial activity of the crude ethanolic extract of *H. martiana* Hayne, *C. leptophloeos* (Mart.) Gillett, *O. campechianum* Mill. and *M. nigra* L., as well as the hexanic and ethyl acetate fractions of the last one, in addition to three commercial compounds – gallic acid (GA), *p*-Coumaric acid (*p*-CA) and benzoic acid (BA) – and one synthesized conducting polymer, polypyrrole (PPy), against clinical isolates of *E. coli*, *K. pneumoniae*, and *P. mirabilis* obtained from domestic animals.

MATERIALS AND METHODS

Ethical approval. The study was approved by the Animal Ethics Committee of the “Universidade Federal do Oeste da Bahia” (UFOB) under protocol number 00172019. The clinical isolates are registered with the National Genetic Heritage Management System and Associated Traditional Knowledge (SisGen) under the number AF98CB8.

Location of the experiment. The study was carried out at the “Laboratório de Microbiologia e Imunologia Animal” (Laboratory of Animal Microbiology and Immunology - LAMIA) of the “Campus Ciências Agrárias” (Agricultural Sciences Campus - CCA) of the “Universidade Federal do Vale do São Francisco” (UNIVASF), located on Highway BR-407 Km 12, Lot 543, Projeto de Irrigação Nilo Coelho, PE, Brazil.

Bacterial clinical isolates. The clinical isolates evaluated came from domestic animals, including pigs, dogs, cats, and horses, which showed a variety of infectious processes, e.g., gastrointestinal, respiratory and urinary infections, pyoderma, and abscesses with purulent secretion, without distinction by age or sex, attended from May 2018 to December 2019 at the Veterinary University Hospital (HVU) of UFOB, Multidisciplinary Center of Barra/BA, and kindly donated for the experiment. Six isolates were used in total, two of the following species: *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*, previously identified by morphotinctorial features through Gram staining and biochemical tests (Markey et al. 2013), followed by confirmation using the matrix-assisted laser desorption ionization–time of flight (MALDI-TOF).

Origin of the test substances. The crude ethanolic extracts (CEEs) of *Hymenaea martiana* Hayne (jatobá) and *Morus nigra* L. (amora miura), the ethyl acetate (AE) and hexanic (FH) fractions of *M. nigra* L. leaves were produced at the Pharmacy Laboratory of UNIVASF. The CEE of *Ocimum campechianum* Mill. (leaf, seeds, and stem) (alfavaca) was extracted at the Bromatology and Animal Nutrition Laboratory of UNIVASF. The CEE of *Commiphora leptophloeos* (Mart.) JB Gillett (imburana) leaves were extracted at the “Instituto Federal do Sertão Pernambucano” (Federal Institute of Education, Science, and Technology Sertão Pernambucano - IF Sertão), Campus Zona Rural. To obtain the CEE of the analyzed plant species, the plant material (leaves of *H. martiana* Hayne, *M. nigra* L. and *C. leptophloeos*; leaf, seeds and stem of *O. campechianum* Mill.) was dried in a forced circulation oven (temperature of 40 °C) for three days. Subsequently, the material was processed in a grinder and subjected to exhaustive maceration with 95% ethanol for 72 hours. The extractive solution was subjected to a solvent distillation process in a rotary evaporator at reduced pressure (average temperature of 45 °C), thus obtaining the CEE, according to Peixoto et al. (2015) and Silva et al. (2012). The CEE of *M. nigra* L. was used in order to obtain AE and FH fractions. For this, the CEE was suspended in a mixture of H₂O:MeOH (7:3 v/v) and extracted successively with hexane and ethyl acetate (AcOEt) in crescent order of polarity (Souza et al. 2018). The gallic, benzoic, and *p*-Coumaric acids were obtained commercially from Sigma Aldrich®. Polypyrrole was produced at a concentration of 2 mg/mL by chemical oxidation according to Silva Júnior et al. (2016) at the “Laboratório de Espectroscopia de Impedância e Materiais Orgânicos” (Laboratory of Impedance Spectroscopy and Organic Materials – LEIMO) of UNIVASF, Campus Juazeiro, BA.

Antimicrobial activity of the substances. All CEEs and acetate and hexanic fractions were used at a stock concentration of 25,000 µg/mL; for this, 0.025 g of the CEE of each species was diluted in 1.000 µL. The CEEs of *H. martiana* and *C. leptophloeos* were diluted in 10% Tween 20 and 90% distilled water. The CEE of *O. campechianum*

Mill. and *M. nigra*, as well as the ethyl acetate fraction of *M. nigra* were diluted in 10% ethyl alcohol and 90% distilled water. However, the hexanic fraction of *M. nigra* was diluted in 20% dimethyl sulfoxide (DMSO, Neon) and 80% of distilled water. Gallic and *p*-Coumaric acid were used at a stock concentration of 10,000 µg/mL. To obtain this concentration, gallic acid was diluted in sterile distilled water and *p*-Coumaric acid in 10% Tween 20 and 90% distilled water. For benzoic acid, the stock concentration was 8,000 µg/mL diluted with 50% dimethyl sulfoxide (DMSO, Neon) due to its low solubility in water. PPy was prepared and used at a stock concentration of 2,000 µg/mL (standard solution). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by following the protocol M07-A9 (CLSI 2012).

To determine the MIC, 100 µL of Mueller-Hinton broth (MH, Kasvi, São José dos Pinhais, Brazil) was added to 96-well microplates (OLEN, São José dos Pinhais, Brazil), followed by 100 µL of the test substance (at respective stock concentration). Subsequently, successive microdilutions were carried out, thus obtaining a 1:2 dilution. This yielded final concentration ranges of 12,500 to 97.65 µg/mL for CEEs and fractions; 5,000 to 39.06 µg/mL for gallic and *p*-Coumaric; 4,000 to 31.25 µg/mL for benzoic acid; 1,000 to 7.81 µg/mL for polypyrrole. For the test, six bacteria were initially spiked in brain heart infusion (BHI, Kasvi, São José dos Pinhais, Brazil) and incubated for 24 hours at 37 °C in a bacteriological oven. After that, a bacterial suspension was prepared in 5 mL of saline solution, adjusted to 0.5 tube in the McFarland standards (approximately 1.5×10^8). From the bacterial suspension, a 15 µL aliquot was transferred to microtubes containing 1,485 mL of MH broth, obtaining a solution with a concentration of approximately 1.5×10^6 . Next, 10 µL of this solution was inoculated, using a micropipette, in microplate wells (in triplicate for each dilution) containing the previously homogenized substances. The microplates were incubated at 37 °C for 24 hours in a bacteriological oven. The MBC was determined after 24 hours of incubation. For this, an aliquot of each microplate well was inoculated with a replicator on a plate containing MH agar (Kasvi), which was incubated at 37 °C for 24 hours. Next, 20 µL of 2,3,5-151 triphenyl-tetrazolium chloride (TTC, Dinâmica, Indaiatuba, Brazil) was added to each microplate well, which was incubated again at 37 °C for 1 hour. Color change of TTC suggests bacterial viability. The MIC was considered the lowest concentration of a

substance capable of inhibiting bacterial growth, whereas the MBC was considered the lowest concentration of a substance capable of causing bacterial death. The tests were carried out in triplicate (technical and biological).

RESULTS

The CEEs of *Hymenaea martiana* and *Ocimum campechianum* Mill. showed inhibitory activity over *Proteus mirabilis*, with an MIC of 3,125 µg/mL and 12,500 µg/mL, respectively. Both extracts also showed bactericidal activity (MBC of 12,500 µg/mL) over *P. mirabilis*. However, the CEE of *Commiphora leptophloeos* did not show this activity (MBC > 12,500 µg/mL). The CEE and hexanic and ethyl acetate fractions of *Morus nigra* showed no antimicrobial activity over the Gram-negative pathogens *Escherichia coli*, *Klebsiella pneumoniae*, and *P. mirabilis*, tested in the current study (Table 1).

With regard to phenolic compounds, only gallic acid did not show antimicrobial activity over any isolates tested (Table 2). Benzoic acid showed inhibitory (MIC of 2,000 µg/mL) and bactericidal activity against *K. pneumoniae* (MBC of 2,000 µg/mL). However, despite having an MBC of 4,000 µg/mL, the other isolates did not show bactericidal activity since, in the diluent control test, this concentration led to the death of *P. mirabilis* and *E. coli* isolates. *p*-Coumaric acid showed inhibitory activity (MIC of 5,000 µg/mL) against all pathogenic isolates tested, as well as bactericidal activity (MIC of 5,000 µg/mL) against 66.66% (4/6) of isolates, being effective against *E. coli* and *K. pneumoniae*. Polypyrrole was the substance that showed the best antibacterial activity, inhibiting 100% of the isolates, and also showing bactericidal activity against 83.33% (5/6) of pathogenic clinical isolates tested (MIC and MBC of 1,000 µg/mL) (Table 2).

DISCUSSION

The antimicrobial potential against clinical isolates of *Proteus mirabilis* demonstrated by the CEE of *Hymenaea martiana* may be related to the possible presence of plant phenolic compounds, such as flavonoids, steroids, and terpenoids, previously found in the phytochemical screening of the

Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of crude ethanolic extract of *Hymenaea martiana*, *Commiphora leptophloeos*, *Ocimum campechianum* Mill, and ethyl acetate and hexanic fraction of *Morus nigra* over isolates of *Proteus mirabilis* (1 and 17), *Escherichia coli* (3 and 15) and *Klebsiella pneumoniae* (13 and 14), also over ATCC BAA 2452 (*E. coli*), ATCC 13883 (*K. pneumoniae*) and ATCC 7002 (*P. mirabilis*)

Isolates	Crude ethanolic extract 25,000 µg/ml											
	<i>Hymenaea martiana</i>		<i>Commiphora leptophloeos</i>		<i>Ocimum campechianum</i>		<i>Morus nigra</i>		Ethyl acetate fraction of <i>M. nigra</i>		Hexanic fraction of <i>M. nigra</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	3,125	12,500	12,500	> 12,500	12,500	12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500
3	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500
13	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500
14	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500
15	> 12,500	> 12,500	N.T.	N.T.	> 12,500	> 12,500	> 12,500	> 12,500	N.T.	N.T.	> 12,500	> 12,500
17	3,125	12,500	12,500	> 12,500	12,500	12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500
ATCC BAA 2452	12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500
ATCC 13883	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	> 12,500	N.T.	N.T.	> 12,500	> 12,500	> 12,500	> 12,500
ATCC 7002	3,125	12,500	N.T.	N.T.	12,500	12,500	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.

N.T. = Not tested.

crude ethanol extract on the research of Peixoto et al. (2015). Likewise, the growth inhibition of this bacterium caused by the CEE of *Ocimum campechianum* Mill. is possibly due to the presence of several classes of secondary metabolites, e.g., phenolic compounds, flavonoids, polyphenols, tannins and monoterpenes, such as eugenol and 1,8-cineole, which have been related to antimicrobial properties (Tacchini et al. 2021, Dharsono et al. 2022).

However, even when finding antibacterial activity against *P. mirabilis*, a Gram-negative bacterium, according to Palaniappan & Holley (2010), natural products usually exhibit their best action against Gram-positive bacteria, acting differently on microorganisms. The absence of antimicrobial activity against *Escherichia coli* and *Klebsiella pneumoniae* suggests that these CEEs contained very low concentrations of active antibacterial compounds, or at the same time, effective compounds capable of inhibiting antibacterial activity (Abdel-Massih et al. 2010). Furthermore, the results also suggest that *E. coli* and *K. pneumoniae* show higher tolerance to the antimicrobials of the plant extract than *P. mirabilis*.

The absence of inhibitory and bactericidal activity by *Commiphora leptophloeos* CEE against Gram-negative pathogens tested in the current study – *E. coli*, *K. pneumoniae*, and *P. mirabilis* – highlights the lower susceptibility of this bacterial group to this CEE. Furthermore, it also highlights the greater resistance of this group of bacteria compared to the Gram-positive group, which has been reported as susceptible to this extract in different studies (Clementino et al. 2016, Silva et al. 2019). The reduced susceptibility of Gram-negative bacteria may be attributed to the unique organization of their cell envelope, which differs substantially from that of Gram-positive microorganisms. While Gram-positive bacteria have a cytoplasmic membrane composed of a thick peptidoglycan layer (20–80 nm), Gram-negative bacteria have a relatively thin peptidoglycan layer (≈ 10 nm) that is covered by an additional outer membrane composed of lipopolysaccharides (LPS) and phospholipids (Mai-Prochnow et al. 2016). This additional structure functions as a selective permeability barrier, restricting the diffusion of many bioactive molecules.

Furthermore, synergistic interactions between the cytoplasmic and outer membranes make Gram-negative bacterial cells essentially impermeable to several classes of

compounds, including plant-derived extracts and antibiotics (Leus et al. 2023). These structural features not only confer intrinsic resistance to antimicrobials but also increase tolerance to environmental stresses such as ultraviolet radiation, heat, and desiccation (Mai-Prochnow et al. 2016). Therefore, the results of the present study suggest that the compounds present in the CEE of *C. leptophloeos* were unable to effectively cross the outer membrane of the Gram-negative bacteria tested, reinforcing the idea that these structural barriers play an important role in resistance among Gram-negative microorganisms.

Likewise, as observed for CEE of *C. leptophloeos*, the hexanic and ethyl acetate fractions of *Morus nigra* (25,000 $\mu\text{g}/\text{mL}$) also did not show inhibitory and bactericidal activity against the isolates tested in the current study. However, the antibacterial activity of *M. nigra* has been studied in various organic fractions (Tahir et al. 2017). Souza et al. (2018) found inhibitory and bactericidal activity against a Gram-negative strain of *E. coli* by both the CEE of *M. nigra* and the hexanic fraction, with the former showing a more pronounced activity compared to the latter. Still, the bioactive principles and mechanisms responsible for this activity have not yet been elucidated.

Medicinal plants have been widely used worldwide in the treatment of infectious diseases, including those caused by Gram-negative bacteria (Garvey et al. 2011). Nonetheless, increasing evidence shows that most plant-derived compounds exhibit limited or no antibiotic activity against these microorganisms. This low efficacy is generally attributed to the permeability barriers and efflux mechanisms characteristic of Gram-negative pathogens, which reduce intracellular accumulation of phytochemicals (Leus et al. 2023). Additionally, the outer membrane that surrounds the cell wall of these bacteria can reduce the diffusion of hydrophobic compounds and create a barrier to the penetration of numerous molecules, while also activating enzymes capable of hydrolyzing these molecules (Saha et al. 2013). In addition, factors related to the plant, such as the plant parts used, the harvesting season, growing region, and type of processing, can influence the composition of the extracts, the aging mechanism, and the levels of compounds responsible for antimicrobial activity (Vaou et al. 2021). From this perspective, numerous and variable factors can influence the antimicrobial action of plant extracts.

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the gallic acid, benzoic acid, p-coumaric acid and polypyrrole over isolates of *Proteus mirabilis* (1 and 17), *Escherichia coli* (3 and 15) and *Klebsiella pneumoniae* (13 and 14), also over ATCC BAA 2452 (*E. coli*), ATCC 13883 (*K. pneumoniae*) and ATCC 7002 (*P. mirabilis*)

Isolates	Gallic acid		Benzoic acid		p-coumaric acid		Polypyrrole	
	10,000 $\mu\text{g}/\text{ml}$		8,000 $\mu\text{g}/\text{ml}$		10,000 $\mu\text{g}/\text{ml}$		2,000 $\mu\text{g}/\text{ml}$	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	N.T.	N.T.	2,000	4,000	5,000	> 5,000	1,000	1,000
3	> 5,000	> 5,000	2,000	4,000	5,000	5,000	1,000	1,000
13	> 5,000	> 5,000	2,000	2,000	5,000	5,000	1,000	1,000
14	> 5,000	> 5,000	2,000	2,000	5,000	5,000	1,000	1,000
15	5,000	> 5,000	2,000	4,000	5,000	5,000	1,000	> 1,000
17	N.T.	N.T.	2,000	4,000	5,000	> 5,000	1,000	1,000
ATCC BAA 2452	5,000	5,000	2,000	4,000	2,500	> 5,000	1,000	> 1,000
ATCC 13883	5,000	> 5,000	2,000	4,000	5,000	5,000	1,000	> 1,000
ATCC 7002	5,000	5,000	2,000	4,000	5,000	> 5,000	1,000	> 1,000

N.T. = Not tested.

Of the compounds evaluated, only gallic acid (> 5,000 µg/mL) did not affect any of the isolates tested, unlike the results observed by Díaz-Gómez et al. (2014), who investigated the effect of gallic acid in cultures of *E. coli* JM109 and found notable inhibitory effects against Gram-negative bacteria. However, the authors reported that the effects observed depend directly on the dose, type, and time of exposure to the substance. These factors may justify the absence of gallic acid activity over *E. coli* isolates studied here. In the current scenario, the *E. coli* isolates from the present study were apparently more resistant compared to those studied by Díaz-Gómez et al. (2014).

The bactericidal ability shown by benzoic acid (2,000 µg/mL of MIC and MBC) against *K. pneumoniae* isolates shows promise since, in recent decades and with the widespread use of antibiotics, the incidence of multidrug-resistant *K. pneumoniae* isolates has increased throughout the world, representing a major public health threat and a with this microorganism constituting a priority pathogen for research and development of new therapeutic approaches (Li et al. 2024). In this scenario, benzoic acid, either isolated or associated with other antimicrobial compounds, could be an alternative against these bacteria.

p-Coumaric acid (5,000 µg/mL of MIC and MBC) showed antibacterial activity against 66.66% (4/6) of pathogenic isolates tested, showing effectiveness over *E. coli* and *K. pneumoniae*. Probably, *p*-Coumaric acid promoted irreversible alterations in the permeability of the membrane cell, which led the cells to lose their capacity to maintain cytoplasm macromolecules and to bind to DNA to inhibit cellular functions, promoting the death of the isolates (Lou et al. 2012). In a study by Ojha & Patil (2019), it was observed that *p*-Coumaric acid inhibited the biochemical activities of the RecA enzymes from *E. coli* (EcRecA) and *Listeria monocytogenes* (LmRecA), including the DNA binding, chain changing, and coprotease functions. The authors also observed that *p*-Coumaric acid potentiated ciprofloxacin activity and reduced cellular viability of *L. monocytogenes*, inhibiting the expression of RecA induced by ciprofloxacin and blocking the SOS response, which is a DNA repair mechanism activated when the bacteria are exposed to stress conditions, e.g., oxidative stress, hunger, and treatment with antibiotics. Thus, the authors' findings suggest that *p*-Coumaric acid is a potent RecA inhibitor and can be used as an adjuvant to antibiotics to reduce resistance of the food pathogen *L. monocytogenes*, as well as in other pathogenic bacterial strains, including *E. coli*. Therefore, the results obtained in this study corroborate those by Ojha & Patil (2019) regarding antibacterial activity against pathogenic strains of *E. coli*. Since *E. coli* and *K. pneumoniae* represent a growing concern for healthcare due to the increase of antibiotic resistance (Patil et al. 2023), *p*-Coumaric acid activity against these etiological agents makes it an available alternative to combat them.

In the current study, polypyrrole (1,000 µg/mL of MIC and MBC) was the substance that stood out the most in terms of antibacterial activity, being effective against 83.33% (5/6) of pathogenic clinical isolates, but unable to kill the *E. coli* isolate 15. It is believed that the bioactivity of PPy is related to the presence of positive charges produced during polymerization along its main chain, thus providing an important antibacterial effect (Silva Júnior et al. 2020, Rocha et

al. 2023). Their positive charges bind to the negatively charged bacterial membrane, causing bacterial death. The interaction of cationic groups with the bacterial cell membrane, which has a negative charge, promotes the formation of surfactants that disrupt the essential functions of the cell membrane, stopping protein activity and preventing bacterial DNA from multiplying (Almeida et al. 2021). According to Silva Júnior et al. (2016), the electrostatic addition of PPy to negatively charged bacteria occurs firstly by PPy adsorption on the cell surface, followed by the diffusion of nanoparticles along the cell wall, nanoparticles binding to cytoplasmic membrane, the release of intracellular components, and the release of K⁺ ions, thus leading to bacterial death. These results highlight the antibacterial potential of polypyrrole against Gram-negative bacteria such as *E. coli*, *K. pneumoniae* and *P. mirabilis*. The results are promising and deserve to be emphasized since, as previously highlighted, this is a group of bacteria that represents a growing concern for healthcare due to the increase in antibiotic resistance (Patil et al. 2023).

CONCLUSION

The results obtained indicate that the crude ethanolic extracts (CEE) of *Hymenaea martiana* and *Ocimum campechianum*, as well as the compounds benzoic acid, *p*-Coumaric acid, and mainly polypyrrole, represent a promising alternative to combat resistant microorganisms such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*.

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Conflict of interest statement.- On behalf of all authors, the corresponding author states that there is no conflict of interest.

Credit author statement.- Jôiciglecia P. Santos: Study conception and design, carrying out experiments, data analysis, interpretation of results and writing of the manuscript. Natilene S. Santos and Aline S. Sant'ana: Support in data collection and organization, assistance in carrying out laboratory procedures, and contribution to the scientific discussion of the findings. Jônatas J. Silva: Supports in data collection and organization, assistance in carrying out laboratory procedures. Nayara Andreo: Critical review of the manuscript and contribution to the scientific discussion of the findings. Marcos Wilker C. Santos: Data analysis, interpretation of results and contribution to the scientific discussion of the findings. Jackson Roberto G.S. Almeida: Provided the tested extracts. Mateus M. Costa: General supervision of the project, methodological guidance, obtaining financing and laboratory infrastructure

Data availability statement.- The entire dataset supporting the results of this study was published in the article itself.

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