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Original Article

Antibacterial activity of *Ilex paraguariensis* (Yerba Mate) against Gram-positive and Gram-negative bacteria

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Abstract

Introduction: The stems and leaves of *Ilex paraguariensis* are popularly used for tea infusions in South America and the Middle East. The health benefits have been previously studied, revealing anti-mutagenic, anti-oxidant, hepatoprotective, hypocholesteremic and glycemic improvement. Limited research was performed on the antibacterial activity of the aqueous extract of Yerba Mate on standard and clinical isolates of Gram-positive and Gram-negative bacteria.

Methodology: Commercial *Ilex paraguariensis* stems and leaves were ground and extracted with sterile deionized water at 70°C. Four ATCC bacterial strains and twenty-five bacterial clinical strains were used for testing. To obtain the minimal inhibitory concentration (MIC), the Yerba Mate aqueous solution was serially diluted according to the microdilution method. For the minimal bactericidal concentration (MBC), the tubes with clear broth were sub-cultured. To identify the types of ESBLs present in the clinical isolates, a multiplex PCR was performed.

Results: An antibacterial activity was observed against most of tested strains, with a greater activity against Gram-positive bacteria. MIC and MBC values ranged between 0.468 mg/mL and 15 mg/mL of aqueous extract of Yerba Mate.

Conclusion: The aqueous extract of the stems and leaves of *Ilex paraguariensis* extracted at 70°C showed a significant antibacterial activity. There was no correlation found between the different molecular resistance profiles and the antibacterial activity range.

Key words: Yerba Mate; antibacterial; MIC/MBC.

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Introduction

Dried leaves and stems of the plant *Ilex paraguariensis*, also known as Yerba Mate, are popularly used in South America and some Middle Eastern countries in preparation of tea infusions [1-3]. Approximately 50 g of dried leaves and stems are packed into a cup; hot water is then repeatedly added for 3-4 minutes and drunk with a metal straw, the bombilla, which has small holes at its bottom preventing the passage of particles of the herb [3].

Previous experiments done on *Ilex paraguariensis* display a wide range of health benefits, such as anti-mutagenic [4,5], anti-glycation [6,7], and anti-oxidant effects [8-10]. It was also found to have hepatoprotective, hypocholesteremic [10] and vasodilation effects [11]. Several other studies have reported improvement in lipid profile and weight reduction in mice after Yerba Mate intake [12]. Recent studies suggest that habitual consumption of Yerba Mate has shown neuroprotective, anticonvulsant and anti-depressant effects [13].

Antibiotic resistance is becoming a major threat to our health with the rapid spread of bacteria with multidrug resistance; hence, the production of new antimicrobials to combat these Multi-Drug-Resistant (MDR) bacteria is of crucial importance. Several publications studied the antibacterial activity of the methanol fraction of Yerba Mate against Gram-positive and Gram-negative bacteria [14-16]. Limited research has been performed on antibacterial effect of the aqueous extract of this plant. The purpose of this study is to determine and evaluate the anti-bacterial activity of the aqueous extract of Yerba Mate against Gram-positive and Gram-negative bacterial strains with different susceptibility profiles, including organisms with high levels of resistance.

Methodology

Microorganisms

The test organisms used in this study were American Type Culture Collections *Acinetobacter baumannii* ATCC 17978, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213,

and *Escherichia coli* ATCC 25922. In addition to 25 clinical isolates including: *Klebsiella pneumoniae* (n = 6), *Enterococcus faecalis* (n = 2), *Enterobacter agglomerans* (n = 1), *Enterobacter aerogenes* (n = 1), *Serratia marcesens* (n = 1), *Escherichia coli* (n = 11), *Staphylococcus aureus* (n = 2) and *Proteus mirabilis* (n = 1) that were isolated from patients at the Centre Hospitalier Du Nord Hospital (CHN)-Lebanon. Bacteria were cultured on non-selective media and incubated overnight at 37°C for the experiments.

Extraction method

Dried leaves and stems of the commercial brand of Yerba Mate (Amanda; Argentina; Stems and Leaves 100%; *Ilex paraguariensis*) purchased from a local supermarket were finely ground to small particles using a commercial food blender. Sterile deionized water was added to the ground leaves and stems at a ratio of 3.6 mL to 1 g of ground Yerba Mate and heated at 70°C for 2 hours with occasional stirring. The temperature was determined as advised by the product label. The extraction method was modified from previous extractions [17] to increase its resemblance to the method of consumption.

The extract was subsequently filtered through filter paper Whatmann (1M) and then centrifuged at 5000 g for 30 minutes at 4°C. The supernatant was concentrated by rotary evaporation and then dialyzed at 4°C against deionized water for 48 hours. The dialyzed extract was centrifuged at 3325 g for 30 minutes at 4°C. The supernatant was dried using a SpeedVaccum Concentrator (Thermo Fisher Scientific, Milford, MA, USA). The SpeedVac-extract was weighed, dissolved in deionized water at a concentration of 120mg/mL and stored at 4°C.

MIC and MBC determination

For the determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of Yerba Mate extract, the microdilution method was performed as recommended by the CLSI (2017) [18]. Briefly, a solution of aqueous Yerba Mate extract with a concentration of 120mg/mL (50µL) was added to a 150µL solution of Mueller Hinton Broth. It was then serially diluted into 7 sterile tubes according to the microdilution method to determine the minimal inhibitory concentration (MIC). Positive (bacteria with culture media without Yerba Mate) and negative (only culture media) control tubes were added to each series. A bacterial suspension (10^6 CFU/mL) of 100ul was added to each tube and incubated for 24 hours. The experiments were done in duplicates and repeated three independent times. In case of discrepancy, the same values provided by 2 experiments out of three were considered and the third was discarded. When the three experiments gave three different values, the experiments were repeated. The MICs were read after 24 hours of incubation for visual growth (turbidity), and the clear tubes with no visual growth were sub cultured for MBC determination (the tube with the lowest concentration of Yerba Mate yielding no growth of bacteria was considered as MBC tube).

Phenotypic and Genotypic Detection of β-Lactamases

Double Disk Synergy method for ESBLs

The double disk synergy has been well documented in the literature and is considered a valid and reliable method for the detection of ESBL-producing organisms [19-21]. This test uses disks containing different extended spectrum β-lactam antibiotics to facilitate the detection of different types of ESBLs. A culture of a 0.5 McFarland's suspension of test bacteria was inoculated onto Mueller-Hinton agar plates and disks containing cefoxitin (30µg), cefotaxime (30µg), ceftazidime (30µg), cefpodoxime (10µg), cefepime (30µg) or aztreonam (30µg) were placed on the agar surface at a distance of 25mm from a centrally located amoxicillin/clavulanic acid disk. The plates were then incubated aerobically for 16 hours at 37°C. Isolates are considered ESBL-producers when the zone of inhibition around any of the extended spectrum cephalosporin disks shows a visible expansion towards the centrally located antibiotic disk that contains amoxicillin/clavulanic acid (keyhole effect) [22]. Resistance to 3rd generation cephalosporins accompanied by susceptibility to cefoxitin was considered indicative for ESBL-production whereas resistance to cefoxitin was considered indicative of chromosomal AmpC production [23].

E-test for ESBL production confirmation

The E-test was performed on all presumptive ESBL-producing organisms as a phenotypic confirmatory test for ESBL production. E-test strips were obtained from BD (AB Biodisk, Solna Sweden) and consist of a plastic strip with a gradient concentration of ceftazidime at one end and ceftazidime plus clavulanic acid at the other. The strips were placed on a Mueller-Hinton agar plate (Bio-Rad, Marnes-La-Coquette, France) previously spread with a 0.5 McFarland's suspension of the test organism. The plates were then incubated aerobically for 16 hours at 37°C. ESBL-production was considered positive if the

Table 1. Multiplex PCR primer sets and conditions for ESBL genes.

Genes	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	Amplicon size (bp)	Reference
bla_{SHV}	CTTTATCGGCCCTCACTCAA	AGGTGCTCATCATGGGAAAG	237	[31]
bla_{TEM}	CGCCGCATACACTATTCTCAGAATGA	ACGCTCACCGGCTCCAGATTTAT	445	[31]
bla_{TX-M}	ATGTGCAGYACCAGTAARGTKATGGC	TGGGTRAARTARGTSACCAGAAYCAGCGG	593	[31]
bla_{OXA}	ACACAATACATATCAACTTCGC	AGTGTGTTTAGAATGGTGATC	813	[31]

ratio of the minimum inhibitory concentration (MIC) of ceftazidime to the MIC of ceftazidime plus clavulanic acid was ≥ 8 [24]. The presence of a phantom zone (rounded zone) or a deformation around the ceftazidime strip was directly indicative of ESBL activity [25].

Phenotypic detection of Plasmidic AmpC by Phenyl Boronic Acid disk method

All clinical isolates were tested for the production of plasmidic AmpC β-lactamases using the phenyl boronic acid disk method (Sigma-Aldrich, Milwaukee, Wisconsin, USA) [26,27]. A culture of a 0.5 McFarland's suspension was inoculated onto Mueller-Hinton agar plates. Two cefoxitin disks were positioned at opposite sides of the agar plate and to one of the disks; 10µL of phenyl boronic acid solution (400µg) was added. The plates were left to dry for approximately 15 minutes before being inverted and incubated for aerobically 16 hours at 37°C. Following incubation, an increase of ≥ 5 mm in zone diameter around the cefoxitin disk containing phenyl boronic acid versus that seen around the cefoxitin disk alone was considered positive for plasmidic AmpC production [26].

Phenotypic detection of carbapenem resistance

All clinical isolates were tested for resistance to ertapenem, imipenem, and meropenem using antibiotic discs. Presumptive phenotypic identification of the mechanism of resistance was done using plain, Phenyl-Boronic Acid (PBA), EDTA, and Cloxacillin embedded agars, in addition to Temocillin discs [28-30]. Increase of at least 5mm of the inhibition zones in presence of PBA or EDTA agars were considered as indicators for KPC and MBL production respectively. Cloxacillin was considered as indicator for both KPC and Oxacillinases, and resistance to Temocillin suggested an Oxa-48.

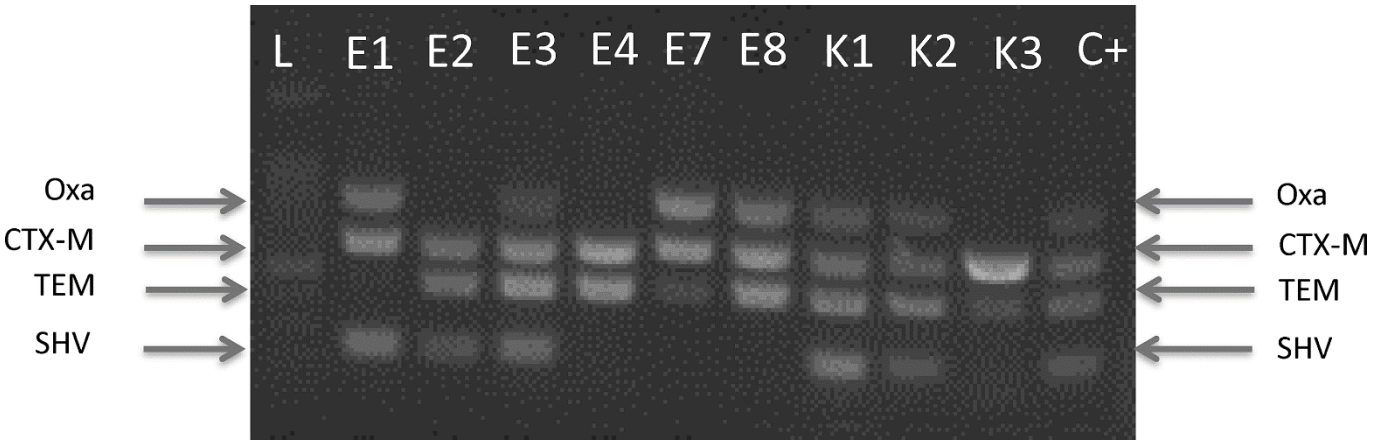
Genetic detection of ESBLs and OXA-48

Multiplex PCR was performed in order to identify the type(s) of ESBLs present in the clinical isolates. Bacterial DNA was prepared by re-suspending 1-2 colonies of each test strain in 200µL dH2O and heating the solution at 95°C for 10 minutes. The presence of blaCTX-M, blaSHV, blaTEM and blaOXA genes was tested for using previously published primer sets and conditions [31,32]. The primer sequences and the expected amplicon size for each gene are listed in Tables 1 and 2. Each reaction tube contained 10µL of Master mix (Qiagen, Hilden, Germany), 4µL of primers, 1µl of DNA and was made up to 20µL total volume with sterile H_2O. The PCR reaction conditions consisted of a 15 minutes denaturation step at 95°C, followed by 30 amplification cycles of 30 seconds at 94°C, 90s at 62°C and 60 seconds at 72°C, with a final extension step of 10 minutes at 72°C. In order to visualize the PCR amplicons, samples were mixed with 4 µL of Thermo Scientific loading dye and loaded into the wells of a 1% agarose gel. The gel was prepared by melting 1g of agarose in 100mL Tris-acetate-EDTA (TAE) buffer. After allowing the solution to cool, 5 µL of Red Safe (ABM, Richmond, Canada), a DNA chelating agent, was added before casting the gel in a gel tray. Gels were run at 120 volts for 60 minutes. Amplicons were visualized using an Ultra-Violet transilluminator system (DIGI DOC-IT System TM) for analysis. For OXA-48 detection, 100 bp of DNA ladder (Fermentas Life Sciences, St. Leon-Rot, Germany) was used as size marker for the amplicons. 13 µL of the PCR product with 2 µL loading dye (Fermentas Life Sciences, St. Leon-Rot, Germany) were loaded into each well. A positive control strain was used.

Table 2. Multiplex PCR primer sets and conditions for carbapenemase gene.

Gene	Primers	Primer Sequences	Amplicon size (bp)	Reference
OXA-48	OXA-48 A	5'TTGGTGGCATCGATTATCGG3'	744	[32]
	OXA-48 B	5'GAGCACTTCTTTTGTGATGGC3'		

Figure 1. Gel showing multiplex PCR amplification of DNA for the detection of the different β-lactamases present in *Escherichia coli* and *Klebsiella pneumoniae* strains. The expected product sizes are: blaOXA 813 bp, blaCTX-M 593 bp, blaTEM 445 bp and blaSHV 237 bp, respectively. The gel shows 9 test strains, a positive control (far right hand lane) and a 100bp DNA ladder (far left lane of gel). C+: positive control, L: 100bp Ladder.



Results

Phenotypic and genotypic resistance

There was full agreement between the results obtained by phenotypic and by genotypic methods for the detection of bacterial resistance. The phenotypic tests suggested that E1, E2, E3, E4, K1, K2, and K3 were ESBL producers, E5 and E6 were AmpC producers. E7 and E8 were positive for both ESBL and AmpC, in addition, E9 and K4 were resistant to carbapenems however with negative tests for Metallo-Beta-Lactamase (MBL) and Klebsiella Producing Carbapenrmase (KPC). The rest of bacterial strains did not show a mechanism of multi-Drug-Resistance.

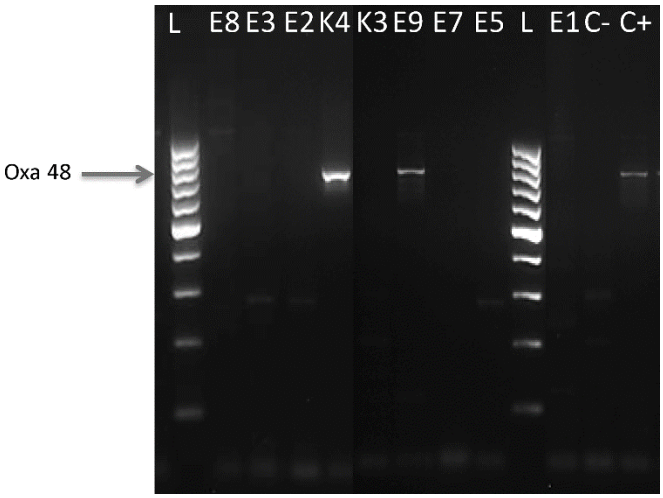
E-test results showed that the ratios of the MIC ceftazidime to the MIC ceftazidime plus clavulanic acid was ≥ 8 for all the strains showing a keyhole effect, confirming therefore ESBL production.

To identify the types of ESBLs present in the clinical isolates, multiplex PCR was performed. All resistant isolates of *Klebsiella* and *E. coli* with positive ESBL phenotypic tests were positive for CTX-M (three *Klebsiella pneumoniae* clinical isolates K1, K2, K3; six *Escherichia coli* clinical strains E1, E2, E3, E4, E7, E8) (Figure 1). Three *Escherichia coli* clinical strains (E1, E2, E3) and two *Klebsiella pneumoniae* clinical strains (K1, K2) were positive for SHV (Figure 1). Five *Escherichia coli* clinical strains (E2, E3, E4, E7, E8) and three *Klebsiella pneumoniae* (K1, K2, K3) were positive for TEM (Figure 1). Four *Escherichia coli* clinical strains (E1, E3, E7, E8) were positive for bla OXA (Figure 1). Among the tested strains, only *Klebsiella pneumoniae* clinical isolate K4 and *Escherichia coli* clinical isolate E9 were positive for bla OXA-48 (Figure 2). Positive controls confirmed the specificity of the PCR primers used (Figure 1, 2).

Antibacterial activity of Yerba Mate

An antibacterial effect was observed against all the tested strains; however, it varied between Gram-positive and Gram-negative organisms, showing a greater activity against Gram-positive bacteria. Yerba Mate had an MIC and MBC of 0.468 mg/mL against *Staphylococcus aureus* (ATCC29213) and an MIC of 0.468 mg/mL and MBC of 1.875 mg/mL against *Enterococcus faecalis* (Table 3). Yerba Mate's aqueous extract also displayed a high antibacterial activity against Gram negative *Acinetobacter baumannii* (ATCC 17978) with MIC and MBC of 0.468 mg/mL (Table 3).

Figure 2. Gel showing multiplex PCR amplification of DNA for the detection of the OXA-48 present in *Escherichia coli* and *Klebsiella pneumoniae* strains. The expected products are: blaOXA-48 744bp. The gel shows 9 test strains, a positive control and negative control (far right hand lane, and a 100bp DNA ladder (far left lane of gel). C+: positive control, C-: Negative control, L: 100bp Ladder.



The results in Table 3 suggest that Yerba Mate's MICs against *Klebsiella pneumonia* were slightly higher than those against *E. coli*. In addition, the presence and the variety of beta-lactamase genes did not seem to influence the activity of Yerba Mate that showed even higher MICs against the susceptible strains than the ESBL or AmpC producers. Moreover, the aqueous extract of Yerba Mate had almost the same effect (MIC 3.75mg/mL) on the two Oxa-48 producer strains *E. coli* (E9) and *Klebsiella* (K4) whether producing beta lactamases or not.

Discussion

Yerba Mate aqueous extract exhibited an antibacterial activity against the tested strains. In general, the MIC and MBC values in this study ranged between 0.468 mg/mL and 15 mg/mL (Table 3).

The antibacterial activity of the extract did not show any correlation with the profile of resistance of the tested bacteria (beta-lactamase production). The resistance to beta-lactams is usually increased with the number of beta-lactamases found in a specific strain, however, this was not observed with Yerba Mate extract (Table 3). Moreover, ESBLs, AmpCs, and OXA-48 *Escherichia coli* clinical isolates showed more or less the same results of MIC and MBC, the differences being within a maximum of one or two dilutions. The same observation can be seen with *S. aureus* where Methicillin resistance did not have any effect on the MIC of Yerba Mate that was 1.875 mg/mL for both strains (Table 3). Thus Yerba Mate extract in

Table 3. Antibacterial activity of aqueous extract of *Ilex paraguariensis*.

Bacterial strains	MIC (mg/mL)	MBC (mg/mL)	Beta-lactamase
Escherichia coli (ATCC 25922)	1.875	3.75	
Staphylococcus aureus (ATCC 29213)	0.468	0.468	
Pseudomonas aeruginosa (ATCC 27853)	1.875	1.875	
Acinetobacter baumanii (ATCC 17978)	0.468	0.468	
Escherichia coli ESBL (E1)	1.875	3.75	CTX-M, SHV, OXA
Escherichia coli ESBL (E2)	7.5	7.5	CTX-M, SHV, TEM, OXA
Escherichia coli ESBL (E3)	7.5	15	CTX-M, SHV, TEM
Escherichia coli ESBL (E4)	3.75	15	CTX-M, TEM, OXA
MIC and MBC means of ESBL producing E.coli	**5.15**	**10.30**	
Escherichia coli AmpC (E5)	7.5	15	
Escherichia coli AmpC (E6)	3.75	3.75	
MIC and MBC means of AmpC producing E.coli	**5.60**	**9.37**	
Escherichia coli ESBL + AmpC (E7)	3.75	7.5	CTX-M, OXA
Escherichia coli ESBL + AmpC (E8)	15	15	CTX-M, OXA
MIC and MBC means of ESBL+AmpC producing E.coli	**9.37**	**11.25**	
Escherichia coli OXA-48 (E9)	3.75	3.75	Oxa-48
Escherichia coli (E10)	7.5	7.5	Susceptible strain
Escherichia coli (E11)	7.5	15	Susceptible strain
MIC and MBC means of susceptible E.coli	**7.5**	**11.25**	
Klebsiella pneumoniae ESBL (K1)	1.875	1.875	CTX-M, SHV, TEM
Klebsiella pneumoniae ESBL (K2)	15	15	CTX-M, SHV, TEM
Klebsiella pneumoniae ESBL (K3)	15	15	CTX-M, TEM
MIC and MBC means of ESBL producing K. pneumoniae	**10.62**	**10.62**	
Klebsiella pneumoniae OXA-48 (K4)	3.75	3.75	Oxa-48
Klebsiella pneumoniae (K5)	7.5	15	Susceptible strain
Klebsiella pneumoniae (K6)	7.5	15	Susceptible strain
MIC and MBC means of susceptible K. pneumoniae	**7.5**	**15**	
Serratia marcesens	7.5	7.5	
Enterobacter aerogenes	3.75	7.5	
Enterobacter agglomerans	7.5	7.5	
Proteus mirabilis (clinical strain isolate	3.75	7.5	
Enterococcus faecalis (EF1)	1.875	1.875	
Enterococcus faecalis (EF2)	0.468	1.875	
Methicillin-Sensitive *Staphylococcus aureus*	1.875	3.75	
Methicillin-Resistant *Staphyloccocus aureus*	1.875	1.875	

ATCC: American Type Culture Collection; ESBL: Extended Spectrum Beta-lactamase; AmpC: AmpC-type beta-lactamase; OXA-48: OXA-type beta-lactamase.

this study showed a higher antibacterial effect on Methicillin Resistant *S. aureus* (MRSA) than in a study conducted by Burris *et al.* (2014) [33], where the MIC against MRSA was found to be 4 mg/mL.

The MIC and MBC of Yerba Mate, in this study, against *Pseudomonas aeruginosa* (ATCC 27853) were found to be 1.875 mg/mL and against clinical strain *Enterobacter aerogenes* to be 3.5 mg/mL and 7.5 mg/mL, respectively (Table 3). *Pseudomonas* spp. and *Acinetobacter* spp. are both Gram negative non fermenters with a high ability to acquire resistance by beta-lactamase production or porin loss. They also have very potent efflux pumps. Surprisingly, none of the two organisms showed an exceptional resistance to Yerba Mate in our experiments reinforcing therefore the possibility proposed here above that the mechanism of activity of Yerba Mate is totally independent from the classical mechanisms of bacterial resistance, regardless of the MIC value.

In general, a greater antibacterial activity of Yerba Mate was observed in this study against Gram-positive bacteria than Gram-negative bacteria (Table 3). In a similar study, HEPES buffer protein extraction of Yerba Mate had an MIC and MBC of 150 µg/mL and 25 µg/mL against *Escherichia coli* O157:H7 (ATCC 43894) and *Staphylococcus aureus* (ATCC 27708) respectively [16]. Both studies showed a higher antibacterial activity against standard *Staphylococcus aureus* than *Escherichia coli*.

A hexane extract of Yerba Mate exhibited a weaker antibacterial activity against Gram-negative ATCC strains *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Escherichia coli* [14] as compared to the aqueous extracts of Yerba Mate conducted in this study. The main components of the distillate did not exhibit any antibacterial activity at 800 mg/mL (highest concentration used in the experiment) against *Pseudomonas aeruginosa* and *Enterobacter aerogenes*, while exhibited an MIC of 400 mg/mL against *Escherichia coli* [14]. In addition, Martin *et al.* (2013) [34] found that the methanol and ethanol extracts did not inhibit the growth of *Escherichia coli* (ATCC 25922) strain, but showed an MIC of 1.56 mg/mL and an MBC of 3.13 mg/mL against *Staphylococcus aureus* (ATCC 25923). In comparison to our results, the aqueous extract of Yerba Mate exhibited an MIC of 1.875 mg/mL and MBC of 3.75 mg/mL against the same strain *Escherichia coli* (ATCC 25922) and an MIC and MBC of 0.468 mg/mL against *Staphylococcus aureus* (ATCC 29213). Limited studies were done to evaluate the effect of Yerba Mate extracts on Gram-negative bacteria, particularly *Klebsiella pneumoniae*. In this study, the MIC and MBC against clinical strains of *Klebsiella pneumoniae* of different profiles of resistance ranged between 1.875 and 15 mg/mL (Table 3).

The comparison of data shows a change in the MIC and MBC of Yerba Mate with variation in extraction protocols. Bastos *et al*. (2007) [35], showed greater phenolic content in aqueous extraction of Yerba Mate extracted at 97°C compared to ethanol solvent based extraction extracted at 76°C. This can be contributed to the variation of antibacterial components extracted at each temperature.

In previous studies, Yerba Mate was found to be rich with saponins, xanthines, minerals polyphenols, and caffeoyl derivatives that attributed to its health benefits [35,36,17]. Caffeic acid, caffeine, caffeoyl derivatives, caffeoyl shikimic acid, chlorogenic acid, feruloylquinic acid, kaempferol, quercetin, quinic acid, rutin, and theobromine have been identified in Yerba Mate [36,37]. Pure forms of these compounds have been isolated and correlated with antibacterial activity against Gram-positive and Gram-negative bacteria [38-41]. Specifically, pure forms of caffeic and chlorogenic acids have yielded antibacterial activity against Gram-negative bacteria [39,42]. 3,4-dihydroxybenzaldehyde showed the highest antibacterial activity against Methicillin-Resistant *Staphyloccocus aureus*; in addition, citric acid, 3-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, caffeic acid, quinic acid, quercetin, and new unknown compounds were the top 10 identified compounds for antibacterial activity dependent on computational ranking methods utilizing GC-MS data [43]. Filip *et al*. (2010) [44], identified caffeoyl derivatives, methylxanthines, and rutin from Yerba Mate aqueous extracts with antifungal activity. Despite the identification of many of the pure forms known to have antibacterial activity in a variety of Yerba Mate extracts, there is limited evidence to the main compound or synergistic effect of several compounds responsible for this activity. Further studies are needed to distinguish the contributing compounds, which may explain the variation in results between studies as well as contribute in formulating a new antibacterial agent.

Conclusion

In conclusion, the aqueous extract of the stems and leaves of *Ilex paraguariensis* extracted at 70°C showed a significant antibacterial activity. There was no correlation found between the different molecular resistance profiles and the antibacterial activity range. A more in-depth analysis to identify the molecules

responsible for this activity as well as testing a wider range of bacterial isolates is important for a better understanding of the potential role of Yerba Mate in developing new antibacterial agents that are certainly needed nowadays.

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