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Original Article

Antibacterial Activity of Tinctures from Tree leaves belonging to the *Bignoniaceae* family and their Synergistic Effect with Antibiotics

Torres Carola Analía[1,2], Nuñez María Beatriz[1], Isla María Inés[3,4], Castro Marcela Paola[1,2], Gonzalez Ana María[1,2,5] and Zampini Iris Catiana[3,4]

[1]Departamento de Ciencias Básicas y Aplicadas, Universidad Nacional del Chaco Austral (UNCAUS), Comandante Fernández 755-Presidencia Roque Sáenz Peña, Chaco, Argentina.
[2]Laboratorio de Microbiología de los Alimentos (UNCAUS), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.
[3]Instituto de Química del Noroeste Argentino (INQUINOA-CONICET), Universidad Nacional de Tucumán. Argentina.
[4]Cátedra de Química Orgánica y Biológica, Facultad de Ciencias Naturales e IML y Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 471, San Miguel de Tucumán, Tucumán, Argentina.
[5]Instituto de Botánica del Nordeste IBONE (CONICET), Sargento Cabral 2131, Corrientes. Argentina.

ABSTRACT

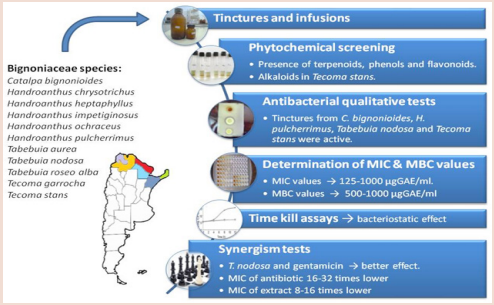
Context: Some species of *Bignoniaceae* are widely used in medicinal practice by the natives of South America. **Aims:** Tinctures and infusions from twelve tree species of this family were evaluated for *in vitro* antibacterial activity against pathogenic bacteria. The effect of interactions between the four most active extracts and conventional antibiotics was also evaluated. **Methods and Material:** Bioautography and disc diffusion methods were used to select the most active extracts, then agar macrodilution and broth microdilution method were used to determine the minimal inhibitory and minimal bactericidal concentration (MIC and MBC). Time-kill assay and checkerboard method were employed to determine the type of antimicrobial effect and synergism, respectively. **Results:** It could be determined that tinctures from *Catalpa bignonioides*, *Handroanthus pulcherrimus*, *Tabebuia nodosa* and *Tecoma stans* were able to inhibit bacterial growth. The MIC and MBC observed were between 125-1000 µg GAE/ml and 500-1000 µg GAE/ml, respectively. The tested extracts were more effective against Gram-positive microorganisms. Time-kill experiments indicated bacteriostatic activity. Phytochemical screening showed terpenoids, phenols and flavonoids. Alkaloids were detected only in *Tecoma stans*. Among these combinations, the best was *Tabebuia nodosa* extract plus gentamicin. In most cases, MIC values were reduced 16-32 times for antibiotics, and even 8-16 times for extracts. **Conclusion:** These results revealed that some of the selected combinations could efficiently inhibit the growth of tested strains at lower concentrations than those required for the lonely use of the antimicrobial. These extracts would improve the efficacy of antibiotics against resistant bacteria, hence they could be used for anti-infective therapy.

Key words: Checkerboard method, Fractional inhibitory concentration (FIC), Gentamicin, *Tabebuia nodosa*, Time-kill experiments.

Key Messages: Plant extracts and antibiotics: a new tool to fight against bacterial infections.

SUMMARY

- The tinctures of *Catalpa bignonioides*, *Handroanthus pulcherrimus*, *Tabebuia nodosa* and *Tecoma stans* inhibited bacterial growth.
- MIC and MBC values were between 125-1000 µg GAE (Gallic acid equivalent)/ml and 500-1000 µg GAE/ml, respectively.
- Phytochemical screening showed terpenoids, phenols and flavonoids. Alkaloids were detected only in *Tecoma stans*.
- The combination of *Tabebuia nodosa* extract and gentamicin was the most effective.



PICTORIAL ABSTRACT

Abbreviations used: MIC: Minimal concentration inhibitory, MBC: Minimal bactericidal concentration, GAE: Gallic acid equivalent, INQUINOA: Instituto de Química del Noroeste Argentino (Institute of Chemistry of Northwest from Argentina), IBONE: Instituto de Botánica del Nordeste (Northeast Institute of Botany), CONICET: Consejo Nacional de Investigaciones Científicas y Técnicas (National Council of Scientific and Technical Research), ASTM: Association for Testing Materials of United States, ATCC: American Type Culture Collection, DMSO: Dimethyl sulfoxide, CFU: Colony-forming unit, FIC: Fractional inhibitory concentration.

Correspondence:

Ms. Zampini Iris Catiana, Ayacucho 471, San Miguel de Tucumán, Argentina.

Phone no: +54 0381-4203062, Fax no: +54 0381-4203062

Email: zampini@csnat.unt.edu.ar
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INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. These compounds can be terpenoids, glycosteroids, flavonoids and polyphenols. Most of these molecules have weak antibiotic activity; however, plants fight infections successfully. Hence, it becomes apparent that plants adopt a synergistic strategy to combat infections.[1]

Bignoniaceae Juss. is a family of trees, shrubs or climbers, which is made up of about 100 genera and 800 species. Some species of this family are widely used in medicinal practice by the natives of South America.[2,3] The well-known medicinally important members of this family are *Tecoma*, *Catalpa*, *Handroanthus*, *Tabebuia* and *Jacaranda*. Several species traditionally used have demonstrated to be useful for microbial infections.[4-7] Torres *et al.*, 2013[8] have previously analyzed the antibacterial properties of 20 climbers of this family.

The aim of this work has been to evaluate the antibacterial effect of the infusions and tinctures of leaves from twelve species of trees belonging to the *Bignoniaceae* family against pathogenic bacteria. Besides, a study

of synergistic effects resulting from the combination of tinctures with commercial antibiotics was conducted.

SUBJECTS AND METHODS

Plant material

Plant material was collected from the North of Argentine. The voucher specimens were deposited and conserved in the Herbarium of the *Instituto de Botánica del Nordeste* (IBONE-CONICET), Corrientes, Argentina.

Microorganisms

Microorganisms used in this work were *Staphylococcus aureus* ATCC 29213, *S. epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, four clinical isolates of methicillin-sensitive *S. aureus* (F13, F29, F32 and F33), two methicillin-resistant clinical isolates of *S. aureus* (F7 and F22), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, antibiotic-resistant clinical isolates of *Enterobacter cloacae* (F302 and F338), *Klebsiella pneumoniae* (F364), *P. aeruginosa* (F305), *Proteus mirabilis* (F304), and *Morganella morganii* (F339).

Extraction and preliminary phytochemical investigation

Leaves were dried at room temperature. Then, dry leaves were crushed and milled until they reached a size between 1.70 mm and 710 µm using ASTM (Association for Testing Materials of United States) sieves. Afterwards, tinctures and infusions were prepared with the obtained powder. Tincture: 20 g of powder was macerated in 80° ethanol (100 ml) for 7 days in a dark place at room temperature. Infusion: 5 g of plant powder was put in contact with boiling water (100 ml) for 20 min. Finally, all extracts were filtered through Whatman N°1 filter paper, centrifuged at 3000 rpm for 5 min and were stored at -20°C in the dark.

Phytochemical analysis

The presence of secondary metabolites was assessed according to qualitative standard methods.[9,10] The total phenolic content was quantified according to Singleton *et al*., 1999.[11] Flavonoid content was determined according to Woisky and Salatino, 1998.[12]

Antimicrobial assays

The qualitative determination of antibacterial activity was assessed by the agar disc diffusion method[13] and the bioauthography assay[14] using a spot containing 30 µg phenolic compounds of each extract. MIC values of the most active extracts were determined by serial agar macrodilution and broth microdilution method.[15] The microdilution method was also used to determine MBC values.

The extracts were dried and re-suspended in dimethyl sulfoxide (DMSO, Sigma Aldrich, USA). Dilutions of crude extracts in DMSO (range concentration between 62.5 and 1000 µg GAE/ml) were prepared. A growth control of each tested strain and a DMSO control were included. MIC was defined as the lowest concentration of extract at which bacterial growth was not observed after incubation. MBC was defined as the lowest extract concentration at which 99.9% of the bacteria have been killed. All experiments were carried out in duplicate.

Antibacterial effect was evaluated by the time-kill assay. In this test, a standardized suspension of bacteria (5×10^5 colony-forming unit-CFU-/ml) was added into Müeller–Hinton broth containing the extracts to give a final concentration between 500–2000 µg/ml. These mixtures were then incubated at 37°C for 12 h shaking at 200 rpm. Aliquots of 0.01ml of the diluted samples were withdrawn at time intervals (4 h) for the determination of the number of CFU/ml.[16] Crude extracts were considered to be bactericidal at the lowest concentration which reduced the original inoculum by ≥3 $\log_{10}$ CFU/ml (99.9% reduction in bacterial population) in 4 h.

Estimation of synergy between plant extracts and antibiotics

Synergy between extracts and selected antibiotics (ampicillin, gentamicin and oxacillin) was studied by the checkerboard assay method.[17] Combinations of oxacillin and the extracts were tested only for Gram-positive bacteria. The concentrations used in the combinations for each antibiotic ranged from 0.05 to 204.8 µg/ml and for each extract between 15.62 and 500 µg GAE/ml.

MIC values were determined for each antibiotic and for each of these combinations to establish any interaction effect. The FIC (Fractional in-

Table 1: Phenolic and flavonoid content in tinctures and infusions of 12 selected tree species of *Bignoniaceae* family

Samples	Tinctures		Infusions	
	Phenolic compounds (mg GAE/g DE)	Flavonoids (mg QE/g DE)	Phenolic compounds (mg GAE/g DE)	Flavonoids (mg QE/g DE)
Catalpa bignonoides	133.13 ± 5.8^{a}	3.07 ± 0.6^{a}	124.30 ± 1.26^{a}	10.24 ± 1.32^{a}
Handroanthus chrysotrichus	73.53 ± 0.43^{b,c}	14.23 ± 0.5^{b}	59.08 ± 0.83^{b}	1.35 ± 0.32^{b,c}
H. heptaphyllus	98.90 ± 7.9^{i}	34.29 ± 2.21^{c}	90.35 ± 3.2^{c}	12.90 ± 2.21^{e}
H. impetiginosus (wf)	66.80 ± 1.8^{c,d}	17.87 ± 0.38^{d}	29.99 ± 2.88^{d}	1.39 ± 0.17^{b,c}
H. impetiginosus(pf)	63.05 ± 1.70^{c}	17.64 ± 0.25^{d}	35.55 ± 7.28^{d,e}	3.66 ± 0.14^{c}
H. lapacho	160.73 ± 7.80^{e}	18.78 ± 1.35^{d}	145.62 ± 1.30^{f}	15.37 ± 1.65^{f}
H. ochraceus	46.71 ± 0.58^{c}	6.14 ± 0.99^{e}	41.04 ± 1.56^{e}	3.02 ± 0.94^{c,d}
H. pulcherrimus	121.41± 10.33^{a}	36.54 ± 0.68^{f}	120.47 ± 1.70^{a}	13.51 ± 3.23^{e,f}
Tabebuia aurea	25.35 ± 1.26^{f}	14.99 ± 0.15^{b}	40.21 ± 6.88^{e}	0.14 ± 0.02^{b}
Tabebuia nodosa	276.91 ± 2.36^{g}	47.67 ± 2.32^{g}	42.79 ± 4.00^{e}	5.47 ± 1.40^{g}
Tabebuia roseoalba	131.12 ± 5.87^{a}	29.95 ± 1.94^{h}	192.17 ± 4.76^{g}	23.35 ± 2.72^{h}
Tecoma garrocha	135.18 ± 1.69^{a}	1.76 ± 0.003^{a}	84.10 ± 1.96^{c}	2.67 ± 0.40^{c,d}
Tecoma stans	84.61 ± 4.15^{h,i}	10.27 ± 0.37^{i}	63.85 ± 4.26^{b}	1.23 ± 0.11^{b,c}

*Mean values ± standard deviation. wf: white flowers; pf: pink flowers; GAE: Gallic Acid Equivalent; DE: Dry extract; QE: Quercetin equivalent. Different letters within a column indicate significant difference at $p<0.05$.

Table 2: Antibacterial activity, MIC and MBC (µg GAE/ml) values of tinctures against pathogenic bacteria

Strains	*C. bignonoides*	*H. pulcherrimus*	*T. nodosa*	*T. stans*
Gram positive				
***S. aureus* ATCC 29213**	250/500	500/1000	500/1000	250/500
***S. aureus* (F13)**	500/1000	500/1000	500/R	500/1000
***S. aureus* (F29)**	500/R	500/R	500/R	500/R
***S. aureus* (F32)**	500/1000	500/R	500/R	500/1000
***S. aureus* (F33)**	500/R	500/1000	500/R	500/1000
***S. aureus* (F22)**	1000/R	1000/R	1000/R	1000/R
***S. aureus* (F7)**	500/R	500/R	500/R	500/R
***S. epidermidis* ATCC 12228**	250/1000	500/R	500/R	125/1000
***E. faecalis* ATCC 29212**	1000/R	1000/1000	1000/R	1000/R
Gram negative				
***E. coli* ATCC 35218**	R	R	R	R
***P. aeruginosa* ATCC 27853**	R	R	R	R
***E. cloacae* (F302)**	R	R	R	R
***E. cloacae* (F338)**	R	1000/R	R	R
***K. pneumoniae* (F364)**	R	R	R	R
***M. morganii* (F339)**	1000/R	R	R	1000/R
***P. aeruginosa* (F305)**	R	R	R	R
***P. mirabilis* (F304)**	1000/R	1000/R	R	1000/R

R: Resistant; not detected within the tested concentrations (62.5 to 1000 µg GAE/ml).

hibitory concentration) index was then calculated as the sum of each component FIC in a combination and interpreted as either synergistic (≤0.5), additive (>0.5 and ≤1.0), indifferent (>1 and ≤4.0) or antagonistic (>4.0).[18] The assays were performed in triplicate and as independent tests; mean values were calculated.

RESULTS

Alkaloids were only detected in leaves of *Tecoma stans* while saponins were not detected, but all the plants showed the presence of terpenoids, phenols and flavonoids. The results of the total phenolics and flavonoids content are presented in Table 1.

The qualitative screening of antimicrobial activity showed that 30 µg of phenolic compounds from infusions were not active whereas the same amount of ethanolic extracts of many plants were able to inhibit bacterial growth. The tinctures of *C. bignonioides*, *H. pulcherrimus*, *Tabebuia nodosa* and *Tecoma stans* were selected for the determination of MIC, MBC values and synergistic effect. The four extracts were active in varying degrees against Gram-positive strains. *Catalpa* and *Tecoma* inhibited the growth of *Proteus* and *Morganella morganii*, *Handroanthus* was active against *P. mirabilis* and one clinical isolate of *E. cloacae*, while *Tabebuia* had no effect against Gram-negative bacteria (Table 2).

In the time-kill assay, the extracts significantly inhibited bacterial growth when compared with the growth control; however, the reduction in growth was ≤3 $\log_{10}$ CFU/ml for all isolates, indicating a bacteriostatic effect (Figure 1).

FIC indices for extracts and antibiotic combinations–calculated according to the checkerboard test– are shown in Tables 3 and 4. The combination of the extracts with oxacillin diminished the MIC values of antibiotic against methicillin resistant clinical isolates of *Staphylococcus* (F7 and F22). The MIC value of the oxacillin against strain F7 was 25.6 µg/ml and 204.8 µg/ml for strain F22 (Table 3). However, in the combinations with extracts, the antibiotic concentrations required to obtain the same effect

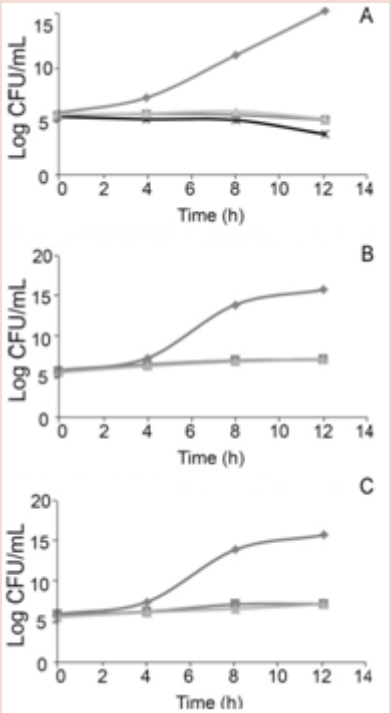

Figure 1: Time kill assay of A) *S. aureus*, B) *M. morganii* and C) *P. mirabilis* in presence of *Tecoma stans* extract. Control (rhombus), MIC (triangle), 2MIC (square) and 4MIC (cross).

were much lower (sometimes 16-32 times lower). *Morganella morganii*, *K. pneumoniae* and *P. mirabilis* were the most sensitive to combinations within Gram negative bacteria isolates (Table 4). The best combination

Table 3: MIC of conventional antibiotics and FIC Index values of combinations between plant extracts and antibiotics against Gram positive bacteria

	MIC antibiotic (µg/ml)			Concentrations of extract and antibiotic used in the combination expressed in µg/ml and FIC index of different combinations											
	A	G	O	A/Cb	G/Cb	O/Cb	A/Hp	G/Hp	O/Hp	A/Tn	G/Tn	O/Tn	A/Ts	G/Ts	O/Ts
Sa	0.4	1.6	1.6	0.1/62.5[#] (0.5[a])	0.4/31.3 (0.37[a])	0.1/62.5 (0.31[a])	0.1/15.6 (0.31[a])	0.4/15.6 (0.31[a])	0.2/31.3 (0.25[a])	0.2/31.3 (0.62[b])	0.05/62.5 (0.37[a])	0.2/31.3 (0.25[a])	0.05/62.5 (0.37[a])	0.2/125 (0.62[b])	0.4/125 (0.75[b])
F13	25.6	25.6	1.6	12.8/250 (1.00[b])	0.8/62.5 (0.16[a])	0.8/125 (0.75[b])	3.2/62.5 (0.25[a])	1.6/62.5 (0.19[a])	0.2/62.5 (0.19[a])	3.2/125 (0.37[a])	0.8/62.5 (0.16[a])	0.8/250 (1.00[b])	6.4/125 (0.50[a])	1.6/62.5 (0.19[a])	0.8/250 (1.00[b])
F29	51.2	12.8	1.6	12.8/31.3 (0.37[a])	1.6/31.3 (0.19[a])	0.8/500 (1.50[c])	25.6/125 (0.75[b])	1.6/31.3 (0.19[a])	25.6/250 (1.00[b])	12.8/125 (0.50[a])	1.6/62.5 (0.25[a])	0.8/250 (1.00[b])	12.8/125 (0.50[a])	3.2/125 (0.50[a])	0.4/250 (0.75[b])
F32	0.4	0.8	0.8	0.05/62.5 (0.25[a])	0.2/125 (0.50[a])	0.4/31.3 (0.62[b])	0.1/31.3 (0.31[a])	0.2/125 (0.75[b])	0.2/62.5 (0.62[b])	0.05/62.5 (0.25[a])	0.1/62.5 (0.37[a])	0.4/31.3 (0.56[b])	0.1/62.5 (0.37[a])	0.1/62.5 (0.25[a])	0.1/250 (0.62[b])
F33	0.4	0.8	0.8	0.2/125 (0.75[b])	0.2/125 (0.50[a])	0.05/31.3 (0.19[a])	0.2/31.3 (0.56[b])	0.05/15.6 (0.16[a])	0.1/250 (0.62[b])	0.2/62.5 (0.62[b])	0.1/62.5 (0.37[a])	0.2/125 (0.19[a])	0.05/250 (0.62[b])	0.2/125 (0.50[a])	0.2/125 (0.50[a])
F7	25.6	25.6	25.6	3.2/250 (0.62[b])	1.6/62.5 (0.37[a])	0.2/125 (0.26[a])	3.2/62.5 (0.25[a])	3.2/125 (0.37[a])	3.2/125 (0.37[a])	3.2/31.3 (0.19[a])	1.6/31.3 (0.12[a])	0.2/62.5 (0.13[a])	12.8/125 (0.75[b])	1.6/125 (0.31[a])	0.4/500 (1.02[c])
F22	102.4	102.4	204.8	25.6/250 (0.50[a])	25.6/125 (0.31[a])	25.6/125 (0.25[a])	25.6/250 (0.50[a])	6.4/62.5 (0.12[a])	12.8/500 (0.56[b])	25.6/250 (0.50[a])	12.8/62.5 (0.19[a])	51.2/62.5 (0.50[a])	51.2/125 (0.62[b])	6.4/62.5 (0.12[a])	25.6/125 (0.25[a])
Ef	0.4	3.2	1.6	0.1/125 (0.37[a])	1.6/125 (0.62[b])	0.05/125 (0.28[a])	0.1/62.5 (0.31[a])	0.8/500 (0.75[b])	0.1/62.5 (0.12[a])	0.05/62.5 (0.19[a])	0.4/500 (0.75[b])	0.05/125 (0.16[a])	0.05/125 (0.19[a])	1.6/125 (0.62[b])	0.4/125 (0.19[a])

[#]µg GAE/ml of extracts. FIC Index values are shown in brackets. a Synergistic effect; b Additive effect; c Indifferent effect; ND: not detected. Sa: *Staphylococcus aureus* ATCC 29213; Ef: *Enterococcus faecalis* ATCC 29212. A: Ampicillin; Cb: *Catalpa bignonioides*; G: Gentamicin; Hp: *Handroanthus pulcherrimus*; O: Oxacillin; Tn: *Tabebuia nodosa*; Ts: *Tecoma stans*.

was *T. nodosa* extract and gentamicin, synergism obtained in 81.25% of bacteria tested. Only the combination of *Tecoma stans* extract and ampicillin had a synergistic effect for both clinical isolates of *E. cloacae*. No antagonism was observed for any of the combinations evaluated.

DISCUSSION

The tinctures tested had higher antibacterial activity against Gram positive bacteria than against Gram negative ones. The latter bacteria are usually less susceptible to the action of plant extracts.[19,20] However, it is noteworthy that among the species studied in this work *C. bignonioides*,

Table 4: MIC of conventional antibiotics and FIC Index values of combinations between plant extracts and antibiotics against Gram negative bacteria

	MIC antibiotic (µg/ml)		Concentrations of extract and antibiotic used in the combination expressed in µg/ml and FIC index of different combinations							
	A	G	A/Cb	G/Cb	A/Hp	G/Hp	A/Tn	G/Tn	A/Ts	G/Ts
Ec	204.8	12.8	25.6/125[#] (0.19[a])	1.6/125 (0.25[b])	3.2/500 (0.27[a])	1.6/62.5 (0.16[a])	101.4/500 (0.75[b])	1.6/62.5 (0.16[a])	51.2/250 (0.37[a])	3.2/62.5 (0.28[a])
Pa	ND	1.6	ND	1.6/62.5 (1.03[c])	ND	1.6/62.5 (1.03[c])	ND	1.6/62.5 (1.03[c])	ND	1.6/500 (1.25[c])
F302	204.8	204.8	ND	51.2/250 (0.37[a])	ND	102.4/125 (0.56[b])	ND	51.2/62.5 (0.28[a])	51.2/500 (0.25[a])	102.4/125 (0.56[b])
F338	204.8	25.6	ND	12.8/62.5 (0.53[b])	ND	25.6/250 (1.12[c])	ND	3.2/62.5 (0.16[a])	51.2/125 (0.31[a])	25.6/250 (1.12[c])
F364	204.8	204.8	51.2/500 (0.50[a])	ND	51.2/500 (0.50[a])	51.2/125 (0.31[a])	102.4/125 (0.56[b])	25.6/500 (0.37[a])	ND	1.6/250 (0.13[a])
F339	102.4	12.8	25.6/125 (0.31[a])	3.2/62.5 (0.28[a])	6.4/250 (0.19[a])	3.2/250 (0.37[a])	6.4/62.5 (0.09[a])	3.2/125 (0.31[a])	25.6/250 (0.50[a])	1.6/62.5 (0.19[a])
F304	102.4	204.8	102.4/62.5 (1.03[c])	51.2/125 (0.31[a])	ND	25.6/250 (0.37[a])	ND	51.2/62.5 (0.28[a])	ND	102.4/125 (0.62[b])
F305	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[#]µg GAE/ml of extracts. FIC Index values are shown in brackets. a) Synergistic effect; b) Additive effect; c) Indifferent effect; ND: Not detected. Ec: *Escherichia coli* ATCC 35218; Pa: *Pseudomonas aeruginosa* ATCC 27853. A: Ampicillin; Cb: *Catalpa bignonioides*; G: Gentamicin; Hp: *Handroanthus pulcherrimus*; O: Oxacillin; Tn: *Tabebuia nodosa*; Ts: *Tecoma stans*.

H. pulcherrimus and *T. stans* were able to inhibit the growth of some Gram negative strains.

Some studies have demonstrated that plants of the *Handroanthus* genus have antibacterial activity against *Helicobacter pylori*[21] and *S. aureus*.[22] Most of these results referred to studies carried out on barks, while this work considered leaves as the objects of study. Though, different extracts of leaves of *T. stans* have been reported for antimicrobial effects on some human pathogenic bacteria,[7,19,23,24] which partially support our results.

The results of synergism tests suggest the potential use of some of these plants to improve the effect of antibiotics. MIC values were reduced 16-32 times for antibiotics, in most cases, and even eight or sixteen times for extracts, depending on the tested bacterium. No antagonism was observed for any of the combinations evaluated and when indifference or additivity were observed, a substantial decrease in the MIC of the antibiotic in the combination was detected, while the MIC extract remained unchanged or decreased only 2 times. These data are also relevant. Although no synergism was observed for these combinations, they decreased the dose of the antibiotic with a consequent reduction of side effects.

The interaction of plant extracts with antibiotics is one of the novel ways to overcome the resistance mechanisms of bacteria. Several studies on the interaction between plant extracts and antibiotics indicated a synergistic interaction with antibiotics.[25,26]

It is remarkable that, to our knowledge, there are not data in worldwide literature about studies of synergism between these species and commercial antibiotics. Our results revealed that some of the combinations selected can effectively inhibit the growth of tested strains at lower concentrations than those required for the individual agents.

CONCLUSION

The results suggest that the extracts of the studied *Bignoniaceae* species possess some compounds with antimicrobial properties; besides, they could enhance the efficacy of antibiotics against resistant bacteria. Consequently, these extracts could be used as antimicrobial agents for infectious diseases therapy in humans. Chemical studies are required to determine the compounds responsible for antibacterial effect of these species in leaves; in this sense, investigations to identify the structures of active principles are being conducted in our laboratory.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

1. Hemaiswarya SH, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against diseases. Phytomed. 2008; 15(8): 639-52.
2. Alonso J, Desmarchelier C. Indigenous medicinal plants Argentina: scientific bases for use in primary health care [Plantas medicinales autóctonas de la Argentina: bases científicas para su aplicación en atención primaria de la salud]. 1st ed. Buenos Aires: Fitociencia; 2006.
3. Bastos ML, Lima MRF, Conserva LM, Andrade VS, Rocha EMM, Lemos RPL. Studies on the antimicrobial activity and brine shrimp toxicity of *Zeyheria tuberculosa* (Vell.) Bur. (*Bignoniaceace*) extracts and their main constituents. Ann Clin Microbiol. Antimicrob. 2009; 8(1): 16.
4. Agra MF, Baracho GS, Nurit K, Basilio IJ, Coelho VP. Medicinal and poisonous diversity of the flora of *Cariri paraibano*. Brazil. J Ethnopharmacol. 2007; 111(2): 383-95.
5. Gómez Castellanos JR, Prieto JM, Heinrich M. Red Lapacho *(Tabebuia impetiginosa)*-A global ethnopharmacological commodity?. J Ethnopharmacol. 2009; 121(1): 1-13.
6. Castillo L, Rossini C. *Bignoniaceae* metabolites as semiochemicals. Molecules 2010; 15(10): 7090-105.
7. Senthil kumar CS, Suresh kumar M, Pandian MR. *In vitro* antibacterial activity of crude leaf extracts from *Tecoma stans* (L.) Juss. exKunth, *Colues forskohlii* and *Pogostemon patchouli* against human pathogenic bacteria. Int J Pharm Tech Res. 2010; 2(1): 438-42.
8. Torres CA, Zampini IC, Nuñez MB, Isla MI, Castro MP, Gonzalez AM. *In vitro* antimicrobial activity of 20 selected climber species from the *Bignoniaceae* family. Nat Prod Res. 2013; 27(22): 2144-8.
9. Dominguez X. Phytochemical research methods [Métodos de investigación fitoquímica]. México: Limusa; 1988.
10. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. Berlin: Springer-Verlag; 1996.

11. Singleton V, Orthofer R, Lamuela-Raventos R. Analysis of total phenols and other oxidation susbstrates and antioxidants by means of Folin Ciocalteu reagent. Methods Enzimol. 1999; 299 (1): 152-78.
12. Woisky RG, Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control. J Apic Res. 1998; 37(2): 99-105.
13. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk susceptibility tests; M2-A9. 26:1. Wayne, PA: CLSI; 2006.
14. Nieva Moreno MI, Isla MI, Cudmani NG, Vattuone MA, Sampietro AR. Screening of antibacterial activity of Amaicha del Valle (Tucuman, Argentina) propolis. J Ethnopharmacol. 1999; 68(1): 97-102.
15. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; M7-A7. Wayne, PA: CLSI; 2006.
16. Chattopadhyay D, Mukherjee T, Pal P, Saha B, Bhadra R. Altered membrane permeability as the basis of bactericidal action of methdilazine. J Antimicrob Chemother. 1998; 42(1): 83-6.
17. Moody J. Synergism testing: Broth microdilution checkerboard and broth macrodilution methods. In: Isenberg Henry D, García Lynne S, editors. Clinical Microbiology Procedures Handbook. Washington D.C: ASM Press; 2007. 5.12.1-23.
18. Schelz Z, Molnar J, Hohmann J. Antimicrobial and antiplasmid activities of essential oils. Fitoterapia 2006; 77(4): 279-85.
19. Salem MZM, Gohar YM, Camacho LM, El-Shanhorey NA, Salem AZM. Antioxidant and antibacterial activities of leaves and branches extracts of *Tecoma stans* (L.) Juss. Ex Kunth against nine species of pathogenic bacteria. Afr. J. Microbiol. Res. 2013; 7(5): 418-26.
20. Pagès JM, James CE, Winterhalter M. The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. Nat. Rev. Microbiol. 2008; 6(12): 893-903.
21. Park BS, Lee HK, Lee SE, Piao XL, Takeoka GR, Wong RY, *et al*. Antibacterial activity of *Tabebuia impetiginosa* Martius ex DC (Taheebo) against *Helicobacter pylori*. J. Ethnopharmacol. 2006; 105(1): 255-62.
22. Martínez M, Mancuello C, Brítez F, Pereira C, Arrúa J, Franco G, *et al*. Caracterización química y actividades biológicas de lapachol aislado de *Handroanthus heptaphyllus* (Vell.) Mattos. Steviana 2012; 4: 47-64.
23. Singh V, Kumar L, Chakraborthy GS, Mazumder A. Pharmacological and phytochemical findings of *Tecoma stans*- a review. Japhr. 2011 Jan; 1(3): 75-81.
24. Govindappa M, Sadananda TS, Channabasava R, Vinay VR. *In vitro* anti-inflammatory, lipoxygenase, xanthine oxidase and acetycholinesterase inhibitory activity of *Tecoma stans* (L.) Juss. Ex Kunth. Int. J. Pharm. Bio Sci. 2011; 2(2): 276-85.
25. Rosato A, Vitali C, De Laurentis N, Armenise D, Antonietta Milillo M. Antibacterial effect of some essential oils administered alone or in combination with norfloxacin. Phytomed. 2007; 14(11): 727-32.
26. Olajuyigbe OO, Afolayan AJ. Evaluation of combination effects of ethanolic extract of *Ziziphus mucronata* Willd. subsp. *Mucronata* Willd. and antibiotics against clinically important bacteria. Scientific World Journal 2013. http://dx.doi.org/10.1155/2013/769594

ABOUT AUTHORS

Torres Carola Analía: Obtained her Ph. D. degree in 2015 from College of Natural Sciences and Miguel Lillo Institute, National University of Tucuman (UNT- Argentina). She is also an Adjunct Professor at the Pharmacy Course, National University of Chaco Austral. She is professor of Microbiology and Immunology. Carola A. Torres, Ph. D., is working mainly in Medicinal plants from the North of Argentina, antibacterial natural products, isolation and structural elucidation by HPLC-MS.

Zampini Iris Catiana: Is an Adjunct Professor of Organic and Biological Chemistry at the Facultad de Ciencias Naturales, Universidad Nacional de Tucumán (UNT- Argentina) and Assistant Researcher at the Instituto de Química del Noroeste Argentino (INQUINOA-CONICET, National Council for Scientific and Technical Research in Argentina). Iris C. Zampini, Ph. D., supervised the doctoral thesis of Carola A. Torres. Her research is focused on the evaluation of biological activities and toxicity of medicinal plants from Northwest of Argentine.