

Short communication

Antioxidant capacity and antibacterial activity of phenolic compounds from argentinean herbs infusions

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ARTICLE INFO

Article history:

Received 1 September 2009

Received in revised form 21 October 2009

Accepted 30 October 2009

Keywords:

Phenolic compounds

Herbs infusions

Antimicrobial and antioxidant properties

ABSTRACT

Argentinean herbs infusions have been studied for their phenolic content, antioxidant and antibacterial activities in order to find out new natural products. Among the 13 infusions assayed *Ilex paraguayensis* present the highest amount of total phenolic compounds and flavonoid fraction. The high correlation between antioxidant capacities and total phenolic contents indicate that phenolic compounds are the major contributors of these capacities. *I. paraguayensis* infusion is the most effective to reduce the viability of *Escherichia coli* and *Staphylococcus aureus*. The antibacterial effect is related with phenolic compounds concentration but also with its profile. These results permit propose these herbs as new sources of safe natural antioxidants and preservatives for food industry.

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1. Introduction

Herbal infusion is one of the most commonly consumed beverages in the world and is rich in polyphenolic compounds collectively known as the tea flavonoids (Hertog, Kromhout, & Aravanis, 1995; Langley-Evans, 2000; Lie & Xie, 2000). The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities and low toxicity (Sharma, Hanna, Kauffman, & Newman, 1992). Bioactive compounds commonly found in herbs, and other plants have been shown to have possible health benefits with antioxidative, anticarcinogenic, antihypertensive, antimutagenic, and angiogenesis inhibitory activities (Cao & Cao, 1999; Geleijnse, Launer, Hofman, Pols, & Witteman, 1999; Kahkonen et al., 1999; Yen, Duh, & Tsai, 2002). Interestingly, many herbs are known to contain large amounts of phenolic antioxidants other than well-known vitamin C, vitamin E, and carotenoids. Phenolic antioxidants in herbs are mainly composed of phenolic acids (Cao & Cao, 1999) and flavonoids (Madsen & Bertelsen, 1995).

Medicinal plants and herbs were also investigated for antimicrobial activities against important pathogenic bacteria (Dorman & Deans, 2000; Sagdic, 2003; Sagdic, Kuscu, Ozcan, & Ozcelik, 2002). However, the antioxidant and antimicrobial effects of herbs

commonly consumed in our country during centuries have not been studied until now.

The current focus is toward natural compounds with antioxidants and antibacterial properties, especially polyphenols of herbs.

The aim of the present study was to investigate the total phenolic, flavonoid and non-flavonoid fractions content, antioxidant and antibacterial properties of herbs infusions prepared in common way in which they are consumed in Argentina.

2. Materials and methods

2.1. Samples

Common herbs, commercially available from pharmacy in Argentinean, were used for this study. *Lippia integrifolia*, *Mentha piperita*, *Lippia turbinata*, *Wendtia calysina*, *Chenopodium ambrosioides*, *Minthostachys verticillata*, *Peumus boldus*, *Aloysia citriodora* and *Ilex paraguayensis* herbs were selected. Herb combinations obtained commercially as digestive, contain: *L. integrifolia* 20%, *L. turbinata* 20%, *W. calysina* 20%, *C. ambrosioides* 20% and *M. verticillata* 20%.

2.2. Chemicals

Gallic acid was obtained from Merck. Ciocalteús phenol reagent and sodium carbonate were from Merck. Iron (III) chloride 6-hydrate, iron (II) sulfate 7-hydrate, potassium acetate and ascorbic acid were purchased from Cicarelli. Hydrochloric acid and methanol were purchased from Merck (Germany). 2,4,6-Tri(2-pyr-

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idyl)-s-triazine (TPTZ) was purchased from Fluka Chemie AG (Buchs, Switzerland). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma–Aldrich (St. Louis, MO).

2.3. Preparation of the herbs infusions

Boiling water (250 ml) was added to 2 g of individual herbs in a conical flask and stirred by a magnetic bar on a hot plate at 90 °C for 10 min. Then, the solution was filtered through cotton wool. Herb infusions were clarified by the addition of 30 mg/l of activated charcoal. All samples were filter-sterilized. The same procedure was carried out to prepare the infusions with the selected herb mixtures, with the addition of 1 g of each herb to 250 ml of boiling water. For the antimicrobial assay concentrated herb infusions were prepared with the addition of 4 g of individual herbs to 250 ml of boiling water.

2.4. Phenolic compounds determinations

2.4.1. Colorimetric determination of total phenolic compounds

Colorimetric determination of total phenolics was based on the procedure of Singleton and Rossi (1965). A standard curve of gallic acid was used. Results are expressed as milligram per liter gallic acid equivalents (GAE).

2.4.2. Non-flavonoids and flavonoids concentration

The 10.0 ml of wine sample was mixed with 10.0 ml of diluted HCl (1:3) and 5.0 ml of an 8.0 mg/ml formaldehyde solution and incubated 24 h at room temperature in order to precipitate the flavonoid fraction (Zoecklein, Fugelsang, Gump, & Nury, 1990, chap. 7). The non-flavonoid phenol contents were determined in the filtrate using the procedure of Singleton and Rossi. The flavonoid content was obtained by the difference between total phenol and non-flavonoid content. All determinations were carried out in triplicate. Results are expressed as milligram per liter of gallic acid equivalents (GAE).

2.5. Antioxidant capacity

2.5.1. Ferric-reducing antioxidant power assay (FRAP)

The FRAP assay was carried out according to the procedure of Benzie and Strain (1996) with slight modification. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe (II)-tripirydyltriazine compound from colorless oxidized Fe (III) form by the action of electron donating antioxidants. Briefly, the FRAP reagent was prepared from acetate buffer (pH 3.6), 10 mmol TPTZ solution in 40 mmol HCl and 20 mmol iron (III) chloride solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37 °C in a water bath prior to use. Fifty microliters of sample were added to 1.5 ml of the FRAP reagent. The absorbance of the reaction mixture was then recorded at 593 nm after 4 min. The standard curve was constructed using iron (II) sulfate solution (100–3500 µM), and the results were expressed as µmol/l FeSO₄. All the measurements were carried out in triplicate and the mean values were calculated.

2.5.2. Free radical scavenging ability by the use of a stable DPPH radical (1,1-diphenyl-2-picrylhydrazyl)

The DPPH radical scavenging activity of herbs infusions was determined using the method proposed by Von Gadov, Joubert, and Hansmann (1997). Aliquot (50 µl) of the tested sample was placed in a cuvette, and 2 ml of 6×10^{-5} M methanolic solution of DPPH radical was added. Absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined after 15 min for all samples. Methanol was used to

zero spectrophotometer. The absorbance of the DPPH radical without antioxidant (control) was measured daily. Methanolic solutions of trolox were tested. All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994):

$$\% \text{ inhibition} = [(A_{C(0)} - A_{A(t)}) / A_{C(0)}] \times 100$$

where $A_{C(0)}$ is the absorbance of the control at $t = 0$ min, $A_{A(t)}$ is the absorbance of the antioxidant at $t = 15$ min.

2.6. Antibacterial activity

2.6.1. Bacterial strains and culture conditions

The bacterial strains used as test organism were *Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 25923. Bacteria were cultured at 37 °C in nutrient broth and agar medium (contain in g/l: beef extract, 3; peptone, 5; sodium chloride, 8 and for solid medium, agar, 15). Before experimental use, cultures from solid medium were sub cultivated in liquid media, incubated for 24 h and used as the source of inoculums for each experiment.

2.6.2. MIC and MBC determinations

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was determined in Mueller–Hinton broth (MHB), using a macrobroth dilution method as described by the Clinical and Laboratory Standards Institute (CLSI) (2007) (CLSI). The final concentration of bacteria in each macrobroth dilution tube was approximately 5×10^5 cfu/ml of MHB. Serial dilutions of herb infusions were used. The MIC was defined as the lowest concentration of phenolic compound that resulted in no visible growth after 24 h of incubation at 37 °C. Samples (50 µl) from clear tubes were plated on Mueller–Hinton agar (MHA) plates. The MBC was defined as the lowest concentration of phenolic compounds that resulted in $\geq 99.9\%$ kill of the initial inoculums. The MIC and MBC of clarified herb infusions were also carried out and were used as control. The studies were conducted in triplicate.

2.6.3. Influence of phenolic compounds of herb infusions on bacteria viability

The liquid growth medium used in this experiment was nutrient broth. Concentrated herbs infusions were added to the medium to obtain the same phenolic compound concentrations that were found in each herb infusion. The media were inoculated 7% with overnight culture. Bacterial growth was followed by incubation for 16 h at 37 °C and was determined by enumerating the number of viable cells by plating serial dilutions in the nutrient agar medium.

2.7. Statistical analysis

All experiments were carried out at least in triplicate. Statistical analysis was performed using MS-Excel software.

3. Results and discussion

3.1. Phenolic compounds concentrations

Table 1 shows the total phenolic compounds, non-flavonoid and flavonoid compounds concentrations in selected herbs infusions and their mixtures.

The total phenolic content ranged in a wide range from 50.3 to 925.0 mg GAE/l. *I. paraguayensis*, *L. integrifolia* and *M. piperita* had the highest concentration of total phenolic compounds among the 13 herbs infusions assayed. The phenolic compound concentration in *A. citriodora*, *W. calysina*, *P. boldus* and *M. verticillatta* infusions

Table 1

Total phenolic compounds (TP), non-flavonoid (NF) and flavonoid (F) compounds concentrations in herbs infusions.

	Infusions			Clarified infusions		
	TP	NF	F	TP	NF	F
<i>Lippia integrifolia</i>	916 ^a	53	864	0.7	0	0.7
<i>Mentha piperita</i>	830	68	761	3.6	0	3.6
<i>Lippia turbinata</i>	123	41	82	2.6	0	2.6
<i>Wendtia calysina</i>	521	41	480	2.5	0	2.5
<i>Chenopodium ambrosioides</i>	50	11	40	0.2	0	0.2
<i>Minthostachys verticillata</i>	202	22	180	2.9	0	2.9
<i>Peumus boldus</i>	409	23	387	2.4	0	2.4
<i>Aloysia citriodora</i>	533	54	479	1.3	0	1.3
<i>Ilex paraguayensis</i>	925	45	880	0.6	0	0.6
Digestive herbs	118	49	69	0	0	0
Li/MP	860	54	810	0	0	0
Li/IP	910	44	860	0	0	0
MP/IP	832	21	811	0	0	0

^a Milligram of equivalent of gallic acid (GAE)/l.

were 42.4%, 43.7%, 55.7% and 78.2% lower than *I. paraguayensis* infusion, respectively. The infusions of *L. turbinata* and *C. ambrosioides* had the lowest phenolic compounds concentration.

With regard to infusions of herbs mixtures the higher concentration of total phenolic compounds was observed with *L. integrifolia*/*M. piperita* (Li/MP), *L. integrifolia*/*I. paraguayensis* (Li/IP) and *M. piperita*/*I. paraguayensis* (MP/IP) combinations and the lowest with the commercial herbs mixture.

The total phenolic content in clarified infusions ranged from 0.0 to 3.57 mg of GAE/l, indicating that the clarification process was effective to remove phenolic compounds.

In all infusions, flavonoid fraction was between 66.6% and 95.1% higher than non-flavonoid fraction. *I. paraguayensis*, *L. integrifolia* and *M. piperita* infusions possess the higher concentration of flavonoid compounds.

With respect to the infusions of herbs mixtures, the higher concentration of flavonoid compounds was observed with Li/MP, Li/IP and MP/IP combinations and the lowest with the commercial herbs infusion.

3.2. Antioxidant capacity

Two methods have been used to measure the antioxidant activities of infusions: FRAP and DPPH radical scavenging assays (Fig. 1).

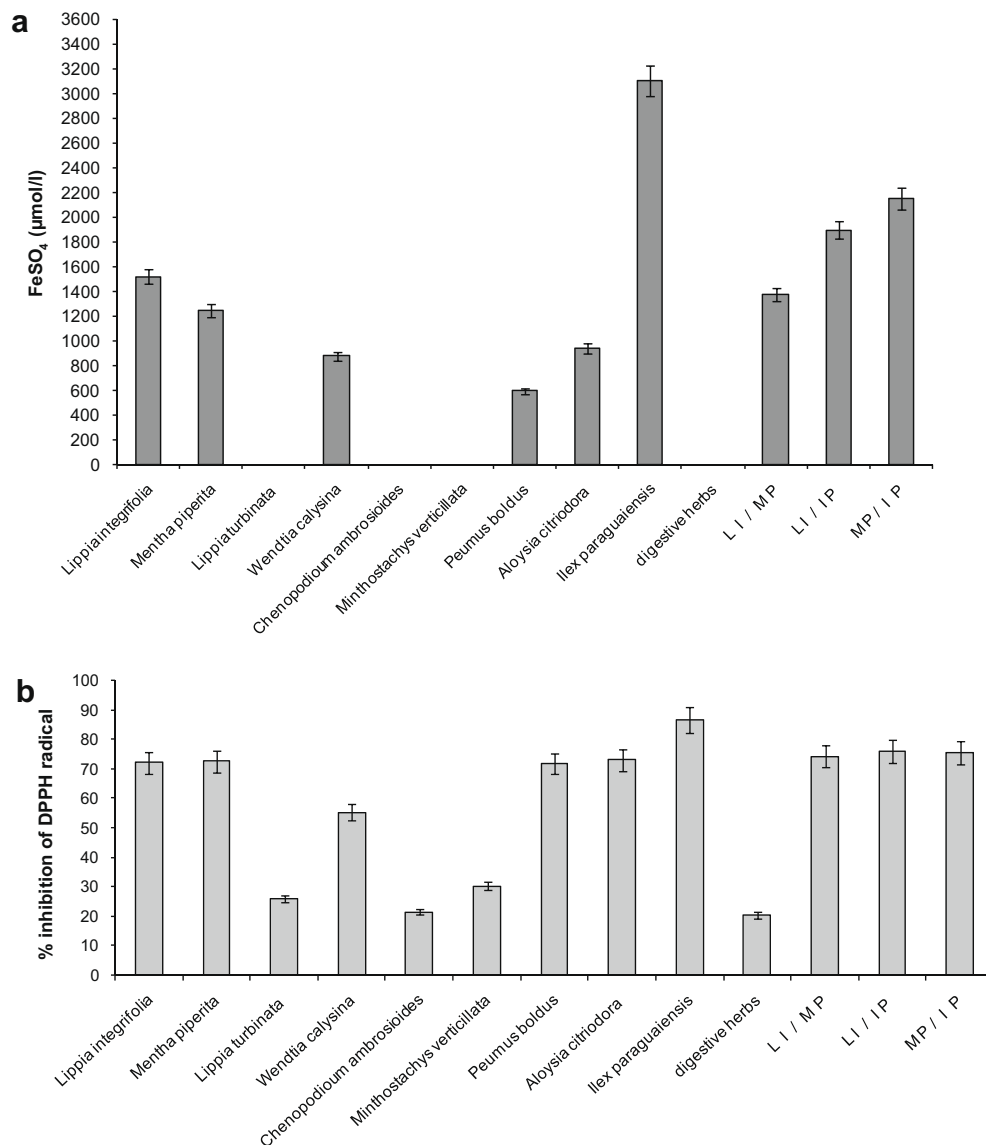


Fig. 1. Antioxidant capacity of herbs infusions. Ferric-reducing antioxidant power of herb infusions ($\mu\text{mol/l FeSO}_4$) (a) and percentage inhibition of DPPH radical (%) (b).

Results in Fig. 1a shows that among 13 infusions, *I. paraguayensis* poses the higher ferric reducing power (3100 $\mu\text{M/l FeSO}_4$). Lower ferric reduced power values were found in *L. integrifolia* (1520 $\mu\text{M/l FeSO}_4$), *M. piperita* (1250 $\mu\text{M/l FeSO}_4$), *A. citriodora* (940 $\mu\text{M/l FeSO}_4$), *W. calysina* (880 $\mu\text{M/l FeSO}_4$) and *P. boldus* (600 $\mu\text{M/l FeSO}_4$) infusions. Ferric reducing power was not detected in *L. turbinata*, *C. ambrosioides* and *M. verticillata* infusions. Among herbal combinations, *MP/IP* poses the higher ferric reducing power (2150 $\mu\text{M/l FeSO}_4$) and *Li/IP* and *Li/MP* infusions showed a ferric reduced power 12.0% and 35.8% lower, respectively. Ferric reducing power was not detected in the commercial herbs infusion.

The results of DPPH radical scavenging activity showed (Fig. 1b) that the greatest free radical scavenging activity was found with *I. paraguayensis* (86.5%), followed by *A. citriodora* (73.0%), *M. piperita* (72.5%), *L. integrifolia* (72.0%) and *P. boldus* (71.8%). *L. turbinata*, *C. ambrosioides* and *M. verticillata* showed a lowest radical scavenging activity, with values between 21.5% and 55.3% of radical scavenging activity. Among herb mixtures, *Li/IP* poses the highest radical scavenging activity (75.9%), followed by *MP/IP* (75.5%) and *Li/MP* (74.2%). The com-

mercial herbs infusion showed the lowest radical scavenging activity (20.4%).

The radical scavenging activity on clarified infusions were very lower (0.1–7.8%).

To correlate the phenolic compounds concentrations with the antioxidant capacities, the correlation coefficients (R^2) were calculated for the 13 herbs infusions (Fig. 2).

The R^2 between the antioxidant capacities obtained from FRAP assay and phenolic contents was 0.8117 (Fig. 2a) and the R^2 between the antioxidant capacities obtained from DPPH assay and phenolic contents was 0.8566 (Fig. 2b). Therefore, high phenolic content is an important factor in determining the antioxidant capacities of these herbs.

3.3. Antibacterial activity of phenolic compounds of herb infusions

The MIC and MBC of each selected herb infusions against *E. coli* are presented in Table 2. Inhibition of growth (MIC) was observed with *I. paraguayensis*, *L. integrifolia* *M. piperita* and *P. boldus* infusions, among them only *I. paraguayensis* (925 mg GAE/l) or *L. integrifolia* (916 mg GAE/l) infusions produce cellular death (MBC).

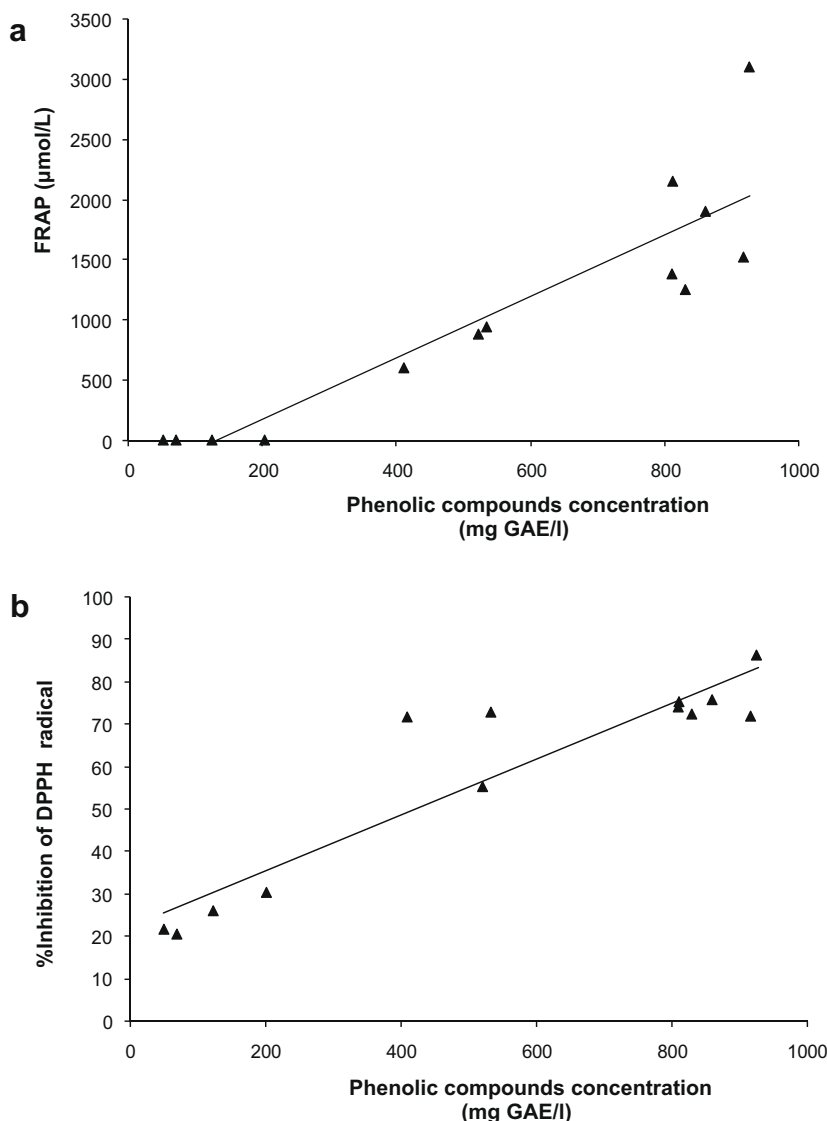


Fig. 2. Correlation between the antioxidant capacities and total phenolic compounds. Antioxidant capacities were measured by the FRAP (a) and DPPH (b) assays. $R^2 = 0.8117$ in Fig. 2a, and $R^2 = 0.8566$ in Fig. 2b.

Table 2
MIC and MBC of selected infusions against *E. coli*.

Selected herbs	Infusions		Clarified infusions	
	MIC	MBC	MIC	MBC
<i>Lippia integrifolia</i>	916 ^a	916	–	–
<i>Mentha piperita</i>	830	–	–	–
<i>Wendtia calysina</i>	–	–	–	–
<i>Peumus boldus</i>	409	–	–	–
<i>Aloysia citriodora</i>	–	–	–	–
<i>Ilex paraguayensis</i>	925	925	–	–

^a Milligram of equivalent of gallic acid (GAE)/l.

There were not observed inhibition of the growth with clarified infusions, so the antibacterial effect was attributed to the phenolic compounds in the infusions.

For *S. aureus*, a turbidity difference was observed between the different dilutions prepared, but total bacteria growth inhibition was not observed.

The number of viable cells of *E. coli* in nutrient broth supplemented with different infusions and clarified infusions, at the

end incubation were determined (Fig. 3a). All infusions reduced the number of viable cells of *E. coli*, between 0.58 and 1.5 log cycles, respect to the control. *I. paraguayensis* infusion was the most effective reducing 1.5 log cycles the number of viable cells of *E. coli*. *L. integrifolia*, *M. piperita*, *W. calysina*, *Li/IP*, *MP/IP* and *Li/MP* infusions reduced the number of viable cells by 1.02, 1.05, 1.09, 1.12, 1.01 and 1.09 log cycles respectively. *P. boldus* and *A. citriodora* infusions were the lowest effectives, reducing the number of viable cells 0.59 and 0.58 log cycles respectively. Clarified infusions were fewer effectives to reduce the viable cells number than their corresponding infusion. No differences were observed in the reduction of viable cells number between *A. citriodora* infusion and its corresponding clarified infusion, whereas all the others infusions reduced the number of viable cells between 0.4 and 1.16 log cycle, respect their clarified infusions.

Fig. 3b shows the number of viable cells of *S. aureus* at the end incubation. Significantly difference was not observed between the control and the clarified infusions. *L. integrifolia*, *P. boldus*, *I. paraguayensis*, *Li/MP*, *Li/IP* and *MP/IP* infusions, decreased 1.05, 3.25, 3.35, 1.55, 2.85 and 2.05 log cycles the viability of *S. aureus*, respect to the control. *I. paraguayensis* and *P.*

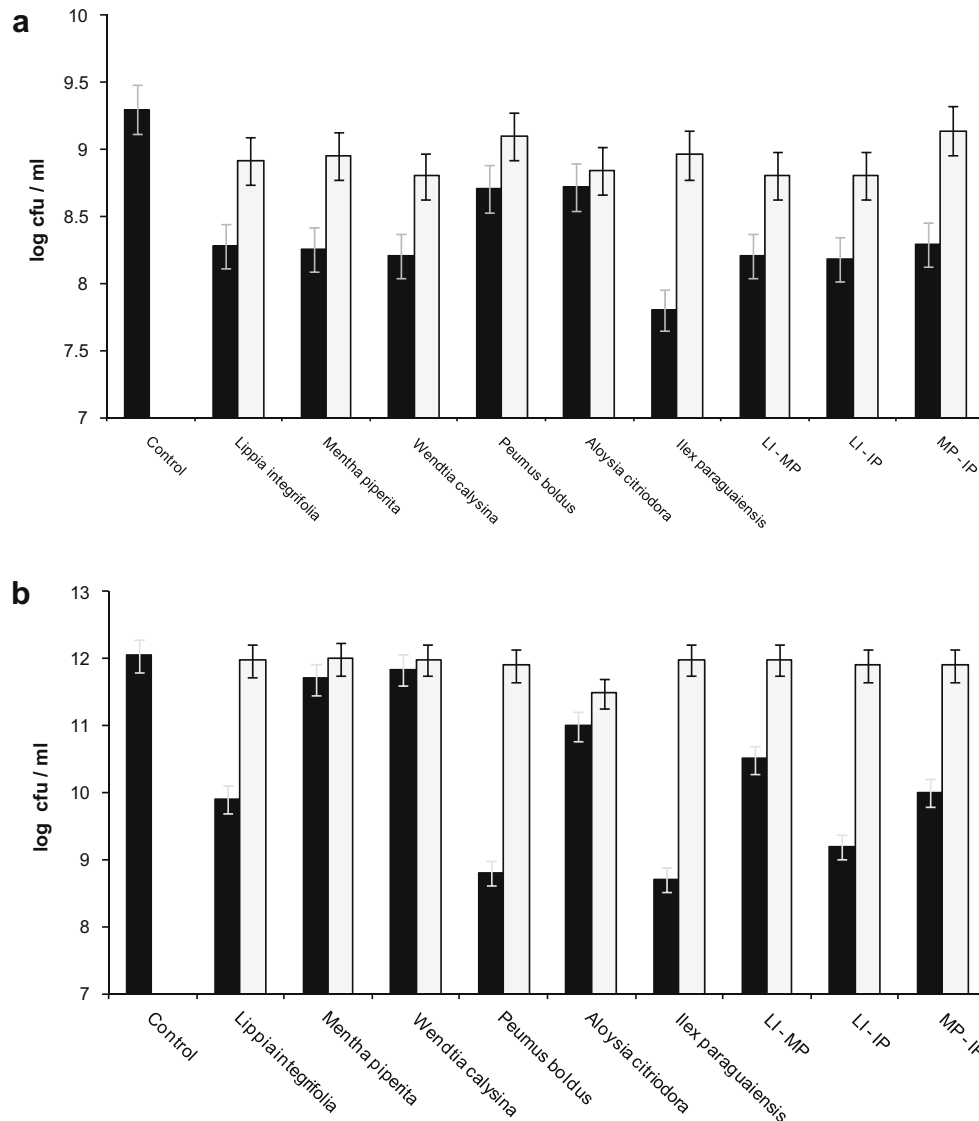


Fig. 3. Log of the number of viable cells (log cfu/ml) of *Escherichia coli* ATCC 35218 (a) and *Staphylococcus aureus* ATCC 25923 (b) in media supplemented with different herbs infusions, at the end incubation. (■) Infusion and (□) clarified infusions. Each point represented the average value of three determinations.

boldus infusions were the most effective to reduce the viable cell number of this species.

There were not observed significant differences between *M. piperita* and *W. calysina* infusions with their corresponding clarified infusions, being the less effective to reduce the number of viable cells.

4. Discussion

The herbs used in this study, characteristic of this region of the world, were traditionally consumed as infusion in Argentina from centuries since the original peoples attributed it, several effects related with the medicinal properties. In the present study, we investigated the relation between the phenolic content with the antioxidant and antibacterial properties of several herbs and herbal combinations infusions.

There was observed that the infusions with the highest amount of total phenolic compounds were *I. paraguayensis*, *L. integrifolia* and *M. piperita* and its combinations. In all infusions, flavonoid fraction was greater than non-flavonoid fraction, and the higher values were observed in the above mentioned infusions.

The best values of antioxidant activity were found in the infusions containing *I. paraguayensis*, *L. integrifolia* and *M. piperita*.

Ours results indicates that antioxidants in these herbs were capable of scavenging free radicals and reducing oxidants. The significant relationship between the antioxidant activities and total phenolic compounds suggest that phenolic compounds, particularly flavonoid fraction, are the major contributors of antioxidant capacities of these herbs.

Ours results are in agreement with those observed by Li, Wong, Cheng, and Chen (2008) with phenolic compounds of Chinese medicinal plants and with those of Yoo, Lee, Lee, Moon, and Lee (2008) that found a higher correlation between antioxidant capacity and total phenolic and total flavonoids content in commercial herbs. This antioxidant activity of some phenolic compounds in herbs was related to its capacities to quench lipid peroxidation, prevent DNA oxidative damage, and scavenge reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals (Cao & Cao, 1999; Kahkonen et al., 1999).

This correlation between the antioxidant activity and total phenolic compounds was too reported by other authors for the phenolic compounds present in the fruits, grains and red wine (Heim, Tagliaferro, & Bobilya, 2002; Javanmardi, Stushnoff, Locke, & Vivanco, 2003; Kahkonen et al., 1999; Lee, Kim, Lee, & Lee, 2003; Yoo, Lee, Park, Lee, & Hwang, 2004).

Concerning antibacterial activity, all infusions reduced the number of viable cells of *E. coli*, being *I. paraguayensis*, *L. integrifolia* and *M. piperita* and these combinations the most effective.

The more effective infusions to reduce the viable cells number of *S. aureus* were those prepared with *I. paraguayensis* and *P. boldus* and the combinations *Li/IP* and *MP/IP*.

The results with *I. paraguayensis* infusions on the viability of both bacteria are in according to the polyphenols concentration.

However *P. boldus* infusion that has a half of phenolic compounds concentrations than *I. paraguayensis* infusion was too effective to inhibit *S. aureus* growth, suggesting that the antibacterial effect of selected herbs is related with the concentration of phenolic compounds but also with the phenolic compounds profile in each infusion.

While there is little information about the antibacterial activity of Argentinean herbal infusions, we demonstrated the antibacterial effect of phenolic compounds on several pathogens bacterial, with phenolic compounds from Argentine wines (Rodríguez Vaquero, Alberto, & Manca de Nadra, 2007a; Rodríguez Vaquero, Alberto, &

Manca de Nadra, 2007b; Rodríguez Vaquero & Manca de Nadra, 2008).

From our results we can conclude that the phenolic compounds of commonly consumed Argentinean herbs have important antioxidant and antibacterial activities. On the basis of the knowledge of these properties will be possible the use of these herbs, extensively distributed in our country, as raw material to formulate new products to be used in food industry as natural antioxidants, replacing synthetic antioxidant and also as natural food preservatives.

Acknowledgments

This work was supported by grants from Consejo de Investigaciones de la Universidad Nacional de Tucumán, Argentina (CIUNT) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

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