ANTIOXIDANT CAPACITY OF *ILEX PARAGUARIENSIS* EXTRACTS BY USING HRP-BASED BIOSENSOR

L.D. MELLO† and L.T. KUBOTA‡

† *Universidade Federal do Pampa, Travessa 45, 1650, 96413-170, Bagé, RS, Brasil*
lucilene.unipampa@gmail.com
‡*Instituto de Química - Unicamp, 13083-970, Campinas, SP, Brasil. kubota@iqm.unicamp.br*

***Abstract* — Phenol compounds are the major constituents in vegetables and can be correlated to the antioxidant capacity of plants. Thus, the relationship between total antioxidant activity (TAA) and total phenol content of *Ilex paraguariensis* extracts were evaluated using a Horseradish peroxidase-based biosensor. Antioxidant activities of these extracts were investigated by the 1,1-diphenyl-2-pycrylhydrazyl (DPPH) radical scavenging method. TAA of the investigated *Ilex paraguariensis* samples were well correlated to the phenol content, presenting correlation coefficients R > 0.9. The measured antioxidant capacity expressed in terms of relative antioxidant capacity of tea and yerba mate of different origin were listed in relation to the 10 mmolL^{-1} Trolox solution. According to these observations the biosensor reading can be applied to determine the TAA of *Ilex paraguariensis* samples.**

***Keywords* — Biosensor, Antioxidant Activity, Polyphenols, Tea, *Ilex paraguariensis*.**

I. INTRODUCTION

Ilex paraguariensis St. Hill or popularly known as "mate tree" is a native small tree which is widely cultivated in South American Countries, mainly Argentine followed by Brazil and Paraguay. Yerba mate is a product constituted exclusively by the dried, slightly roasted and milled leaves of *I. paraguariensis* according to Argentinian and Brazilian legislation and consumed as aqueous extract (Cogoi *et al.*, 2013). In Brazil, *Ilex paraguariensis* leaves can also be industrialized in two forms; as *Mate* Tea (known as *Ilex paraguariensis* tea) that is a product prepared with roasted leaves of the plant, and it can be commercially packed in individual tea bags (1 or 2 g) or as *Mate* Tea concentrate for use as ingredient in the food or dietary supplement industries (Heck and De Mejia, 2007). *Ilex paraguariensis* cultivation has great important economic and social considering that it is carried through by a great number of small producers and cooperatives. (Tormen, 1995). These extracts are rich in alkaloids, vitamins, minerals, additional compounds as panthotenic acid, fatty acids, amino acids, saponins and mainly polyphenols. *Ilex* leaves contain significant amounts of hydrosoluble polyphenols, such as isochlorogenic acid, caffeic acid and chlorogenic acid (Efing *et al*., 2009; Burris *et al*., 2012), which present high antioxidant capacities and are considered to exhibit anti-cancer effect in mammals by strengthening an organism's natural defenses and protecting it against cellular destruction. Polyphenols are a class of phytochemicals found in high concentration and they have been associated to the slight adstringent and bitter taste of tea (Heck and De Mejia, 2007). *Ilex paraguariensis* is also used in popular medicine for the therapeutic properties attributed to the high caffeoyl-derivates content and flavonoids (Anesini *et al*., 2012; Sardi *et al*., 2007).

The chemical structure of these compounds is suitable for free radical-scavenging activities providing protection from reactive attacks, because they are excellent hydrogen or electron donor and their intermediates radical are relatively stable due to resonance delocalization and lack of suitable sites for attack by molecular oxygen (Silva *et al*., 2000).

Therefore, it is of great interest to evaluate the antioxidant potential of plants in relation to their phenolic constituents. Methods have been proposed for the detection of antioxidants *in vivo* and *in vitro* characterization. Characterization of antioxidant *in vitro*, such as photometric (Jayachita and Krithiga, 2012), fluorimetric (Nikokavoura *et al*., 2011), chromatographic (Boudier *et al*., 2012) and electrochemical methods (Anidi *et al*., 2012; Gorjanovic *et al*., 2012) has been developed. The photometric methods are based on the detection of absorption of radical scavenger such as cytochrome c or artificial radicals. One of the most common methods based on free radical is stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) method, used to compare antioxidant activity with the Trolox standard antioxidant. The test of scavenging chemically stable $DPPH^{\bullet}$ radical is a method widely used to evaluate antioxidant capacity in a relatively short time compared to other methods (Blois, 1958).

For all that, the determination of polyphenols in plants and the correlation of this content with the antioxidant activity may be interesting for the investigation of natural source as chemotherapeutic agents. The amperometric modified electrodes that combines redox enzymatic reactions with electrochemical detection have been reported as an alternative suitable for the detection of polyphenols in plant extracts (Rawal *et al*., 2011; Nadifiyine *et al*., 2013). Oxi-reductases enzymes-based biosensors exhibit a relatively specificity for electrons donor as the phenolic compounds and can be used for this purpose (Prehn *et al*., 2012).

The aim of the present work is the evaluation of the correlation between the total polyphenols content in *Ilex paraguariensis* leaves determined initially by an elec-

trochemical peroxidase based-biosensor and the antioxidant capacity of these extracts.

II. MATERIALS AND METHODS

A. Chemicals

DPPH was purchased from Sigma (St. Louis, USA); Trolox was supplied by Aldrich (Milwaukee, USA). All aqueous solutions were prepared using water purified with a Milli-Q system (Millipore, System).

B. Preparation of the samples

Ilex paraguariensis tea and yerba mate were obtained from local supermarkets and all samples are produced by Brazilian companies. Although the yerba mate and tea to be consumed as two different infusions, in this experiment they were prepared by the same way which allowed the almost complete extraction of the water soluble plant constituents. Amount of 0.1g samples were dissolved in 10.0 mL of hot water in triplicate.

C. Determination of polyphenols content by the biosensor method

The development and optimization of the conditions for the biosensor was studied in a previous work (Mello *et al*., 2003). The carbon paste electrode was prepared by immobilization of the 0.1mg of DNA additive and 200μL of a solution of 1mg.mL^{-1} HRP enzyme on 25mg silica-titanium using glutaraldehyde 5%(v/v). This mixture was dried at room temperature and after mixed with 25mg of graphite powder dropping 35μL of mineral oil until get a homogeneous paste. This paste was put into a cavity of 1mm deep consisting of platinum disk sealed into the extremity of a glass tube (4mm i.d.) and pressed to smooth the surface. The biosensor was used to measure the total polyphenol content in mate extracts without pretreatments. The chlorogenic acid (CGA) was used as the reference compound. Measurements were carried out using a biosensor as working, Pt wire as auxiliary and Ag/AgCl saturated KCl as reference electrode. The experiments made at constant potential, the current response was recorded as function of the time, following the addition of CGA. The response of the biosensor was measured as the difference between total and residual current.

D. Evaluation of the free radical scavenging capacity

In order to evaluate the efficiency of the vegetables extracts as radical scavengers, they are allowed to react with commercially available free radical DPPH ($C_{18}H_{12}N_5O_6$) (Blois, 1958). The colorimetric test for free radical relies on the reaction (DPPH$^{\bullet}$ + AH $\rightarrow$ DPPH-H + A$^{\bullet}$) of specific antioxidant (AH) with DPPH adapted from Ohnishi *et al* (1994). Aliquots of *Ilex* samples were added into 0.1mmol L^{-1} of DPPH ethanol solution. Reduction of DPPH$^{\bullet}$ was followed by monitoring the decrease of the absorbance after 10 min in its characteristic wavelength (517nm) of this purple-blue DPPH$^{\bullet}$ solution. In the radical form, DPPH$^{\bullet}$ present a maximum absorption at 517nm, but upon reduction by an antioxidant, the absorption disappears and the pale-yellow non-radical form is produced. The radical scavenging effects was expressed as % of the absorbance of the DPPH control solution without antioxidant and was compared on the basis of IC_{50} (IC_{50} represents the antioxidant concentration needed to reduce 50% of the initial amount of DPPH$^{\bullet}$). All assays were carried out in triplicate and 10mmol L^{-1} trolox solution was used as the reference compound.

DPPH absorbance in all assays was measured in a quartz curvette of ten mm path using a spectrophotometer UV/VIS from Pharmacia Biotech® Ultraspec 2000 connected to a PC (software Wavescan®).

III. RESULTS AND DISCUSSION

A. Free radical scavenging capacity

The accepted way to evaluate the effect of antioxidants is through their antiradical activity. The decrease of DPPH$^{\bullet}$ concentration is an index to estimate radical scavenger capacity of plants extracts. The remaining DPPH$^{\bullet}$ concentration in the reaction medium was calculated from the following calibration curve determined experimentally.

$$A_{517nm} = -0.01\ (\pm\ 0.01) + 0.0056\ (\pm\ 0.0002)\ [DPPH^{\bullet}] \quad (1)$$

The concentration of phenol necessary to decrease 50% of the initial radical concentration (IC_{50}) in 10 minutes was also calculated. This parameter is widely used to measure the antioxidant power. A low IC_{50} indicates strong antioxidants compounds present in vegetables (Berté *et al*., 2011). Mate tea is better antioxidant (IC_{50} = 12.0 ± 0.3 μmol L^{-1}) in comparison to the yerba mate (IC_{50} = 35.0 ± 0.1 μmol L^{-1}). This difference can be due to the different commercial preparations of the two extracts. Mate tea is constituent of *Ilex paraguariensis* roasted leaves and yerba mate is constituent of *Ilex paraguariensis* blanching leaves which can have others parts of the plant. Tea and yerba mate vary in chemical composition, the types and amount of phenolic compounds present will differ depending on the maturity, local of production, agricultural practices as well as many other environmental factors, manufacturing and infusion preparation (Isolabella *et al.*, 2010; Burris *et al*., 2012).

B. Correlation between polyphenols content and antioxidant capacity

In order to evaluate if there is a correlation between antioxidant capacity and polyphenols content determined by HRP-based biosensor, tea and yerba mate of different origin were used. The content of total phenols obtained for tea varied from 2.30mmol L^{-1} to 11.23mmol L^{-1} and for yerba mate varied from 4.0mmol L^{-1} to 18.0mmol L^{-1} (calculated values in triplicate for both samples). The used parameter was total antioxidant activity (TAA), calculated by:

$$TAA = \frac{Abs_{standard}}{Abs_{sample} - Abs_{standard}}$$

where, $Abs_{standard}$ is the absorbance of the $DPPH^{\bullet}$ solution in the presence of a known Trolox concentration and Abs_{sample} is the absorbance of the $DPPH^{\bullet}$ solution in the presence of the sample.

A linear model expressed the total antioxidant activity (TAA) against polyphenols compound concentration determined by peroxidase-based biosensor in *Ilex* samples. The results are shown in Fig. 1 and Fig. 2, for the tea and yerba mate, respectively. This means that the antioxidant activity may be obtained directly from following relationships:

$$\text{TAA to mate tea} = 0.8\ (\pm 0.1)\ [\text{total phenol}] + 2.8\ (\pm 0.6)\ (R = 0.986) \quad (2)$$

$$\text{TAA to yerba mate} = 0.3\ (\pm 0.1)\ [\text{total phenol}] - 0.4\ (\pm 0.6)\ (R = 0.973) \quad (3)$$

The total antioxidant activities of the investigated extracts were well correlated with total phenols content. The correlation coefficients were $R > 0.9$, in both cases, as can be seen the correlation varied slightly from kind of extracts, considering the same plant. It is important to emphazise that each kind of sample will have a determined correlation between TAA and total phenol concentration. It suggests that, a simple reading with biosensor is possible to evaluate the TAA of the plants extracts. However, for different kind of samples a new correlation should be performed.

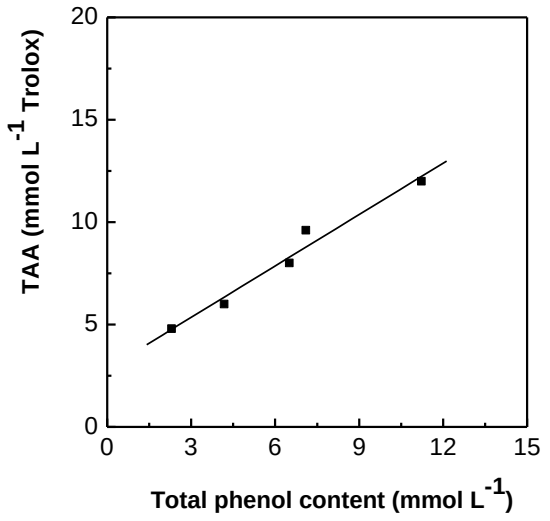

Fig. 1. Correlation between total phenol content presents mate tea samples and total antioxidant activity.

Compounds like ascorbate or carbohydrates showed no response as interference in the biosensor response (Mello *et al*., 2003), thus the phenol content is the main source of the antioxidants in plants. It suggests that even through vegetables contain other compounds which can act as antioxidant, these do not interfere significantly in the antioxidant activity determination with the biosensor. In other words, the antioxidant compounds that are not phenols would be present in a great proportion varying as the phenols content. As a result of this behavior the measuring with the presented biosensor was used to list this property in *Ilex* extracts, expressed in terms of relative antioxidant capacity. The results are listed on Table 1. Infusions prepared from the leaves of tea and yerba mate of the different origin showed good antioxidant index. Tea showed a relative antioxidant capacity of more than 40% and yerba mate between 20% and 30% in relation to a 10mmol L^{-1} Trolox solution. It is important to comment that the phenolic compounds concentration measured by the biosensor were much lower than used Trolox solution concentration and the results of the antioxidant index showed a very good antioxidant capacity.

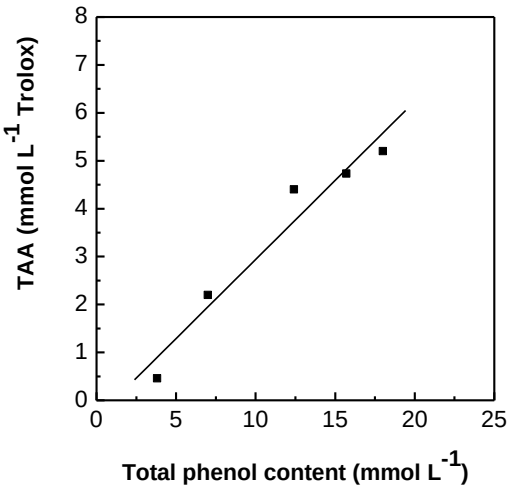

Fig. 2. Correlation between total phenol content present in yerba mate samples and total antioxidant activity.

Table 1. Relative antioxidant capacity of the *Ilex paraguariensis* extracts measured by HRP-biosensor in relation to the 10 mmol L^{-1} Trolox solution.

Sample	Total phenol (mmol L^{-1}) ± SD*	Relative antioxidant capacity (%) ± SD*
tea 1	3.0 ± 0.1	45.0 ± 1.0
tea 2	1.9 ± 0.1	39.0 ± 0.1
tea 3	2.60 ± 0.03	44.3 ± 0.1
tea 4	2.7 ± 0.1	45.0 ± 1.0
yerba mate 1	4.0 ± 0.3	30 ± 4
yerba mate 2	3.8 ± 0.5	30 ± 1
yerba mate 3	3.8 ± 0. 1	29.0 ± 1.4
yerba mate 4	2.9 ± 0.1	20.0 ± 1.4
yerba mate 5	3.6 ± 0.4	27 ± 4
yerba mate 6	3.5 ± 0.1	26.4 ± 1.2

*SD = standard deviation for three replicates.

The antioxidant capactity of plant leaves has been compared to those observed for fruits and vegetables in in tea some studies. Polyphenols in plants, mainly catechins have shown higher antioxidant protection than vitamin C and E (Du Toit *et al*., 2001; Cao *et al*., 1996).

Although several biosensors for phenol compounds have been described in the literature in the last years, the study described show a new application of enzyme-

based biosensor, in the evaluation of antioxidant capacity of natural extracts.

IV. CONCLUSION

The determination of the total phenol content by the biosensor was representative in terms of antioxidants compounds. The performance in the assay of the antioxidant potential of *Ilex paraguariensis* aqueous infusions and its correlation with the phenol content determined by the biosensor was demonstrated. A good correlation was obtained with the antioxidant properties of the extracts and total phenol content. The measured antioxidant capacity expressed in terms of the relative antioxidant capacity of tea and yerba mate of different origin were listed. The results showed good relative antioxidant capacity when compared with a 10mmol L^{-1} concentration Trolox solution. According to these results it can be considered that the simple reading of the biosensor is possible to know the total antioxidant activity (TAA) and an index degree of antioxidant capacity of different samples. The use of biosensor in this case provides some important advantages as easily manipulation, selective response and fast evaluation of antioxidant capacity of plants extracts.

ACKNOWLEDGMENTS

The authors thank FAPESP for financial support.

REFERENCES

Anesini, C., S. Turner, L. Cogoi and R. Filip, "Study of the participation of caffeine and polyphenols on the overall antioxidant activity of mate (*Ilex paraguariensis*)," *Food Sci. Technol.*, **45**, 299-304 (2012).

Anidi, S., F. Mojab, A. Bayandori, K. Tabib and F. Kobarfard, "A simple electrochemical method for the rapid estimation of antioxidant potentials of some selected medicinal plants," *Iranian J. Pharm. Res.*, **11**, 117-121 (2012).

Berté, K.A., M.R. Beux, P.K. Spada, M. Salvador and R. Hoffmann-Ribani, "Chemical composition and antioxidant activity of yerba-mate (*Ilex paraguariensis* A. St. Hil, Aquafoliaceae) extract as obtained by spray drying." *J. Agric. Food Chem*., **25**, 5523-5527 (2011).

Blois, M.S., "Antioxidant determinations by the use of a stable free radical," *Nature*, **181**, 1199-1200 (1958).

Boudier, A., J. Tournelize, B. Grzegorz, S. El Hani, R. Bengeddour, A. Sapin-Minet and P. Leroy, "High-performance liquid chromatography method to evaluate the hydrogen atom transfer during reaction between 1,1-diphenyl-2-picryl-hydrazyl radical and antioxidants," *Anal. Chim. Acta*, **711**, 97-106 (2012).

Burris, K., F.M. Harte, M.S. Davidson, C. Stewart and S. Zivanovic, "Composition and bioactive properties of yerba mate (Ilex paraguariensis A. St. Hil)," *Chilean J. Agric. Res.*, **72**, 268-274 (2012).

Cao, G., E. Sofic and R.L. Prior, "Antioxidant capacity of tea and common vegetables," *J. Agric. Food Chem*., **44**, 3426-3431 (1996).

Cogoi, L., M.S. Giacomino, N. Pellegrino, C. Anesini and R. Filip, "Nutritional and phytochemical study of Ilex paraguariensis fruits," *J. Chem*., Article ID 750623 (2013).

Du Toit, R., Y. Volsteedt and Z. Apostolides, "Comparison of the antioxidant content of fruits, vegetables and teas measured as vitamin C equivalents," *Toxicology*, **166**, 63-69 (2001).

Efing, L.C., T.K. Caliari, T. Nakashima and R.J.S. De-Freitas, "Chemical, characterization and antioxidant capacity of mate (Ilex paraguariensis St. Hil)," *Boletim do Centro de Pesquisa de Processamento de Alimentos*, **27**, 241-246 (2009).

Gorjanovic, S., D. Komer, F. Pastor, A. Belscak-Cvitanovic, L. Pezo, I. Hecinovic and D. Seeznjevic, "Antioxidant capacity of teas and herbal infusions: Polarographic assessment," *J. Agric. Food Chem*., **60**, 9573-9580 (2012).

Heck, C.I. and E.G. De Mejia, "Yerba mate (*Ilex paraguariensis*): A comprehensive review on chemistry, health implication and technological consideration," *J. Food Sci*., **72**, R138-R151 (2007).

Isolabella, S.L., L. Cogoi, P. Lopez, C. Anesini, G. Ferraro and R. Filip, "Study of the bioactive compounds variation during yerba mate (*Ilex paraguariensis*) processing," *Food Chem*., **122**, 695-699 (2010).

Jayachita, A. and N. Krithiga, "Study on antioxidant property in selected medicinal plant extracts," *Int. J. Med. Arom. Plants*, **2**, 495-500 (2012).

Mello, L.D., M.D.T. Sotomayor and L.T. Kubota, "HRP-based amperometric biosensor for the polyphenols determination in vegetables extracts," *Sens. Actuator B-Chem*., **96**, 636-645 (2003).

Nadifiyine, S., C. Calas-Blanchard, A. Amine and J. Marty, "Tyrosinase biosensor used for the determination of catechin derivatives in tea: Correlation with HPLC/DAD method," *Food Nutr. Sci.*, **4**, 108-118 (2013).

Nikokavoura, A., D. Christodouleas, E. Yannakopoulou, K. Papadopoulos and A.C. Calokerinos, "Evaluation of antioxidant activity of hydrophilic and lipophilic compounds in edible oils by a novel fluorimetrc method," *Talanta*, **84**, 874-880 (2011).

Ohnishi, M., H. Morishita, H. Iwahashi; S. Toda, Y. Shirataki, M. Kimura and R. Kido, "Inhibitory effects of chlorogenic acids on linoleic acid peroxidation and haemolysis," *Phytochem*., **36**, 579-583 (1994).

Prehn, R., J. Gonzalo-Ruiz and M. Cortina-Puig, "Electrochemical detection of polyphenolic compounds in foods and beverages," *Curr. Anal. Chem.*, **8**, 472-484 (2012).

Rawal, R., S. Chawla and C.S. Pundir, "Polyphenol biosensor based on laccase immobilized onto silver nanoparticles/multiwalled carbon nanotube/ polyaniline gold electrode," *Anal. Chem*., **419**, 196-204 (2011).

Sardi, F., N. Turkmen, G. Polat and Y. Velioglu, "Total polyphenol, antioxidant and antibacterial activities of black mate tea," *Food Sci. Technol. Res*., **13**, 265-269 (2007).

Silva, F.A.M., F. Borges, C. Guimarães, J.L.F.C. Lima, C. Matos and S. Reis, "Phenolic acids and derivates: Studies on the relationships among structure, radical scavenging activity and physiochemical parameters," *J. Agric. Food Chem*., **48**, 2122-2126 (2000).

Tormen, M.J., "Economia ervateria brasileira," *Erva Mate: Biologia e Cultura no Cone Sul*, eds. H. Winge, A.G. Ferreira, J.E.A. Mariath and L.C. Tarasconi, Editora da Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil (1995).

Received: April 16, 2013
Accepted: November 27, 2013
Recommended by Subject Editor: M. Luján Ferreira