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Evaluation of the Antioxidant Activity as an Additional Parameter to Attain the Functional Quality of Natural Extracts

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SUMMARY. Due to differences in the functional quality of natural extracts, we have also faced differences in their effectiveness. So, it was intended to assess the antioxidant activity of natural extracts in order to attain their functional quality. It was observed that all the extracts (brown and green propolis, *Ginkgo biloba* and Isoflavin Beta®) and the standard used (quercetin) showed antioxidant activity in a dose-dependent manner with IC_{50} values ranging from 0.21 to 155.28 μg mL^{-1} (inhibition of lipid peroxidation and scavenging of the DPPH$^{\bullet}$ assays). We observed a high correlation (r^2= 0.9913) among the antioxidant methods; on the other hand, the antioxidant activity was not related to the polyphenol and flavonoid content. As the DPPH$^{\bullet}$ assay is a fast method, presents low costs and even has a high correlation with other antioxidant methods, it could be applied as an additional parameter in the quality control of natural extracts.

RESUMEN. "Evaluación de la actividad antioxidante como parámetro adicional para lograr la calidad funcional de extractos naturales". Debido a las diferencias en la calidad funcional de extractos naturales, nosotros hemos advertido también diferencias en su efectividad, por lo que se pretende estimar la actividad antioxidante de extractos naturales para lograr su calidad funcional . Fue observado que todos los extractos (propolis marrón y verde, *Ginkgo biloba* e Isoflavin Beta®) y el patrón usado (quercetina) mostraron actividad antioxidante de una manera dosis-dependiente con valores de IC_{50} que van de 0,21 a 155,28 al μg mL^{-1} (inhibición de la peroxidación lipídioca y captación de radicales libres del DPPH$^{\bullet}$). Se observó una alta correlación (r^2 = 0,9913) entre los métodos antioxidantes y por otro lado la actividad antioxidante no estuvo relacionada con el contenido del polifenoles ni de flavonoides. Como el análisis del DPPH$^{\bullet}$ es un método rápido, presenta costos bajos e incluso tiene una correlación alta con otros métodos antioxidantes, este método podría utilizarse como un parámetro adicional en el control de calidad de extractos naturales.

INTRODUCTION

Natural extracts have been used in many domains including medicines, nutrition, flavoring, beverages, dyeing, repellents, fragrances, cosmetics, smoking, and other industrial purposes. Since the prehistoric era, herbs have been the basis for nearly all medicinal therapy [1], and nowadays, according to a 1983 World Health Organization (WHO) estimate, a majority of the population in developing countries depend on traditional and herbal medicine as their primary source of health care [2]. In recent years there is an intensive interest in researches objecting the evaluation and the standardization of natural/herbal extracts and other materials from natural sources. Many of such researches have been aimed to the evaluation of the antioxidant activity of these natural sources [1, 3-5], since antioxidant compounds and/or extracts could be applied to treat and prevent human diseases. Moreover, natural antioxidants could be even used in foods and cosmetics, etc. to replace synthetic antioxidants, which are being restricted due to their carcinogenicity [1,6].

Epidemiological and animal studies and *in vitro* experiments revealed that polyphenols pre-

KEY WORDS: Antioxidant activity, Lipid Peroxidation, Natural extracts, Scavenging of DPPHü radicals.
PALABRAS CLAVE: Actividad antioxidante, Captación de radicales libres del DPPH, Extractos Naturales, Peroxidación lipídica.

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sent in these natural extracts, and in fruits and vegetables possess antioxidant properties including ROS quenching and inhibition of lipid peroxidation, and exert anticarcinogenic, antimutagenic, antitumoral, antibacterial, antiviral and anti-inflammatory effects [7].

An antioxidant can be defined as any substance that when present at low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate [3]. So, the physiological role of antioxidants, as this definition suggests, is to prevent damage to cell components arising as a consequence of chemical reactions involving free radicals. Fortunately, free radical formation is controlled naturally by endogenous and exogenous antioxidants [8]. However, in some situations such as cell injury, radiation, when the antioxidant system is overwhelmed and a stress situation is established, there is a need for external antioxidants to try to control and prevent the tissue damage. Under physiological conditions of the organism, various reactive oxygen species (ROS, e.g. $O_2^{\bullet -}$, H_2O_2, $^{\bullet}OH$) are formed and decomposed by enzymatic and non-enzymatic reactions. Increased levels of ROS can initiate lipoperoxidation, damage or development of various diseases. Fe and other transient metals participate in ROS formation and lipoperoxidation. The $^{\bullet}OH$ radical is very reactive and it may be formed in the Fenton reaction [7]:

$$H_2O_2 + Fe^{2+} \rightarrow {}^{\bullet}OH + OH^- + Fe^{3+}$$

So, due to their high applicability, one must take into account the quality of natural products that can affect their efficacy and/or safety. However, we face a lack of regulatory practices worldwide, which has led to some difficulties in the standardization of natural extracts and even it has led to product quality differences.

Therefore, the objectives of this work were to estimate the polyphenol and flavonoid content and to evaluate the antioxidant activity of several natural extracts by two different methods. It was also intended to search for correlation among these parameters. In addition, it was intended to assess if the antioxidant activity could be properly applied as a parameter to attain the functional quality of natural extracts. Brown and Green propolis, *Ginkgo biloba,* Isoflavin Beta® and also a potent isolated antioxidant, quercetin (standard) were chosen to be evaluated, due to their potential antioxidant activity [9-12].

MATERIAL AND METHODS

Chemicals

Brazilian brown propolis ethanolic extract (BPE) was purchased from Apis Flora (Ribeirão Preto - SP, Brazil. Patent number PI 0405483-0, published in Revista de Propriedade Industrial N° 1778 from 01/02/2005). Brazilian green propolis ethanolic extract (GPE) was a gift from Bioessens Ltda (Cotia - SP, Brazil). Both BPE and the GPE presented 11% dry weight. *Ginkgo biloba* dried extract was purchased from SP Farma (São Paulo - SP, Brazil). Isoflavin Beta® extract from France was donated by Galena (Campinas - SP, Brazil). Thiobarbituric acid (TBA) and 2,2-Diphenyl-1-picryl-hydrazyl (DPPHü) were purchased from Sigma Chemical Co. (St. Louis - MO, USA). Quercetin was purchased from Acros (New Jersey, USA), Gallic Acid and Folin-Ciocalteau were purchased from Merck (Darmstadt, Germany). All other chemicals were of reagent grade and were used without further purification.

Total Polyphenol and Flavonoid content in the extracts

The extracts were accurately weighted and diluted in the hydroalcoholic solution of each experiment. The dried extracts were firstly extracted as further described.

Total polyphenol content in the extracts was determined by the Folin-Ciocalteau colorimetric method. To 0.5 mL of 50% hydroalcoholic solution of each extract was mixed 0.5 mL of the Folin-Ciocalteau reagent and 0.5 mL of 10% Na_2CO_3, and the absorbance was measured at 760 nm after 1 h incubation at room temperature, with a Hitachi spectrophotometer U-2001. Total polyphenol content was calculated as gallic acid equivalents (mg g^{-1}) from an analytical curve [13]. The experiment was performed in triplicate.

Total flavonoid content was determined using the aluminum chloride colorimetric method. To 0.5 mL of 80% hydroalcoholic solution of each extract, 0.5 mL of 2% $AlCl_3$ ethanol solution was added. After 1 h at room temperature, the absorbance was measured at 420 nm, with a Hitachi spectrophotometer U-2001. Total flavonoid content was calculated as quercetin (mg g^{-1}) from an analytical curve. The amount of 2% aluminum chloride was substituted by the same amount of distilled water in blank [13]. The experiment was performed in triplicate.

Assessment of the antioxidant activity

Preparation of Samples

Brown Propolis extract. 500 mg (accurate weighted) of the ethanolic extract of propolis was firstly solubilized with propylene glycol (1:10) and then diluted using the medium of each reaction. The final concentration range in the reaction medium was 5.8-92.8 μg mL^{-1} for the inhibition of lipid peroxidation assay, and 37.1-371.3 μg mL^{-1} for the scavenging of the DPPH• radical assay.

Green Propolis extract. 500 mg (accurate weighted) of the ethanolic extract of propolis was firstly solubilized with propylene glycol (1:10) and then diluted using the medium of each reaction. The final concentration range in the reaction medium was 4.1-33.0 μg mL^{-1} for the inhibition of lipid peroxidation assay, and 33.4-330.0 μg mL for the scavenging of the DPPH• radical assay.

Ginkgo biloba extract. 500 mg (accurate weighted) of the dry extract was weighted and it was first dissolved with 10 mL of 80% ethanol. After shaking the suspension for 30 min and centrifugation at 3000 rpm for 20 min, the supernatant was collected and the precipitate was then extracted with 10 mL of 80% ethanol following the same steps. Finally, the supernatant was combined with previous supernatant and adjusted to 25 mL with 80% ethanol. Then this solution was diluted with the medium of each reaction. The final concentration range in the reaction medium was 5.0-200.0 μg mL^{-1} for the inhibition of lipid peroxidation, and 10.0-400.0 μg mL^{-1} for the scavenging of the DPPH• radical assay.

Isoflavin Beta® extract. 100 mg (accurate weighted) of the dry extract was stirred with 80% ethanol for 15 min. The ethanol suspensions were centrifuged at 1660g for 10min and supernatant fractions collected. The precipitate was again extracted with 5 mL of 80% ethanol. Finally, the supernatant fractions were combined and the volume adjusted to 25 mL with deionized water obtaining the ethanol extracts. Then, this solution was diluted with the medium of each reaction. The final concentration range in the reaction medium was 12.5-300.0 μg mL^{-1} for the inhibition of lipid peroxidation, and 31.2-500.0 μg mL^{-1} for the scavenging of the DPPH• radical assay.

Quercetin. 10 mg of quercetin was first solubilized and diluted with propylene glycol rending the final concentration range in the reaction medium of 0.04-2.5 μg mL^{-1} for the inhibition of lipid peroxidation. For the scavenging of the DPPH• radical assay, 10 mg of quercetin was solubilized and diluted with methanol, rending the final concentration range in medium of 0.1-10 μg mL^{-1}.

Inhibition of lipid peroxidation assay

10 μL of each sample was added to 1 mL of a reaction medium containing KCl (130 mM) and Tris-HCl (10 mM), (pH 7.4), and mitochondria was added to yield a final concentration of 1 mg of protein. Then, plus 50 μM ferrous ammonium sulfate and 2 mM sodium citrate was added and the samples were incubated at 37 °C for 30 mins. Mitochondria was isolated by differential centrifugation from livers of male Wistar rats as described for Pedersen *et al.* [14] and mitochondrial protein content was determined by the biuret reaction [15]. For TBA-reactive compound determination, 1 mL of 1% thiobarbituric acid (TBA) (prepared in 50 mM NaOH), 0.1 mL of NaOH (10 M) and 0.5 mL of H_3PO_4 (20%) were added, followed by incubation for 20 min. at 80 °C. The TBA-reactive compounds were extracted with 2 mL of n-butanol. The samples were then centrifuged at 9.800 *g* for 10 min. The measurement was performed on the supernatant at 535 nm, with a Hitachi spectrophotometer U-2001 [16,17]. The experiments were performed in triplicate.

Scavenging of the DPPHü radical assay

The scavenging activity of the DPPH• free radical was performed as described by Blois [18], modified. In a tube it was additioned 1 mL of sodium acetate buffer (100 mM) pH 5.5, 1 mL of ethyl alcohol 95%, 100 μL of sample and 500 μL DPPH• alcoholic solution (250 μM). The reaction was incubed for 10 min at 25 °C. The DPPH• reduction was determined from the change in absorbance measured at 517 nm (spectrophotometer Hitachi U2001). The blank was prepared from the reaction mixture without DPPH• solution. All measurements were performed in triplicate.

Evaluation of the functional activity of green propolis extracts by using the scavenging of the DPPH• radical assay and the standard quercetin

Some green propolis extracts were obtained from Brazilian market (Bioessens - Cotia -São Paulo or Oficina das Ervas - Ribeirão Preto - São Paulo) and were evaluated in the scavenging of the DPPH• radical assay in order to estimate

their activity as quercetin equivalents. In this study, we evaluated fluid extracts (Samples 1-5) and also a dried extract. The dried extract was extracted with hydroalcoholic solution 80% (Sample 6) or hydroalcoholic solution 50% (Sample 7) in order to choose the best solubilizing solvent. The extraction procedure was performed by agitating the suspensions formed in vortex (one min), then they were kept in ultrasound bath for 10 mins, agitated again in vortex for more one min and centrifuged to 1660 g for 5 min. These solutions and the fluid extracts were then diluted using hydroalcoholic solution 80% and assayed in the scavenging of the DPPH• radical assay. Quercetin was prepared as previously described. The final concentrations obtained in the reaction medium ranged from 40 to 320 μg mL^{-1}.

RESULTS AND DISCUSSION

Many research groups are examining the chemical nature and activity of natural antioxidants in fruits, vegetables, grains, herbs and other foods. Most antioxidants isolated from higher plants are polyphenols, which show biological activity as antibacterial, anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic, and immune-stimulating effects [3]. The antioxidant activity of phenolics is mainly due to their redox properties which allow them to act inhibiting chemiluminescence reactions [11,19], breaking the chain reaction of lipid and scavenging several free radicals [20], etc. Typical examples of polyphenols in order of complexity are hydroxyl stilbenes, such as resveratrol, oligomeric catechol structures based on caffeic acid moieties, the large group of flavonoids, monomeric and oligomeric flavan-3-ols and hydroxilyzable tannins (Fig. 1) [20].

The Folin-Ciocalteau method and the $AlCl_3$ coloration to determine the total polyphenol and flavonoid contents, respectively, are currently used to analyze plants and food materials, therefore, they were applied to evaluate the natural extracts in this study. The content of polyphenol and flavonoid present in the marketed extracts are shown in Table 1. It was observed that the polyphenol content ranged from 13.3 to 205.5 mg g^{-1}, while the flavonoid content ranged from 2.27 to 66.3 mg g^{-1}. This large concentration range observed in the composition of the marketed extracts may be completely acceptable due to several points: they were obtained from several sources, they were extracted by different manners, rending different content of dry weight and they were also marketed in different physical forms (powder or liquid).

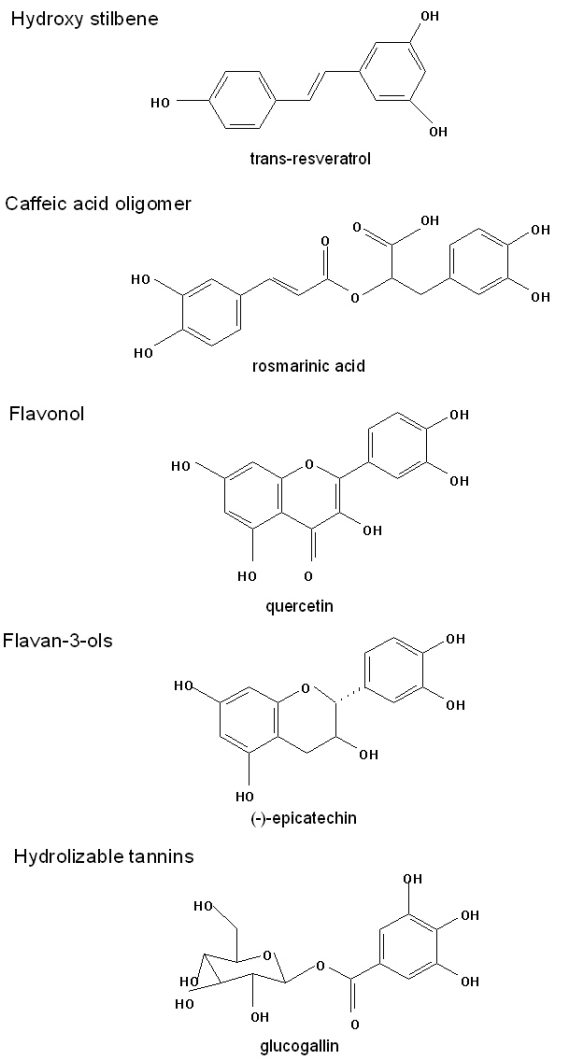

Figure 1. Typical polyphenols of various structural classes.

	Polyphenol content (mg g^{-1})	Flavonoid content (mg g^{-1})
BPE	13.30 ± 0.15	4.70 ±0.52
GPE	17.84 ± 0.32	2.27 ± 0.03
Ginkgo biloba	205.5 ± 5.00	66.3 ± 0.00
Isoflavin Beta®	75.0 ± 0.00	6.8 ± 0.00

Table 1. Flavonoid and polyphenol content in the extracts.The results represent the mean of 3 determinations ± Standard deviation.

Despite the importance of the characterization of polyphenol and flavonoid content in the samples, it is noteworthy that in natural extracts, which are a mixture of different polyphenols and flavonoids, the antioxidant activity may not be directly related to these chemical com-

pounds. For example, two different polyphenols can react with the Folin-Ciocalteau reagent forming products with similar absorbencies, but with very different antioxidant activities. Therefore, the evaluation of the antioxidant activity seems a very useful tool to evaluate the functional quality of marketed natural extracts too.

In order to evaluate the antioxidant activity of the samples, they were evaluated against several free radicals by two different methods. The antioxidant activity of the marketed extracts (Brown and Green propolis, *Ginkgo biloba,* Isoflavin Beta) and of quercetin were evaluated in the inhibition of the lipid peroxidation in the mitochondria/Fe^{+2} system and in the scavenging of the DPPH• radical. Quercetin was used as a standard antioxidant in order to estimate the activity of each extract as quercetin equivalent.

Based on the measurement of the inhibition of both systems assessed for brown propolis extract, green propolis extract, *Ginkgo biloba* extract, Isoflavin Beta® extract and quercetin (Fig. 2), we estimated the relative - inhibitory activity of each sample tested at different concentrations. The percentage of inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = 100 - \frac{100 \times A_S}{A_0} \quad \textbf{Equation 1}$$

Where A_S is the absorbance observed when experimental sample was added, and A_0 the absorbance of the positive control (sample absence).

The percent of inhibition in each antioxidant method was plotted against different concentrations of the extracts examined and the concentration which caused 50% inhibition was taken as the IC_{50} value. The IC_{50} values were determined using GraphPad Prism® software.

The detection and measurement of lipid peroxidation is the evidence most frequently cited to support the involvement of free radical reactions in toxicology and in human disease [21]. For this reason, this method has been used worldwide and due to the presence of mitochondria as lipid source, one can suggests this method represents some physiological conditions.

Lipid peroxidation was assayed by malondialdehyde (MDA) generation [16,17] in the presence of different concentrations of brown and green propolis, *Ginkgo biloba,* Isoflavin Beta® and quercetin. Regarding to lipid peroxidation in-

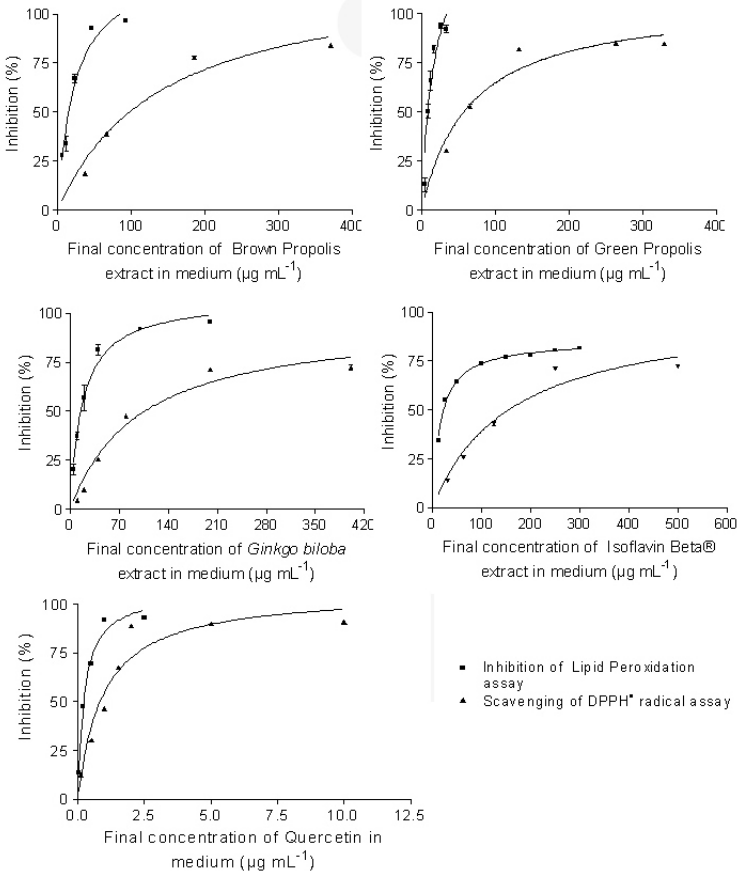

Figure 2. Percentage of inhibition found for different concentrations of Brown Propolis extract, Green Propolis extract, *Ginkgo biloba* extract, Isoflavin Beta® extract and Quercetin in the Lipid Peroxidation assay and in the Scavenging of the DPPH• radical assay. Results are represented by means ± SEM.

	Inhibition of lipid peroxidation assay (IC_{50} µg mL^{-1})	Quercetin equivalent (mg of quercetin . gram of $extract^{-1}$)	Scavenging of the DPPH• radical assay (IC_{50} µg mL^{-1})	Quercetin equivalent (mg of quercetin gram of $extract^{-1}$)
BPE	15.00	14.33	98.00	8.51
GPE	8.40	25.60	57.00	14.63
Ginkgo biloba	17.50	12.29	106.14	7.86
Isoflavin Beta®	22.72	9.46	155.28	5.37
Quercetin	0.21	-	0.83	-

Table 2. IC_{50} values found for the Inhibition of lipid peroxidation assay and for the scavenging of the DPPH• radical assay and estimation of quercetin equivalent.

duced by Fe^{2+}/ascorbate, the initiation of peroxidation sequence of a membrane in a free lipid peroxide system refers to the attack of any species that has sufficient reactivity to abstract a hydrogen atom from a polyunsaturated fatty acid.

Considering that membrane fractions (mitochondria) isolated from disrupted cells, as used in this experiment, may contain some lipid peroxides, which are formed enzymatically in tissues by cyclooxygenase and lypoxygenase enzymes when iron salts are added, these lipid peroxides can be decomposed to generate peroxyl (LOO•) and alkoxyl (LO•) radicals that can abstract hydrogen from polyunsaturated acyl chains and propagate lipid peroxidation [21]. The peroxides breaking down will produce carbonyl compounds known as TBA-reactive compounds, such as malondialdehyde (MDA), which forms a characteristic chromogenic adduct with 2 molecules of thiobarbituric acid (TBA), that is evaluated in 535nm.

It was found that all the extracts evaluated and also the quercetin standard showed antioxidant activity in a dose-dependent manner, as it can be seen in Fig. 2. The IC_{50} values can be seen in Table 2.

In this method we can observe that quercetin showed the lower IC_{50} (0.21 µg mL^{-1}), and among the extracts, the green propolis showed the lower IC_{50} (8.40 µg mL^{-1}). The antioxidant activity of the extracts was expressed in quercetin equivalents by comparing the IC_{50} of the natural extracts with IC_{50} of the quercetin standard (Table 2). We observed that one gram of extract range from 9.46 to 25.60 mg of quercetin. One gram of green propolis extract is equivalent to 25.60 mg of pure quercetin in terms of inhibiting lipid peroxidation.

The scavenging activity of the DPPH• radical is a very simple method, which is currently used in laboratory, due to its rapidity and low costs. DPPH• is a stable free radical that potentially reacts with the compounds able to donate H^+. In this method, it is prepared the positive control without the samples, and it indicates the maximum odd electrons of DPPH•, which was considered 100% of free radicals in the solution to calculate inhibition (%) in the system caused by the samples.

It was also found that all the extracts evaluated and also the quercetin standard showed antioxidant activity in this method, in a dose-dependent manner, as it can be seen in Fig. 2. The IC_{50} values can be seen in Table 2.

In this method we can observe that quercetin showed again the lower IC_{50} (0.83 µg mL^{-1}), and among the extracts, the green propolis showed the lower IC_{50} (57.00 µg mL^{-1}). Regarding to the quercetin equivalent, we observed that one gram of extract ranged from 5.37 to 14.63 mg of quercetin. So, one gram of green propolis extract is equivalent to 14.63 mg of pure quercetin in terms of scavenging DPPH• radical.

In order to correlate the polyphenol and flavonoid content with the antioxidant methods, a regression model was used. The correlation between polyphenol or flavonoid content with the IC_{50} values from both the Lipid peroxidation and DPPH• assays presented $r^2 \leq 0.15$. So, none correlation was observed when relating the phenolic composition and the antioxidant activity. As already mentioned, it is well known that the antioxidant properties of single compounds within a group can vary remarkably so that the same levels of phenolics and flavonoids do not necessarily correspond to the same antioxidant responses. Moreover, the response of phenolics in the Folin-Ciocalteau assay also depends on their chemical structure, and the radical-scavenging capacity of an extract cannot be predict-

	Sample 1 [a]	Sample 2 [a]	Sample 3 [a]	Sample 4 [a]	Sample 5 [a]	Sample 6 [b]	Sample 7 [b]
Scavenging of the DPPH• radical assay (IC50 μg mL^{-1})	57.0	87.9	65.3	38.1	70.	70.0	106.0
Quercetin equivalent (mg of quercetin gram of extract^{-1})	14.6	9.4	12.7	21.8	11.9	11.9	7.8

Table 3. Evaluation of the antioxidant activity of several green propolis extracts by the scavenging of the DPPH• radical assay and estimation of quercetin equivalent. [a] Fluid extract. [b] Dry extract extracted with hydroalcoholic solutions (80 or 50%).

ed on the basis of its polyphenolic content [3]. However, a good significant correlation coefficient (r^2 = 0.93) was observed between the polyphenol and flavonoid content, which is not a surprising result, since flavonoid is a class of chemical compounds present in the polyphenolic group.

On the other hand, a high significant correlation coefficient (r^2 = 0.9913) was observed between the IC_{50} values of the lipid peroxidation and the DPPH• methods evaluating not only all the extracts, but also quercetin (Fig. 3). A high correlation using the antioxidant activity was also observed by Atoui and collaborators [3]. This group found a correlation coefficient of r^2 = 0.97 between the DPPH• and a chemiluminescent method in the evaluation of several herbs.

So, knowing that the DPPH• assay is a fast method, presents low costs and even has a good correlation with lipid peroxidation and chemiluminescent methods, we can suggest that this method could be properly applied in the future as an additional parameter in the quality control of natural extracts, which are supposed to have antioxidant activity. Moreover, the precision of this method was also evaluated using the concentrations which presented 50% of the system inhibition, showing values lower than the literature recommendations [22]. We observed 1.66% within-assay precision and 1.92% between-day repeatability for *Ginkgo biloba* extract, 2.16% within-assay precision and 4.90% between-day repeatability for Isoflavin Beta® extract, 9.54% within-assay precision and 3.63% between-day repeatability for brown and green propolis extracts, 2.74% within-assay precision and 5.14% between-day repeatability for quercetin.

The concept of functional quality of natural extracts and medicines based on their biological activity has already been reported for some researchers for the evaluation of propolis extracts [23,24] and for the evaluation of food [5]. Notewor-

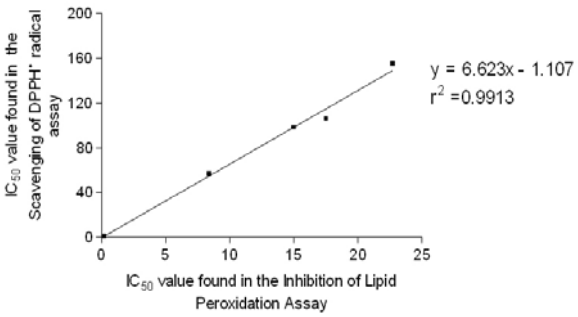

Figure 3. Correlation observed between the IC_{50} values found in the Inhibition of Lipid Peroxidation and the Scavenging of DPPH• radical assays. For this evaluation it was used the IC_{50} values of Brown propolis, Green propolis, Isoflavin Beta® and *Ginkgo biloba* extracts and Quercetin.

thy, the characterization of such material must also be undertaken, e.g. phenolic and flavonoid content.

The evaluation of the functional quality by using the DPPH• method could also be a good tool to standardize natural extracts by using quercetin as standard. So, the applicability of this method was assessed by evaluating several samples of marketed Green propolis extracts and estimating them as quercetin equivalents. The results are shown in Table 3. We observed that one gram of the fluid extract ranged from 9.4 to 21.8 mg of quercetin. Regarding the dried extract, we observed that the extraction with 80% hydroalcoholic solution (Sample 6) was able to reach the same antioxidant activity as one of the fluid extracts (Sample 5), suggesting that this solvent is the best one to solubilize the antioxidant compounds of this extract. The data obtained in this study show that the green propolis extracts available in the Brazilian market may present different antioxidant activities, suggesting that these extracts should be used in different concentrations when added to pharmaceutical formulations.

CONCLUSION

The scavenging of the DPPH• free radical method presents a lot of advantages, such as rapidity, low cost, good precision, good correlation with other antioxidant methods, so it could be properly applied as an additional parameter to attain the functional quality of natural extracts. In addition, we observed that quercetin could be used as standard to lead to the standardization of these extracts. So, besides performing the characterization of these extracts by evaluating the polyphenol, flavonoid and other chemical compounds, the antioxidant activity by the DPPH• method could be employed.

With this kind of standardization and with the implementation of regulatory practices worldwide, it would be easier the establishment of natural extracts supposed to functionalize formulations to treat and prevent the human damages occurring due to free radicals and also to replace synthetic antioxidants in industry. In addition, we would have less product quality differences and the finished products would yield the same therapeutically effectiveness.

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