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Green and roasted mate: phenolic profile and antioxidant activity

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Abstract: Mate infusions have been recently and frequently used as dietary supplements because of their health effects to prevent atherosclerosis and coronary heart disease and to be antioxidant. The aim of this study was to determine phenolic compounds and antioxidant activities of green mate (GM) and roasted mate (RM) extracts and compare them with green tea (GT). Total polyphenol content of GM and RM extracts was found to be similar and to range from 237.20 to 438.50 mg gallic acid equivalent (GAE) g^{-1} dry extract and from 256.72 to 473.11 mg GAE g^{-1} dry extract depending on the solvent used and type of fraction, respectively. Polyphenol yield of GM and RM extracts were in the range of 20.94-101.72 mg GAE g^{-1} dw leaf and 22.08-74.56 mg GAE g^{-1} dw leaf, respectively. GM and RM extracts exhibited similar antioxidant activity when measured by reducing power. However, RM extracts had significantly higher protection factor (PF) values determined by rancimat assay and radical scavenging activity (RSA) determined by DPPH radical scavenging compared to GM extracts. In general, RM extracts had higher antioxidant activity than GM extracts although both mate leaves were found to have similar phenolic profiles and contain mainly chlorogenic acid and isomers as a phenolic matter.

Key words: Mate, green tea, antioxidant, phenolic, HPLC

Yeşil ve kavrulmuş mate: fenolik profili ve antioksidatif aktivitesi

Özet: Mate infüzyonları son zamanlarda kalp ve damar rahatsızlıklarını önleyici ve antioksidan etkileri nedeniyle besin desteği olarak sıklıkla kullanılmaktadır. Bu çalışmanın amacı, yeşil mate (GM) ve kavrulmuş mate (RM) ekstraktlarının antioksidan aktivitesini ve fenolik bileşiklerini belirlemek ve ulaşılan bulguları yeşil çayla karşılaştırmaktır. GM ve RM ekstraktlarının toplam polifenol içeriği birbirine yakın bulunmuş olup, kullanılan solvent ve fraksiyon tipine bağlı olarak 1 g kuru ekstrakt içinde gallik asit eşdeğeri (GAE) olarak sırasıyla, 237.20 - 438.50 mg ve 256.72 - 473.11 mg bulunmuştur. GM ve RM ekstraktlarının polifenol verimleri ise 1 g kuru ağırlık içinde GAE olarak sırasıyla, 20.94-101.72 mg GAE ve 22.08-74.56 mg değerleri arasında değişim göstermiştir. GM ve RM ekstraktları, indirgeme gücü yöntemine göre benzer antioksidan aktivite göstermişlerdir. Ancak, RM ekstraktlarının, ransimat testi ile belirlenen koruma faktörü (PF) değerleri ve DPPH yöntemiyle belirlenen radikal giderme kapasitesi değerleri GM ekstraktları ile karşılaştırıldığında daha yüksek bulunmuştur. Her iki mate yaprağının benzer fenolik içeriğe sahip olmasına ve başlıca fenolik madde olarak klorojenik asit ve izomerlerini içermesine rağmen, RM ekstraktları GM ekstraktlarından genellikle daha yüksek antioksidan aktivite göstermiştir.

Anahtar sözcükler: Mate, yeşil çay, antioksidan, fenolik, HPLC

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Introduction

Mate (*Ilex paraguariensis*) tea is widely consumed for its peculiar flavour and stimulating properties (Filip et al., 2001) all around the world, especially in South America. GM is obtained after the processes of scorching, crushing and drying of leaves and stems (Bastos et al., 2006) whereas RM is obtained by the roasting of green mate leaves (Kawakami and Kobayashi, 1991; Clouatre, 2004). Mate infusions have been shown to inhibit the peroxidation of isolated human low density lipoproteins (LDL) (Gugliucci and Stahl, 1995), hence their use has recently been proposed as a dietary supplement for the prevention of clinical expression of atherosclerosis and coronary heart disease (Carini et al., 1998). Antioxidative properties have also been found in mate tea (Chandra and Gonzalez de Mejia, 2004; Bravo et al., 2007). These have been attributed to the fact that mate leaves contain many bioactive compounds, such as phenolic acids, which seem to be responsible for the antioxidant activity of GM infusions in both vivo and vitro (Bastos et al., 2006). It is reported that mate tea contains a significant amount of chlorogenic acids (9.8%) (Carini et al., 1998).

The antioxidant activity of phenolics is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Osman et al., 2004). These methods are based on different chemical and physical principles in monitoring oxidation. Thus, the activity of antioxidants may vary according to the assay used (Schwarz et al., 2001).

Demand for natural antioxidants has increased due to consumer concerns about the safety of synthetic antioxidants in food (Anagnostopoulou et al., 2006; Sun and Ho, 2005). For this reason, the emergence of natural extracts possessing antioxidation properties will help in reducing the current dependency on synthetic antioxidants in food applications. Some natural antioxidants, including buckwheat, green and black tea, rosemary, oregano, thyme and rice koji extracts, were reported to be effective against lipid oxidation in edible fat and oils (Simandi et al., 2001; Gramza et al., 2006; Sun and Ho, 2005), frying and storage of fried products (Houhoula et al., 2004) and meat products (Tang et al., 2001; Lin and Lin, 2005). On the other hand, in the literature, there have been some reports on antioxidant activity of mate (Chandra and Gonzalez de Mejia, 2004; Bastos et al., 2006; Bravo et al., 2007), but there hasn't been not enough information on comparison of antioxidant activities of GM and RM polyphenols. For this reason, the objectives of this study were to estimate polyphenol contents, to evaluate antioxidant activities and to determine phenolic profiles of GM and RM extracts. For a more reliable assessment, antioxidant activities of tea extracts were evaluated by applying three different methods (DPPH radical scavenging assay, reducing power and rancimat assay). Phenolic contents and compositions and antioxidant activities of mate extracts were further compared with GT, a well known antioxidant.

Materials and methods

Plant materials

GM and RM (both are Brazilian originated and same brand) were purchased from a local market in Sydney, Australia. GT was purchased from a local market in Ankara, Turkey. Mate and green tea leaves were ground using a coffee grinder and sieved to obtain a particle size of 150-300 μm and stored at + 4 °C until they are used.

Chemicals

Folin-Ciocalteu's reagent, (+)-Catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-) epigallocatechingallate (EGCG), (-)- epicatechin-gallate (ECG), (-)-catechingallate(CG), (+)-gallo-catechin (GC), chlorogenic acid (5-O-caffeoyl-quinic acid), DPPH (2,2-diphenyl-1-picryhydrazyl) and TCA (trichloroacetic acid) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Caffeine, theobromine and quercetin-3-glucoside (Q3G) were purchased from Fluka (Biochemica-Fluka Cheme-Switzerland). Rutin (Quercetin 3-rhamnoglucoside, Q3RG) was purchased from Wako (Pure Chem. Co., Japan) and kaempferol 3-rhamnoglucoside (K3RG) was purchased from Chromadex (Santa Ana, USA). All other chemicals and solvents were either HPLC or analytical grade.

Extraction of polyphenol

Extracts from mate and green tea leaves were prepared using 80% methanol as a solvent. The

ground dried sample was mixed with the solvent in the ratio of 1:50 (w/v) and extracted for 2 h on an orbital shaker (Biosan, OS-10) at room temperature in the dark. After extraction, the mixture was filtered through Whatman No.1 and clear filtrate was evaporated to dryness under vacuum at 40 °C to get crude extract. The dry extract was weighed to calculate the yield and dissolved in a mixture acetone:water/ 1:2 (v/v). For HPLC analysis, crude methanolic extracts were further diluted with deionised water and filtered through a 0.45 μm membrane filter before injection.

For antioxidant assays, the crude extracts dissolved in aqueous acetone were fractionated by liquid-liquid extraction with ethyl acetate which has been commonly used for selectively removing and concentrating phenolic compounds from various plants including tea (Larger et al., 1998; Satoh et al., 2005; Anagnostopoulou et al., 2006). In this process, the solution was mixed with an equal volume of ethyl acetate. After shaking on orbital shaker using a separating funnel and phase separation, the organic layer was collected and the water layer was extracted with ethyl acetate for one more time. The combined ethyl acetate solution was dried with anhydrous sodium sulphate, filtered and then evaporated under vacuum to dryness. The dry residue was weighed to determine the yield, dissolved in acetone to a concentration of 5 mg mL^{-1} and then stored in amber glass in a freezer for subsequent analysis. The whole process was carried out in triplicate. The extraction yield was calculated gravimetrically.

Total polyphenol determination

Total polyphenols were determined applying the Folin-Ciocalteu method (Obanda and Owuor, 1997). Results were expressed as gallic acid equivalent (GAE). In this method, 1 mL of tea extract diluted with deionised water to a concentration of 100 mg mL^{-1} (to obtain absorbance in the range of the prepared calibration curve) was mixed with 1 mL of 3-fold-diluted Folin-Ciocalteu phenol reagent. 2 mL of 35% sodium carbonate solution was added to the mixture, shaken thoroughly and diluted to 6 mL by adding 2 mL of water. The mixture was allowed to stand for 30 min and blue color formed was measured at 700 nm using a spectrophotometer (Shimadzu UV-VIS 1601).

Antioxidant assays

In the present study, the antioxidant activity of mate and tea extracts was evaluated in terms of radical scavenging activity, reducing power and rancimat assay.

DPPH radical scavenging activity

The radical scavenging activity of tea samples was measured by using the DPPH assay (Katalinić et al., 2004; Atoui et al., 2005) with some modifications. 50 mL of tea extract (50-300 mg mL^{-1}) was mixed with an aliquot of 1950 mL of 6×10^{-5} M DPPH radical in methanol. The reaction mixture was vortexed and let to stand at 25 °C in the dark for 60 min. The decrease in the absorbance at 517 nm was determined with the help of a spectrophotometer using methanol as blank. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation:

$$\%RSA = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where $Abs_{control}$ is the absorbance of the DPPH solution without sample and $Abs_{control}$ is the absorbance of the test sample.

Reducing power

Reducing power of the samples was determined according to the Yuan et al. (2005) with slight modifications. 0.5 mL of the leaf extract (50-300 mg mL^{-1}) was mixed with 1.25 mL phosphate buffer (0.2 M, pH 6.6) and 1.25 mL potassium ferricyanide (1%) and the mixture was incubated at 50 °C in a water bath for 20 min. Samples were then cooled and mixed with 1.25 mL of 10% TCA. Afterwards, 1.25 mL of sample aliquot was mixed with 1.25 mL distilled water and 0.25 mL of 0.1% ferric chloride and then left to react at room temperature for 10 min. Sample absorbances were read at 700 nm. Increase in the absorbance (A) of the reaction mixture indicated an increase in the reducing power.

Rancimat assay

Rancimat assay to assess the antioxidant activity of the samples was carried out using the Rancimat 743 (Metrohm, Herisau- Switzerland) according to AOCS (1989). The extracts dissolved in acetone were

individually added to refined sunflower seed oil. Free fatty acid as oleic acid: 0.16%, peroxide value: 8.62 meqO_2 kg^{-1}oil, fatty acid composition: 9.34% saturated, 27.45% mono-unsaturated, 63.21% poly-unsaturated) to give final concentrations of 0.05-0.35% and 0.03% (w/w), respectively. In the control of sunflower oil, a representative quantity of pure acetone was added. The oxidation process was followed in 3 ± 0.0001 g of oil samples at 110 °C and the air velocity of 20 L h^{-1}. Induction periods, IP (h), of the sunflower oil samples were recorded automatically and the relative activity of the extracts was expressed by the protection factor (PF) calculated by dividing the induction period of sunflower oil with added extract by the induction period of the control oil sample.

Phenolic profile determination of the tea extracts

The identification of individual phenolic compounds in the extracts of the leaves was performed on a HPLC system which was equipped with: LC-10 ADVP Shimadzu pumps, a CTO-10 ASVP column oven and an SPD-MIOA VP photo diode array (PDA) detector, a Shimadzu DGU-14A degasser and SLC-10 A VP system controller. A computer-controlled system with Class-VP software was employed in order for data analysis. The column used was a C_{18} reversed phase Teknokroma Extrasil ODS2 (250 × 4.6mm ID, 5 µm) and was operated at 40 °C. UV spectra were recorded from 190-370 nm and peak areas were measured at 325 nm. The two mobile phases used for gradient HPLC elution were (A) 0.1% orthophosphoric acid in water (w/v) and (B) acetonitrile. The gradient elution profile was 8% B (isocratic) for 5 min. B was gradually increased to 11% at 40 min, to 18% at 62 min and to 23% at 88 min. The column (C_{18} reversed phase Teknokroma Extrasil ODS2, 250 × 4.6mm ID, 5 µm) was re-equilibrated with the initial conditions for 15 min before the next injection. The flow rate was 1.0 mL min^{-1}. The injection volume was 20 µL.

Chromatographic peaks in the samples were identified by comparing their retention times and UV spectra with those of their reference standards and by co-chromatography with added standards. Some of the phenolic acids were identified only by polarity and spectral data from literature. In order to obtain reference compounds for the identification of caffeoylquinic acid derivatives, namely neo- and cryptochlorogenic acid isomerization of pure chlorogenic acid was performed as described previously (Fritshe et al., 2002). Quantification was performed from the peak area of each component and its corresponding calibration curve. Caffeoylquinic acid derivatives were quantified as chlorogenic acid. GT extract was analysed according to the method described by Turkmen and Velioglu (2007).

Data analyses

Statistical analysis was conducted with SPSS for Windows (ver.10.1) and experimental results were expressed as means ± standard errors of triplicate measurements. Analysis of variance was performed by one-way ANOVA procedure. Significant differences between means were determined by Duncan's multiple range test. Differences were considered significant at $P < 0.05$.

Results

As shown in Table 1, total polyphenol content of GM and RM extracts were similar and they were in the range of 237.20-423.88 mg GAE g^{-1}dry extract and 256.72-473.11 mg GAE g^{-1} dry extract, respectively, depending on the fractions obtained. Polyphenol yield of ethyl acetate fractions from GM (20.94 mg GAE g^{-1}dw leaf) and RM (22.08 mg GAE g^{-1}dw leaf) was comparable as a result of their similar extraction yields. However, polyphenol yield of crude extracts from GM (101.72 mg GAE g^{-1}dw leaf) had significantly higher values than those from RM (74.56 mg GAE g^{-1}dw leaf) because of the higher extraction yield. For both mate leaves, the higher phenolic content was detected in the ethyl acetate fractions (423.88- 473.11 mg GAE g^{-1}dry extract) compared to crude extracts (237.20-256.72 mg GAE g^{-1}dry extract).

According to the results shown in Figure 1, the scavenging activity of mate extracts on DPPH radical increased with increasing concentration in the range of 0.05-0.3 mg mL^{-1}. Statistical analysis showed that RM extracts (14.19-79.47%) had higher RSA compared to GM extracts (11.53-61.05%) in the concentration range used in this study. Compared with that of green tea, the RSA of mate extracts was lower in correspondence with their low polyphenol

Table 1. Total polyphenol content (mg GAE g^{-1} dry extract), polyphenol yield (mg GAE g^{-1} dw leaf) and extraction yield (%) of different fractions from green and roasted mate and green tea.

Parameter	Fraction	Green Mate	Roasted Mate	Green Tea
Polyphenol content	Crude	237.20 ± 4.02[a*]	256.72 ± 7.05[a]	367.95 ± 5.84[b]
	Ethyl acetate	423.88 ± 9.10[a]	473.11 ± 8.60[b]	580.96 ± 6.36[c]
Polyphenol yield	Crude	101.72 ± 3.63[b]	74.56 ± 2.37[a]	136.73 ± 2.54[c]
	Ethyl acetate	20.94 ± 0.67[a]	22.08 ± 1.14[a]	101.06 ± 3.10[b]
Extraction yield	Crude	42.86 ± 0.92[c]	29.11 ± 1.48[a]	37.16 ± 0.21[b]
	Ethyl acetate	4.95 ± 0.08[a]	4.66 ± 0.17[a]	17.40 ± 0.63[b]

* Means sharing different letter (a-c) in the same row are significantly different (P < 0.05) according to Duncan's test.

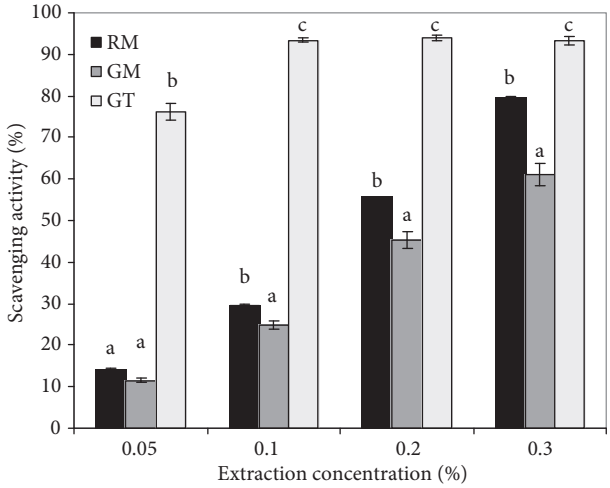

Figure 1. Scavenging activity for DPPH free radicals of GM, RM and GT extracts.*

* Bars with different letters are significantly different at P < 0.05 level, according to Duncan's test.

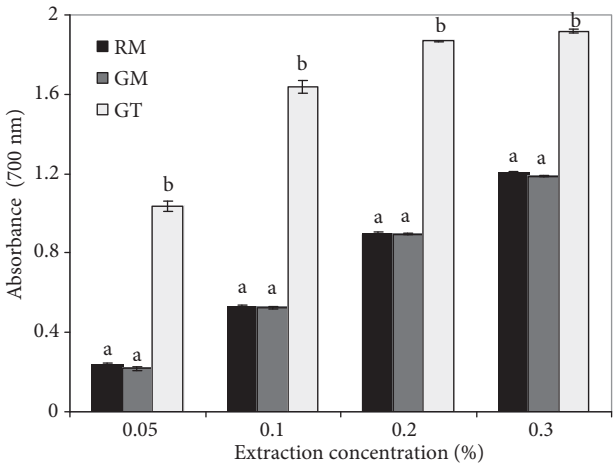

Figure 2. Reducing power of GM, RM and GT extracts.*

* Bars with different letters are significantly different at P < 0.05 level, according to Duncan's test.

content. Reducing power of mate extracts were also increased along with the increased concentrations (Figure 2).

The antioxidant activity of mate extracts determined by the Rancimat method was evaluated on sunflower oil, in comparison with GT extract. The effectiveness of mate extracts in sunflower oil, measured as PF, ranged between 1.13 and 1.96. RM extracts had greater activity (P < 0.05) to suppress oil oxidation at all concentrations compared to GM extracts, indicating that RM extracts have the best response in rancimat assay. By increasing the concentration, protection factor of RM extracts increased markedly and reached value which was about 1.9 times control. However, in the case of GM extracts, a slow increase in the ratio of protection factor was observed (Figure 3). Furthermore, the concentration of 0.35% resulted in decreased PF, indicating that GM extract may act as a pro-oxidant instead of antioxidant at a higher concentration.

Individual phenolic compounds in mate extracts are shown in Table 2. Figure 4 shows typical chromatograms of the extracts of mate leaves. As can be seen, the majority of phenolic compounds could efficiently be separated and the UV profile indicated that most constituents, 6 of 9 peaks detected, in mate

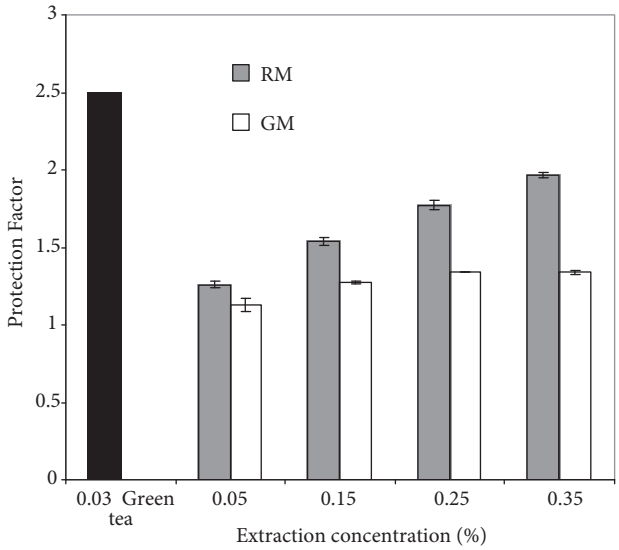

Figure 3. Protection factor (PF) determined by rancimat assay of GM, RM and GT extracts.

extracts showed a similar spectral behaviour with maximum absorbance at 325 nm and a shoulder at 310 nm, characterizing them caffeoylquinic acid derivatives known as phenolic acids. Peaks 2-4 were identified as neochlorogenic acid, chlorogenic acid and cryptochlorogenic acid, respectively. Peaks 7-9 were assigned as unidentified dicaffeoylquinic acid derivatives considering the elution profile of these compounds from mate and other plants reported in the literature (Filip et al., 2001; Yoshimoto et al., 2002; Bravo et al., 2007; Krizman et al., 2007). The only flavonoid determined in both mate leaves was rutin.

Discussion

The extractability of polyphenols from RM was poor (Table 1), which was may be due to the formation of insoluble polyphenol–protein and polyphenol–carbohydrate including cell wall polysaccharide complexes (Siddhuraju, 2006) as a result of processing. It is known that these insoluble compounds have significant antioxidant activities. (Serpen et al., 2007). Bastos et al. (2006) reported that total polyphenol yield of water extracts from GM was higher when compared with RM extracts, which supports our results. Bravo et al. (2007) reported that total polyphenol yield of the extracts of 3 brands of GM was 8.10-9.77%, which is consistent with our results (10.17%).

Both of mate leaves contained higher phenolic content in ethyl acetate fractions (Table 1), which is in accordance with findings of Termentzi et al. (2005) and Anagnostopoulou et al. (2006). Because of the higher total polyphenol content of ethyl acetate fractions, which is approximately 2-fold higher than their respective crude extracts (Table 1), these extracts

Table 2. Individual phenolic compounds (mg g^{-1} dry weight leaf) of the extracts from green and roasted mate and green tea.

Compound	Extracts		
	Green Mate	Roasted Mate	Green Tea
Neochlorogenic acid	23.19	4.82	nd*
Chlorogenic + Cryptochlorogenic acid	19.67	14.73	nd
Total unidentified caffeoyl derivatives	16.50	11.20	nd
Rutin	7.37	0.46	0.76
EGC	nd	nd	25.20
EC	nd	nd	5.62
EGCG	nd	nd	43.40
ECG	nd	nd	18.43
Q3G	nd	nd	0.31
K3RG	nd	nd	0.51
Total individual phenolic compounds	66.73	31.21	94.23

nd : Not detected

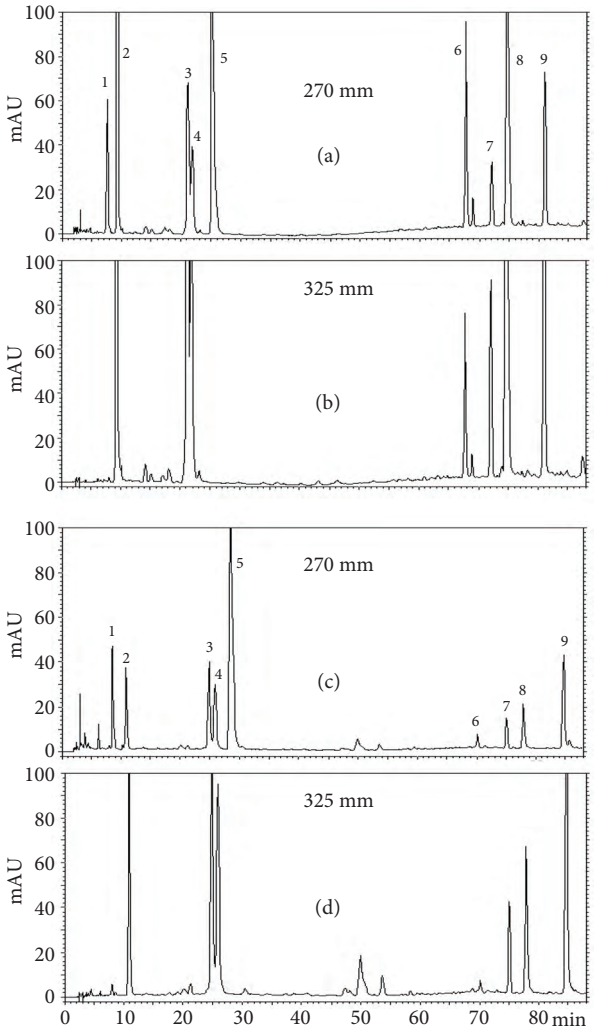

Figure 4. HPLC chromatograms of methanol extracts at 270 and 325 nm from GM (a, b) and RM (c, d), where 1-Theobromine 2-Neochlorogenic acid, 3-Chlorogenic acid, 4-Cryptochlorogenic acid, 5-Caffeine, 6-Rutin, 7, 8 and 9-Unidentified caffeoyl derivatives.

were selected for further determinations. When compared with GT extracts, both GM and RM extracts had significantly lower polyphenol content and yield (Table 1). Similar results were reported by some previous studies (Chandra and Gonzalez de Mejia, 2004; Bravo et al., 2007). On the other hand, the polyphenol content of ethyl acetate fractions from mate was still higher than that from *Sorbus domestica* fruits (Termentzi et al., 2005), sweet orange peel (Anagnostopoulou et al., 2006). For this reason, mate leaves can be considered as a good source of polyphenols.

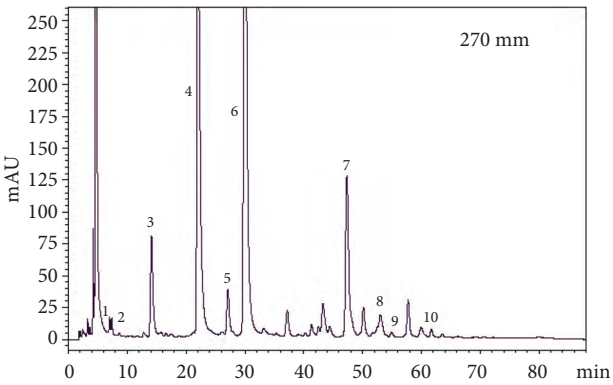

Figure 5. HPLC chromatogram of methanol extract from GT, where 1-GC, 2-Theobromine, 3-EGC, 4-Caffeine, 5-EC, 6-EGCG, 7-ECG, 8-Rutin, 9-Q3G, 10-K3RG.

The RSA of mate extracts (Figure 1) is thought to be due to a synergistic intervention of all the polyphenols, mainly chlorogenic acids, in the extracts (Carini et al., 1998). Because polyphenols possess ideal structural chemistry for free, RSA (Rice-Evans et al., 1997) and their potential scavenging abilities might be due to the active hydrogen donor ability of hydroxyl substitution in the aromatic rings (Siddhuraju, 2006). On the other hand, components other than polyphenols in RM extracts such as maillard reaction products (e.g. pyrazines, pyrroles and furans) having been formed during roasting process (Kawakami and Kobayashi, 1991) may also contribute to their antioxidant activity. These products exhibit strong antioxidant activity (Yanagimoto et al., 2002; Siddhuraju and Becker, 2007) and they have begun to receive much attention as antioxidants recently (Yanagimoto et al., 2004).

On the contrary of our findings on RSA (Figure 1), Bixby et al. (2005) found that *Ilex paraguariensis* extracts displayed higher DPPH radical scavenging activity than green tea and also had higher polyphenol concentration. The differences can be due to differences in the variety of mate and green tea leaves.

At 0.05-0.3 mg mL^{-1}, GM and RM extracts had comparable reducing power (0.22-1.19 and 0.23-1.20, respectively) (Figure 2), which has a direct and a positive correlation with antioxidation properties (Osman et al., 2004). Similarly, Bastos et al. (2006) reported that GM and roasted GM infusions showed comparable antioxidant activities determined by ferric thiocyanate method. Reducing power of mate extracts

can be attributed to their phenolic compounds due to the fact that chlorogenic acid possesses iron-reducing ability (Psarra et al., 2002; Makris et al., 2003). Previous studies (Clifford, 1990; Filip et al., 2001) and this study showed that mate leaves contained high content of chlorogenic acids. Consistent with DPPH radical scavenging (Figure 1), green tea had the highest reducing power. Other authors also reported that antioxidant activity of mate leaves was lower than that of green tea using FRAP (Bravo et al., 2007) and oxygen radical absorbance capacity (ORAC) assay (Chandra and Gonzalez de Mejia, 2004). The PF in the range of 1.13-1.96 indicated that mate extracts had inhibitory effects against lipid peroxidation in the oil sample with regard to the control because a PF higher than 1.0 suggests better protection from autoxidation (Gramza et al., 2006) and the effects increased with increasing concentration in the range of 0.05-0.35%. However, GT extract showed the strongest inhibitory activity with PF value of 2.5 at a rather low concentration (0.03%).

Stronger suppression of RM extracts on sunflower oil was in close agreement with that measured from RSA (Figure 1) but not reducing power (Figure 2). It can be due to the nature of the assays and the variety of radicals applied (Sun and Ho, 2005). Yu et al. (2002) showed that 3 different methods to measure antioxidant activity provided different rankings of the antioxidant activity of wheat extracts, which supports our results. Similarly, in the study by Schwarz et al. (2001), ranking antioxidant activities within the different extract groups resulted in different trends depending on the assay (Oxygen consumption, Conjugated dienes and Rancimat) used. Polyphenols of GM and RM can contribute to their antioxidant activity by Rancimat assay because of the fact that the antioxidant activity of tea extracts in sunflower oil strongly correlated with the total polyphenol content ($r = 0.74$) (Gramza et al., 2006). Fritshe et al. (2002) also reported that individual artichoke compounds -chlorogenic acid, cynarin, luteolin, luteolin-7-O-glucoside- showed a remarkable antioxidant activity by Rancimat method.

The relationship between concentration of extract and increased protection factor (Figure 3) was also reported by Yu et al. (2002), who studied antioxidant properties of extracts from 3 different wheat varieties by means of different methods. The researchers also found that the only Platte variety showed a pro-oxidative effect at a higher extract concentration in rancimat assay. In addition, it was reported that in low concentrations allicin can be responsible for the antioxidative properties of garlic, although it can also act as a pro-oxidant in high concentrations (Fernández-López et al., 2005).

The results on phenolic profiles (Table 2, Figure 4) obtained from our study are in accordance with the previous reports (Carini et al., 1998; Filip et al., 2001; Pomilio et al., 2002; Filip and Ferraro, 2003; Bravo et al., 2007). The same individual phenolic compounds were found in GM and RM extracts but their concentrations were different in both extracts (Table 2). The amount of total individual phenolics determined was almost 2 times higher in GM (66.73 mg g^{-1}dw leaf) compared with RM(31.21 mg g^{-1} dw leaf), which is consistent with the pattern observed in total polyphenol yield of crude extracts. Similarly, Clifford (1990) reported that green mate and roasted mate provided 107-133 and 16-41 mg chlorogenic acids per approx. 200 mL, respectively.

On the basis of the results of this study, phenolic composition of mate leaves is different from that of GT. It is clear that the main phenolics detected in both mate extracts were chlorogenic acid isomers whereas those in GT were catechins, with EGCG as the major compound (Figures 4 and 5). Similarly, Bixby et al. (2005) reported that HPLC polyphenol fractions differed significantly between green tea and *Ilex paraguariensis* (Ip) extracts and Ip contained high levels of chlorogenic acid which were low or absent in green tea. The content of total individual phenolics in GT extract was higher than those in mate extracts as observed in total polyphenol. For all samples, the lower amount of total individual phenolics compared to total polyphenols could be due to the presence of other phenolics which was not accounted for in HPLC analyses, but detected by Folin-Ciocalteu reagent.

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