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Note

Total Polyphenol, Antioxidant and Antibacterial Activities of Black Mate Tea

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Black mate tea was extracted with different 50% solvents (acetone, N,N-dimethyl formamide (DMF), ethanol and methanol) for 2, 8 and 18 h. The extracts were screened for polyphenol content, antioxidant and antibacterial activities. Total polyphenol content of the extracts ranged from 97.01 to 119.28 mg gallic acid equivalent (GAE)/g dry weight (dw) tea depending on the solvent used and extraction time applied. In general, methanol was the least efficient solvent for polyphenol extraction from black mate tea and the efficiency of the others was found to be similar. All extracts showed antioxidant activity by 2,2-diphenyl-1-picryhydrazyl (DPPH) radical and reducing power. Different trend was observed for each method with respect to solvents used. The extracts possessed antibacterial activity depending on the solvent used and bacterium tested and the results sowed that black mate tea extracts had strong antimicrobial activity against selected bacteria, except for *E. coli* O157: H7. While *S. aureus* was found to be the most sensitive to all extracts, *E. coli* was the most resistant among bacteria tested.

Keywords: black mate tea, extraction, solvent, antioxidant, polyphenol, antibacterial

Introduction

Ilex paraguariensis is widely used for the preparation of the most popular tea-like beverage of South America (Filip *et al.,* 2001). The commercial product made with it, named *Mate* or *Yerba Mate* is recognized worldwide for its nutritional and medicinal value being included in important national food codes and Pharmacopoeias (Anesini *et al.,* 2006). Green mate is obtained after scorching, crushing, and drying leaves and stems, and can be stored for up to 1 year before commercialization, depending on the consumer's preference. Black mate (BM) tea is obtained by the roasting of green mate at 160℃ for approximately 12 min (Bastos *et al.* 2006). Mate tea was first used as a tonic and stimulating drink (Mazzafera, 1997). Nowadays, it is also considered functional food (Zuin *et al.,* 2005) due to its some of the pharmalogical activities such as hepatoprotective, cholerectic, antioxidant and hypocholesteremic (Filip *et al.,* 2001). Gugliucci and Stahl (1995) showed that water extracts of mate were capable of inhibiting the initiation and the propagation of low density lipoprotein (LDL) oxidation. Therefore, their use has been proposed as a dietary supplement for the prevention of the clinical expression of atherosclerosis and coronary heart disease (Carini *et al.,* 1998). Mate tea is also used against mental and physical fatique (owing to the presence of xantines: caffeine and theobromine) (Anesini *et al.,* 2006). This tea contains a significant amount of phenolic compounds, mainly caffeoylquinic acids, such as chlorogenic acid (Clouatre, 2004; Carini *et al.,* 1998; Mazzafera, 1997) and these compounds have many favourable effects on human health such as the lowering of human low-density lipoprotein and reduction of hearth disease and cancer (Baydar *et al.,* 2004) due to their well-known abilities to scavenge free radicals, i.e. antioxidant power (Pinelo *et al.,* 2004). The use of plants and herbs as antioxidants in processed foods is becoming of increasing importance in the food industry as an alternative to synthetic antioxidants (Proestos *et al.,* 2006).

Microbial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety. Currently, there is a growing interest to use natural antibacterial compounds for the preservation of foods (Jayaprakasha *et al.,* 2003).The antimicrobial activity of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds (Dupont *et al.,* 2006).

Although there have been some reports on antioxidant activity of BM tea (Bastos *et al.,* 2006; Turkmen *et al.,* 2005; Mello *et al.,* 2005) little or no information is available on its antimicrobial activity. Therefore, the aim of this study was to clarify not only the antioxidant activity but also the antibacterial activity in BM tea extracts against different food-borne pathogens. In the present study, antioxidant activity of tea extracts was determined by using two methods based on different mechanism. One is DPPH radical scavenging assay most widely used for polyphenol from plant and the other is reducing power which was chosen due to the fact that iron-polyphenol complex was demonstrated to cause the inhibition of formation of oxygen radicals associated with many pathological conditions (Yoshino and Murakami, 1998)

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and also probably growth of some pathogen microorganisms (Chung *et al.,* 1998).

Materials and Methods

Plant materials Brasilian originated BM tea (*Ilex paraguarensis*) samples were purchased from local market in Sydney-Australia. Tea samples were ground to pass a 710 μm screen and stored at + 4℃ before experiments.

Chemicals DMF (min 99.0%), ethanol (min 99.8%) and methanol (min 99.9%) were either analytical or HPLC grade from Fluka (BioChemica-Fluka Cheme GmbH Buchs-Switzerland). Acetone (min 99.5%) was from Aldrich (St. Louis, MO, USA). Folin-Ciocalteu's reagent was from Merck (Darmstadt-Germany). DPPH and TCA (trichloroacetic acid) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were analytical grade and from Merck.

Extraction of tea polyphenol Extracts from BM tea were prepared using aqueous solutions (50%) of methanol, ethanol, DMF, acetone as solvents. The selection of these solvents was because our previous finding showed that 50% solvents were the most efficient for the polyphenol content and antioxidant activity of teas among various solvent systems included water and DMF, acetone, methanol, ethanol at various concentrations in water (50, 80 and 100%) (Turkmen *et al.,* 2005). The ground BM tea sample (0.2 g) with 10 ml of solvent was extracted at 23±2 ℃ for different times (2, 8 and 18 h) on horizontal shaker. The sample was filtered through Whatman No.1 filter paper to remove rough particles and then centrifuged (10 min, 10,000×g). The supernatant was stored at −18℃ until analyzed. Each solvent extraction was carried out in triplicate.

Determination of total polyphenol The amount of total polyphenol was determined using the Folin-Ciocalteu method (Obanda and Owuor, 1997). A calibration curve of gallic acid (range from 0.005 to 0.05 mg/ml) was prepared and the results determined from regression equation of the calibration curve ($y=62.94x-0.67$, $R^2=0.99$) were expressed as mg gallic acid equivalents (GAE)/g tea on dry weight basis. In this method, BM tea extract (1 ml) diluted 10–75 times with de-ionized water (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 double beam spectrophotometer (Shimadzu UV-VIS 1601, Kyoto, Japan).

Antioxidant activity determination by DPPH radical scavenging assay The antioxidant activity of BM tea samples was measured by using the DPPH assay (Katalinic *et al.,* 2004; Atoui *et al.,* 2005) with some minor modification. The extract (80 μl) diluted 15-fold with distilled water was mixed with an aliquot of 1185 μl of 6×10^{-5} M DPPH radical in methanol. Distilled water was used as a control instead of extract. The reaction mixture was vortex-mixed and let to stand at 25℃ in the dark for 60 min. Absorbance at 517 nm was measured using a spectrophotometer using methanol as blank. Antioxidant activity was expressed as percentage inhibition (% I) of the DPPH radical and was determined by the following equation (Yen and Duh, 1994):

$$\%\,Inhibition=\frac{Abs_{control}-Abs_{sample}}{Abs_{control}}\times100$$

Antioxidant activity determination by reducing power Reducing power of BM tea extracts was determined according to the Yuan *et al.* (2005) with slight modifications. The extract (0.5 ml) diluted 15-fold with distilled water was mixed with 1.25 ml of phosphate buffer (0.2 M, pH 6.6) and 1.25 ml of potassium ferricyanide (1%) and the mixture was incubated at 50℃ in a water bath for 20 min. Samples were then cooled and mixed with 1.25 ml of 10% TCA. Afterwards, sample aliquot (1.25 ml) was mixed with 1.25 ml of distilled water and 0.25 ml of 0.1% ferric chloride and then left to react at room temperature for 10 min. Sample absorbances (A) were read at 700 nm. Increase in the absorbance of the reaction mixture indicated increase in the reducing power.

Antibacterial activity to determine antibacterial activity, *Staphylococcus aureus, Listeria monocytogenes* (ATCC 7644, Oxoid, UK), *E. coli* O157: H7, *Hafnia alvei, Yersinia enterocolitica* 0: 3 and *Bacillus cereus* were used as test bacteria. *Y. enterocolitica* 0: 3 was grown in Tyriptic Soy Broth (Merck, Germany) at 30℃ for 18–24 h. The other bacteria were grown in the same medium at 37℃ for 18–24 h. Test microorganisms were obtained from the culture collections of Ankara University, Department of Food Engineering, Ankara, Turkey. Antibacterial activity was determined by the disc diffusion method (Bauer *et al.,* 1966). A sterilize 6 mm diameter antibacterial susceptibility blank disc (Oxoid, Basinstoke, UK) was loaded with 200 μl (4 mg) of each extract of BM tea and left to dry in an open sterile Petri dish in a laminar air flow (Forma Scientific, Turkey). The test bacterium was transferred onto a 9 cm diameter Petri dish containing Nutrient Agar (Merck, Germany) using a sterile cotton swab and spread over the whole surface of the medium as a thin film. The inhibition of bacterial growth was evaluated by measuring the diameter of the transparent inhibition zone around each disc. Control disc was loaded with the same solvent and dried using the same method as the treated disc.

Statistical analysis 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 and Discussion

Polyphenol content The polyphenol content of BM tea extracts was examined and presented in Table 1. Total polyphenol content of the extracts ranged from 97.01 to 119.28 mg GAE/g dw tea depending on the solvent

Table 1. Total polyphenol content and antioxidant activity of BM tea aqueous extracts[a].

Parameter	Extr. time (h)	Extraction Solvent: 50 % Acetone	50 % DMF	50 % Ethanol	50 % Methanol
Total polyphenol (mg GAE / g dw tea)	2	117.62±2.25$^{a\,B}$	101.10±0.71$^{a\,A}$	98.58±1.72$^{a\,A}$	97.01±0.77$^{a\,A}$
	8	111.64±2.44$^{a\,B}$	119.28±4.14$^{b\,B}$	111.17±1.76^{bB}	97.09±1.47$^{a\,A}$
	18	115.03±5.06$^{a\,B}$	119.28±2.06$^{b\,B}$	112.27±0.91^{bB}	99.76±0.34$^{a\,A}$
Antioxidant activity Reducing power (Absorbance)	2	1.31±0.04^{aC}	1.26±0.01^{aC}	1.15±0.01$^{a\,B}$	1.06±0.02$^{a\,A}$
	8	1.26±0.00^{aC}	1.27±0.01^{aC}	1.17±0.01$^{a\,B}$	1.08±0.01$^{a\,A}$
	18	1.21±0.07$^{a\,BC}$	1.28±0.01^{aC}	1.15±0.00$^{a\,AB}$	1.07±0.00$^{a\,A}$
Radical scavenging activity (% Inhibition)	2	93.00±0.49$^{a\,A}$	92.39±0.32$^{a\,A}$	92.69±0.12$^{a\,A}$	92.39±0.44$^{a\,A}$
	8	92.37±0.19$^{a\,A}$	92.26±0.22$^{a\,A}$	92.10±0.34$^{a\,A}$	92.00±0.17$^{a\,A}$
	18	93.06±0.26$^{a\,A}$	92.19±0.37$^{a\,A}$	92.93±0.23$^{a\,A}$	92.54±0.31$^{a\,A}$

[a]: Data are expressed as means±SE of triplicate experiments. Means in a column ($^{a,\,b}$across extraction times) not having a common letter are different ($p<0.05$).
Means in a row ($^{A,\,B,\,C}$across solvent types) not having a common letter are different ($p<0.05$).

used and extraction time. Anesini *et al.,* (2006) found that total polyphenol content of *Yerba Mate* was 6.89 g/100 g dried plant material, respectively, which is lower than our results (9.70–11.93 g GAE/100 g dw tea). The difference can be due to variation in the extraction procedure. In general, ethanol, DMF and acetone almost yielded similar polyphenol content at 2, 8 and 18 h of extraction but the lower amount of total polyphenol content was recorded in methanol extracts. As seen in Table 1, the increase in extraction time from 2 h to 8 h significantly increased polyphenol content of BM tea extracts when using DMF and ethanol as solvent. Lapornik *et al.* (2005) found that with increase in extraction time (from 1 up to 24 h) total phenolics in methanol and ethanol extracts of grape strongly increased, which supports partly our results. On the other hand, with increasing time from 8 to 18 h, polyphenol content of all extracts did not changed ($p<0.05$). The results showed that variation in polyphenol content with increasing extraction time varied depending on the solvent used, which was previously reported for *Orthosiphon stamineus* plant (Akowuah *et al.,* 2005).

Antioxidant activity BM tea extracts possessed reducing power ranging between 1.06–1.31 (Table 1) much higher than the value of control sample (0.123±0.12). Bravo *et al.* (2007) reported that mate infusions (10 mg/ml) had a reducing power slightly higher than black tea, but lower than green tea at the same concentrations, which supports partly our results. Regardless of extraction time, reducing power of the extracts was significantly affected by the extraction solvents with the following order from high to low: acetone=DMF>ethanol>methanol. This result was also in consistent with the results of total polyphenol content. Negi *et al.* (2005) also noted differences in reducing power of various extracts of seabuckthorn seed. On the other hand, increase in extraction time (from 2 to 18h) did not have the effect on the reducing power of the extracts (Table 1). *Ilex paraguariensis* contains significant level of caffeoylquinic acid isomers such as chlorogenic acid (Filip and Ferraro, 2003; Bixby *et al.,* 2005; Anesini *et al.,* 2006) which was shown to posses iron-reducing ability and protect Fe^{+2} ion from auto-oxidation completely (Yoshino and Murakami, 1998). Therefore it would be concluded that polyphenols, such as chlorogenic acid, in BM tea extracts contribute to their reducing powers.

The results of the free radical scavenging activity of BM tea extracts at the different extraction times were shown in Table 1. The activities obtained for the extracts were found between 92.00–93.06%, which indicates that tea extracts have a noticeable effect on scavenging free radical. Consistent with our results, Bixby *et al.* (2005) found that mate tea showed higher radical scavenging activity than red wines and green tea. Contrary to the results of reducing power, there was no statistically significant difference among solvent extracts with respect to radical scavenging activity. This can be due to the fact that different methods to measure antioxidant activity with various mechanisms may lead to different observations (Sun and Ho, 2005). However, the literature includes the studies reporting the effect of the extracting solvent on DPPH scavenging activity (Canadanovic-Brunet *et al.,* 2005; Negi *et al.,* 2005; Yuan *et al.,* 2005). The difference between the literature and our results can be due to variation in material used. Extraction time also did not affect the activity of the extracts, which is consistent with the results of reducing power. Phenolic compounds are known to reduce strongly DPPH radical due to the high reactivities of their functional groups, i.e. hydroxyl substituents (Heim *et al.,* 2002; Rice-Evans *et al.,* 1997). Additionally, Mello *et al.* (2005) reported that a good correlation was obtained between the radical scavenger capacity and total phenol content of mate tea ex-

Table 2. Antibacterial activity of BM tea aqueous extracts.

	Mate tea				
	Extr. time (h)	Diameter of inhibited zone (mm) [a]			
		50 % Acetone	*50 % DMF*	*50 % Ethanol*	*50 % Methanol*
S. aureus	2	20.00 ± 0.29^{aC}	19.67 ± 0.33^{aBC}	18.67 ± 0.17^{aA}	19.00 ± 0.00^{aAB}
	8	22.67 ± 0.33^{bD}	20.00 ± 0.00^{aC}	18.00 ± 0.00^{aA}	19.17 ± 0.17^{aB}
	18	24.83 ± 0.17^{cD}	21.00 ± 0.00^{bC}	18.33 ± 0.33^{aA}	19.50 ± 0.29^{aB}
H. alvei	2	16.67 ± 0.33^{abC}	nz	16.00 ± 0.00^{aB}	12.83 ± 0.17^{aA}
	8	16.17 ± 0.17^{aB}	nz	16.33 ± 0.33^{aB}	12.50 ± 0.29^{aA}
	18	17.00 ± 0.00^{b}	nz	17.00 ± 0.00^{b}	13.00 ± 0.00^{a}
Y. enterocolitica	2	9.00 ± 0.00^{aAB}	8.67 ± 0.33^{aA}	9.67 ± 0.33^{aBC}	10.00 ± 0.00^{aC}
	8	11.67 ± 0.17^{bB}	11.67 ± 0.33^{bB}	12.00 ± 0.00^{bB}	10.50 ± 0.29^{aA}
	18	12.00 ± 0.00^{c}	12.00 ± 0.00^{b}	13.00 ± 0.00^{c}	12.00 ± 0.00^{b}
L. monocytogenes	2	15.00 ± 0.00^{C}	15.00 ± 0.00^{C}	13.33 ± 0.33^{aB}	11.33 ± 0.17^{aA}
	8	16.00 ± 0.00^{C}	16.00 ± 0.00^{C}	15.00 ± 0.00^{bB}	13.67 ± 0.33^{bA}
	18	16.00 ± 0.00	16.00 ± 0.00	15.00 ± 0.00^{b}	14.00 ± 0.00^{b}
B. cereus	2	9.00 ± 0.00	8.00 ± 0.00	7.00 ± 0.00	8.00 ± 0.00
	8	10.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	9.00 ± 0.00
	18	9.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	7.00 ± 0.00
E. coli O157:H7	2	nz	nz	nz	nz
	8	nz	nz	nz	nz
	18	nz	nz	nz	nz

[a]: Data are expressed as means±SE of triplicate experiments. Means in a column ([a, b, c] across extraction times) not having a common letter are different ($p<0.05$).
Means in a row ([A, B, C, D] across solvent types) not having a common letter are different ($p<0.05$).
nz: No inhibition zone detected.

tract. Therefore, polyphenols in BM tea extracts may be considered to be the main contributors to radical scavenging activity of BM tea extracts observed in our study.

Antibacterial activity The inhibitory effect of BM tea extracts on the growth of selected bacteria is presented in Table 2. Extraction solvents used as control had no inhibitory effects on the six bacteria tested. The extracts inhibited the growth to variable extents, except for *E. coli,* depending on the bacterium tested and solvent used. Acetone was found to be the most effective solvent against test bacteria but others were also effective although DMF extracts did not show any activity against *H. alvei.* While *S. aureus* was the least resistant to mate tea extracts *E. coli* was not inhibited by any of BM tea extracts. In general, increase in extraction time from 2 to 18 h significantly increased antibacterial activity of the extracts depending on the bacterium tested and the solvent used (Table 2).

With respect to the solvent used, the difference among antibacterial activity of the extracts was significant ($p<0.05$), depending on the test bacterium and extraction time. There is no data on the same materials in the literature. However, differences in antibacterial activity of various solvent extracts have been reported in previous studies with seabuckthorn seed (Negi *et al.,* 2005) and grape seed (Baydar *et al.,* 2004). A significant correlation was not observed between amounts of polyphenol and antibacterial activity of the extracts. However, antibacterial activity of plant extracts can be attributed to their individual phenolic compounds by previous studies (Sakanaka *et al.,* 2000; Jayaprakasha *et al.,* 2003; Ozkan *et al.,* 2004). On the other hand, an additional research on phenolic composition of each solvent extract is required for comprehensive assessment of individual compounds exhibiting antibacterial activity.

According to the results, it was surprising to find that DMF extracts of mate tea were not effective against *H. alvei* while other three extracts inhibited this bacterium. Determination of which compound necessary for inhibition of the bacterium is absent in DMF extracts will be also the subject of further work.

Conclusions

Polyphenol content of BM tea was influenced by solvents used and extraction time. All extracts showed remarkable antioxidant activity by DPPH radical and reducing power. Radical scavenging activity of the extracts was not affected by solvent used and extraction time while reducing power of the extracts was affected by only solvent. BM tea extracts exhibited antimicrobial activity against bacteria tested, except for *E. coli.* In medicine comprehensive studies on antibiotics have been carried out to enhance immune system in human. On the other hand, the effects of natural supplies such as plant extracts on pathogens have been investigated. The present study showed that BM tea could be a potential source for inhibitory substances for some food-borne pathogens.

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