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Antioxidant activity of various extracts of old tea leaves and black tea wastes (*Camellia sinensis* L.)

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Abstract

The antioxidant activity of various extracts of old tea leaves (OTL) and black tea wastes (BTW) in comparison with that of green tea leaves (GTL) was evaluated. The highest extraction yield and antioxidant activity were found in hot water extracts of GTL. Hot water extracts of OTL and BTW showed the same statistically significant antioxidant activities ($P < 0.05$). Also, there was no statistically significant difference between the antioxidant activities of OTL and BTW extracts obtained by the other two extraction methods (ethyl acetate and methanolic methods) and, in some cases, BTW extracts acted even better than OTL extracts. The antioxidant activity of tea extracts was not concomitant with the development of their reducing power. This suggested that the antioxidant activity of tea extracts likely involves other mechanisms in addition to those of reductones. Totally, BTW extracts had antioxidant activities comparable with or even better than those of OTL extracts. Hence, OTL and BTW, which are often considered as agricultural wastes, can be used as potent natural antioxidative sources.
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Keywords: Antioxidant activity; Reducing power; Tea extracts; Extraction method; Green and old tea leaves; Black tea wastes

1. Introduction

Green tea leaves (GTL) (*Camellia sinensis* L.) contain 10–30% (dry leaf weight) of polyphenols, including catechins, flavonols, flavanones, phenolic acids, glycosides and the aglycones of plant pigments (Pan, Niu, & Liu, 2003). Tea polyphenols are natural antioxidants (Tanizawa et al., 1984) and considered to be responsible for the anticarcinogenic and antimutagenic properties of tea, as well as protective action against cardiovascular diseases (Shahidi & Wanasundara, 1992; Tijburg, Mattern, Folts, Weisgerber, & Katan, 1997; Wiseman, Balentine, & Frei, 1997). They also have a stronger antioxidative activity than butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and DL-α-tocopherol; and the toxicity of tea polyphenols is lower than that of BHA, BHT and DL-α-tocopherol (Chen & Wan, 1994).

Tea extracts are powerful antioxidants, mainly owing to the presence of (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epigallocatechin gallate and (−)-epicatechin gallate (Salah et al., 1995). Catechins are known to be non-volatile taste compounds of green tea (Nwuha, Nakajima, Tong, & Ichikawa, 1999) and present at 8–15% of dry leaf weight (Goto, Yoshida, Amano, & Horie, 1996). These compounds are effective free radical-scavengers (Salah et al., 1995) and also effective by metal chelation (Shahidi, Ke, Zhao, Yang, & Wanasundara, 1992).

Both green and black teas are manufactured from young shoots, mainly the first 2–4 leaves and a bud. The extracts of green tea have been reported in the literature as an antioxidant in animal and vegetable oils (Das, Grosh, Bhattacharyya, & Guha, 1965; Matsuzaki & Hara, 1985). The other tea leaves (old tea leaves, OTL), which are not used in tea manufacture, are considered to be agricultural wastes. Zandi and Gordon (1999) showed that the extracts

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from OTL have potential as natural antioxidants. After various processing stages, such as withering, rolling, fermentation, drying, and finally packing, GTL are converted to black tea. Investigations have indicated that black teas containing smaller amounts of polyphenols also have antioxidant activities (Frei & Higdon, 2003; Retvield & Wiseman, 2003). Black tea production is inevitably accompanied with some wastes, but no report has appeared on the antioxidant activity of extracts of black tea wastes (BTW).

Various extraction methods affect the extraction yield and antioxidant activity of tea extracts. Some studies on the extraction conditions of active antioxidant components from GTL have been reported (Agarwal, Katiyar, Zaidi, & Mukntar, 1992; Chen & Chan, 1996; Ge & Jin, 1994; Hu, Jiang, & Zhu, 1997; Li & Feng, 1996; Mai, Chamber, & McDonald, 1990; Pan et al., 2003; Sakanaka, Tachibana, & Okada, 2005), but there is no comparable study on the effect of various extraction methods on the antioxidant activity of OTL and BTW extracts. The purpose of this work was to investigate the effect of three conventional extraction methods (ethyl acetate, hot water and methanolic methods) on the extraction yield and antioxidant activity of OTL and BTW extracts, and to compare them with those of GTL.

2. Materials and methods

2.1. Materials

Samples of GTL and OTL were collected from a tea garden in Ramsar (northern Iran). BTW were supplied by a tea factory in Ramsar. Polyphenol oxidase of GTL and OTL was inactivated by plunging the leaves into boiling water for 3 min. The leaves were then drained, dried at room temperature and ground to a fine powder (Zandi & Gordon, 1999).

Linoleic acid was purchased from Sigma Aldrich Company, England. All chemicals and solvents were of analytical reagent grade and purchased from Merck Company, Germany.

2.2. Preparation of tea extracts

2.2.1. Ethyl acetate method

In brief, 10 g of dried sample were extracted three times with 150 ml of hot distilled water (80 °C, 30 min). The infusion was cooled to room temperature, filtered, and then extracted with an equal volume of chloroform to remove caffeine and pigments. The remaining aqueous layer was saved and twice extracted with an equal volume of ethyl acetate. The ethyl acetate phase was then removed using a rotary evaporator under reduced pressure below 40 °C. The resulting concentrated extracts were dried in vacuo below 40 °C and weighed to determine the yield (Agarwal et al., 1992; Chen & Chan, 1996).

2.2.2. Hot water method

In brief, 4 g of dried sample were extracted with 40 ml of distilled water at a temperature from 80 to 105 °C in an autoclave for 20 min to give an initial extract (fraction I). The residues were extracted with 60 ml of distilled water at a temperature from 100 to 130 °C for 30 min to give fraction II. After cooling to room temperature and then filtering, the two fractions were combined and dried in vacuo below 40 °C and weighed to determine the yield (Mai et al., 1990).

2.2.3. Methanolic method

In brief, 10 g of dried sample were twice extracted with 70 ml of methanol with shaking at room temperature for 1 h. The extracts were filtered through a filter paper and the filtrates were pooled. The residues were re-extracted with 60 ml methanol at room temperature overnight. Pooled filtrates were condensed with a rotary evaporator under reduced pressure below 40 °C and were then dried in vacuo below 40 °C and weighed to determine the yield (Sakanaka et al., 2005; Zandi & Gordon, 1999).

2.3. Determination of antioxidant activity

Antioxidant activity was determined by the thiocyanate method. In brief, a mixture of 200 mg/l tea extract (2.0 ml) in distilled water, 2.51% linoleic acid in absolute ethanol (2.0 ml), 0.05 M phosphate buffer (pH 7.0) (4 ml), and distilled water (2.0 ml) was placed in a vial ($\phi = 38$ mm, $h = 75$ mm) with a screw cap and then placed in an oven at 40 °C in the dark. To this solution (0.1 ml) was added 75% ethanol (9.7 ml) and 0.02 M ferrous chloride in 10% hydrochloric acid (0.1 ml). Precisely 3 min after adding of 30% ammonium thiocyanate (0.1 ml) to the reaction mixture, the absorbance was measured at 500 nm every 24 h until the absorbance reached maximum. The control (distilled water) was subjected to the same procedures as the tea extract (Xu, Yang, Chen, Hu, & Hu, 2003).

The influence of tea extracts on oxidation of the lipid system was estimated on the basis of two kinetic parameters characterizing the lipid oxidation during its initial stage: stabilization factor F and oxidation rate ratio ORR. F is a measure of the effectiveness

$$F = \mathrm{IP}_{\mathrm{inh}}/\mathrm{IP}_0,$$

where $\mathrm{IP}_{\mathrm{inh}}$ is the induction period in the presence of an inhibitor, and IP_0 is the induction period of the non-inhibited oxidation. ORR is an inverse measure of strength

$$\mathrm{ORR} = W_{\mathrm{inh}}/W_0,$$

where W_{inh} is the oxidation rate in the presence of an inhibitor, and W_0 is the initial oxidation rate of the non-inhibited oxidation. The method of estimating the needed kinetic values for calculating these two parameters is shown in Fig. 1 (Farhoosh, 2005).

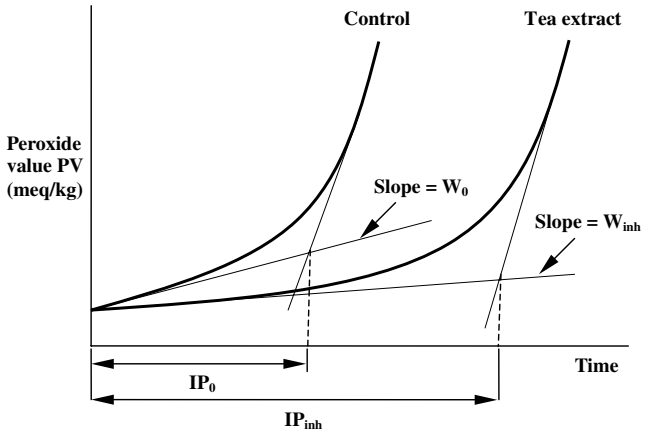

Fig. 1. A schematic kinetic curve of peroxide accumulation during oxidation of lipid systems, and the kinetic values which can be derived from it.

2.4. Determination of reducing power

Reducing power on iron ion was measured according to the method of Yen and Chen (1995). Extracts (100 μg) in 1.0 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [$K_3Fe(CN)_6$] (1%), then the mixture was incubated at 50 °C for 30 min. Afterwards, 2.5 ml of trichloroacetic acid (10%) were added to the mixture, which was then centrifuged at 1000*g* for 10 min (Heraeus Labofuge 200). Finally, 2.5 ml of the upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm (Jenway 6105 UV–Vis spectrophotometer). Increased absorbance of the reaction mixture indicated increased reducing power. The control and standard were subjected to the same procedures as the sample except that, for the control, only distilled water was added, and, for the standard, 100 μg extract was replaced with 100 μg of ascorbic acid.

2.5. Statistical analysis

All determinations were carried out in three triplicate and data were subjected to analysis of variance. Analysis of variance was performed using the ANOVA procedure. Statistical analyses were performed according to the MSTATC software. Significant differences between means were determined by Duncan's multiple range test. *P* values less than 0.05 were considered statistically significant.

3. Results and discussion

Fig. 2 illustrates the yield of different tea extracts. As can be seen, the yield of hot water extracts (30.56 ± 5.06%) is significantly higher than that of methanolic extracts (14.53 ± 8.67%), which is in turn significantly higher than that of ethyl acetate extracts (5.17 ± 4.38%). This can be attributed to different affinities of the extraction solvents for total tea leaves constituents in terms of their different extraction conditions, such as polarity of extracting solvents, and temperature (Moure et al., 2001). The yield in each of the three extraction methods significantly decreased with increasing age of tea leaves. This may be due to leaf physiological changes and/or material transport in the plant during the growth period (Taiz & Zeiger, 2001). Unlike the ethyl acetate method, both hot water and methanolic methods created statistically significant differences between the extraction yields of OTL and BTW.

The effect of extraction method and raw material on inhibited lipid oxidation of linoleic acid emulsion at 40 °C is shown in Table 1. Except for the BTW extract from the ethyl acetate method, induction period of all the

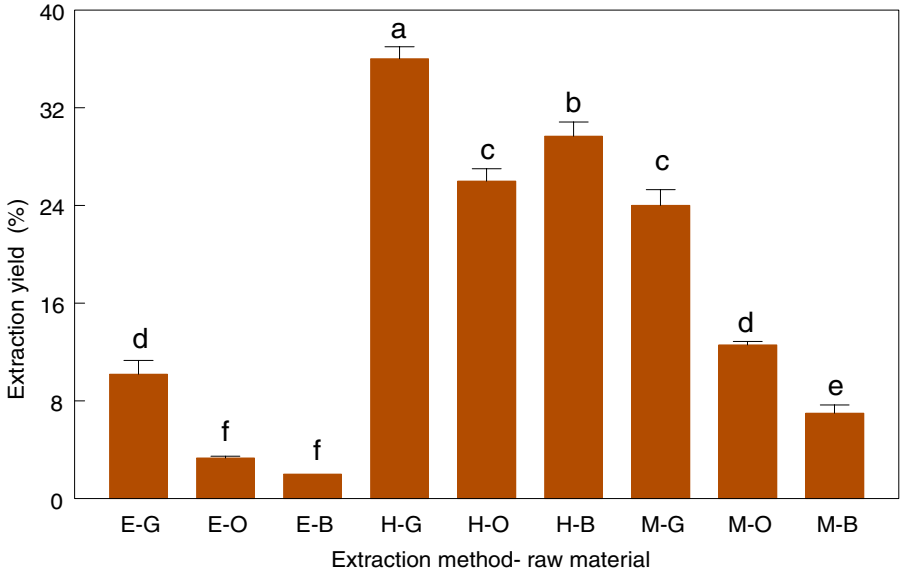

Fig. 2. The yield of different tea extracts as the means ± standard deviation (SD). Means with the same letters are not significantly different at $P < 0.05$. E, ethyl acetate method; H, hot water method; M, methanolic method; G, green tea leaves; B, black tea wastes; O, old tea leaves.

Table 1
Two kinetic parameters characterizing the effect of the extraction method and raw material on the inhibited lipid oxidation of linoleic acid emulsion at 40 °C, $PV_0 = 0$ meq/kg, $IP_0 = 59.224$ h, $W_0 = 0.006$ meq/kg/h

Extraction method–raw material	Kinetic parameter[a]	
	F (IP_{inh}/IP_0)	ORR (W_{inh}/W_0)
Ethyl acetate–green tea leaves	3.883 ± 0.349 c	0.556 ± 0.155 cd
Ethyl acetate–old tea leaves	2.524 ± 0.932 e	0.583 ± 0.032 cd
Ethyl acetate–black tea wastes	2.290 ± 0.369 e	0.694 ± 0.140 d
Hot water–green tea leaves	18.620 ± 0.793 a	0.003 ± 0.003 a
Hot water–old tea leaves	14.350 ± 0.981 b	0.005 ± 0.001 a
Hot water–black tea wastes	12.940 ± 0.588 b	0.014 ± 0.002 a
Methanol–green tea leaves	3.245 ± 0.287 d	0.589 ± 0.043 cd
Methanol–old tea leaves	2.576 ± 0.515 e	0.435 ± 0.065 c
Methanol–black tea wastes	2.697 ± 0.569 e	0.256 ± 0.005 b

Means within a column with the same lower case letters are not significantly different at $P < 0.05$.
[a] Values are means ± standard deviation (SD).

tea extracts was significantly higher than that of the control. Among the tea extracts, hot water extract of GTL was the most significantly effective. Roedig-Penman and Gordon (1997) reported that the extraction of GTL with hot water provided an extract that was highly effective as an antioxidant for an oil-in-water emulsion. Despite the fact that both ethyl acetate and methanolic methods provide a higher purity of catechins as the most important cause of the antioxidant activity in tea extracts, hot water tea extracts showed a statistically significant higher stabilization factor. Manzocco, Anese and Nicoli (1998) showed that thermal treatment caused an increase in radical-scavenging properties of tea extracts. They ascribed this to the progressive oxidation of polyphenols, a process leading to the formation of macromolecular compounds with stronger radical-scavenging power. This may be attributable to the increased resonance delocalization as well as to the higher stability of the aryloxyl radicals incurred by hydrogen bonding (Kikugawa, Kunugi, & Kureki, 1990). Mai et al. (1990) found, when black tea leaves are extracted by aqueous media at temperatures from 120 to 210 °C, certain extracts are formed which contain appreciable quantities of gallic acid. These extracts have an antioxidant activity comparable with or superior to synthetic antioxidant systems. The significantly higher effectiveness of ethyl acetate green tea extract than methanolic green tea extract can be attributed to the higher purity of its polyphenolic compounds. It was interesting to find that there was no statistically significant difference between the stabilization factor of the extracts of OTL and BTW in each three extraction methods.

Oxidation rate ratio, as a measure of the oxidation rate during the induction period, was significantly lower for hot water tea extracts than those of the other two extraction methods. Also, there was no statistically significant difference between oxidation rate ratios of all hot water tea extracts. It was interesting to find that the methanolic extract of BTW acted as an inhibitor of oxidation of the lipid system during the induction period and was even stronger than the methanolic extracts of GTL and OTL.

Fig. 3 shows the reducing power of the different tea extracts. The reducing power of all the different tea extracts was significantly higher than it was for control. On average, hot water tea extracts had reducing power lower than those of methanolic tea extracts, which were in turn lower than those of ethyl acetate tea extracts. Only ethyl acetate tea extracts had reducing activities the same or higher than that of ascorbic acid. Except for hot water extracts of

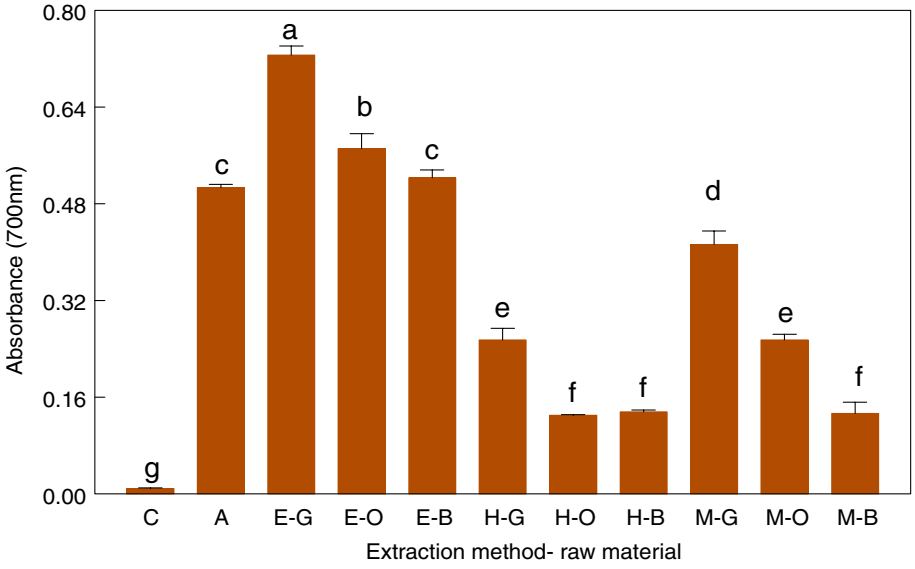

Fig. 3. Reducing power of different tea extracts as the means ± standard deviation (SD). Means with the same letters are not significantly different at $P < 0.05$. C, control; A, ascorbic acid; E, ethyl acetate method; H, hot water method; M, methanolic method; G, green tea leaves; B, black tea wastes; O, old tea leaves.

OTL and BTW, the reducing power of OTL extract was significantly higher than that of BTW in both ethyl acetate and methanolic extraction methods.

It was interesting to find that, although hot water tea extracts had the highest antioxidant activity, they were the least effective ones in reducing power. Also, it appeared that ethyl acetate tea extracts had the highest reducing power. The reducing capability of a compound may serve as a significant indicator of its potential antioxidant activity (Meir, Kanner, Akiri, & Hadas, 1995; Tanaka, Kuie, Nagashima, & Taguchi, 1988). However, this pattern was not observed in this research. Yildirim et al. (2000) found a negative correlation between antioxidant and reducing power activities of sage extract. They concluded that there may not always be a positive correlation between antioxidant and reducing power activities. Furthermore, the antioxidant activities of putative antioxidants have been attributed to various mechanisms. Among these are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging (Diplock, 1997).

The reducing properties are generally associated with the presence of reductones (Duh, 1998), such as ascorbic acid (a potent reducing agent), which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990). Reductones are also reported to react directly with peroxides (Shimada, Fujikawa, Yahara, & Nakamura, 1992) and also with certain precursors of peroxides, thus preventing peroxide formation (Xing et al., 2005). Our data on the reducing power of different tea extracts suggested that the antioxidant activity of tea extracts likely involves other mechanisms in addition to those of reductones.

4. Conclusions

The results of the present study indicated that the tea extracts obtained by the hot water method provided the highest extraction yield and antioxidant activity. In this method, the extraction yield of the BTW was higher than that of the OTL. On the basis of the measured kinetic parameters, the antioxidant activity of BTW extract was the same or even better than that of OTL extract. The antioxidant activity of the tea extracts was not concomitant with the development of their reducing power. This suggested that the antioxidant activity of the tea extracts likely involves other mechanisms than those of reductones. Totally, OTL and BTW, which are often considered as agricultural wastes, could be used as potent natural antioxidative sources.

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