

Antioxidant Activity of Extracts of Pu-erh Tea and Its Material

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Abstract: In this study, we reported an experimental study on the *in vitro* antioxidant activity of various extracts of pu-erh tea as well as its fermented materials (*Camellia sinensis* (L.) var. *assamica* (Masters) Kitamura). The antioxidant activity of the tea extracts were measured using *in vitro* assays including the reducing power assay and the assay evaluating the free radical scavenging capacity such as the hydroxyl, 1,1-diphenyl-2-picrylhydrazyl (DPPH), the superoxide, and the nitrogen dioxide radical. All tea extracts showed a dose dependent scavenging radical capacity as well as the reducing power activity. Particularly, the ethanol precipitation and water extract of pu-erh tea as well as the ethanol precipitation and ethyl acetate extract of pu-erh tea material, with the IC₅₀ value of 3.5 µg/mL, 27.9 µg/mL, 13.5 µg/mL and 18.1 µg/mL, respectively, showed the highest antioxidant capability against all four free radicals studied here. The experimental results also revealed that there existed a significant difference in *in vitro* antioxidant capability among the tea extracts, and there was a strong correlation between the antioxidant capacity and the antioxidant species. Particularly, not only the content of the total catechins but the content of the total phenolics, the thearubigins, the theabrownins, and the tea polysaccharide contributed to the tea extract antioxidant activity. In addition, the ferric reducing/antioxidant power assay was used to measure the total antioxidant activity of eight tea extracts from fresh infusions. The ethyl acetate extract from pu-erh tea as well as its showed a relative high reducing power. The experimental results reported here suggested that pu-erh tea could be an important dietary antioxidant source.

Key words: Pu-erh tea, tea extracts, antioxidant activity and IC₅₀, solid state fermentation

INTRODUCTION

Pu-erh tea, produced mainly in Yunnan Province of China, is well known for its special flavor and potential healthy benefits to human beings. It is a traditional beverage in Hongkong, Taiwan as well as many other areas in Southeast Asia. Sano *et al.* (1986) reported that Pu-erh tea significantly reduced the plasma cholesterol ester and triglyceride levels in rat plasma and the similar results have been reported by Miura *et al.*, 1995).

It was commonly believed that the longer the preservation period, the better the quality and taste of Pu-erh tea. Almost all pu-erh tea are produced from the fresh leaves of arge leaf tea via the following process: Fresh leaves → Sterilized enzyme → Rolling → Sun drying → Solid state fermentation → Dry → Pu-erh tea. Specifically, the fresh leaves of *Camellia sinensis* (L.) var. *assamica* (Masters) Kitamura are drum-fried to inactivate the polyphenol oxidases. Then, the fixed leaves are rolled and sunshine dried. Once dry, the leaves sundried green tea are piled up in a humid environment a few weeks for the solid state fermentation, in which the tea polyphenols are further oxidized by the action of microorganisms under humid and warm conditions, resulting in the low tea polyphenol and catechin concentrations (Gong *et al.*, 2005).

The antioxidant activity of green tea has been reported by many groups. Green tea has been credited with providing a wide variety of health benefits, and in particular, its ability to prevent free radical damage, which has been attributed to the significant scavenging and antioxidative properties of high concentration unpolymerized catechins as well as their gallates (Wei *et al.*, 2002; Coyle *et al.*, 2008). There are only a few studies in the literature regarding the biological action of pu-erh tea. Duh *et al.* (2004) confirmed that pu-erh tea had significant inhibitions on both lipid and non-lipid oxidation similar to that of green tea extract, as well as their direct nitric oxide (NO) scavenging activities. Wang *et al.* (2007) reported that the water extract of pu-erh tea can significantly scavenge H₂O₂ in a concentration dependent manner and inhibit the xanthine oxidase activity.

However, it is still not clear what the main antioxidant components in pu-erh tea are as well as their free radical scavenging effects, how to improve their antioxidant activity and so on.

In this study, both the *in vitro* antioxidant activities and the reducing power of pu-erh tea and its fermented materials (*Camellia sinensis* (L.) var. *assamica* (Masters) Kitamura) as well as their various solvent extracts were

measured and compared for the purpose of evaluating their *in vivo* antioxidant activities. In addition, the antioxidant activities of main antioxidant components in tea extracts were also studied.

MATERIALS AND METHODS

The Pu-erh tea was produced through solid state fermentation of *Aspergillus niger* using green tea dried by sunshine as its materials in 2006 year. Pu-erh tea and its fermented materials were purchased from Guoyang Tea Factory in Menghai County at Xishuangbanna, China. 1, 2, 3-trihydroxybenzene, sulfanilic acid and N-(1-Naphthyl) ethylenediaminedihydrochloride were purchased from China International Pharmaceutical Group Corporation. 1,1-diphenyl-2-picrylhydrazyl (DPPH) dextran MW 10000 Coomassie brilliant Blue G-250 and epicatechin (EC) were purchased from Sigma-Aldrich. All chemicals were analytical grade.

Preparation of tea extracts: Pu-erh tea or its material powders (40–80 mesh) were first steeped in ethanol (tea/ethanol = 1:10) at 45 °C for 12 hours and then filtered. The clear solution were collected and concentrated under a reduced pressure and lyophilized (the dried product was called the ethanol steep extract here), while the solid was recovered. The process was repeated for three times. Then, the solid retentate was further extracted with the distilled water at 1:10 ratio of retentate to distilled water for 3 hours at 50 °C. The process was repeated 3 times. The filtrate was collected. One-third of the filtrate was concentrated under a reduced pressure followed by lyophilization (the leading product was termed as the ethanol extract). The other filtrate was extracted with ethyl acetate for 5 times at a ratio of filtrate to ethyl acetate 1:1, and the upper layer was concentrated under a reduced pressure and then lyophilized (termed as the ethyl acetate extract while the lower layer or the water rich layer was further extracted using chloroform (the volume ratio of the water layer to chloroform is 1:1) for 5 times. The leading chloroform rich layer was concentrated under a reduced pressure and lyophilized (termed as the chloroform extract), while the water rich layer was extracted with butanol at a water layer to butanol ratio of 1:1 for 3 times and the butanol rich layer was concentrated under a reduced pressure followed by lyophilization (the leading product was called the butanol extract). Finally, the water rich layer was precipitated by ethanol for 24 hours at a water layer to ethanol ratio of 1:3 at the room temperature. After the filtration, the solid was lyophilized (termed as the ethanol precipitation) while the solution was concentrated under a reduced pressure followed by lyophilization (the leading product was termed as the ethanol extract).

Chemiluminescence assay for evaluating hydroxyl radical scavenging activity: The scavenging activity of hydroxyl radical OH^\cdot was determined by the

chemiluminescence method as described by Cheng *et al.* (2003), with a slight modification.

The OH^\cdot were generated by a Fenton-type reaction at the room temperature. Specifically, the 1.0 ml reaction mixture contained: 600 μl luminol (0.1 mM, diluted in the carbonic acid-buffered saline solution (CBSS), pH 10.2), 100 μl sample solution of different concentrations (CBSS was used in the control), 200 μl Fe^{2+} -EDTA (3 mM), and 100 μl (96) H_2O_2 (1.2 mM). The reaction was initialized by adding Fe^{2+} -EDTA then H_2O_2 to the mixture. The integral intensity of chemiluminescence intensity (CL) was recorded and the scavenging rate was calculated by

$$\text{Scavenging rate(\%)} = \frac{(\text{CL}(\text{control}) - \text{CL}(\text{sample}))}{\text{CL}(\text{control})} \times 100$$

in which CL(control) and CL(sample) are the CL integral intensity of the control and sample, respectively. The concentration of the sample giving 50% inhibition (IC_{50}) was determined from a dose response curve.

Assay for evaluating DPPH radical scavenging activity: The scavenging effects of tea extracts on DPPH radicals *in vitro* were determined by the method as described by Peng (Peng *et al.*, 2007). First, the DPPH buffer solution was prepared by dissolving 0.04g DPPH into toluene and then being diluted to the concentration of 0.04g/L with 50% (volume ratio) ethanol aqueous solution. Then, 0.1 ml tea extracts of various concentrations was mixed with 4.9 ml DPPH buffer solution. The mixture was held for 20 min at the room temperature. The scavenging effect was determined from the UV adsorption intensity (Systronic 2202 UV-Vis spectrophotometer) at the 517 nm of the sample, the control, and the blank:

$$\text{Scavenging effect \%} = \left[\frac{A - B}{A_0} \right] \times 100$$

where A_0 is the absorbance of the control (DPPH solution with 4.9 ml DPPH buffer solution and 0.1ml 50% aqueous solution of ethanol, no tea polysaccharide), A and B are the absorbance of the reaction mixture and the blank (0.1ml polysaccharide and 4.9ml 50% ethanol aqueous solution), respectively.

The concentration of the sample giving 50% inhibition (IC_{50}) was determined from a dose response curve.

Assay for determining superoxide radical O_2^\cdot scavenging activity: The scavenging effects of tea extracts on superoxide radicals *in vitro* were analyzed by the method as described by Zhao *et al.*, (2003). Specifically, the autoxidation rate of 1,2,3-trihydroxybenzene without the tea extracts was measured by first mixing 4.5 ml Tris-HCl buffer (50mmol/L, pH = 8.2) with 4.2 ml distilled water and kept at the temperature 25 °C for 20 min, then after adding 0.3 ml 1,2,3-trihydroxybenzene solution (3 mmol/L, 25 °C)

into the mixture, the UV absorbance of the mixture at 420 nm was measured every 30 seconds, from which the value of A per minute can be calculated. The autoxidation rate of 1,2,3-trihydroxybenzene in the presence of the tea extracts was determined in a similar way except that the tea extracts was added into the solution before adding the 1,2,3-trihydroxybenzene. In both cases, the 10 mmol/L HCl solution was used as the control. The scavenging effect was determined by

$$\text{Scavenging effect \%} = \frac{A_1/t - A_2/t}{A_1/t} \times 100\%$$

in which A_1/t and A_2/t are the autoxidation rate of 1,2,3-trihydroxybenzene with and without the tea extracts, respectively. The concentration of the sample giving 50% inhibition (IC_{50}) was determined from a dose response curve.

Assay for determining NO_2^- radical scavenging activity: The scavenging effects of tea extracts on NO_2^- radical *in vitro* were evaluated by the method as described by Yu *et al.* (2007). Specifically, 1 ml 5 μ g/ml $NaNO_2$ solution was mixed with different concentrations of the tea extracts in a 25 ml tube and kept at 37 °C for 30 min. Then, 1 ml 0.4 % sulfanilic acid and 0.5ml color reagent N-(1-Naphthyl)ethylenediamine dihydrochloride (0.2%) were added into the mixture. After being diluted to 25 ml using distilled water, the leading mixture was kept at the room temperature for 1 min. The UV absorbance (A_1) of the mixture at 540 nm was then measured (Systronic 2202 Uv-Vis spectrophotometer). The distilled water was used as the control. The scavenging effect was calculated by

$$\text{Scavenging effect \%} = \frac{A_0 - A_1}{A_0} \times 100$$

Here, A_0 and A_1 are the absorbance without and with the tea extracts, respectively.

The concentration of the sample giving 50% inhibition (IC_{50}) was determined from a dose response curve.

Ferric reducing antioxidant power (FRAP): The reducing power of the tea extracts was determined by the method of Oyaizu (Oyaizu, 1986). Specifically, 10–1000 μ g tea extracts were first dissolved into 1 ml distilled water and then mixed with 2.5 ml 0.2 M phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The leading mixture was incubated at 50 for 20 min. Then, a portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture followed by the centrifugation at a speed of 3000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml 0.1 % $FeCl_3$, then its absorbance at 700 nm was measured using 1.0 cm cuvette. The increase of the absorbance indicated the increase of the reducing power.

Analysis of tea polyphenols, total catechins, theaflavins, thearubigins, and theabrownins: The content of the tea

polyphenols, the total catechins, the theaflavins (TF), the thearubigins (TR), and the theabrownins (TB) were determined by the method of Liang *et al.* (2005).

HPLC analysis of gallic acid and catechins: 0.1 g tea powders were first dissolved into 40 ml distilled water and then filtered using a piece of ‘Double-ring’ No 102 filter paper (Hangzhou Xinhua Paper Ltd, China). The filtrate was diluted to 50 ml and filtered through a 0.45 μ m membrane for HPLC analysis. The content of both gallic acid and catechins were determined by HPLC as described by Lin *et al.* (1996).

Analysis of soluble tea polysaccharides: 0.5g tea extracts were first dissolved into 100 ml distilled water at the room temperature and then filtered through a piece of ‘Double-ring’ No 102 filter paper (Hangzhou Xinhua Paper Ltd, China). The filtrate was mixed with the ethanol until the final ethanol concentration was 80%. The precipitated tea polysaccharides were collected by the centrifugation and then dissolved into the distilled water to determinate their concentration by the method of Dubois *et al.* (1956). Dextran (Pharmacia AR-grade, molecular weight 10000) was used as the standard to calibrate the concentration of the soluble tea polysaccharides, which were measured by the absorbance at 490 nm using 1.0 cm cuvette.

RESULTS

Hydroxyl radical scavenging activity: The IC_{50} of all extracts of both pu-erh tea and its materials were listed in Table1. As shown by the IC_{50} value, the water extract of pu-erh tea ($IC_{50} = 27.9 \mu$ g/ml) and pu-erh tea material exhibited the greatest and the second greatest the hydroxyl radical scavenging activity, respectively, while the hydroxyl radical scavenging activity of the chloroform extract of the pu-erh tea as well as its material was relatively small. In addition, it was clear that the scavenging hydroxyl radical capacity of the water extract of pu-erh was significantly higher than that of the pu-rh tea materials. On the contrary, both the butanol and ethanol steep extract of pu-erh tea showed a much lower hydroxyl radical scavenging activity than that of pu-erh tea materials. However, there was no significant difference in hydroxyl radical scavenging activity between pu-erh tea and its materials in all other extracts. The observed difference in scavenging activity could be attributed to the concentration difference in main antioxidant components in various tea extracts. Specifically, as shown in both Table1 and Table2, the content of theabrownin as well as the tea polysaccharides in the pu-erh tea water extract was higher than other extracts. Moreover, as reported by Yang *et al.* (2007), the hydroxyl radical scavenging activity of thearubigins was high, and in oxidized phenolic compounds, the hydroxyl radical scavenging activity decreased in the order of

Table 1: IC₅₀ value and the main antioxidant species in the extracts of pu-erh tea as well as its fermented materials

Tea extracts	IC ₅₀ values of scavenging different free radicals			
	Hydroxyl radical (OH [•])	DPPH radical	Superoxide anion (O ₂ ^{•-})	Nitrogen dioxide radical (NO ₂ [•])
Water extract of pu-erh tea	27.9 ^d	58.5 ^d	234.7 ^{de}	59.5 ^c
Water extract of material	34.2 ^c	69.3 ^d	16.2 ^e	57.4 ^c
Ethyl acetate extract of pu-erh tea	38.1 ^c	107.2 ^b	53.9 ^f	30.9 ^d
Ethyl acetate extract of material	41.28 ^c	61.6 ^d	131.4 ^c	18.1 ^e
Ethanol extract of pu-erh tea	104.5 ^b	39.6 ^e	224.3 ^{de}	91.6 ^b
Ethanol extract of material	41.4 ^c	156.2 ^a	381 ^c	120.9 ^a
Ethanol precipitation of pu-erh tea	37.0 ^c	3.5 ^e	63.4 ^f	36.1 ^d
Ethanol precipitation of material	42.1 ^c	21.4 ^f	13.5 ^e	132.5 ^a
Chloroform extract of pu-erh tea	132.4 ^a	158.5 ^a	257 ^d	89.7 ^b
Chloroform extract of material	136.8 ^a	88.4 ^c	129.7 ^c	66.1 ^c
Butanol extract of pu-erh tea	110.5 ^b	16.5 ^f	314.5 ^{cd}	53.8 ^{cd}
Butanol extract of materia	139.4 ^c	42.1 ^c	217.6 ^{de}	63.4 ^c
Ethanol steep extract of pu-erh tea	55.5 ^c	38.3 ^c	536.5 ^b	70.6 ^{bc}
Ethanol steep extract of materia	143.7 ^c	41 ^e 8	76.5 ^a	34.7 ^d
Vc	27.3 ^d	/	18.3 ^e	/
Rutin	/	103.3 ^b	14.4 ^f	/

Table 1: Continued

Tea extracts	Main antioxidant species					
	Total catechins	Total polyphenols	Theaflavins	Thearubigins	Theabrownins	Tea
Water extract of pu-erh tea	4.74±0.07	4.74±0.07	0.22±0.001	07±0.0214	.80±0.02	1194±0.02
Water extract of material	2.35±0.04	2.35±0.04	0.16±0.00	3.76±0.01	3.47±0.01	3.93±0.05
Ethyl acetate extract of pu-erh tea	12.41±0.25	23.46±0.61	0.50±0.01	7.11±0.04	2.35±0.03	4.25±0.34
Ethyl acetate extract of material	19.39±0.21	55.33±0.26	1.34±0.02	10.89±0.10	0.93±0.05	3.84±0.24
Ethanol extract of pu-erh tea	3.16±0.04	24.21±0.19	0.10±0.00	2.80±0.23	8.20±0.06	8.35±0.22
Ethanol extract of material	1.11±0.01	21.99±0.33	0.14±0.01	1.47±0.12	2.97±0.05	6.08±0.32
Ethanol precipitation of pu-erh tea	3.40±0.01	15.00±0.38	0.08±0.02	0.48±0.09	14.24±0.11	1.08±0.04
Ethanol precipitation of material	0.54±0.01	9.00±0.02	0.11±0.01	0.03±0.03	2.87±0.05	6.81±0.02
Chloroform extract of pu-erh tea	0.53±0.00	10.42±0.17	0.30±0.01	3.09±0.02	0.87±0.03	0.64±0.01
Chloroform extract of material	0.04±0.02	3.26±0.57	0.22±0.01	1.05±0.07	0.00±0.00	0.78±0.02
Butanol extract of pu-erh tea	3.08±0.02	31.45±0.71	0.30±0.00	12.52±0.06	5.00±0.02	12.51±0.90
Butanol extract of materia	16.51±1.85	44.97±0.17	0.37±0.01	12.76±0.09	8.01±0.27	5.38±0.13
Ethanol steep extract of pu-erh tea	3.83±0.24	16.87±0.10	0.97±0.01	8.99±0.36	1.82±0.04	2.31±0.12
Ethanol steep extract of materia	11.22±0.92	35.28±0.24	0.32±0.00	7.69±0.00	2.08±0.02	3.97±0.03
Vc	/	/	/	/	/	/
Rutin	/	/	/	/	/	/

Note: The superscripts a to g indicated the statistically significant difference as analyzed by ANOVA (p<0.05).

Table 2: Monomer catechin and gallic acid content of some extracts of pu-erh tea and its materials (g/100g dry mass)

Sample	Gallic acid	C	EGC	EGCG	EC	ECG
Water extract of pu-erh tea	8.21	0.72	0.35	0.28	1.35	0.25
Ethanol precipitation of pu-erh tea	0.55	0.05	ND	0.04	0.19	0.03
Ethyl acetate extract of material	0.38	3.11	4.81	13.91	9.74	15.06

Note: ND denoted "no detect".

theaflavins (TF) > theabrownin (TB) > thearubigins (TR) (Yang *et al.*, 2007). In addition, Chen *et al.* (2005) reported that the tea polysaccharides (TPS) exhibited the significant *in vitro* inhibitory effects on both hydroxyl (IC₅₀ = 101 µg. ml⁻¹) and superoxide radical (IC₅₀ = 145 µg. ml⁻¹) as well as the lipid peroxidation (IC₅₀ = 238 µg. ml⁻¹) (Chen *et al.*, 2005). The results reported here indicated that both theabrownin and tea polysaccharide in pu-erh tea may play a crucial role in hydroxyl radical scavenging activity.

DPPH radical scavenging activity: The effects of various tea extracts on DPPH radical scavenging capacity were summarized in Table 1. Specifically, the DPPH radical scavenging activity decreased in an order of the ethanol precipitation extract (IC₅₀ = 3.5 µg/ml) > the butanol extract > the ethanol steep extract > the ethanol extract > the water extract > the ethyl acetate extract > the chloroform extract. It may be due to the tea polyphenols

and theabrownin content difference in tea extracts, particularly, the tea polyphenols and theabrownin content in the ethanol precipitation extract was as high as 15.0% and 14.24%, respectively. In addition, both gallic acid and EC were also found in the ethanol precipitation extract as shown by HPLC analysis (Table 2). Similarly, among pu-erh tea material extracts, the DPPH radical scavenging activity of the ethanol precipitation extract was also the greatest, with a IC₅₀ value as high as 21.4 µg/ml. The DPPH radical scavenging activity in all other tea material extracts decreased in an order of the ethanol steep extract > the butanol extract > the ethyl acetate extract > the water extract > the chloroform extract > the ethanol steep extract. Particularly, as shown in Table 1, the tea polysaccharide content in the ethanol precipitation extract was found as high as 6.81%, indicating that the tea polysaccharide may have a strong DPPH-scavenging capability. On the other hand, the strong DPPH-scavenging capability observed in the ethanol steep extract, the butanol extract and the acetate extract of pu-erh tea materials may be attributed to the high content of the total catechins, the tea polyphenols, and the thearubigins in them.

Superoxide radical (O₂^{•-}) scavenging activity: The superoxide radical scavenging activity of all extracts was summarized in Table 1. Specifically, the superoxide

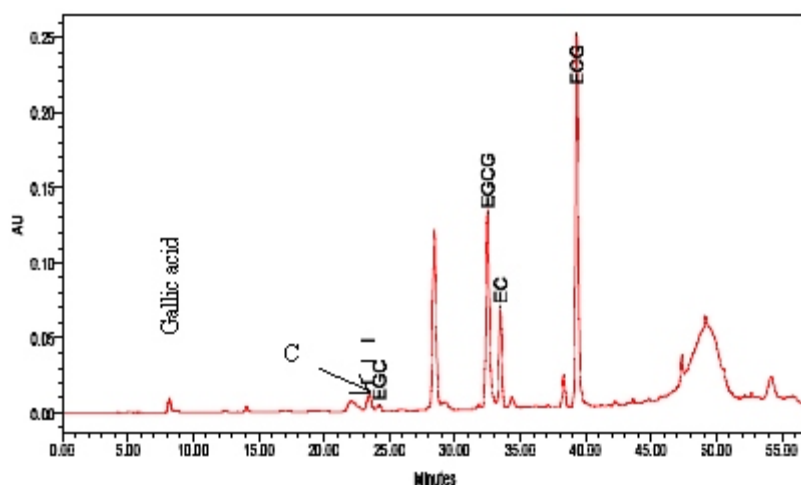


Fig 1: HPLC chromatogram of ethyl acetate extract of pu-erh tea material

radical scavenging activity of pu-erh tea extracts decreased in an order of the ethyl acetate extract > the ethanol precipitation > the ethanol extract > the water extract > the chloroform extract > the butanol extract > the ethanol steep extract. This may be due to the high content of the total catechins (12.41%), the tea polyphenols (23.46%), the thearubigins (7.11%), and the tea polysaccharide (4.25%) in the ethanol precipitation extract of pu-erh tea. On the contrary, the superoxide radical scavenging activity of pu-erh tea material extracts decreased as the ethanol precipitation > the water extract > the chloroform extract > the ethyl acetate extract > the butanol extract > the ethanol extract > the ethanol steep extract, among which the ethanol precipitation extract exhibited the greatest scavenging activity with a IC50 value 13.5 μ g/ml while the water extract showed second strongest activity with a IC50 value 16.2 μ g/ml. In addition, the content of the tea polysaccharide and tea polyphenols in the ethanol precipitation extract of pu-erh tea material was 6.81% and 9.0%, respectively.

Nitrogen dioxide radical (NO₂⁻) scavenging activity:

The effects of pu-erh tea extracts on the nitrogen dioxide radical scavenging activity were listed in Table 1. Specifically, in pu-erh tea extracts, the scavenging activity was found to decrease in an order of the ethyl acetate extract > the ethanol precipitation > the butanol extract > the water extract > the ethanol steep extract > the chloroform extract > the ethanol extract, while in pu-erh tea material extracts, the scavenging activity decreased as the ethyl acetate extract > the ethanol steep extract > the water extract > the butanol extract > the chloroform extract > the ethanol extract > the ethanol precipitation. The ethyl acetate extract of both pu-erh tea and its material demonstrated the strongest nitrogen dioxide radical scavenging (NO₂⁻) capability, which may be attributed to its high total catechins, tea polyphenols and thearubigins content as revealed by HPLC analysis (Fig. 1) that showed the evidence of high monercatechins content such as EGCG, EC and ECG in

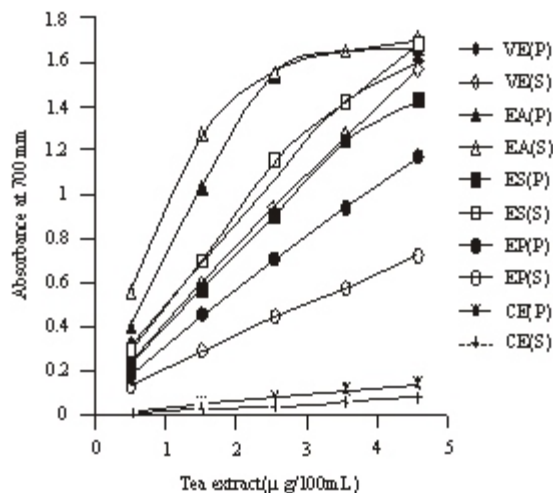


Fig 2: Reducing power of tea extracts. Here, P and S denoted the pu-erh tea and pu-erh tea material (green tea dried by sunshine), respectively

the ethyl acetate extract of pu-erh tea material (Table 2). This result was consistent with that of Miao *et al.* (2001). They reported a detail study about the reactions of the tea polyphenol derivatives such as the epicatechin (EC) and epigallocatechin gallate (EGCG), with the nitrogen dioxide radical (NO₂⁻) using time-resolved pulse radiolysis technique, in which both EC and EGCG showed a significant NO₂⁻ radical scavenging activity (Miao *et al.*, 2001). These results indicated that, besides the total catechins, both the tea polyphenols and thearubigins had the strong nitrogen dioxide radical scavenging activity.

Reducing power of tea extracts: The variation of the reducing power (the absorbance at 700 nm) as a function of tea extract concentration was plotted in Fig. 2, in which the reducing 9Year 8Month 9Day power increased with the concentration. Particularly, the ethyl acetate extract of pu-erh tea as well as its materials exhibited

relatively strong reducing power in comparison with other tea extracts, which may be due to their high content of the total catechins, the tea polyphenols and the thearubigins (Table 1), partially because it has been reported by Lunder *et al* (1992) that there was a certain correlation between the antioxidant activity and the epigallocatechin gallate (EGCG) content (Lunder, 1992).

DISCUSSION

As shown by the experimental results, the extracts of pu-erh tea as well as its materials exhibited an obvious difference in free radical scavenging activity toward all four kinds of free radicals studied here. The observed difference in pu-erh tea extracts may be attributed to not only the catechins and tea polyphenols content but also the theabrownin and tea polysaccharide content. However, the experimental results also showed that there was a free radical scavenging activity difference between the same extracts of pu-erh tea and its materials. It may be explained as follows. The pu-erh tea was produced from green tea *Camellia sinensis* (L.) var. *assamica* (Masters) Kitamura via a long period of solid state fermentation in which *Aspergillus niger* was generated as the starter culture. During the fermentation, a large amount of *Aspergillus niger* were generated and the temperature increased significantly (Gong *et al.* 2005). As a result of the high temperature (30 ~ 60 °C) as well as the high humidity (85%), the reaction rate of both the oxidized and decomposed reactions in sundried green tea leaves increased. Particularly, after 40 days, a large amount of polyphenols such as the catechins in pu-erh tea material have been found to convert into the thearubigins and theabrownins, which may be responsible for the observed difference (Zhou *et al.*, 2004).

In this study, the antioxidant activity as well as the reducing power has been measured *in vitro* in various extracts of both pu-erh tea and its materials. Particularly, it has been found that the ethanol precipitation and the water extract of pu-erh tea as well as the ethanol precipitation and ethyl acetate extract of pu-erh tea materials showed the highest antioxidant activity against the 1,1-diphenyl-2-picrylhydrazyl (DPPH), the hydroxyl (OH[•]), the superoxide (O₂^{•-}), and the nitrogen dioxide (NO₂[•]) radical. The strong antioxidant activity observed in the tea extracts may be due to not only the content of the total catechins but also the content of the total polyphenols, the thearubigins, the theabrownins and the tea polysaccharide. The experimental results indicated that the antioxidant power of pu-erh tea was stronger than that of its materials. Furthermore, it was found that the antioxidant power showed a strong correlation with the total theabrownins and polysaccharide content. The results reported here suggested that pu-erh tea could be an important dietary antioxidant source.

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