

Comparative Analysis of the Antioxidant and Free-Radical Scavenging Activities of Different Water-Soluble Extracts of Green, Black and Oolong Tea Samples

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Abstract

Extracts of 40 green, black or oolong high quality tea samples from China, Japan or India among others, have been prepared according to the manufacturer instructions and assayed for total polyphenols content using the Folin-Ciocalteu phenol reagent. In addition, the antioxidant activity was assessed by the CUPRAC method and the free-radical scavenging activity was determined using the antioxidant-promoting decay of the stable free-radical DPPH. Caffeine and the most important catechins were identified using RP-HPLC previously validated method. Results from this study suggest that teas, even though they content similar amounts of caffeine, differ considerably in polyphenolic content and therefore in antioxidant and free-radical scavenging activities, depending on the part of the plant used and the process applied to the material for the preparation of the final tea presentation. Correlations among Folin-Ciocalteau's reactivity of tea samples, the neocuproine reactivity, the DPPH decolourization and the HPLC analysis suggest that the antioxidant activity is due essentially to polyphenolic compounds present in teas, mainly EGCG. The best extraction method of tea powder was the one indicated by dealers. In general, the richest samples belong to green teas and the poorest samples belong to the black teas.

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Keywords

Tea, Polyphenols, Antioxidants, Free-Radical Scavenging

1. Introduction

The benefits of tea polyphenols consumption have been well established. Tea polyphenols are a source of valuable phytochemical compounds whose consumption may affect health and be beneficial for pathologies, like cardiovascular disorders and cancer [1] [2]. Teas may be classified in at least three categories depending on the degree of fermentation. Unfermented teas are known as green teas, partially fermented teas are known as oolong teas and highly fermented teas are known as black teas. Independently of the fermentation degree, teas may be enriched with aroma from plants leading to a broad variety of teas featured by their flavour. From a health point of view green tea has been the most studied due to its richness in phenolic compounds known as phenolics. Among complex phenolics are flavanols and related compounds (Figure 1). The major flavanic polyphenols in green tea are (-)epicatechin-3-gallate (ECG), (-)epicatechin (EC), (-)epigallo-catechin (EGC) and (-)epigallocatechin-3-gallate (EGCG) [3]. The black tea also contains tearubigins and theaflavins [4]. Green tea polyphenol catechins (GTPs) are highly active molecules acting as antioxidant [2], pro-oxidant [5], anti-inflammatory [6] and anti-cancer [7] agents. EGCG is the most widely studied catechin [1]. Among the standardized preparations enriched in green tea polyphenols is Polyphenon[®] which has been used in studies into inflammation-related colon carcinogenesis in mice [6] and in studies of catechin pharmacokinetics in healthy human beings [3] [5]. The combination of EGCG and sulindac significantly enhanced apoptosis in rat colon, reducing the aberrant crypt foci [8]. Despite these positive effects, administration of very large doses of concentrated green tea extracts has been shown to trigger certain adverse effects that rise concerns from a food safety point of view [9]-[11]. The aim of the present research was to compare the total phenolic content and the antioxidant and free-radical scavenging activities of a number of samples of different green, oolong and black teas from Asia and Africa prepared as water extracts as indicated by the dealers.

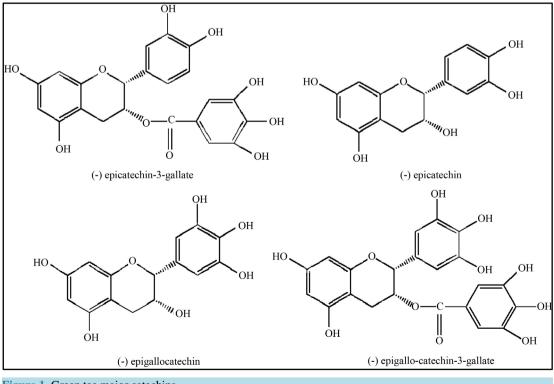


Figure 1. Green tea major catechins.

2. Materials and Methods

2.1. Materials

All common reagents were of the highest grade commercially available. 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteau's phenol reagent (FC), FeCl₃ and neocuproine were purchased to Sigma-Aldrich Química S.A. (Tres Cantos, Spain). Tea samples were purchased from different commercial sources as indicated in the tables. Preparation of the water extracts was performed as indicated by the tea distributor.

2.2. Tea Samples and Preparations of Teas

1 (3 years Kukicha, Japan, green); 2 (Sencha, Japan, green); 3 (Kukicha, Japan, green); 4 (3 years Hojicha, Japan, green); 5 (Bancha, Japan, green); 6 (Bancha leaf, Japan, green); 7 (second Matcha, Japan, green); 8 (Gyokuro, Japan, green); 9 (first Matcha, Japan, green); 10 (Wild Green, Japan, green); 11 (Special Gunpowder, China, green); 12 (Bi Luo Chung, China, green); 13 (Black, Ethiopia, black); 14 (Long Jing, China, green); 15 (Tai Ping China, green); 16 (Chun Mee, China, green); 17 (Jade Rings, China, green); 18 (Mo Li Feng Yan, China, green); 19 (Long Jing Dragon Well, China, green); 20 (Orange Pekoe, Portugal, black); 21 (Yunnan Supreme, China, black); 22 (Keemun, China, black); 23 (LapsangSouchong, China, black); 24 (Wen Shan BaoZhong, China, oolong); 25 (Ba Xian, China, oolong); 26 (Tung Ting, China, oolong); 27 (Da Hong Pao, China, oolong); 28 (Ro Gui (Cassia), China, oolong); 29 (BaiHaooolong, China, oolong); 30 (Ding Dong, China, oolong); 31 (Yabao (Silver Pu-er bud raw), China, Pu-er); 32 (Old Pu-er, China, Pu-er); 33 (block Pu-er, China, Pu-er); 34 (FTGFOP1, India, black); 35 (SFTGFOP, India, black); 36 (First Flush, India, black); 37 (Second Flush, India, black); 38 (First Flush, India, black); 39 (Black, Tanzania, black); 40 (Black, Madagascar, black). For the preparation of water extracts 1 g of the corresponding tea samples were mixed with 50 mL of hot water according to the manufacturer's instructions. The resulting water extract was used immediately for the assays.

2.3. Total Phenols Content

Total phenolic compounds present in tea extracts were determined with the Folin-Ciocalteau's phenol reagent for phenolics [12]. Reaction mixtures of 1.5 mL contained 0.6 mL of sodium carbonate saturated solution, 0.2 mL of FC and 0.1 mL of sample. The resulting mixtures were incubated for 10 min at 50°C and cooled prior the measurement of the absorbance at a wavelength of 760 nm. Gallic acid was used as standard for calibration. The values obtained in the reaction of the samples with the FC were expressed as mg of gallic acid equivalents (GAE) per gram (wet weight) of plant material.

2.4. CUPRAC Assay

Antioxidant activity was determined by the CUPRAC assay as reported elsewhere [13]. Reaction mixtures containing 0.02 mL of sample, 1 mL of 10 mM CuCl₂, 1 mL of 7.5 mM neocuproine in ethanol and 1 mL of 1 M ammonium acetate (pH 7.0) were prepared and kept for 60 min to allow colour development and after were read at A_{450} . Gallic acid was used as standard for calibration. The results were expressed as gallic acid equivalents (GAE) per gram (wet weight) of plant material.

2.5. DPPH Assay

The capacity to scavenge free radicals was assessed by DPPH colour decay after 10 min of incubation [14]. Reaction mixtures containing 0.03 mL of sample and 2.9 mL of DPPH dissolved in methanol were kept in the dark for colour development and the reduction in colour was determined by measuring the absorbance signal at a wavelength of 515 nm. Trolox[®] was used as standard and results were expressed as Trolox[®] equivalents per gram (wet weight) of plant material.

2.6. Determination of Polyphenols

The contents of polyphenols and caffeine in the tea varieties were analysed in duplicate using RP-HPLC with UV detector. Polyphenols and caffeine were first extracted in hot water as indicated in the envelop of the tea and the aqueous extract was immediately filtered through cotton-wool. 8 mL of filtrate were mixed with 2 mL of

methanol and the mix was filtered under vacuum through a 0.45 μ m membrane. A Waters 2690 Alliance (Waters, Milford, MA, USA) separation module equipped with a Supelcosil LC-18 analytical (5 μ m, 150 mm × 4.6 mm I.D.; Bellefonte, PA, USA), protected by a C18 (10 μ m, 30 mm × 4.6 mm) pre-column guard cartridge, and coupled with a Waters 996 Photodiode Array Detector (Waters) were used in the analysis. The separation of compounds in the column and detection were carried out at 20°C. Compounds were eluted undergradient in water (0.1% formic acid)/methanol (0.1% formic acid) in which the concentration of water varied as follows: 0 min, 80%; from 0 to 15 min, decreased to 50%; from 15 to 25 min, 50%. The flow rate was 1 mL/min, the detection of compounds was achieved at 280 nm, and injection volume was 20 μ L. Quantification was achieved using Polyphenon 60 as standard and confirmed with either caffeine or catechins alone as standard (Sigma Chemical Co., St. Louis, MO, USA).

3. Results and Discussion

The administration of large oral doses of a concentrated green tea known as Polyphenon 60 increases the toxicity of the ribosome-inactivating protein ebulin f due to a synergic proapoptopic action of tea catechins with ebulin f [11]. During these studies, we found different effects of tea depending on the source. In order to gain insights on these differences we analysed forty tea samples from Asia, Europe and Africa countries obtained from different manufacturers for total phenolic content, antioxidant and anti-free-radical scavenging activities. They belong to green, oolong, Pu-er and black categories.

Total phenolics were determined with the Folin-Ciocalteau's reagent for phenols. The reaction is a typical electron transfer assay based on the oxidation/reduction reaction of samples [15] [16]. FC reacts also with non-phenolic compounds like ascorbic acid, cysteine, fructose, sucrose, uric acid, and xanthine among a number of organic substances and iron sulphate, sodium phosphate and tin chloride among the inorganic ones [15]. Therefore, our measures may account not only for phenolics but also for undetermined amounts of other water-soluble reductive substances present in the extracts. Our results indicate clearly that the total content of phenolics was dependent on the type of tea. In general, the richest samples belong to green teas (**Table 1** and **Table 2**). The poorest samples belong to the black teas. Oolong teas showed intermediate values. Noteworthy, the richness in phenolics was very different among the same tea type depending on the part of the plant used and the process applied to the material for the preparation of the final tea presentation. Water extraction of tea samples with hot water was highly dependent on the form of industrial preparation of tea. As expected, phenolic extraction of tea powder was better than stalks and leaves for the times indicated by the dealers.

The antioxidant activity of tea samples was determined with neocuproine (2,9-dimethyl-1,10-phenantroline) in the assay known as CUPRAC. In the original procedure it was reported that Cu^{2+} is reduced by the sample and the resulting Cu^+ forms a complex with two molecules of neocuproine that absorbs at 450 nm [17]. As shown in Table 1 and Table 2, the antioxidant activities of teas were dependent on the tea type.

The free-radical scavenging activity of teas was studied using the decolourization assay of the stable free-radical 2,2-diphenyl-1-picrylhydrazyl known as DPPH [15] [18]. The chemical process is an electron-transfer reaction in which the deep purple colour of DPPH is time-dependent decoloured by the biological sample to be tested by following the absorbance at 515 nm with a colorimeter [15] [19]. As shown also in **Table 1** and **Table 2**, tea-dependent decolourization of DPPH run in parallel to the content in polyphenols and the anti-oxidant activity of the samples. In this line, we analysed the correlation between the values obtained in the different procedures to ascertain whether quantitative relationships exists between the content in phenols and the anti-oxidant and free-radical scavenging activities.

The water extracts of teas were prepared as indicated by the manufacturers, which differ among teas. Most of them recommend 2 - 3 min of contact between tea sample and hot water. To ascertain whether longer times could improve extraction, we selected two teas samples of each tea type and extracted phenolics with hot water after 5 and 10 min of the addition of boiling water. As **Figure 2** shows, extraction for 10 min led to an increase of material that reacted with FC in the range of 14% to 28%. In the case of reaction with DPPH the differences were much higher reaching 56% in the Pu-er tea. At room temperature, the temperature of water used for the extractions decrease from 100° C to 70° C in 2 min and to 50° C in 10 min, independently of the recipient used for extraction (glass or steel vessel like that used in cafeteria). For most teas with high content of polyphenolic material, 2 - 3 min of extraction with hot water ensures a good recovery of polyphenols with a minimum of aroma loss.

Теа	Total phenols content		Antioxidant activity		Free-radical scavenging activity	
Code	GAE (mg)/Wet weight of tea (g)	SD	GAE (mg)/Wet weight of tea (g)	SD	TE (mg)/Wet weight of tea (g)	SD
7	149.55	1.54	62.40	4.14	218.46	1.06
2	146.49	2.92	45.73	0.80	203.24	4.96
9	142.70	2.85	53.00	2.53	193.46	1.27
8	121.08	3.51	35.23	0.96	116.60	11.01
19	97.66	3.13	30.93	0.59	105.80	2.56
17	93.33	0.41	29.53	0.16	94.86	1.09
16	78.20	0.68	30.27	2.86	128.79	7.10
11	54.59	2.31	19.70	0.41	72.42	4.72
12	53.78	2.31	21.57	2.82	74.36	1.27
15	52.16	0.97	19.03	3.11	76.71	6.57
6	46.94	0.68	20.17	1.55	68.22	6.19
3	46.76	0.27	13.93	0.21	51.18	1.24
14	38.83	0.83	15.60	1.07	64.67	17.60
5	36.76	1.24	11.27	1.00	50.42	2.16
10	33.78	1.62	8.90	0.74	34.20	4.79
18	22.07	2.45	4.53	0.89	25.00	0.89
1	19.01	1.36	4.67	0.26	16.08	2.81
4	16.58	0.83	5.57	1.01	14.82	1.52

 Table 1. Total phenolics, antioxidant and free radical scavenging activities of green tea extracts (GAE, gallic acid equivalents; TE, Trolox equivalents; SD, standard deviation).

 Table 2. Total phenolics, antioxidant and free radical scavenging activities of black tea extracts (GAE, gallic acid equivalents; TE, Trolox equivalents; SD, standard deviation).

Tea	Total phenols content		Antioxidant activity		Free-radical scavenging activity	
Code	GAE (mg)/Wet weight of tea (g)	SD	GAE (mg)/Wet weight of tea (g)	SD	TE (mg)/Wet weight of tea (g)	SD
37	59.64	0.95	18.83	0.43	68.33	1.31
36	58.47	0.95	18.07	0.26	62.47	2.36
39	55.77	1.09	16.93	0.21	52.05	1.08
38	51.62	0.54	15.97	0.16	49.05	1.31
21	48.38	1.50	13.10	0.27	55.12	3.01
40	46.31	0.68	15.33	0.16	36.20	1.78
35	45.86	1.02	14.87	0.16	24.77	1.50
34	44.95	0.56	14.33	0.16	21.63	1.50
13	41.44	1.09	13.63	0.16	21.92	0.86
22	35.14	1.77	9.37	0.36	17.61	4.08
20	24.86	2.74	4.83	1.13	31.27	1.98
23	14.23	1.09	3.27	0.42	9.62	2.36
25	50.45	0.95	17.00	0.64	73.23	1.54
28	49.46	1.89	12.50	0.21	45.56	2.20
27	38.38	1.24	10.60	0.47	25.45	1.73
24	32.70	0.81	8.13	0.31	13.47	1.73
30	24.50	0.95	7.10	0.41	12.20	1.08
29	12.16	0.81	5.43	0.26	9.63	0.65
26	6.67	0.56	1.60	0.27	6.20	0.89
33	29.19	0.97	11.83	0.33	14.20	0.86
32	10.90	0.68	2.47	0.49	7.21	0.65
31	5.59	0.41	0.47	0.16	3.06	1.96

In order to establish a quantitative relationship between the polyphenols content and the antioxidant and free radical scavenging activities of the different tea samples we carried on a study of correlation. As it is shown in

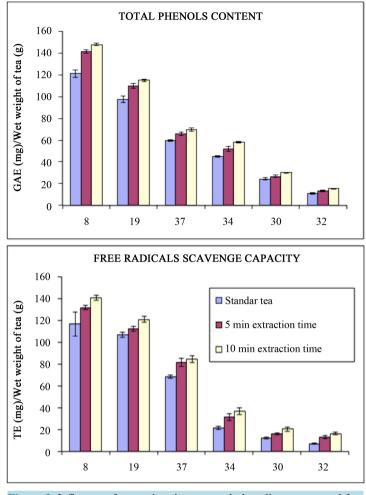


Figure 2. Influence of extraction time on total phenolics content and free radical scavenging activities of some selected teas.

Figure 3, the match of reactivity of teas water extracts with FC, with the values of the CUPRAC assay led to a straight line corresponding to the equation y = 0.3628x - 1.7827 with a R² value of 0.9528. The match of FC assay with the values of the DPPH decolourization assay gave also a straight line corresponding to the equation y = 1.3857x - 13.979 with R² value of 0.9068. These results indicated that the antioxidant capacity of the water extracts of tea is due to a great degree to the polyphenols content. On the other hand, we found also a good correlation between the antioxidant activity assessed by the CUPRAC procedure and the free radical scavenging activity assessed as loss of colour of DPPH with the equation y = 3.7872x - 6.632 and a R² value of 0.9358.

We further analysed the different tea samples by HPLC and compared with the catechins content of Polyphenon 60. As shown in **Figure 4**, Polyphenon[®] 60 contents the four major catechins plus a significant content of caffeine. The most abundant catechin in the Polyphenon[®] 60 we used was EGCG, followed by EGC, ECG and ECC. The HPLC analysis of the tea samples indicated that the content of catechins run in parallel with the result obtained in the reaction of water extracts with FC; the major reaction the higher content in catechins is. It is noteworthy that some tea samples were rich in caffeine and very poor in catechins as determined by reaction with the phenol reagent and by HPLC. Such tea samples showed a very low reaction with the phenol reagent.

Daily tea drinking is considered currently as a healthy practice due to the antioxidant substances therein contained especially polyphenols [1] [2] [20]-[23]. Such compounds positively affect many biological functions. Therefore, if its consumption is aimed to improve the antioxidant state of the consumer the content in polyphenols should be taken into account. Nonetheless, tea is appreciated not only by antioxidant effects but also by its flavour and taste.

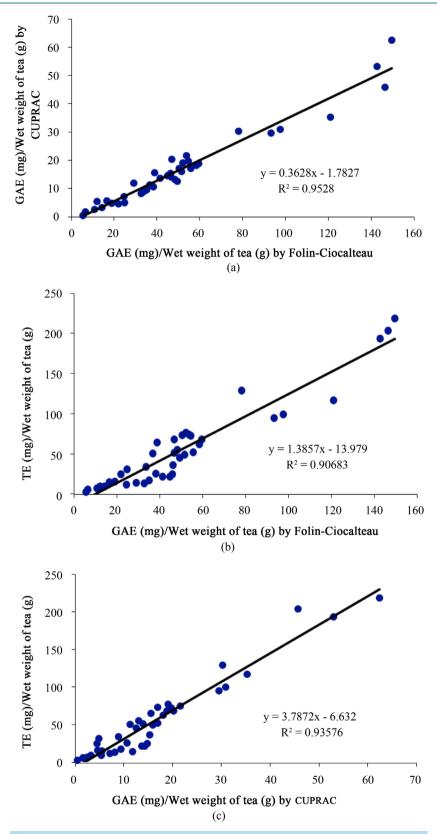


Figure 3. Correlation plots between the polyphenols content and antioxidant and free radical scavenging activities of the different tea samples.

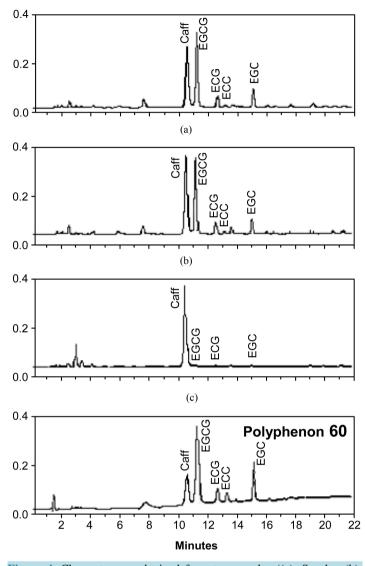


Figure 4. Chromatogram obtained from tea samples ((a): Sencha; (b): Mat cha1^a; (c): Da Hong Pao) and Polyphenon 60 (Caff: Caffeine; EGCG: Epigallo-catechin-3-gallate; ECG: Epicatechin-3-gallate; ECC: Epicatechin; EGC: Epigallocatechin).

4. Conclusion

Our results indicate that teas, even though they content similar amounts of caffeine, differ considerably in polyphenolic content and therefore in antioxidant and free-radical scavenging activities. Antioxidant and free radical scavenging quality was dependent on the industrial processing of tea samples. In general, the less processed and fermented teas were the richest ones. Optimal extractions of phenolics from tea samples were observed following the indications of tea dealers. The form of tea presentation, powder, stalks or leaves, had also a deep influence in the extraction of antioxidants. Correlations between Folin-Ciocalteau's reactivity of tea sample, the neocuproine reactivity and DPPH decolourization suggest that the antioxidant activity is due essentially to polyphenolic compounds present in teas, mainly EGCG.

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