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## Determination of Antioxidant Activity in Tea Extracts, and Their Total Antioxidant Content

Alexander Yashin<sup>1</sup>, Yakov Yashin<sup>1</sup>, Boris Nemzer<sup>2\*</sup>

<sup>1</sup> Scientific Research Center “Chromatographia”, Moscow, Russia

<sup>2</sup> Department of Research & Development, VDF FutureCeuticals, Inc., Monence, IL, USA

**\*Corresponding Author**

Boris Nemzer

Director of Research and Development

FutureCeuticals, Inc.

Monence, IL 60914

USA

Email: [bnemzer@vandrunen.com](mailto:bnemzer@vandrunen.com)

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### Abstract

Antioxidant activity of different types of tea (green, oolong, black, pu-erh ) were measured using different modern methods. Several types of commercially available teas, from various manufacturers were tested for antioxidant content using the amperometric method, the data is displayed here. Data gathered about antioxidant content of these different tea samples can be used to estimate quality and type of tea. The data collected using this method is also important when trying to account for the normal daily consumable antioxidant of healthy people and also patients using clinical antioxidant therapy.

**Keywords:** Tea, antioxidant activity, amperometric method, antioxidants, tea polyphenols.

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### 1. Introduction

In the last decade, determination of antioxidant activity and the total content of antioxidants in foods, beverages, dietary supplements and herbal extracts has been in wide demand. This relates to the fact that antioxidants can prevent free radicals, primarily highly reactive oxygen and nitrogen species, from damaging human health.

The steady increase of free radicals in cells creates the conditions for so-called oxidative

stress, wherein free radicals oxidize blood vessel walls, protein molecules, DNA, and lipids. These radicals are particularly active in interacting with membrane lipids that contain unsaturated bonds, and thus alter the properties of cell membranes. The most active free radicals break bonds in DNA molecules and damage the cells' genetic apparatus regulating their growth, which can result in cancerous cells. Recently a large number of diseases have been associated with oxidative stress. Oxidative stress also plays a key role in aging.

Harmful effects of free radicals and oxidative stress can be reduced by regular consumption of foods and beverages which exhibit antioxidant activity.

The primary natural antioxidants include flavonoids, oxiaromatic acids, vitamins C and E, carotenoids, and other compounds.

In recent years bioflavonoids have become more popular since they possess anticarcinogenic, antisclerotic, antiallergenic properties, and their antioxidant activity is several tens of times greater than  $\alpha$ -tocopherol, vitamin C, and  $\beta$ -carotene. A combination of natural bioflavonoids contained in vegetables, berries, fruits, grains, seeds, nuts, etc. is especially effective. Bioflavonoids are synthesized by plants to protect them from oxidative processes, and during long-term evolution they formed their optimal combinations.

Plant-based bioflavonoids effectively protect the human body from oxidative stress; a conclusion base on substantial epidemiological evidence.

Tea is one of the richest sources of antioxidants and the three major forms of antioxidant tea are green tea, oolong tea, and black tea. These teas are differed in producton methods and chemical composition [1, 2].

The major antioxidants in tea are catechins, then theaflavins, thearubigins, oxyaromatic acids, flavonols, such as kaempferol, myricetin, quercetin; flavones, such as apigenin; derivatives of gallic acid, such as tannins, etc. The most powerful antioxidant tea is green tea which is characterized by the presence of large amount of flavan-3-ols known as catechins. (-) Epigallocatechin-3-gallate (EGCG) is most abundant catechin in green tea and may occure up to 50% of the catechins by weight. Oolong tea is partially oxidized or fermented tea (25-60%) and black tea is fully oxidized. The result of catechins oxidation is the formation of catechins dimers, known as theaflavins. These compounds are responsible for the color and taste and also a key factor in the antioxidant activity.

The known in vitro antioxidant properties of catechins and other polyphenolic compounds in tea have led to interest in the potential health benefits of tea consumption [3]. The evaluation of

their efficacy as antioxidants in vivo is more complex [4]. Numerous epidemiologic studies have addressed the relationships between tea consumption and the incidence of cardiovascular diseases [5-9]. The antioxidant activity of green tea polyphenols and, more recently, the pro-oxidant effects of these compounds, have been suggested as potential mechanisms for cancer prevention [10-12]. The mechanism of action of tea on human health can be characterized not only by potent antioxidant activity (like reduction of LDL oxidation, lipid per oxidation, and DNA oxidation [13,14]) but also anti-inflammatory (skin disorders, arthritis) and thermogenesis (fat oxidation and energy expenditure) activities as well [15].

In [2] has been shown that although tea polyphenols have generally been regarded as antioxidants, the emerging evidence for the pro-oxidant effects of these compounds is interesting and raises many potential questions. Studies using higher doses of EGCG also show that pro-oxidant effects may play a role in the potential toxic effects of EGCG that have been reported in vivo [16]. Most case reports involved the use of non-traditional dosage forms (e.g., pills, or capsules) but there has been a report of green tea beverage causing hepatotoxicology [17]. The existing differences in sensitivity to potential green tea toxicity are unclear and probably related to individual differences in metabolism and bioavailability of green tea polyphenols [2].

Antioxidant activity in tea is measured by well-known methods, such as ORAC, FRAP, ABTS, etc. [18-22].

The Oxygen Radical Absorption Capacity method (ORAC) is based on the measurement of fluorescence quenching in fluorescein when it is exposed to the stable radicals and antioxidants being studied [22].

The Ferric Reducing/Antioxidant Power (FRAP) assay is based on the recovery of an Fe (III)-tripyridyltriazine complex in Fe (II) at low pH, exhibiting an intense color [19,20].

The total content of antioxidants can be determined by an amperometric method and high performance liquid chromatography.

## 2. Determination of Antioxidant Activity

Antioxidant activity of green tea, as measured by different methods, is usually higher than the antioxidant activity of black tea or oolong tea [18, 22-25].

However, it was determined that the theaflavins in black tea and catechins in green tea are equally effective antioxidants [26, 27].

The antioxidant activity of certain types of tea, i.e., green tea [28, 29], black tea [30] and oolong tea [31-33] have also been investigated.

The major hypothesis of the beneficial health effects of tea is associated with its antioxidant properties [34]. In addition to the capturing (quenching) of free radicals, the tea catechins can chelate metal ions such as iron and copper, preventing their participation in Fenton and Haber-Weiss reactions [35].

Antioxidant capacity of various teas and tea polyphenols has been investigated in many studies [36-43]. Using the oxygen radical absorption capacity method (ORAC), green and black teas were found to have a higher antioxidant activity with respect to peroxy radicals than vegetables (garlic, kale, spinach, and Brussels sprouts) [22].

The total antioxidant activity of green tea was shown by FRAP to be higher than that of black tea [19]. Epicatechin and catechin were classified by the trolox equivalent antioxidant capacity (TEAC) method as the best among 24 plant flavonoids [44].

Coulometric evaluation of the antioxidant capacity of several tea extracts was performed using electrogenerated bromine [45].

In this case, the integral antioxidant capacity (AOC) of tea was assessed in kilocoulombs (kC) per 100g of tea. At the same time, the total flavonoid content was determined in terms of rutin and was expressed in mg/100g of tea.

The bromine AOC in black teas ranged from 7.4 to 19.3 kC/100g. The total flavonoid content was within the range of  $29 \pm 1.3$  to  $72 \pm 9.5$ .

The total content of phenols as well as the portion of catechins in them was determined in black and green teas by the Folin-Ciocalteu method [40]. The content of all polyphenols in black teas ranged from 80.5 to 134.9 mg/g of dry tea weight relative to gallic acid. The greatest

amount of total polyphenols were found in Ceylon black tea. The percentage of all catechins in the total content of polyphenols in black tea was determined to range from 10.1 to 37.3%. The largest percent of catechins was detected in black Darjeeling tea.

The total content polyphenols in green tea was within the range of 65.8 to 106.2 mg/g, and the portion of catechins varied from 50.4 to 98.0%. The largest percent of catechins was identified in Japanese green tea (Bandia).

In one of the studies the total antioxidant capacity of 27 different types of tea (green, black, oolong, and Pu-erh) was determined using FRAP assay [19]. Five grams of dry tea powder was brewed in 150 ml of boiling distilled water. The antioxidant capacity ranged from 132  $\mu\text{mol/g}$  for Pu-erh tea and up to 1,144  $\mu\text{mol/g}$  for one grade of green tea. The average values are given in Table 1.

**Table 1. The Total Antioxidant Capacity of Different Teas Using FRAP Assay [19].**

| No. | Type of Tea | Number of Experiments | Value in $\mu\text{mol/g}$ (Dry Powder) |
|-----|-------------|-----------------------|---|
| 1   | Green       | 13                    | 571                                     |
| 2   | Oolong Tea  | 5                     | 373                                     |
| 3   | Black       | 8                     | 365                                     |
| 4   | Pu-erh      | 1                     | 132                                     |

These measurements show that antioxidant activity of green tea is higher than that of black tea.

The values for antioxidant activity varied by 2-3 times—the authors attribute this to the tea grades as well as cultivation, production, and storage conditions.

The antioxidant capacity of brewed teas changed only slightly during storage for 48 hours at 4 °C.

The antioxidant activity of catechins depends on the number of hydroxyl groups, that is epigallocatechin gallate (8 hydroxyl groups), epicatechin gallate (7), gallic catechin (6), and epicatechin (5) [46].

**Table 2. Total Content of Antioxidants (CCA) in Black Tea (Quercetin Used as Reference)**

| No. | Name                                  | Manufacturer/Country                                 | CCA mg/g |
|-----|---------------------------------------|--|----------|
| 1   | Darjeeling Premium                    | Mlesna Tea Naturally, Sri Lanka                      | 186.6    |
| 2   | Darjeeling Tea No.1                   | India, Darjeeling                                    | 150.0    |
| 3   | Darjeeling                            | Darjeeling tea manufacturer, Djukpana Plantation     | 146.5    |
| 4   | Akbar Premium                         | Sri Lanka  | 138.6    |
| 5   | Beta Tea Black Tea (Selected Quality) | Beta Tea Groups, Turkey                              | 118.0    |
| 6   | Presidential Ceylon Baikhovi Tea      | Mlesna, Sri Lanka                                    | 118.0    |
| 7   | Hyleys                                | Sri Lanka  | 109.4    |
| 8   | Bahar                                 | Sri Lanka  | 106.2    |
| 9   | Darjeeling                            | Greenfield, UK                                       | 106.0    |
| 10  | Beseda                                | Unilever   | 106.0    |
| 11  | Darjeeling                            | Chaygorod  | 103.6    |
| 12  | Darjeeling                            | Chaygorod  | 103.0    |
| 13  | Alokozay                              | Dubai, UAE   | 102.1    |
| 14  | English Breakfast Assam               | India  | 97.8     |
| 15  | Mabroc Earl Grey                      | Sri Lanka  | 96.4     |
| 16  | Monzil Pride                          | Sri Lanka  | 94.9     |
| 17  | Estate Pure Ceylon Tea Garden Mark    | Sri Lanka  | 94.3     |
| 18  | Riston Exclusive Quality              | Sri Lanka  | 90.0     |
| 19  | Victorian Tea                         | Curtis&Patridge London, Ltd., England                | 88.4     |
| 20  | Ahmad Earl Grey                       | Ahmad Tea Ltd., Moscow Region, Mytischensky District | 88.0     |
| 21  | Jungle Call                           | Kenya  | 86.6     |
| 22  | White Nights Aromatized Black Tea     | OOO Russian Tea Company                              | 79.9     |

| No. | Name  | Manufacturer/Country  | CCA mg/g |
|-----|---|---|----------|
| 23  | Greenfield Golden Ceylon  | Greenfield, UK  | 79.5     |
| 24  | Beta Tea  | Sri Lanka   | 79.4     |
| 25  | Borodinsky  | India, Ceylon   | 78.5     |
| 26  | St. Clairs 100% Pure Ceylon Tea                                   | Founder St. Clairs Tea Plantation   | 77.7     |
| 27  | Chelton Tea Collection, Gunpowder                                 | Jafferjee Brothers, Sri Lanka   | 76.3     |
| 28  | Curtis Lemon Lane   | Under control of Curtis Partidge London, Ltd.                             | 73.0     |
| 29  | Pearl of China  | Forsman Tea, Finland  | 63.9     |
| 30  | Assam No. 17  | Tea Collection, India   | 63.7     |
| 31  | Impra Black Tea   | Imperial Tea Exports (Pvt) Ltd.   | 58.8     |
| 32  | Darling with Blueberries and Mango (British Royal Academy of Tea) | English present tea 100% Ceylon tea                                       | 57.0     |
| 33  | Just the One Indian   | OOO Moscow Tea Company  | 56.3     |
| 34  | Black Tea   | Indonesia   | 55.2     |
| 35  | Black Tea with Bergamot   | Azercay, Azerbaijan   | 54.6     |
| 36  | Black Tea   | Maryam, Azerbaijan  | 54.1     |
| 37  | Akbar   | Akbar Brothers Ltd., Sri Lanka (OOO Yakovlev Tea-Packing Factory, Russia) | 51.0     |
| 38  | Hyleys Earl Grey  | Sri Lanka   | 46.4     |
| 39  | Riston Premium  | Sri Lanka   | 43.0     |
| 40  | Ceylon Pekoe  | Forsman Tea, Finland  | 39.8     |
| 41  | Darjeeling  | India, packed in Austria  | 39.0     |
| 42  | Darjeeling in Bags  | Ronnefeldt, Schwarzer Tee   | 32.0     |
| 43  | Nadin, S Novym Godom, Large-Leaved Tea                            | OOO Lealanis, Moscow  | 28.0     |
| 44  | Black Baikhovi Tea  | Vietnam   | 28.0     |
| 45  | Cherny Barkhat  | OOO Russian Tea Company   | 23.7     |
| 46  | Georgian  | Mozhaisky House of Commerce   | 17.8     |

### 3. Determination of the Total Antioxidant Content Using an Amperometric Method.

Amperometric method (AM) used for determination of antioxidants is based on measuring an electric current in the detector cell which occurs during oxidation of the analyte on the working electrode surface when certain potentials are applied [18]. The signal is recorded as differential output curves. Using special software, the areas or peak heights (of the differential curves) are calculated for the analyte and for the reference substance. The average value of three to five consecutive measurements is used for the analysis. Well-known antioxidants, such as quercetin, dihydroquercetin, mexidol, trolox, gallic acid, etc., could be used as reference substances.

The amperometric method has several advantages for determining antioxidant activity: not taking into account sample preparation, one determination takes only a few minutes; analysis (data recording and processing) takes place in real time; accuracy and reproducibility of the analysis

is ensured by accurate dosing with a six-way valve; standard deviation (SD) of valve dispensal is less than 0.5%; SD of the successive measurements of the analyzed samples is less than 5%; limit of detection for polyphenols and flavonoids is at the level of nanograms and picograms ( $10^{-9}$  –  $10^{-12}$  g). At such low concentrations, the likelihood of the mutual influence of different jointly present antioxidants, such as by a manifestation of synergy, is significantly reduced.

The amperometric method is the only method which allows for direct measurement of all antioxidants in a sample. Other methods are indirect—they measure the inhibition of reaction mixtures (free radicals) generated by certain reactions.

Using the amperometric method, the total content of antioxidants was determined in many varieties of teas produced by different companies. Table 2 provides data for green tea and Table 3 for black tea.

**Table 3. Total Content of Antioxidants (CCA) in Green Tea (Quercetin Used as Reference)**

| No. | Name                      | Manufacturer/<br>Country | CCA<br>mg/g |
|-----|---------------------------|--------------------------|-------------|
| 1   | T-Sips Ceylon Tea in Bags | Sri Lanka                | 190.0       |
| 2   | Alokozay                  | Dubai, UAE               | 171.2       |
| 3   | Riston Green Exotic       | Sri Lanka                | 155.0       |
| 4   | Lipton                    | Unilever Foodsolutions   | 143.0       |
| 5   | Minamoto                  | Yunako Company,<br>Japan | 143.0       |
| 6   | NamaCha Live Green Tea    | Japan                    | 139.6       |
| 7   | Tea Tang Sour Sap         | Sri Lanka                | 138.0       |
| 8   | Azercay (Yastl Cay)       | Azerbaijan               | 133.2       |
| 9   | Greenfield Flying Dragon  | London                   | 130.0       |
| 10  | Merlin                    | Brand Tea, Sri Lanka     | 126.8       |
| 11  | Green Tea Gift            | Mirax Pharma             | 125.3       |
| 12  | Nadin Super AOX Verbena   | Vitali Tea               | 125.0       |
| 13  | Green Elephant            | Sri Lanka                | 125.0       |

| No. | Name   | Manufacturer/<br>Country                             | CCA<br>mg/g |
|-----|--|--|-------------|
| 14  | Selenium Green Tea   | Wahan Mingcha Tea Industry Ltd, Hubei, China         | 123.6       |
| 15  | Green Tea with Jasmine                                       | Mabroc Teas Ltd., Sri Lanka                          | 120.1       |
| 16  | Tea Tang Green Tea with Mango                                | Tea Tang, China                                      | 117.0       |
| 17  | Ahmad  | Ahmad Tea Inc., Sri Lanka                            | 116.0       |
| 18  | Jaf Tea, Green Tea with Pieces of Strawberries and Kiwi      | Jafferjee Brothers, Sri Lanka                        | 115.0       |
| 19  | Zhong Guo Mig Cha Milk Green Tea                             | China  | 115.0       |
| 20  | Green Tea with Jasmine                                       | Newby, London  | 114.0       |
| 21  | Tian Ren   | ZAO Tian Ren   | 108.1       |
| 22  | Mlesna Green Ceylon Baikhovi Tea with Strawberries           | Sri Lanka  | 107.0       |
| 23  | Dobroe Utro (with Jasmine)                                   | Dobrynya-Rus, Ltd.                                   | 104.0       |
| 24  | Maitre   | Maitre, France                                       | 98.0        |
| 25  | Tian Ren   | ZAO Tian Ren Academy of Chinese Culture and Medicine | 97.0        |
| 26  | Mabroc Earl Grey   | Sri Lanka  | 96.4        |
| 27  | Jaf Tea, Earl Grey Green Tea with Bergamot                   | Sri Lanka  | 95.0        |
| 28  | Super Pekoe No.1   | Tea Tang Ltd, Colombo, Sri Lanka                     | 91.8        |
| 29  | Yu Shan Yin Yellow Tea (Yellow Needles from a High Mountain) | Tea Yard, China                                      | 90.0        |
| 30  | Laurel Flower Tea  | China  | 88.9        |
| 31  | Silver Cilia   | China  | 84.4        |
| 32  | Tian Ren with Jasmine  | ZAO Tian Ren   | 84.4        |
| 33  | Ahmad Baikhovi Leaf Tea with Jasmine                         | Sri Lanka  | 83.0        |
| 34  | Jasmine tea 632  | China  | 82.7        |
| 35  | Hyleys English Large-Leaved Green Tea                        | Sri Lanka  | 80.4        |
| 36  | Jasmine Tea  | China  | 80.4        |
| 37  | Chelton Tea Collection, Gunpowder                            | Jafferjee Brothers, Sri Lanka                        | 76.3        |

| No. | Name  | Manufacturer/<br>Country                  | CCA<br>mg/g |
|-----|---|---|-------------|
| 38  | Royal Sprouts White Tea   | Tea Yard, China                           | 74.0        |
| 39  | Mlesna Green Ceylon Baikhovi Tea with Mango                               | Sri Lanka                                 | 69.0        |
| 40  | Jasmine Tea   | Shin Kong Mitsukoshi<br>Dep.              | 68.1        |
| 41  | Flower of Tenderness with Red Saffron                                     | Nadin Company,<br>Copenhagen              | 66.0        |
| 42  | Mo Li Hua Cha Jasmine Tea   | Tea Yard, China                           | 66.0        |
| 43  | Hyleys Large-Leaved Tea   | Sri Lanka                                 | 63.6        |
| 44  | Hilltop Collection Tea Lemon Green Tea                                    | Tea Collection,<br>Mozhaysk               | 63.3        |
| 45  | Greenfield Green Mekissa  | London                                    | 60.7        |
| 46  | Xinyang Green Tea   | Dabieshan Tea<br>Company, Henan,<br>China | 60.0        |
| 47  | Dr. Green (Jasmine Tea)   | China                                     | 57.2        |
| 48  | Green tea from Pogadaev   | Sochi, Lazorevskoye,<br>Tea House         | 56.4        |
| 49  | Quan Xin Huang Mountain Mao Feng  | China                                     | 56.3        |
| 50  | Organic GreenTea  | China                                     | 55.5        |
| 51  | Yunnan Mao Feng   | China                                     | 53.9        |
| 52  | Bambu Shaolin Strength (Large-Leaved)                                     | China                                     | 52.2        |
| 53  | Green tea No. 371   | China                                     | 51.2        |
| 54  | Indu from Top Two Leaves, Chinese Large-Leaved Tea with Ginseng           | Indu Enterprises, China                   | 46.5        |
| 55  | Camel   | Zhejiang Tea Company<br>in China          | 45.7        |
| 56  | SVAY Fresh Fantasy large-leaved tea with passion fruit, orange, and Lemon | Germany                                   | 44.8        |
| 57  | Imperial Gunpowder Chinese Elite Tea                                      | China                                     | 44.0        |
| 58  | Indu from Top Two Leaves, Chinese Large-Leaved Tea                        | Indu Enterprises, China                   | 43.7        |
| 59  | Indu from Top Two Leaves, Chinese Large-Leaved Tea with Mint              | Indu Enterprises, China                   | 43.7        |
| 60  | Teh Melati Jasmine Tea  | Bali                                      | 31.2        |



#### 4. Determination of the Total Antioxidant Content Using High Performance Liquid Chromatography (HPLC)

Table 4 shows the total content of catechins, all polyphenols, theaflavins, and thearubigins in different varieties of tea from Kenya, Japan, and China [43]. The total content of catechins ranged from 3.07 to 4.62 mg/g for black tea and from 3.31 to 14.93 mg/g for green tea. The total content of polyphenols is rather high for almost all types of tea. Significant content of theaflavins and thearubigins was found in some green teas. This was probably caused by a breach of green tea production technology. Green tea which was

prepared by traditional technology and was properly stored contains virtually no theaflavins or thearubigins. This fact is confirmed by data shown in Table 5. The total content of theaflavins is very small in green teas.

The total content of catechins estimated by HPLC relative to the sum of all peak areas for all detected catechins is provided in Table 6. The measurements were made for the most famous and expensive teas (black, white, yellow, and green).

All types of tea contained epigallocatechin gallate (EGCG) in the greatest amounts, followed by ECG and EGC. Catechin and epicatechin were present in significantly lower amounts.

**Table 4. Total Content of Catechins, All Polyphenols, Theaflavins, and Thearubigins in Different Tea Grades, in mg/g of Dry Tea [44].**

| No.                  | Type of tea                 | Total catechins | Total polyphenols | Theaflavins | Thearubigins |
|----------------------|-----------------------------|-----------------|-------------------|-------------|--------------|
| <u>Kenyan Teas</u>   |                             |                 |                   |             |              |
| 1.                   | Black Tea (Pekoe Dust)      | 5.91            | 20.65             | 11.61       | 10.30        |
| 2.                   | Black Tea (Broken Pekoe)    | 3.07            | 17.45             | 18.75       | 15.65        |
| 3.                   | Black Tea (Orthodox)        | 4.62            | 22.25             | 13.80       | 11.51        |
| 4.                   | Green Tea (Pekoe Fanning's) | 14.93           | 26.85             | 0.46        | 5.59         |
|                      | Green Tea (Broken Pekoe)    |                 |                   |             |              |
| 5.                   | Green Tea (Orthodox)        | 10.04           | 25.70             | 1.63        | 8.38         |
| 6.                   | White Tea (Silvery Tip)     | 11.06           | 27.10             | 0.41        | 5.77         |
| 7.                   | Oolong (Orthodox)           | 10.20           | 21.30             | 0.85        | 1.75         |
| 8.                   |                             | 9.49            | 26.15             | 6.81        | 8.75         |
| <u>Japanese Teas</u> |                             |                 |                   |             |              |
| 9.                   | Green Tea (Yabukita)        | 12.69           | 19.35             | 0.25        | 9.06         |
| 10.                  | Green Tea (Yutakamidori)    | 12.24           | 19.78             | 0.45        | 7.69         |
| <u>Chinese Teas</u>  |                             |                 |                   |             |              |
| 11.                  | Green Tea (Hanlu)           | 11.19           | 18.82             | 0.81        | 9.30         |
| 12.                  | Green Tea (Yinghong)        | 3.31            | 11.42             | 11.57       | 13.55        |

Note: Teas numbered 1, 2, 4, 5, 9, 10, 11, 12 are made by CTC technology (Cut, Tear, Curl).

**Table 5. Determination of Catechins and Theaflavins in Green and Black Tea Purchased in Singapore [27].**

| No. | Tea grade                  | Content, mg/g |       |       |       |                 |             |
|-----|----------------------------|---------------|-------|-------|-------|-----------------|-------------|
|     |                            | EC            | ECG   | EGC   | EGCG  | Total catechins | Theaflavins |
| 1.  | Green Tea<br>Longjing      | 5.27          | 9.97  | 28.09 | 35.46 | 78.77           | 1.50        |
| 2.  | Jasmine<br>green tea       | 6.06          | 12.66 | 23.46 | 29.83 | 72.01           | 1.81        |
| 3.  | Chrysanthemum<br>Green tea | 8.59          | 12.58 | 18.62 | 16.85 | 56.64           | 1.03        |
| 4.  | Iron Buddha<br>Green Tea   | 4.27          | 3.35  | 30.61 | 11.82 | 50.05           | 0.66        |
| 5.  | Japanese Green<br>Tea      | 6.06          | 5.34  | 36.53 | 18.10 | 66.03           | 0.88        |
| 6.  | Oolong tea                 | 1.75          | 3.58  | 7.70  | 8.99  | 47.76           | 0.66        |
| 7.  | Ceylon Black<br>Tea        | 1.41          | 6.82  | 2.84  | 5.52  | 16.59           | 10.70       |
| 8.  | Pu-erh Tea                 | 0.49          | 0.07  | 0.60  | 0.30  | 1.46            | 1.03        |

In one study, the contribution of individual components of green and black tea into the overall antioxidant activity as well as their concentration, was determined. The compounds in tea were identified by HPLC-MS. Antioxidant activity was determined relative to ABTS<sup>+</sup>, which is a stable free radical (2,2<sup>1</sup>-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid). C-trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a reference compound. The green tea samples were from Kenya. 3 g of tea leaves were brewed in 300 ml of boiling water.

Table 7 shows the concentration and antioxidant activity of catechins and theaflavins in green and black teas, and Table 8 shows contribution of individual components to the total antioxidant activity.

As seen in Table 8, catechins contribute the most to the antioxidant activity in green tea,

whereas in black tea antioxidant acids contribute more than theaflavins. For some reason, the contribution made by thearubigins is not mentioned in this study.

The total antioxidant activity measured by HPLC and by ABTS without separation varied greatly: HPLC showed 8340±60 for green tea and 1494±4.0 for black tea; ABTS showed 11302±226 for green tea and 7382±184 for black tea. The difference of antioxidant activity measured by these methods is 2682 for green tea and 5888 for black tea.

Such differences may indicate that most of the high-molecular compounds are not eluted from a chromatographic column. Therefore, HPLC method gives lower results. Thearubigins are not eluted from the column under these conditions.

**Table 6. Content of Catechins in Various Tea Grades (Column 4x100 mm with GLWakosil C<sub>18</sub> AR and Precolumn 5 µm. Amperometric Detector V = 1.3 V).**

| No. | Catechin                                | Peak Area            |                          |                           |                        |                                 |
|-----|---|----------------------|--------------------------|---------------------------|------------------------|---------------------------------|
|     |   | Black Darjeeling Tea | 'White Monkey' White Tea | 'Royal Sprouts' White Tea | Yu Shan Yin Yellow Tea | Mo Li Hua Cha Green Jasmine Tea |
| 1.  | Epigallocatechin (EGC)                  | 165.2                | 1062.7                   | 500.0                     | 818.8                  | 2191.0                          |
| 2.  | Catechin (C)                            | 95.0                 | 272.6                    | 253.0                     | 295.2                  | 185.9                           |
| 3.  | Epicatechin (EC)                        | 143.8                | 549.7                    | 228.2                     | 314.7                  | 950.6                           |
| 4.  | Epigallocatechin-Gallate (EGCG)         | 909.7                | 1724.4                   | 3139.2                    | 3690.9                 | 2350.4                          |
| 5.  | Epicatechin Gallate (ECG)               | 730.9                | 1152.9                   | 1291.6                    | 1482.7                 | 1168.7                          |
| 6.  | The sum of all peak areas for catechins | 2044.6               | 4762.3                   | 4412.0                    | 6602.3                 | 68.46.6                         |

Note. All measurements are made under the same conditions

**Table 7. Concentration and Antioxidant Activity of the Major Components in Green and Black Teas [25].**

| No.                | Compounds                 | Concentration (µm) |           | Antioxidant activity (µm) |           |
|--------------------|---------------------------|--------------------|-----------|---------------------------|-----------|
|                    |                           | Green Tea          | Black Tea | Green Tea                 | Black Tea |
| <i>Catechins</i>   |                           |                    |           |                           |           |
| 1                  | Gallocatechin             | 513±7.2            | -         | 522±29                    | -         |
| 2                  | Epigallocatechin          | 1594±114           | 48±0.3    | 2032±146                  | 36±3.2    |
| 3                  | Epicatechin               | 374±25             | 34±0.3    | 398±3.5                   | 25±0.7    |
| 4                  | Epigallocatechin gallate  | 1202±7.4           | 52±2.9    | 3606±378                  | 175±3.2   |
| 5                  | Epicatechin gallate       | 389±1.7            | 58±2.9    | 1092±32                   | 195±5.9   |
| <i>Theaflavins</i> |                           |                    |           |                           |           |
| 6                  | Theaflavin                | -                  | 117±4.2   | -                         | 54±2.5    |
| 7                  | Theaflavin-3-gallate      | -                  | 168±7.3   | -                         | 157±1.5   |
| 8                  | Theaflavin-3'-gallate     | -                  | 87±3.8    | -                         | 66±1.6    |
| 9                  | Theaflavin-3,3'-digallate | -                  | 194±6.0   | -                         | 132±6.8   |
| 10                 | Caffeine                  | 1194±11            | 1295±20   | -                         | -         |

**Table 8. Contribution of Individual Components—Antioxidants—into the Overall Antioxidant Activity of Green and Black Tea [25].**

| No. | Compound          | Antioxidant Activity, % |           |
|-----|-------------------|-------------------------|-----------|
|     |                   | Green Tea               | Black Tea |
| 1   | Catechins         | 92.1                    | 28.8      |
| 2   | Oxyaromatic acids | 6.6                     | 39.6      |
| 3   | Flavonols         | 0.4                     | 4.6       |
| 4   | Theaflavins       | 0                       | 27.0      |

## 5. Conclusion

This review shows that tea has a high level of antioxidant activity. In overall antioxidant activity, tea takes a place among the highest rank, along with red wine and cocoa.

Antioxidant activity of tea is effected by many natural polyphenols: catechins, oxyaromatic acids, tannins, flavonols, thearubigins, theaflavins, etc.

Antioxidant activity of various tea grades, as identified by modern methods, descends in the following order: green>oolong>black>Pu-erh tea. Antioxidant activity of green tea is effected mainly by catechins (90%), of black tea—by theaflavins and thearubigins. Rapid determination of the total antioxidant content in tea by an amperometric method can be used to assess tea quality and authenticity.

## References

- Balentine D. A., Wiseman S. A., Bouwens L. C. The Chemistry of Tea Flavonoids. *Crit. Rev. Food Sci. Nutr.* 1997, v. 37, p. 693-704 [DOI: 10.1080/10408399709527797](https://doi.org/10.1080/10408399709527797)
- Lambert J. D., Elias R. J. *The Antioxidant and Pro-oxidant Activities of Green Tea Polyphenols: A role in Cancer Prevention.* Archives of Biochemistry and Biophysics. 2010, v. 501, p. 65-72, [DOI: 10.1016/j.abb.2010.06.013](https://doi.org/10.1016/j.abb.2010.06.013)
- Higdon J. J., Frei B. *Tea Catechins and Polyphenols: Health Effects, Metabolism, and Antioxidant Functions.* *Crit. Rev. Food Sci. Nutr.* 2003, v. 43, p. 89-143. [DOI: 10.1080/10408690390826464](https://doi.org/10.1080/10408690390826464)
- Wiseman S. A., Balentine D. A., and Frei B. *Antioxidants in Tea.* *Crit. Rev. Food Sci. Nutr.* 1997, v. 37, p. 705-718. [DOI: 10.1080/10408399709527798](https://doi.org/10.1080/10408399709527798)
- Stensvold I., Tverdal A., Solvoll K., and Foss O. P. *Tea Consumption. Relationship to Cholesterol, Blood Pressure, and Coronary and Total Mortality.* *Pnev. Med.*, 1992, v. 21, p. 546-553. [DOI:10.1016/0091-7435\(92\)90062-M](https://doi.org/10.1016/0091-7435(92)90062-M)
- Woodward M., and Tunstall-Pedoe H. *Coffee and Tea Consumption in the Scottish Heart Health Study Follow up: Conflicting Relations With Coronary Risk Factors, Coronary Disease, and all Cause Mortality.* *J. Epidemiol. Community Health*, 1999, v. 53, p. 481-487. [DOI: 10.1136/jech.53.8.481](https://doi.org/10.1136/jech.53.8.481)
- Sasazuki S., Kodama H., Yoshimasu K., et al. *Relation between Green Tea Consumption and the Severity of Coronary Atherosclerosis among Japanese Men and Women.* *Ann. Epidemiol.*, 2000, v. 10, p. 401-408. [DOI: 10.1016/S1047-2797\(00\)00066-1](https://doi.org/10.1016/S1047-2797(00)00066-1)
- Riemersma R. A., Rice-Evans C. A., Tyrrell R. M., Clifford M. N. and Lean M. E. *Tea Flavonoids and Cardiovascular Health.* *Q.J.M.*, 2001, v. 94, p. 277-282. [DOI: 10.1093/qjmed/94.5.277](https://doi.org/10.1093/qjmed/94.5.277)
- Arts I. C., Hollman P. C., Feskens E. J., Bueno de mesquite H. B., and Kromhout D. *Catechin Intake Might Explain the Inverse Relation Between Tea Consumption and Ischemic Heart*

- Disease: the Zutphen Elderly Study*. Am. J. Clin. Nutr., 2001, v. 74, p. 227-232
10. Zheng W., Doyle T. J., Kushi L. H., Sellers T. A., Hong C. P., and Folsom A. R. *Tea Consumption and Cancer Incidence in a Prospective Cohort Study of Post-Menopausal Women*. Am. J. Epidemiol., 1996, v. 144, p. 175-182
  11. Kinlen L. J., Willows A. N., Goldblatt P., and Yudkin J. *Tea Consumption and Cancer*. Br. J. Cancer. 1988, v. 58, p. 397-401. DOI: [10.1038/bjc.1988.227](https://doi.org/10.1038/bjc.1988.227)
  12. Imai K., Suga K., and Nakachi K. *Cancer-Preventive Effects of Drinking Green Tea Among a Japanese Population*. Prev. Med., 1997, v. 26, p. 769-775. DOI: [10.1006/pmed.1997.0242](https://doi.org/10.1006/pmed.1997.0242)
  13. Liu Z., Ma L. P., Zhou B., Yang L., and Liu Z. L. *Antioxidative Effects of Green Tea Polyphenols on Free Radical Initiated and Photosensitized Peroxidation of Human Low Density Lipoprotein*. Chem. Phys. Lipids, 2000, v. 106, p. 53-63. DOI: [10.1016/S009-3084\(00\)00133-X](https://doi.org/10.1016/S009-3084(00)00133-X)
  14. Lean M. E., Noroozi M., Kelly I., et al. *Dietary Flavonols Protect Diabetic Human Lymphocytes Against Oxidative Damage to DNA*. Diabetes, 1999, v. 48, p. 176-181.
  15. Katiyar S. K., Perez A., and Mukhtar H. *Green Tea Polyphenol Treatment to Human Skin Prevents Formation of Ultraviolet Light B-induced Pyrimidine Dimers in DNA*. Clin. Cancer Res. 2000, v. 6, p. 38640-3869
  16. Galati G., Lin A., Sultan A. M., O'Brien P. J. *Cellular and in Vivo Hepatotoxicity Caused by Green Tea Phenolic Acids and Catechins*. Free Radical Biol. Med. 2006, v. 40, p. 570-580. DOI: [10.1016/j.freeradbiomed.2005.09.014](https://doi.org/10.1016/j.freeradbiomed.2005.09.014)
  17. Jimenez-Saenz M., Martinez-Sanchez M. C. *Acute Hepatitis Associated with Ingestion of Green Tea Infusions*. J. Hepatol. 2006, v. 44, p. 616-617. DOI: [10.1016/j.jhep.2005.11.041](https://doi.org/10.1016/j.jhep.2005.11.041)
  18. Yashin Ya. I., Ryzhnev V. Yu., Yashin A. Ya., Chernousova N. I. *Natural Antioxidants. Their Content in Food Products and Impact on Human Health and Aging*. Translit, Moscow, 2009. 212 pages.
  19. Benzie J. F. F., Szeto Y. T. *Total Antioxidant Capacity of Teas Analyzed by the FRAP Method*. J. Agr. Food Chem. 1999, v. 47, No. 2, p. 633-636. DOI: [10.1021/1021-if9807768](https://doi.org/10.1021/1021-if9807768)
  20. Langley-Evans S. C. *Antioxidant Activity of Green and Black Tea Determined by the FRAP Method*. Int. J. Food Sci. Nutr. 2000, v. 51, p. 181-188. DOI: [10.1080/09637480050029683](https://doi.org/10.1080/09637480050029683)
  21. Robinson E. E., Maxwell S. R. J., Thorpe G. H. G. *Analysis of Black Tea's Antioxidant Activity by Chemiluminescence Method*. Free Radical Res. 1997, v. 26, p. 291-302. DOI: [10.3109/10715769709097807](https://doi.org/10.3109/10715769709097807)
  22. Cao G., Sofic E., Prior R. L. *Antioxidant Activity of Tea*. J. Agr. Food Chem. 1996, v. 44, p. 3426-3431. DOI: [10.1021/1021-if9602535](https://doi.org/10.1021/1021-if9602535)
  23. Yashin Ya. I., Yashin A. Ya., Chernousova N. I. *Tea Chromatography*. Chemistry and Life. 2005, No. 3, p. 50-53.
  24. Yashin Ya. I., Yashin A. Ya. *Chromatographic Methods used for Analysis of Tea Chemical Composition*. Partners and Competitors. 2004, v. 4, p. 11-13.
  25. Yashin Ya. I., Yashin A. Ya., Chernousova N. I. *Chromatographic Determination of Tea Chemical Composition*. Beer and Beverages. 2005, v. 2, pp. 44-49.
  26. Stewart A. J., Mullen W., Crozier A. *On-line HPLC Analysis of the Antioxidant Activity of Phenolic Compounds in Green and Black Tea*. Mol. Nutr. Food Res. 2005, v. 49, p. 52-60. DOI: [10.1002/mnfr.200400064](https://doi.org/10.1002/mnfr.200400064)
  27. Leung L. K., Su Y., Chen R. et al. *Theaflavins in Black Tea and Catechins in Green Tea are Equally Effective Antioxidants*. J. Nutr. 2001, v. 131, p. 2248-2251.
  28. Lee B.-L., Ong C.-N. *Comparative Analysis of Tea Catechins and Theaflavins by HPLC and Capillary Electrophoresis*. J. Chromat. 2000, v. 881, p. 439-447. DOI: [10.1016/S0021-9673\(00\)00215-6](https://doi.org/10.1016/S0021-9673(00)00215-6)
  29. Sung H., Nah J., Chum S., Park H., Jong S. E., Min W. K. *Antioxidant Effect of Green Tea in Vivo*. Eur. J. Clin. Nutr. 2000, v. 54, p. 527-529. DOI: [10.1038/si.ejcn.1600994](https://doi.org/10.1038/si.ejcn.1600994)
  30. Valeic S. et al. *Antioxidant Chemistry of Green Tea Catechins*. Chem. Res. Toxicol. 1999, v. 12, p. 382-386.
  31. Luczay W., Skzydowska E. *Antioxidant Properties of Black Tea*. Prev. Med. 2005, v.

- 40, p. 910-918. DOI: [10.1016/j.ypped.2004.10.014](https://doi.org/10.1016/j.ypped.2004.10.014)
32. Zhu Q. Y., Hockman R. M., Ensunsa J. L., Holt R. R., Keen C. L. *Antioxidant Activity of Oolong Tea*. J. Agr. Food Chem. 2002, v. 50, p. 6929-6934. DOI: [10.1021/af0206163](https://doi.org/10.1021/af0206163)
  33. Su X. G., Duan J., Jiang Y. M., Shi J., Kakuda Y. *Impact of Brewing Conditions on Antioxidant Capacity of Oolong Tea*. J. Food Compos. Anal. 2006, v. 19, p. 348-353. DOI: [10.1016/j.jfca.2005.02.005](https://doi.org/10.1016/j.jfca.2005.02.005)
  34. Su X., Duan J., Jiang Y., Duan X., Chen F. *Polyphenol Profile and Antioxidant Activity of Brewed Oolong Tea at Different Conditions*. Int. J. Mol. Sci. 2007, v. 8, p. 1196-1205. DOI: [10.3390/i8121196](https://doi.org/10.3390/i8121196)
  35. Higdon J. V., Frei B. *Tea Catechins and Polyphenols: Their Impact on Health, Metabolism and Antioxidative Functions*. Crit. Rev. Food Sci. Nutr. 2003, v. 43, p. 89-143. DOI: [10.1080/10408690390826464](https://doi.org/10.1080/10408690390826464)
  36. Kumamoto M., Sonda T., Nagayama K., Tabata M. *Influence of pH and Metal Ions on the Antioxidant Activity of Catechins*. Biosci. Biotechnol. Biochem. 2001, v. 65, p. 126-132. DOI: [10.1271/bbb.65.126](https://doi.org/10.1271/bbb.65.126)
  37. Kondo K., Kurihara M., Fukuhara K. *Mechanism of Antioxidant Action of Catechins*. Methods Enzymol. 2001, v. 335, p. 203-217. DOI: [10.1016/S0076-6879\(01\)35244-8](https://doi.org/10.1016/S0076-6879(01)35244-8)
  38. Cabrera C., Gimenez R., Lopez C. M. *Determination of Antioxidant Activity of Tea Components*. J. Agric. Food Chem. 2003, v. 31, p. 4427-4435. DOI: [10.1021/jf0300801](https://doi.org/10.1021/jf0300801)
  39. Wachira F. N., Kamunya S. *Kenyan Teas are Rich in Antioxidants*. Tea, 2005, v. 26, p. 81-89.
  40. Hara Y. *Antioxidant Activity of Tea Polyphenols*. Intern. Biotechn. Lab. 1994, №2, p.14.
  41. Satoh E., Tohyama N., Nishimura M. *Comparison Between the Antioxidant Activity of Toasted Tea and that of Green, Oolong, and Black Tea*. Int. J. Food Sci. Nutr. 2005, v. 56, p. 551-559. DOI: [10.1080/09637480500398835](https://doi.org/10.1080/09637480500398835)
  42. Yen G. C., Chen H. Y. *Antioxidant Activity of Different Tea Extracts in Connection with their Antimutagenicity*. J. Agr. Food Chem. 1995, v. 43, p. 27-32. DOI: [10.1021/jf00049a007](https://doi.org/10.1021/jf00049a007)
  43. Khokhar S., Magnusdottir S. G. M. *Total Phenol, Catechin, and Caffeine Contents of Teas Most Often Consumed in the United Kingdom*. J. Agric. Food Chem. 2002, v. 50, p. 565-570. DOI: [10.1021/jf0101531](https://doi.org/10.1021/jf0101531)
  44. Karori S. M., Wachira F. N., Wanyoko J. K., Ngure R. M. *Antioxidant Capacity of Different Types of Tea Products*. African J. Biotechnol. 2007, v. 6, p. 2287-2296.
  45. Atoni A. K., Mansouri A. et al. *Tea and Herbal Extracts: Their Antioxidant Activity and Phenolic Profile*. Food Chem. 2005, v. 89, p. 27-36. DOI: [10.1016/j.foodchem.2004.01.075](https://doi.org/10.1016/j.foodchem.2004.01.075)
  46. Abdullin I.F., Turova E.N., Budnikov G.K. *Coulometric Evaluation of the Antioxidant Capacity of Tea Extracts*. J. Anal. Chem. (Russian). 2001. V.56. p.627-629.
  47. Wiseman S. A., Balentine D. A., Frei B. *Antioxidants in Tea*. Crit. Rev. Food Sci. Nutr. 1997, v. 37, p. 705 – 718.