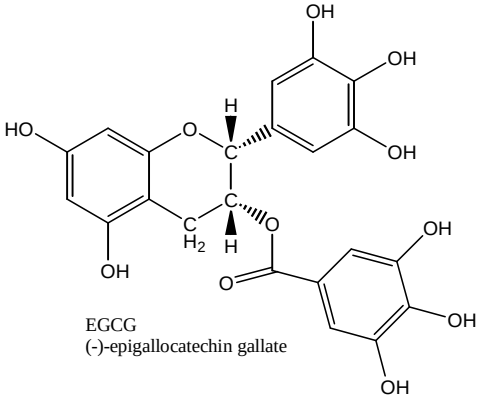
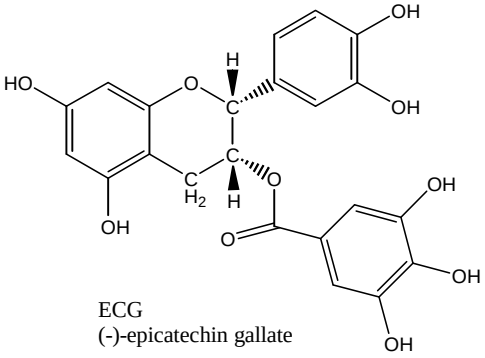
Name _____

This week we will use the skills we developed last week to analyze the antioxidant power of several commercially available bottled teas. The method we are going to use will be very similar to the "Total Antioxidant Capacity of Teas by the Ferric Reducing/Antioxidant Power Assay" article. We will run the gallic acid standard as a comparison method. Depending on the starting concentration of tea, it may be necessary to do more than 4 dilutions. We want to have at least four absorbance (A) readings between 2.000 and .010 A for both the FRAP and Folin-Ciocalteau assays.

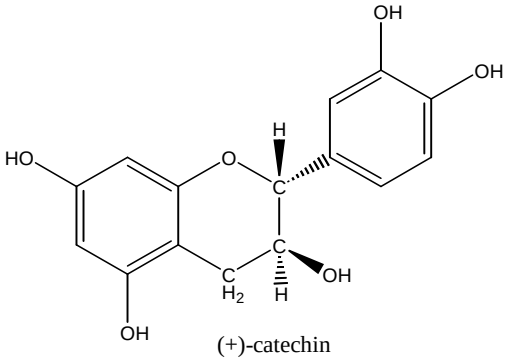
Antioxidant Compounds in Tea



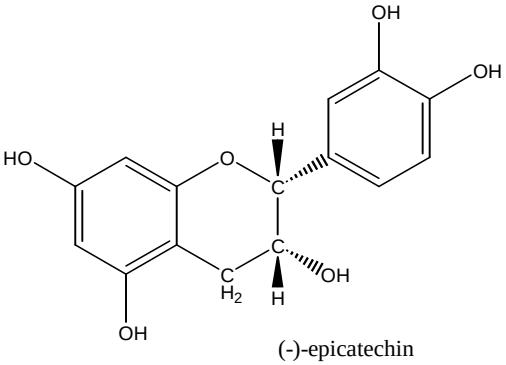
EGCG
(-)-epigallocatechin gallate



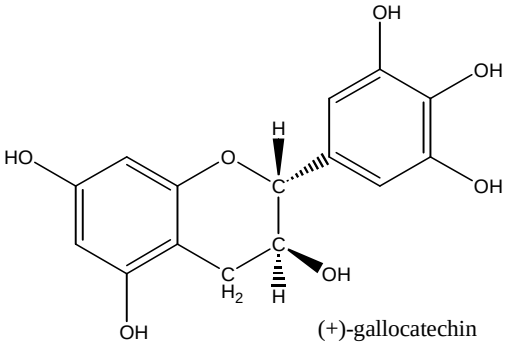
ECG
(-)-epicatechin gallate



(+)-catechin



(-)-epicatechin



(+)-gallocatechin

Total Antioxidant Capacity of Teas by the Ferric Reducing/ Antioxidant Power Assay

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This study aimed to compare in vitro antioxidant power of different types of tea (*Camellia sinensis*). The ferric reducing/antioxidant power (FRAP) assay was used to measure the total antioxidant power of freshly prepared infusions of 25 types of teas. Results showed that different teas had widely different in vitro antioxidant power and that the antioxidant capacity was strongly correlated (r = 0.956) with the total phenolics content of the tea. Expressed as μmol of antioxidant power/g of dried tea leaves, values ranged as 132−654 μmol/g for black ("fermented") teas, 233−532 μmol/g for Oolong ("semifermented") teas, and 272−1144 μmol/g for green ("nonfermented") teas. One cup of tea of usual strength (1−2%), therefore, can provide the same potential for improving antioxidant status as around 150 mg of pure ascorbic acid (vitamin C).

Keywords: *Antioxidant; FRAP; polyphenolics; tea*

INTRODUCTION

Tea (*Camellia sinesis*) is the most widely consumed beverage worldwide and has become an important agricultural product (Balentine, 1992). The type and quantity of tea taken varies in different countries and races (Weisburger, 1996; Kohlmeier, 1997). Black ("fermented") tea is popular in the West; "semifermented" Oolong-type tea is commonly drunk in Taiwan and parts of China; green ("nonfermented") tea is favored in the rest of China, Northern Africa, and Japan (Weisburger, 1996). Tea contains large amounts of polyphenolic compounds with antioxidant properties, and these may prevent oxidative damage of DNA (Zhao et al., 1989; Scott et al., 1993; Cook and Samman, 1996; Rice-Evans et al., 1996; Wiseman et al., 1997; Zhang and Shen, 1997) and inhibit the peroxidation of low-density lipoprotein (Miura et al., 1994; Ishikawa et al., 1997; Luo et al., 1997). Chemical changes such as oxidative damage and peroxidation are related to mutagenesis, to increased risk of cancer (Emerit, 1994; Halliwell, 1996), and to atherogenesis and cardiovascular disease (Maxwell and Lip, 1997). Regular intake of tea may, therefore, improve antioxidant status in vivo and, thereby, help lower risk of certain types of cancer and coronary heart disease (Stensvold et al., 1992; Weisburger, 1996).

Tea antioxidants can protect against strong mutagens in animal models (Yamane et al., 1991; Hasegawa et al., 1995; Leanderson et al., 1997). Moreover, lower incidence of cancer in association with high consumption of tea has been reported in some epidemiological studies (La Vecchia et al., 1992; Baron et al., 1994; Gao et al., 1994; Yu et al., 1995). However, results are not consistent and a protective role for antioxidants in tea has not been clearly demonstrated (Goldbohm et al., 1996; Kohlmeier et al., 1997). This may be due to the form in which tea is taken (Kohlmeier, 1997). Different teas have different antioxidant compositions, and some tea antioxidants may be more active and/or more easily absorbed than others (Graham, 1992; Weisburger, 1996; Cook and Samman, 1996; Rice-Evans et al., 1996; Van Acker et al., 1996; Hollman, 1997; Kohlmeier, 1997). The aim of this study was to measure and compare in vitro antioxidant activities of different types of tea to assess their relative potential for improving in vivo antioxidant status. The relationship of total phenolics content to antioxidant capacity in tea infusions was also studied, and the stability of antioxidant potential in infusions of tea and the effect of dilution on antioxidant potential were investigated.

MATERIALS AND METHODS

Total antioxidant power of a freshly prepared, cooled, filtered infusion (5 g of dry tea leaves/100 mL of boiling, distilled water) of each of 25 different teas was measured using the ferric reducing/antioxidant power (FRAP) assay (U.S. patent pending) (Benzie and Strain, 1996; Benzie and Strain, 1999). Teas were all purchased locally and measured in quadruplicate, after additional dilution in distilled water as appropriate. The FRAP assay was performed as previously described (Benzie and Strain, 1996) using a Cobas Fara centrifugal analyzer (Roche Diagnostics Ltd., Basel, Switzerland). In the FRAP assay, reductants ("antioxidants") in the sample reduce Fe^{3+}/tripyridyltriazine complex, present in stoichiometric excess, to the blue colored ferrous form, with an increase in absorbance at 593 nm. The ΔA is proportional to the combined (total) ferric reducing/antioxidant power (FRAP value) of the antioxidants in the sample. The FRAP assay has a limit of detection of <2 μmol/L reducing/antioxidant power and precision is excellent; within- and between-run CVs are <0.5% and 1.0%, respectively, between 500 and 2000 μmol/L, n > 8 in each case.

In vitro antioxidant power results are expressed as μmol of ferric reducing/antioxidant power (the FRAP value)/g of dry tea leaves, and as μmol of FRAP estimated to be contained in a typical serving (150 mL) of tea in the strength commonly drunk (1.5% w/v). The antioxidant power of freshly prepared, aqueous solutions of ascorbic acid (*d*-α-ascorbic acid, extra pure crystals; Merck, Darmstadt, Germany) was also measured, and the calculated antioxidant power of 1 g of pure ascorbic acid is given as reference.

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Table 1. In Vitro Antioxidant Power of Different Teas Measured Using the FRAP[a] Assay

type of tea	name	FRAP value (μmol/g of dry tea leaves; mean (SEM))[b]	antioxidant power, as μmol of FRAP, estimated to be contained in 1 cup of tea[c]
	pure ascorbic acid (vitamin C)	*11 364 μmol/g*	
black	China Black Tea	379 (39)	853
	Rose Black Tea	378 (16)	851
	Yunnan Tuocha	358 (33)	806
	Marks and Spencers Extra Strong Breakfast Tea	654 (47)	1472
	Pu Li Tea (brand 1)	309 (33)	695
	Pu Li Tea (brand 2)	225 (10)	506
	Pu Li Tea (brand 3)	238 (23)	536
	Pu Li Tea (brand 4)	380 (15)	855
	Premium Yun-Nan Puerh Tea	132 (9)	297
oolong	Oolong Tea (brand 1)	532 (23)	1197
	Oolong Tea (brand 2)	233 (18)	524
	Iron Buddha Tea (brand 1)	472 (26)	1062
	Iron Buddha Tea (brand 2)	340 (25)	765
	Iron Buddha Tea (brand 3)	289 (29)	650
green	China Green Tea (brand 1)	1144 (6)	2574
	China Green Tea (brand 2)	699 (77)	1573
	Jasmine Tea (brand 1)	629 (55)	1415
	Jasmine Tea (brand 2)	526 (29)	1184
	Jasmine Tea (brand 3)	602 (40)	1355
	Jasmine Tea (brand 4)	698 (55)	1571
	Lok On Ngan Jum (brand 1)	727 (48)	1636
	Lok On Ngan Jum (brand 2)	525 (15)	1181
	Shou Mei Tea	386 (20)	869
	Shui Sin Tea (brand 1)	522 (45)	1175
	Shui Sin Tea (brand 2)	272 (5)	612
	Yunnan Green Tea	996 (68)	2241
	Japanese Green Tea	700 (54)	1575

[a] U.S. patent pending. [b] Mean (SEM) of two or three different infusions, prepared on separate days, each infusion measured in quadruplicate. [c] 150 mL of a 1.5% infusion of tea.

For the stability study of antioxidant in tea infusion, tea infusions were kept at 4 °C for 48 h. The infusions were then re-assayed in duplicate and the results compared to those obtained on fresh samples.

For the relationship between antioxidant power and levels of tea polyphenols, FRAP values and the total phenolics content of infusions of 26 different teas were compared in a separate experiment. Total phenolics content was measured by the Folin−Ciocalteu method (Theis and Benedict, 1924; Das, 1971; Singleton et al., 1999).

RESULTS

Different types of tea had widely different antioxidant activities, ranging from 132 μmol/g for one type of black tea ("Premium Yun-Nan Puerh Tea") to 1144 μmol/g for one of the green teas ("China Green Tea") tested (Table 1). From these results it is estimated that a typical cup of green tea contains an amount of antioxidant power similar to that found in 100−200 mg of pure ascorbic acid (vitamin C), highlighting the enormous potential of tea as a dietary source of antioxidant power.

There was no significant change observed in the FRAP values after 48 h storage of tea infusions at 4 °C (paired *t*-test), indicating that antioxidants in tea are stable. There was a linear relationship between the strength of the tea infusion and the FRAP value (Figure 1) over a wide range of values, with close agreement between expected and observed FRAP values ($r = 0.998$, $p < 0.0001$).

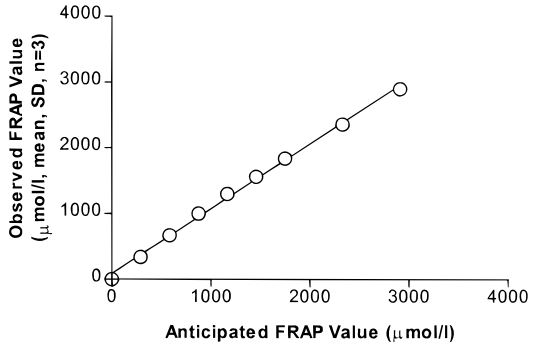

Figure 1. FRAP values of infusion of green tea at different dilutions. Result shows good agreement ($r = 0.998$, $p < 0.0001$) between the anticipated and the observed (measured) FRAP value, indicating no change in antioxidant at different concentrations. Each point represents the mean of triplicate measurement, and while 1 SD error bars are included, reproducibility was high and error bars do not show.

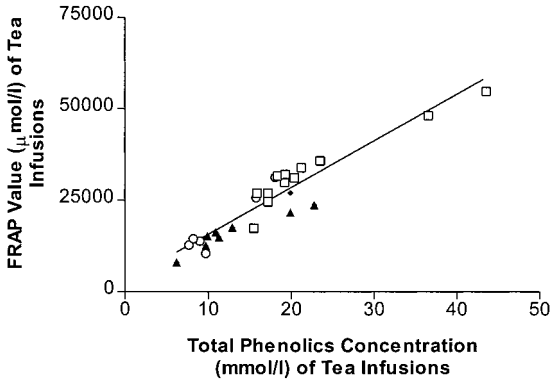

Figure 2. FRAP values and total phenolics content of 26 different tea infusions of 5% (w/v) strength. Green tea infusions are represented by squares, oolong tea by circles, and black tea by triangles. Results showed strong correlation ($r = 0.956$, $p < 0.0001$) between the two parameters. Each point represents the mean of two individual experiments, each experiment with duplicate measurement, i.e., mean of 4 results.

There was strong correlation between FRAP value and total phenolics content of the tea infusions tested ($r = 0.956$, $p < 0.0001$), indicating that the number of phenolic hydroxyl groups is a major determinant of antioxidant power of tea (Figure 2).

DISCUSSION

Tea, particularly green tea, is a potentially rich dietary source of antioxidant power. Various studies have demonstrated in vitro radical trapping antioxidant properties of black tea and green tea, of extracts of tea, and of individual polyphenolic compounds found in tea (Zhao et al., 1989; Scott et al., 1993; Lin et al., 1996; Paganga et al., 1996; Rice-Evans et al., 1996; Zhang and Shen, 1997). This current study presents new data comparing the in vitro antioxidant/reducing power of different types of teas, as they would be drunk, using an objective and highly reproducible measure, the FRAP assay for antioxidant/reducing power. It is worth noting that, while tea polyphenolics are known to bind metal ions (Miller et al., 1996; Paganga et al., 1996; Van Acker et al., 1996), this property does not interfere with the measurement of antioxidant power of tea using the FRAP assay. This was demonstrated by a linear re-

sponse when the antioxidant power of different teas was tested over a wide range of concentrations.

Binding of metal ions, such as iron, in vivo is an antioxidant action of itself, preventing metal ion catalyzed generation of reactive species (Emerit, 1994; Halliwell, 1996). However, tea polyphenolics also have electron-donating antioxidant properties, the relative activity of the different polyphenolic compounds being related to the number and location of the hydroxyl groups and the presence of the galloyl moiety (Lin et al., 1996; Miller et al., 1996; Paganga et al., 1996; Rice-Evans et al., 1996). During the process referred to as fermentation, flavanols in green tea leaves, mainly catechins and their gallic esters, undergo a polyphenol oxidase catalyzed oxidative polymerization which turns the leaves black (Graham, 1992; Weisburger, 1996; Nanjo et al., 1996). During several hours, much of the catechin content of green tea is converted to thearubingens and theaflavins, which give black tea its characteristic astringency. Oolong tea is intermediate in composition between green and black tea. Green tea contains 30−42% catechins of total dry mass, while black tea contains 3−10% and oolong 8−20% (Graham, 1992). Almost 80% of the tea consumed throughout the world each year is black; less than 2% is Oolong, and 20% green tea. (Balentine, 1992; Kong, 1993).

This study has confirmed that the antioxidant power of green tea is considerably higher than black tea, while Oolong tea, expected to be intermediate, appeared similar to black tea. Furthermore, the antioxidant power of all teas correlated strongly with the total phenolics content. Previous studies have shown that tea antioxidants with a greater number of phenolic hydroxyl groups have greater antioxidant power, i.e., epigallocatechin gallate (8 groups) > epicatechin gallate (7 groups) > GC (6 groups) > epicatechin (5 groups) > epigallocatechin (6 groups) (Wiseman et al., 1997). Both FRAP values and phenolics content of different types of teas overlapped, however, with a 2−3-fold difference across different brands of teas of the same type, probably reflecting differences in quality, geographical regions of growth, the time of year when the leaves were picked, and varying storage conditions (Weisburger, 1996; Lin et al., 1996). Nevertheless, the potential for all types of tea to contribute significantly to the dietary intake of antioxidant power is high, as one cup of black tea (1−2%) contains around 800 μmol of antioxidant power and several cups of green tea offer the same antioxidant potential as almost 1 g of pure vitamin C. Vitamin C is an antioxidant of established importance, while the bioavailability and role of tea antioxidants are not yet clear. Nonetheless, tea may be an important dietary source of antioxidant power and tea can be ingested frequently and in relatively large volumes without any known harmful effects. Questions then arise regarding the feasibility of realizing this potential in terms of the effect on antioxidant status and the possible long-term benefits to health associated with increased intake of tea antioxidants.

In summary, this study has shown that green teas have the highest antioxidant power and black teas have the lowest values. Oolong teas, expected to be intermediate between green and black teas, demonstrate activities similar to those of black teas. Also antioxidants in tea are stable for at least 48 h stored at 4 °C. The total antioxidant power correlated strongly with the total phenolics content of tea of all types. Further study into the absorption and effect of tea antioxidants on antioxidant status is needed to evaluate their potential benefit in the maintenance of human health.

ABBREVIATIONS USED

FRAP, ferric reducing/antioxidant power.

LITERATURE CITED

Balentine, D. A. Manufacturing and Chemistry of Tea. In *Phenolics Compounds in Food and Their Effects of Health*; Tang, H. C., Chang, Y. L., Tuan, H. H., Eds.; American Chemical Society: Washington, DC, 1992; Vol. 1, Chapter 8.

Baron, J. A.; de Verdier, M. G.; Ekbom, A. Coffee, tea, tobacco, and cancer of the large bowel. *Cancer Epidemiol. Biomarkers Prev.* **1994**, *3*, 565−570.

Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant" power: the FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.

Benzie, I. F. F.; Strain, J. J. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In *Methods in Enzymolology*; Packer, L., Ed.; Academic Press: Orlando, FL, 1999; Vol. 299, pp 15−27.

Cook, N.; Samman, S. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. *J. Nutr. Biochem.* **1996**, *7*, 66−76.

Das, N. P. Studies of flavonoid metabolism-absorption and metabolism of (+)-catechin in man. *Biochem. Pharm.* **1971**, *20*, 3435−3445.

Emerit, I. Reactive oxygen species, chromosome mutation and cancer: possible role of clastogenic factors in carcinogenesis. *Free Radical Biol. Med.* **1994**, *16*, 99−109.

Gao, Y. T.; McLaughlin, J. K.; Blot, W. J.; Ji, B. T.; Dai, Q.; Fraumeni, J. F., Jr. Reduced risk of esophageal cancer associated with green tea consumption. *J. Natl. Cancer Inst.* **1994**, *86*, 855−858.

Goldbohm, R. A., Hertog, M. G. L., Brants, H. A. M., van Poppel, G.; van den Brandt, P. A. Consumption of black tea and cancer risk: a prospective cohort study. *J. Natl. Cancer Inst.* **1996**, *88*, 93−100.

Graham, H. N. Green tea composition, consumption, and polyphenol chemistry. *Prev. Med.* **1992**, *21*, 334−350.

Halliwell, B. Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in human. *Free Radical Res.* **1996**, *25*, 57−74.

Hasegawa, R.; Chujo, T.; Sai-kato, K.; Umemura, T.; Tanimura, A.; Kurokawa, Y. Preventive effects of green tea against liver oxidative DNA damage and hepatotoxicity in rats treated with 2-nitropropane. *Food Chem. Toxicol.* **1995**, *33*, 961−970.

Hollman, P. C. H. Bioavailability of flavonoids. *Eur. J. Clin. Nutr.* **1997**, *51*, Suppl. 1, S66−S69.

Kong, X. L. Tea leaves production, trade and others. In *Zhongwai Chashi*; Shang-hai Wen Hua Chu Ban She: Shanghai, China, 1993; pp 124−134.

Ishikawa, T.; Suzukawa, M.; Ito, T.; Yoshida, H.; Ayaori, M.; Nishiwaki, M.; Yonemura, A.; Hara, Y.; Nakamura, H. Effect of tea flavonoid supplementation on the susceptibility of low-density lipoprotein to oxidative modification. *Am. J. Clin. Nutr.* **1997**, *66*, 261−266.

Kohlmeier, L. Has the tea been ruined? (guest editorial) *Br. J. Nutr.* **1997**, *78*, 1−3.

Kohlmeier, L.; Weterings, K. G. C.; Steck, S.; Kok, F. J. Tea and cancer prevention: an evaluation of the epidemiological literature. *Nutr. Cancer* **1997**, *27*, 1−13.

La Vecchia, C.; Negri, E.; Francheschi, S.; D'Avanzo, B.; Boyle, P. Tea consumption and cancer risk. *Nutr. Cancer* **1992**, *17*, 27−31.

Leanderson, P.; Faresjo, A. O.; Tagesson, C. Green tea polyphenols inhibit oxidant-induced DNA strand breakage in cultured lung cells. *Free Radical Biol. Med.* **1997**, *23*, 235−242.

Lin, Y. L.; Juan, I. M.; Chen, Y. L.; Liang, Y. C.; Lin, J. K. Composition of polyphenols in fresh tea leaves and associations of their oxygen-radical-absorbing capacity with antiproliferative actions in fibroblast cells. *J. Agric. Food Chem.* **1996**, *44*, 1387−1394.

Luo, M.; Kannar, K.; Wahlqvist, M. L.; O'Brien, R. C. Inhibition of LDL oxidation by green tea extract. (comment, letter) *Lancet* **1997**, *349*, 360−361.

Maxwell, S. R. J.; Lip, G. Y. H. Free radicals and antioxidants in cardiovascular disease. *Br. J. Clin. Pharmacol.* **1997**, *44*, 307−317.

Miller, N. J.; Castelluccio, C. Tijburg, L.; Rice-Evans, C. The antioxidant properties of theaflavins and their gallate esters-Radical scavengers or metal chelators? *FEBS Lett.* **1996**, *392*, 40−44.

Miura, S.; Watanabe, J. Tomita, T. Sano, M.; Tomita, I. The inhibitory effects of tea polyphenols (flavan-3-ol derivatives) on Cu^{2+} mediated oxidative modification of low density lipoprotein. *Biol. Pharm. Bull.* **1994**, *17*, 1567−1572.

Nanjo, F.; Goto, K.; Seto, R.; Suzuki, M.; Sakai, M.; Hara, Y. Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. *Free Radical Biol. Med.* **1996**, *21*, 895−902.

Paganga, G.; Al-Hashim, H.; Khodr, H.; Scott, B. C.; Aruoma, O. I.; Hider, R. C., Halliwell, B.; Rice-Evans, C. A. Mechanisms of antioxidant activities of quercetin and catechin. *Redox Rep.* **1996**, *2*, 359−364.

Rice-Evans C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolics acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.

Scott, B. C.; Butler, J.; Halliwell, B.; Aruoma, O. I. Evaluation of the antioxidant actions of ferilic acid and catechins. *Free Radical Res. Commun.* **1993**, *19*, 241−253.

Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin−Ciocalteu reagent. In *Methods in Enzymolology*; Packer, L., Ed.; Academic Press: Orlando, FL, 1999; Vol. 299, pp 152−178.

Stensvold, I.; Tverdal, A.; Solvoll, K.; Foss, O. P. Tea consumption. Relationship to cholesterol, blood pressure, and coronary and total mortality. *Prev. Med.* **1992**, *21*, 546−553.

Thesis, R. C.; Benedict, S. R. The determination of phenols in the blood. *J. Biol. Chem.* **1924**, *61*, 67−71.

Van Acker, S. A. B. E.; van den Berg, D. J.; Tromp, M. N. J. L.; Griffioen, D. H., van Bennekom, W. P.; van der Vijgh, W. J. F.; Bast, A. Structural aspects of antioxidant activity of flavonoids. *Free Radical Biol. Med.* **1996**, *20*, 331−342.

Weisbuger, J. H. Tea Antioxidants and health. In *Handbook of Antioxidants*; Cadenas, E., Packer, L., Eds.; Dekker: New York, 1996; Chapter 15.

Wiseman, S. A.; Balentine, D. A.; Frei, B. Antioxidants in tea. *Crit. Rev. Food Sci. Nutr.* **1997**, *37*, 705−718.

Yamane, T.; Hagiwara, N.; Tateishi, M.; Akachi, S.; Kim, M.; Okuzumi, J.; Kitao, Y.; Inagake, M.; Kuwata, K.; Takahashi, T. Inhibition of azoxymethane-induced colon carcinogenesis in rat by green tea polyphenol fraction. *Jpn. J. Cancer Res.* **1991**, *82*, 1336−1339.

Yu, G. P., Hsieh, C. C., Wang, L. Y., Yu, S. Z., Li, X. L.; Jin, T. H. Green-tea consumption and risk of stomach cancer: a population-based case-control study in Shanghai, China. *Cancer Cause Control* **1995**, *6*, 532−538.

Zhang, J.; Shen, X. Antioxidant activities of baicalin, green tea polyphenols and alizarin *in vitro* and *in vivo. J. Nutr. Environ. Med.* **1997**, *7*, 79−89.

Zhao, B.; Li, X.; He, R.; Cheng, S.; Wenjuan, X. Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. *Cell Biophys.* **1989**, *14*, 175−185.

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Please work in pairs for this experiment.

Make sure the spectrophotometer is turned on and the wavelength is set at 593 nm.

Part I. Making serial dilutions of a stock solution.

Set up 5 test tubes in a test tube rack

Obtain a stock solution of gallic acid.
Write down the concentration of the gallic acid stock solution _____

With a plastic pipette, remove 3 ml of solution from the stock solution and add it to the first test tube. Add 3 ml of distilled water to the solution in the first test tube. Mix the contents of the first test tube by shaking and/or swirling it.

With a new plastic pipette, remove 3 ml of solution from the first test tube and add it to the second test tube. Add 3 ml of distilled water to the solution in the second test tube. Mix the contents of the second test tube by shaking and/or swirling it.

With a new plastic pipette, remove 3 ml of solution from the second test tube and add it to the third test tube. Add 3 ml of distilled water to the solution in the third test tube. Mix the contents of the third test tube by shaking and/or swirling it.

With a new plastic pipette, remove 3 ml of solution from the third test tube and add it to the fourth test tube. Add 3 ml of distilled water to the solution in the fourth test tube. Mix the contents of the fourth test tube by shaking and/or swirling it.

Add water to the final test tube. This is your water blank.

Part II. Antioxidant measurement of the gallic acid standard using the FRAP method.
Set the spectrometer to 593 nm.
Obtain 6 square plastic spectrophotometer cuvettes.
To each cuvette add 4 drops of the solution from the test tubes using a small (1 ml) plastic pipette following the table below. Use a new small plastic pipette each time.

cuvette	
1	4 drops stock solution
2	4 drops test tube 1
3	4 drops test tube 2
4	4 drops test tube 3
5	4 drops test tube 4
6	4 drops water

Add 3 ml (measured with a plastic pipette) of FRAP solution to each cuvette.
Mix the solution in each cuvette.
Measure the absorbance of each cuvette at 593 nm.
Zero the spectrophotometer with cuvette 6 before each reading.

Complete the following table with you values

Tube	Absorbance	Concentration of antioxidant _____
1		
2		
3		
4		
5		
6	0	0
7		

Add data to the "class data" spreadsheet before you leave the lab.

Observations and comments:

Part III. Total phenolics measurement of the gallic acid standard using the Folin-Ciocalteau reagent.
Set the spectrophotometer to 750 nm.
Set up 6 square plastic cuvettes.
To each cuvette add 10 drops of the solution from the test tubes using a small (1 ml) plastic pipette following the table below. Use a new small plastic pipette each time.

cuvette	
1	10 drops stock solution
2	10 drops from test tube 1
3	10 drops from test tube 2
4	10 drops from test tube 3
5	10 drops from test tube 4
6	10 drops water

Then add 1.5 ml of Folin-Ciocalteau reagent to each cuvette.
Mix the solution in each cuvette by shaking and/or swirling.
Let them set for 7 minutes at room temperature.
Add 1.5 ml of Na_2CO_3 solution to each cuvette.
Mix the solution in each cuvette by shaking and/or swirling.
Allow the cuvettes to sit for 30 minutes.
Read of absorbance of cuvettes 1 – 5 at 750 nm.
Zero the spectrophotometer with cuvette 6 before each reading.

Tube	Absorbance	Concentration of antioxidant _____
1		
2		
3		
4		
5		
6	0 mmol	0

Add data to the "class data" spreadsheet before you leave the lab.
Observations and comments:

Part IV. Making serial dilutions of a commercial tea.
Set up 5 test tubes in a test tube rack
Obtain a sample of commercial bottled tea. What is the brand of you tea? _____

With a plastic pipette, remove 3 ml of solution from the tea and add it to the first test tube. Add 3 ml of distilled water to the solution in the first test tube. Mix the contents of the first test tube by shaking and/or swirling it.

With a new plastic pipette, remove 3 ml of solution from the first test tube and add it to the second test tube. Add 3 ml of distilled water to the solution in the second test tube. Mix the contents of the second test tube by shaking and/or swirling it.

With a new plastic pipette, remove 3 ml of solution from the second test tube and add it to the third test tube. Add 3 ml of distilled water to the solution in the third test tube. Mix the contents of the third test tube by shaking and/or swirling it.

With a new plastic pipette, remove 3 ml of solution from the third test tube and add it to the fourth test tube. Add 3 ml of distilled water to the solution in the fourth test tube. Mix the contents of the fourth test tube by shaking and/or swirling it.

Add water to the final test tube. This is your water blank.

Part V. Antioxidant measurement of the commercial tea using the FRAP method.
Set the spectrometer to 593 nm.
Obtain 6 square plastic spectrophotometer cuvettes.
To each cuvette add 4 drops of the solution from the test tubes using a small (1 ml) plastic pipette following the table below. Use a new small plastic pipette each time.

cuvette	
1	4 drops tea
2	4 drops test tube 1
3	4 drops test tube 2
4	4 drops test tube 3
5	4 drops test tube 4
6	4 drops water

Add 3 ml (measured with a plastic pipette) of FRAP solution to each cuvette.
Mix the solution in each cuvette.
Measure the absorbance of each cuvette at 593 nm.
Zero the spectrophotometer with cuvette 6 before each reading.
Complete the following table with you values

Tube	Absorbance	
1		
2		
3		
4		
5		
6	0	0
7		

Add data to the “class data” spreadsheet before you leave the lab.

Observations and comments:

Part VI. Total phenolics measurement of tea using the Folin-Ciocalteau reagent.
Set the spectrophotometer to 750 nm.
Set up 6 square plastic cuvettes.
To each cuvette add 10 drops of the solution from the test tubes using a small (1 ml) plastic pipette following the table below. Use a new small plastic pipette each time.

cuvette	
1	10 drops tea
2	10 drops from test tube 1
3	10 drops from test tube 2
4	10 drops from test tube 3
5	10 drops from test tube 4
6	10 drops water

Then add 1.5 ml of Folin-Ciocalteau reagent to each cuvette.
Mix the solution in each cuvette by shaking and/or swirling.
Let them set for 7 minutes at room temperature.
Add 1.5 ml of Na_2CO_3 solution to each cuvette.
Mix the solution in each cuvette by shaking and/or swirling.
Allow the cuvettes to sit for 30 minutes.
Read of absorbance of cuvettes 1 – 5 at 750 nm.
Zero the spectrophotometer with cuvette 6 before each reading.

Tube	Absorbance	
1		
2		
3		
4		
5		
6	0 mmol	0

Add data to the “class data” spreadsheet before you leave the lab.
Observations and comments:

Lab Report.
Spreadsheet Data for the class will be available on the MyDU website.

1. Using the data from Part II and Part V calculate the antioxidant power of your tea in terms of mM gallic acid.

2. Using the data from Part III and Part VI calculate the total phenolics of your tea in terms of mM gallic acid.

3. Compare the best linear fit graphs for the 4 different commercial teas tested with the FRAP assay. What are your conclusions?

4. Compare the best linear fit graphs for the 4 different commercial tested with the Folin-Ciocalteau assay. What are your conclusions?

Prelab: Hand in at the beginning of the lab period.
The following data is taken from Part II and Part V of this experiment.
The Gallic acid equivalent (column 6) of the tea is calculated by multiplying column (3), column (4), and column (5) then dividing by column (2)

1. Complete the table

(1) cuvette	(2) A gallic acid	(3) gallic acid mM	(4) A tea	(5) dilution factor	(6) Gallic acid equivalent mM
1	2.438	0.550	1.516	1	0.342
2 (tube 1)	1.569	0.275	0.849	2	
3 (tube 2)	0.774	0.138	0.492	4	
4 (tube 3)	0.366	0.069	0.304	8	
5 (tube 4)	0.191	0.035	0.168	16	

2. What is the average of the values in column (6)?
(This is the antioxidant power of your tea in terms of mM gallic acid.)

3. From page 1 of this handout. What is the stereochemical relationship between (+)-cathechin and (-)-epicatechin?

4. In reference to the article: "Total Antioxidant Capacity of Teas by the Ferric Reducing/Antioxidant Power Assay" Which type of tea black, green, or oolong has the highest antioxidant power?

5. In reference to the previous answer. What was their explanation of their findings?

6. In reference to the article: "Total Antioxidant Capacity of Teas by the Ferric Reducing/Antioxidant Power Assay" What is the correlation between total phenolics (Folin-Ciocalteau) and the FRAP results?