GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

ESTIMATION AND COMPARISON OF TOTAL PHENOLIC AND TOTAL ANTIOXIDANTS IN GREEN TEA AND BLACK TEA

Avneet Kaur, Milandeep Kaur, Prabhjot Kaur, Harpreet Kaur, Sarbjeet Kaur, Khushwinderjit Kaur
GGDSD College, Sector-32C, Chandigarh (UT) India -160047
Corresponding author email: avbawa@yahoo.co.in

ABSTRACT
Indian teas are known for their taste, flavors all over the world. India is the second largest producer of tea in the world after China, including the famous Assam and Darjeeling tea. However, green tea which has potential health benefits is not a popular beverage among Indians. This study was conducted to assess and compare the total polyphenol content (TPC), antioxidant activity in black and green tea. The total polyphenol content in green tea and black tea was determined according to the International Organization for Standardization method (ISO) 14502-1. The antioxidant capacity was determined by Ferric Ion Reducing Antioxidant Power (FRAP) and Polymolybdenum method. The green tea showed a higher polyphenol content and antioxidant levels than black tea ($p < 0.05$). The antioxidant activities were well correlated with the total polyphenol content ($r^2 = 0.9571$ for FRAP method and $r^2 = 0.9287$ for Polymolybdenum assay). The Kangra green tea (North India) had higher TPC and antioxidant levels than Darjeeling green tea (Northeast India). The TPC and antioxidant levels of Kangra tea is at par with China green tea and Ceylon green tea. However, there is a need for larger studies on Indian tea to emphasize about the health benefits of Indian tea especially green tea which will help in its increased consumption as popular beverage in India.

KEYWORDS: Green tea, Black tea, TPC, Total antioxidant levels, FRAP, Polymolybdenum method

INTRODUCTION

Tea is the most popular non-alcoholic beverage in the world. Among tea producing countries the principal producers are China, India, Sri Lanka, Kenya and Indonesia which account for 80% of global production. In India, there are three different geographical areas of cultivation and production of tea. The bulk of tea is produced in the Northeast (West Bengal and Assam), followed by the Northern area (Himachal Pradesh), and small quantities are produced in the Southern India (Tamilnadu). Tea is derived from terminal leaves of shoots of tea plant *Camellia sinensis* family. *Sinensis* species is divided into two distinct varieties *sinensis* and *assamica*. *Camellia sinensis var. sinensis* is indigenous to South east China, Darjeeling, Japan. *Camellia sinensis var assamica* is indigenous to Assam, Thailand, SriLanka (Macfarlane *et al*., 2004). There are essentially three main types of Camellia tea, which are Green, Oolong and Black tea. The difference lies in the 'fermentation' which actually refers to oxidative and enzymatic changes within the tea leaves, during processing (Hicks, 2001). Worldwide, the consumer's preference is approximately 76-78% of black tea followed by green tea (20-22%) and oolong tea (2%). Black tea leaves undergo the highest levels of oxidization and fermentation, as compared to its other tea counterparts. When black tea is oxidized, the catechins are converted into theaflavins and thearubigins, which still act as antioxidants (Ho *et al*., 1994). The theaflavins and thearubigins are sometimes called tannins and are responsible for the darker colour of black tea and more heavily-oxidized oolong teas (Wang *et al*., 2001). Polyphenols found in black tea are very strong antioxidants. The tannins in tea have a therapeutic effect on gastric and intestinal illnesses (Siemann *et al*., 1992). Green tea is rich in chemicals called catechins, which are a form of flavanol monomers, a type of flavonoid. Flavanols are also called flavan-3-ols, and are also found in other plant-based foods and beverages. The catechins include epicatechin (EC), epigallocatechin (EGC), epicatechin-3- gallate (ECG), and epigallocatechin-3-gallate (EGCG). Green tea also contains a small amount of Vitamin C, an antioxidant which is also an essential nutrient (Farhoosh *et al*., 2007). Tea components possess antioxidant, antimutagenic, and anticarcinogenic activity (Liao *et al*., 2001). Green tea consumption has also been linked to the prevention of many types of cancer, including lung, colon, esophagus, mouth, stomach, small intestine, kidney, pancreas, and mammary glands (Koo *et al*., 2004). Several epidemiological studies and clinical trials showed that green tea (black and oolong teas to a lesser extent) may reduce the risk of many chronic diseases (Tsuneki *et al*., 2004). This beneficial effect has been attributed to the presence of high amounts of polyphenols which are potent antioxidants. In particular, green tea may lower blood pressure and thus reduce the risk of stroke and coronary heart disease. Some animal's studies have suggested that green tea might protect against the development of coronary heart disease by reducing blood glucose levels and body weight (Tsuneki *et al*., 2004). India traditionally home to black tea has ignored the potential to produce and propagate green tea. With the increasing knowledge about the health benefits of green tea polyphenols, there is a need to enhance interest in Indian green tea for health benefits. Taking this into

account, the present study was done to estimate the total phenolic content (TPC) and total antioxidant in both black and green teas by Folin-Ciocalteu Method and total antioxidant capacity by Ferric Ion Reducing Antioxidant Power (FRAP) and Polymolybdenum method. An attempt was also made to compare total phenolic content and total antioxidant levels in Indian green tea from Kangra and Darjeeling with those available worldwide (China, Sri Lanka).

MATERIALS & METHODS

10 different brands of black tea (labeled as G-P) available in the local departmental stores of Chandigarh, India were taken. For green tea, 6 different tea samples (labeled as A-F) were taken which included two commercial brands of green tea available locally, one from Kangra (Himachal Pradesh), one from Darjeeling, one from China and one from Ceylon. Each tea sample was studied in triplicate.

Preparation of extract

The preparation of extract was done as described by the International Organization for Standardization (ISO) 14502-1. 0.200 g of each sample was weighed and to it 5 ml of 70% methanol at 70 °C was added. The extract was mixed properly and heated at 70 °C on a vortex for 10 min. After cooling at room temperature, the extract was centrifuged at 200 g for 10 min. The supernatant was collected in a centrifuge tube. The extraction step was repeated twice. Both extracts were pooled and the volume was adjusted to 10 ml with cold 70% methanol. One milliliter of the extract was diluted with water to 100 ml. Analysis of the tea extract was done in triplicate.

Determination of total polyphenol content by folin-ciocalteu method

The total polyphenol content (TPC) was determined by spectrophotometer using tannic acid as standard, according to the method described by the International Organization for Standardization (ISO) 14502-1. 1.0 ml of the diluted sample extract (in triplicate) was added to tubes containing 5.0 ml of a 1/10 dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 ml of a sodium carbonate solution (7.5% w/v) was added and incubated at room temperature for one hour. The absorbance was measured at 765 nm. The TPC was expressed as mg tannic acid equivalents (TAE)/ g. The concentration of polyphenols in samples was derived from a standard curve of tannic acid ranging from 10 to100 µg/ml.

Determination of antioxidant power by using modified ferric ion reducing antioxidant power assay (FRAP)

The total antioxidant capacity was determined by spectrophotometry, using ascorbic acid as standard, according to the modified FRAP assay. 0.1 ml of extract was taken and to it 0.9 ml of ethanol, 5 ml of distilled water, 1.5 ml of HCl, 1.5 ml of potassium fericyanide, 0.5 ml of 1% SDS and 0.5 ml of 0.2% of ferric chloride was added. This mixture was boiled in water bath at 50°C for 20 minutes and cooled rapidly. Absorbance was measured at 750 nm to measure the reducing power of the tea extract. The antioxidants in samples were derived from a standard curve of ascorbic acid ranging from 10 to 100 µg/mL. The total antioxidant power will be expressed as mg ascorbic acid equivalent (AAE)/ g.

Determination of antioxidant capacity by phosphor molybdenum assay

For the determination of total antioxidant capacity the tubes containing 0.2 ml of extract is mixed with 1.8 ml of distilled water, 2 ml of phosphomolybdenum reagent (2.8 mM (40 mg) of sodium phosphate and 4 mM ammonium molybdate (49 mg) in 10 ml of 0.6 M sulphuric acid). The tubes were incubated at 95°Cfor 90 mins. The mixtures are cooled to room temperature and absorbance is measured at 695 nm. The antioxidant capacity is expressed as mg Ascorbic Acid Equivalent (AAE) /g.

Statistical analysis

The assays were carried out in triplicate, and the results were expressed as mean values and the standard deviation (SD). The statistical differences were done by student's t-test ($p < 0.05$). Correlations were established using Pearson's correlation coefficient (r) in bivariate linear correlations using Microsoft office Excel 2007 and SPSS, version 16.

RESULTS & DISCUSSION

India is a major producer, consumer and exporter of tea accounting for 31% of the total global production of tea. Tea from Darjeeling, Assam and Nilgiri are world famous for their taste and flavor. However, in India green tea accounts for only 1% of total production of tea. Since there are very few studies which document the antioxidant potential of India tea, an attempt is made to study total polyphenol content and antioxidant activity in different brands of tea and also the possible correlation between TPC and antioxidant activity was also studied. The total polyphenolic content of 10 samples of black tea and 6 samples of green tea was estimated. The results from this study found that the total polyphenol content in green tea (3.066±1.911 mg TAE/g) was significantly higher than in black tea (0.72±0.55 mg TAE/g) ($p<0.05$). The higher levels of polyphenols in green tea (unfermented tea) than black tea could be due to conversion of the tea polyphenols into thearubigins and theaflavin during the fermentation process of black tea (Jain, 1999) Various other studies have also reported higher total polyphenol content in green tea (Anesini *et al*., 2008; Shrestha *et al*., 2010, Nor Ohairul Izzreen and Mohd Fadzelly, 2013). Among the various green tea studied Cylon tea, China tea and Kangra tea (India) were among the tea with high polyphenolic content. In our study the Kangra tea had higher polyphenol content than Darjeeling tea as reported by Vashisht *et al*. (2007) as the Kangra tea has higher amounts of epigallocatechin-3-gallate as compared to North-East Darjeeling tea. Polyphenols in tea have been reported to have strong antioxidant property which could be attributed to various types of flavan-3-ols present in tea. These flavan-3-ols have been reported to prevent oxidative stress by chelating free ferrous ions which are responsible for the formation of reactive oxygen species by various metabolic processes (Tsai *et al*., 2007). The variations in polyphenolic levels in black tea (2.11- 0.26 mg TAE/g) and green tea (5.20- 1.17 mg TAE/g) observed in our study (Fig.1) could be due to variations in the climate and agronomic practices and other factors such as tea types, commercial brands of tea, tea plantation area, etc (Suteerapataranon *et al*., 2008).

Total Antioxidant power of 10 samples of black tea and 6 samples of green tea were estimated by using modified ferric ion reducing antioxidant power (FRAP) method. The results have shown that the FRAP levels were significantly higher in green tea (5.866±2.73 mg AAE/g) as compared to black tea (2.034±1.39 mg AAE/g) ($p<0.05$) as reported in earlier studies (Su *et al*., 2007, Dutta *et al*. 2013). In black tea, the FRAP levels ranged from 5.58 mg AAE/g to 1.06 mg AAE/g where as in green tea it ranged from 8.85 mg/g to 3.16 mg AAE/g (Fig 2). Benzie and Szeto (1999) have also reported the total antioxidants capacity of teas estimated by FRAP had widely different in vitro antioxidant power. The antioxidant capacity was strongly correlated with the total polyphenolic content of the tea ($r^2 = 0.9571$). Various other studies have also reported strong correlation between TPC and FRAP of different tea (Turkmen *et al*., 2007, Dutta *et al*. 2013).

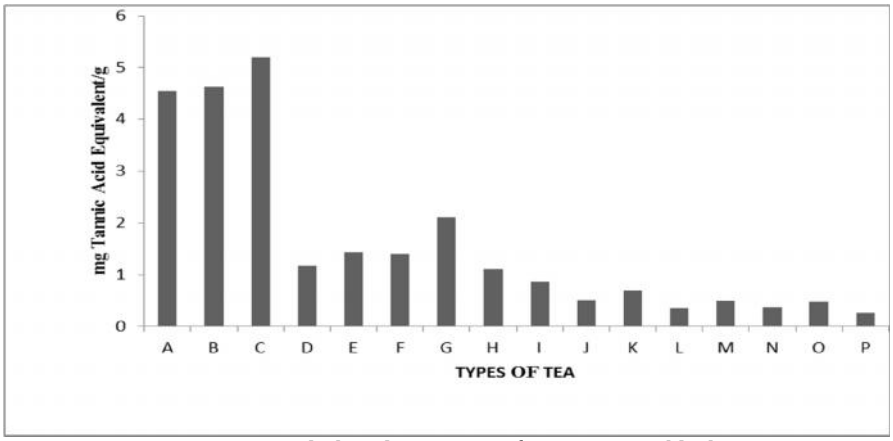

FIGURE 1: Total Phenolic Content of Green Tea & black tea

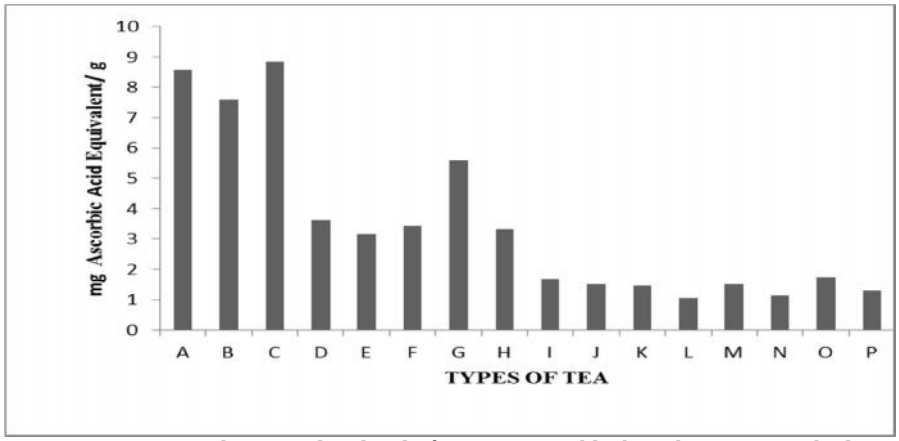

FIGURE 2: Total Antioxidant level of Green Tea & black tea by FRAP method

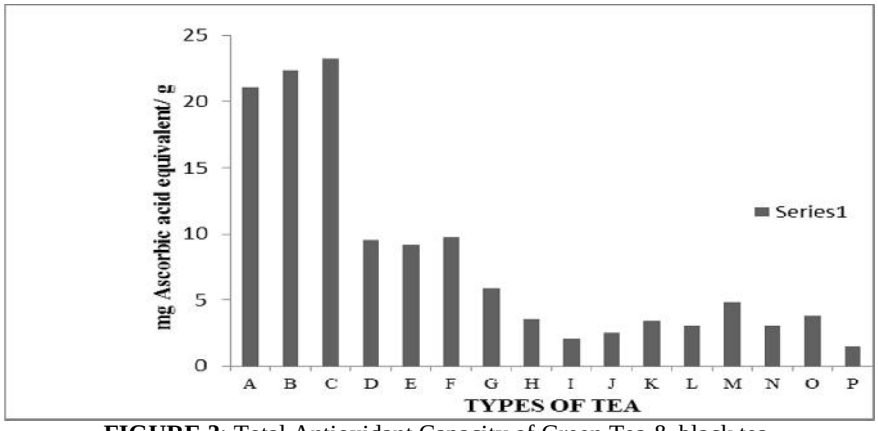

FIGURE 3: Total Antioxidant Capacity of Green Tea & black tea

Total Antioxidant Capacity of 10 samples of black tea and 6 samples of green tea was calculated using phosphomolybdenum assay. This assay usually detects total antioxidants such as ascorbic acid, some phenolics, α-

tocopherol and carotenoids. In the present study the total antioxidant capacity in green tea (15.866±7.036 mg AAE/g) is significantly higher than in black tea (3.386±1.28 mg AAE/g) ($p<0.05$) (Fig 3). Various other studies ha ve reported higher antioxidant activity in green tea than in black tea (Yen *et al*., 1995; Chan *et al*., 2010). The higher antioxidant activity is due to potent antioxidant activities of catechins in green tea are due to their three adjacent hydroxyl (OH) groups on the β-ring as in EGCG, GCG, EGC and GC which are more effective in scavenging free radicals than the two adjacent OH groups as in ECG, CG, and EC. The content of EGCG and EGC in green tea is much higher than in black tea (Almajano *et al*., 2008). In case of black tea the antioxidant properties have been attributed to its chemical components of thearubigins, phenolic acids, catechins, and theaflavins. Theaflavins which impart color, brightness, and astringency to black tea infusion also possess potent antioxidant properties. (Shivaki *et al*., 1994, Miller *et al*., 1996). The total antioxidant capacity showed strong correlation with TPC (and $r^2 = 0.9287$) as reported earlier by Dutta *et al*. (2013) implying that polyphenol possess antioxidant property that provides protection against oxidative stress.

CONCLUSION

This study is based on evaluation of antioxidant activity and the total polyphenolic contents of the different brands of teas available in Indian market. The antioxidant activity in Indian tea showed strong correlation with TPC. It can be concluded that Indian tea especially green tea may act as natural antioxidant substitute. However, furthermore studies on Indian green tea have to be done to make it a popular beverage among Indians.

REFERENCES

Almajano, M. P., Carbó, R., Limenéz, A. L. and Gordon, M. H. (2008) Antioxidant and antimicrobial activities of tea infusions. Food Chem. 108, 55–63.

Anesini, C., Graciela, E. F. and Rosana, F. (2008) Total Polyphenol Content and Antioxidant Capacity of Commercially Available Tea (Camellia sinensis) in Argentina. J. Agric. Food Chem. 56, 9225–9229.

Benzie, I. F. F, Szeto Y. T. (1999) Total antioxidant capacity of teas by the ferric reducing/antioxidant power (FRAP) assay. Journal of Agricultural and Food Chemistry .47, 633 – 636.

Chan, E. W., Lim, Y. Y., Chong, K .L., Tan, J. B. L., Wong, S. K. (2010) Antioxidant properties of tropical and temperate herbal teas. J. Food Compos Anal. 23, 185–189.

Dutta, A. K., Siddiquee, M. A., Hossain, S. and Kabir, Y. (2013) Finlay green tea possesses the highest in vitro antioxidant activity among the 20 commercially available tea brands of Bangladesh. Malaysian Journal of Pharmaceutical Sciences.11, 2, 11–20.

Farhoosh, R., Golmovahhed, G. A. and Khodaparast, M. H. H. (2007) Antioxidant activity of various extracts of old tea leaves and black tea wastes (Camellia sinensis L.). Food Chemistry 100, 231 – 236.

Hicks, A. (2001) Review of Global Tea Production and the Impact on Industry of the Asian Economic Situation. AU J.T. 5(2), October. Also in Asian Int. Tea Conf. ’98, Singapore, 1-2 October 1998.

Ho, C. T., Osawa, T., Huang, M. T., Rosen, R. T. (1994) Food Phytochemicals for Cancer Prevention II Tea Spices & Herbs. Washington D.C. American Chemical Society.

ISO 14502-1: 2005. Determination of substances characteristic of green and black tea. Part 1 Content of total polyphenols in tea. Colorimetric method using Folin-Ciocalteu reagent.

Jain, N. K. (1999) Biosynthesis of precursors for liquor and flavour characteristics in black tea. Global advances in tea science. P 723- 748, Aravali books international (P) Ltd, New Delhi.

Koo, M. W. L., Cho, C. H. (2004) Pharmacological effects of green tea on the gastrointestinal system. European Journal of Pharmacology 500,177–185.

Liao, S., Kao, Y. H. and Hiipakka, R. A. (2001) Green tea biochemical and biological basis for health benefits. Vitamins and Hormones 62, 1–94.

Macfarlane (2004). The Empire of Tea. p 32. The Overlook Press.

Miller, N. J., Castelluccio, C. and Tijburg, L. C. (1996) The antioxidant properties of theaflavins and their gallate esters- radical scavengers or metal chelators? FEBS Lett 1996, 392, 40–44.

Nor Qhairul Izzreen, M . N. and Mohd Fadzelly, A. B. (2013) Phytochemicals and antioxidant properties of different parts of Camellia sinensis leaves from Sabah Tea Plantation in Sabah Malaysia. International Food Research Journal 20(1), 307- 312.

Shivaki, M., Hava, Y., Osawa, T., Kumon, H., Nakayama, T. and Kawakishi, S. (1994) Antioxidative and antimutagenic effects of theaflavins from black tea. Mutat Res 323, 29–34.

Shrestha, R., Lama, J. P. and Shrestha, K. (2010) Total Polyphenols Content and Antioxidant Activity of Different Tea Commercially Produced in Nepal. J. Food Sci. Technol. Nepal 6, 73-79.

Siemann, E . H. and Creasy, L. L. (1992) J Enol. Vitic 43, 49.

Su, X., Duan, J., Jiang, Y., Duan, X. and Chen, F. (2007) Polyphenol Profile and Antioxidant Activity of Brewed Oolong Tea at Different Conditions. Int. J. Mol. Sci. 8, 1196-1205.

Suteerapataranon, S. and Pudta, D. (2008) Flow Injection Analysis-Spectrophotometry for Rapid Determination of

Total Polyphenols in Tea Extracts. J. of Flow Injection Analysis 25, 61-64.

Tsai, P. J., Tsai, T. H., Yu, C. H. and Ho, S. C., (2007) Comparison of NO-scavenging and NO-suppressing activities of different herbal teas with those of green tea. Food Chemistry 103, 181–187.

Tsuneki, H., Ishizuka, M., Terasawa, M., Wu, J. B., Sasaoka, T. and Kimura, I. (2004) Effect of green tea on blood glucose levels and serum proteomic patterns in diabetic (db/db) mice and on glucose metabolism in healthy humans. BMC Pharmacol 4,18–21.

Turkmen, N., Velioglu, Y. S., Sari, F. and Polat, G. (2007) Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea. Molecules 12, 484–496.

Vasisht, K., Sharma, P. D. and Karan, M., et al. (2003) Study to Promote the Industrial Exploitation of Green tea Polyphenols in India. ICS-UNIDO.

Wang, H. and Helliwell, K. (2001) Determination of flavonols in green and black tea leaves and green tea infusions by high-performance liquid chromatography. Food Research International 34, 223 – 227.

Yen, G. C. and Chen, H. Y. (1995) Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agric. Food Chem. 43, 27–32.