

Analysis of Phenolic acids in some samples of Indian and Nepal Tea by High Performance Liquid Chromatography

R Srivastava, A Singh, S Misra, U Singh, A Tiwari

Citation

R Srivastava, A Singh, S Misra, U Singh, A Tiwari. *Analysis of Phenolic acids in some samples of Indian and Nepal Tea by High Performance Liquid Chromatography*. The Internet Journal of Alternative Medicine. 2008 Volume 6 Number 2.

Abstract

The beneficial effects of tea (*Camellia sinensis*) leaves have already been studied in detail. However, the scientists are keen to explore the exact scientific reasons for such effects. The biochemical analysis of tea samples has indicated that they contain mostly polyphenols, mainly catechins. Catechins are present in food and beverages of plant origin. Keeping this in view, High Performance Liquid Chromatographic analysis was performed to estimate phenolic acids in 26 samples of tea (samples of Indian market brand, Indian tea garden and Nepal tea garden such as Janhit green tea™ (JGT), Tata tea premium™ (TT), Brooke bond Taaza™ (BBTZ), Lipton Darjeeling Tea™ (LDT), Tea city™ (TCT), Taj Mahal Tea™ (TAJM), Tetley Tea™ (TET), Assam dana (DASM), Assam dana 1 (ASMD-1), Assam dana 2 (ASMD-2), Assam Tea (ASM), North Bengal tea (NB), North Bengal dana-1 (DNB-1), North Bengal-2 (DNB-2), North Bengal Upper (NBUP), Darjeeling tea-FOP (DARJ-FOP), Darjeeling tea-FOP1 (DARJ-FOP1), Darjeeling tea- brake (DARJ-br), Indian tea garden (Darjeeling-ITGA (DARJ-ITGA), Darjeeling-ITGB (DARJ-ITGB), Darjeeling-ITGC (DARJ-ITGC) and Nepal tea garden (KP-33A, KP-16A, KP-9A, KP-C6 and KP-C2).

INTRODUCTION

Tea, made from the leaves of *Camellia sinensis* (Fam. Theaceae), is one of the most popular beverages worldwide. Green tea is prepared from fresh tea leaves that are pan-fried or steamed and dried to inactivate enzymes. Black tea is prepared by crushing withered tea leaves and allowing enzyme-mediated oxidation, commonly referred to as fermentation leading to the formation of oligomers such as theaflavins and polymers known as thearubigins. Oolong tea is a partially fermented product that contains considerable amount of catechins and oligomerized catechins¹. A number of beneficial effects have been attributed to tea, including the prevention of oral cancer and tooth decay². In animal models the epidemiological studies in relation to tea polyphenols for oral cancer prevention has been sparse and inconclusive^{3,4}. On the other hand, preliminary results from an intervention study have shown that oral and topical administration of a tea preparation significantly reduced the size of oral lesions and the incidence of micronucleated oral mucosa cells in leukoplakia patients compared with a non-treated group^{5,6}. The active constituents of green tea are believed to be the polyphenols, commonly known as tea catechins. The major tea catechins are EGCG, EGC, ECG,

and EC⁷. In brewed green tea, the water extractable material, which usually accounts for one-third of the tea leaves in dry weight, contains about 30% catechins, 3% flavonols, 3-6% caffeine, and other constituents⁸. Studies in many animal models and cell lines have demonstrated that tea and tea polyphenols possess anticarcinogenic activity^{9,10,11}.

Recently, phenolic acid metabolites as biomarkers for tea and coffee-derived polyphenol exposure in human subjects have been used^{9,10}. Good amount of phenolic acids have recently been observed in tea by some other worker also¹².

In this report, we analyzed several samples of tea available in market and tea garden of India as well as Nepal for the presence and amount of phenolic acids and correlated to health. The results are presented here.

MATERIALS AND METHODS

PREPARATION OF SAMPLES FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

The tea samples of the market and tea garden, Indian and Nepal were macerated in a pestle-mortar and extracted by

two methods, viz., hot and cold extractions.

HOT EXTRACTION

Ethanol: water (80:20) was added in the samples, which were heated up to 60-70° C for 30 min. The samples were cooled to room temperature and then centrifuged at 7,500 rpm for 15 min. The supernatants were evaporated at room temperature. Finally dried materials were re-suspended in 1.0 ml HPLC grade methanol by vortexing. Again the solution was filtered through membrane filter (Millipore, pore size 0.45 µm). The prepared samples were stored at 4 ° C for HPLC analysis.

COLD EXTRACTION

Tea samples were macerated in a pestle-mortar and extracted twice with ethanol-water (80:20,v/v, 10 ml). The extracts were kept for overnight in the solvent and then centrifuged at 7,500 rpm for 15 min. The supernatants were evaporated at room temperature. Finally dried materials were re-suspended in 1.0 ml HPLC grade methanol by vortexing. Again the solution was filtered through membrane filter (Millipore, pore size 0.45 µm). The prepared samples were stored at 4 ° C for HPLC analysis.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) ANALYSIS

High performance liquid chromatography (HPLC) of the samples was performed in binary conditions with HPLC system (Shimadzu Corporation, Kyoto, Japan) comprising Shimadzu LC-20 ATVP reciprocating pumps, a variable SPD-20, AVP UV VIS detector, C-18 reverse phase HPLC column (250x4.6 mm) i.d., particle size 5 µm Luna 5µ C-18 (2), (Phenomenex, USA) at 36 ° C. Operating conditions were injection volume (10 µl), mobile phase methanol: water containing 0.4% acetic acid (66:34, v/v) flow rate 1 ml/min and detection at 290 nm and 254 nm. Tannic, gallic, caffeic, ferulic, vanillic, benzoic, cinnamic and salicylic acids were used as internal and external standards. Phenolic acids present in the samples were identified through comparing retention time (Rt. in min.) of standards. E.g. TA (Rt. 2.600), CT (Rt. 2.758), GA (Rt. 2.900), Caf-A (Rt. 3.358), FA (Rt. 3.825), BA (Rt. 5.650), CA (Rt. 6.792) and SA (Rt. 11.400). These phenolic acids were identified by co-injection of internal and external standards for their confirmation. Amount of individual compound was calculated by comparing peak areas of reference compounds with those in the samples run under same elution conditions.

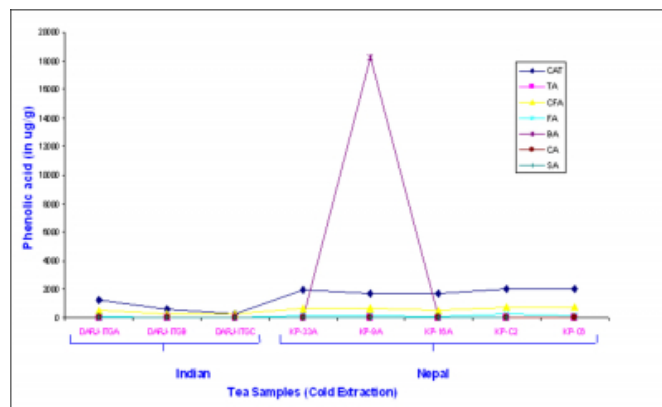
RESULTS

INDIAN TEA GARDEN AND NEPAL TEA GARDEN

In the cold extracted samples, catechin, caffeic and ferulic acids were present in all the samples. Catechin was maximum in KP-C6 (2070.6290 µg/g) and minimum in DARJ-ITGC (227.2576 µg/g). Caffeic and ferulic acids were also maximum in KP-C6 (784.1483 µg/g CfA) (227.7835 µg/g FA) and minimum in DARJ-ITGC (258.2662 µg/g CfA) (35.4526 µg/g FA), respectively. Benzoic acid was present only in KP-9A (18248.0204 µg/g). The amount of catechin was high amount in cold extraction while in hot extraction the amount reduced and caffeic acid also showed this variation in hot and cold extraction in Nepal tea samples (Fig. 1).

Figure 1

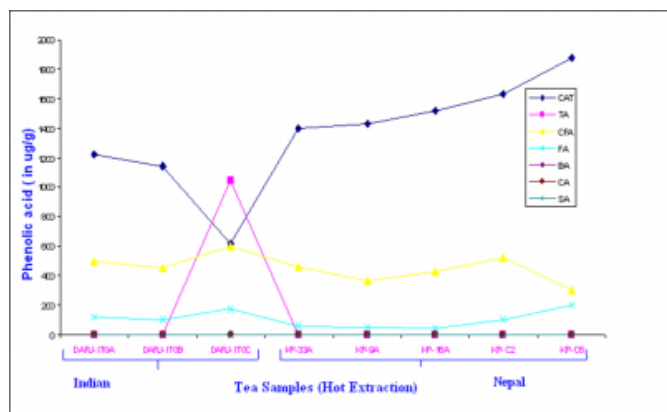
Figure 1: Comparison between Indian Tea Garden and Nepal Tea Garden samples through HPLC analysis (Cold Extraction) Indian tea garden (Darjeeling-ITGA (DARJ-ITGA), Darjeeling-ITGB (DARJ-ITGB), Darjeeling-ITGC (DARJ-ITGC), Nepal tea garden (KP-33A, KP-16A, KP-9A, KP-C6 and KP-C2).



In the hot extracted samples, the amount of catechin was reduced in comparison with cold extracted samples. This may be due to degradation of those compounds during hot extraction. In hot extraction, catechin, caffeic and ferulic acids were present in all the samples. The amount of catechin was maximum in KP-16A (1430.1092 µg/g) minimum in DARJ-ITGC (622.1591 µg/g) and tannic acid was present only in DARJ-ITGC (1050.6685 µg/g). Ferulic acid was also present in all the samples being maximum in KP-C6 (199.9919 µg/g) and minimum in KP-16A (47.7598 µg/g) (Fig.2).

Figure 2

Figure 2: Comparison between Indian Tea Garden and Nepal Tea garden samples through HPLC analysis (Hot Extraction) Indian tea garden (Darjeeling-ITGA (DARJ-ITGA), Darjeeling-ITGB (DARJ-ITGB), Darjeeling ITGC (DARJ-ITGC), Nepal tea garden (KP-33A, KP-16A, KP-9A, KP-C6 and KP-C2).

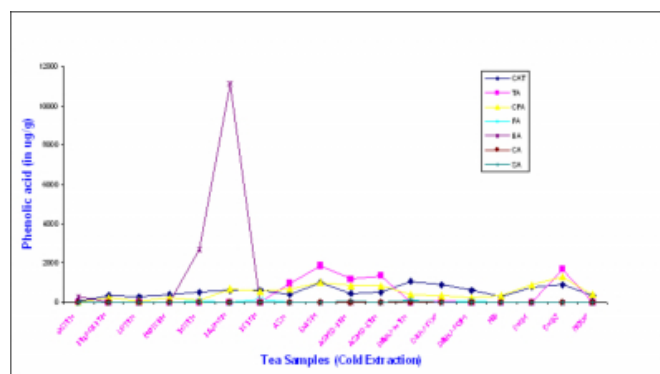


TEA SAMPLES OF INDIAN TEA MARKET

HPLC analysis of other 18 tea samples collected from Indian market indicated that the number and quantity of phenolic acids varied in different tea samples. In cold extraction, catechin was present all the samples being maximum in Darjeeling tea-brake (1090.20 µg/g). Caffeic acid, which was also present in all tea samples was maximum in DNB2 (1270.2038µg/g) and minimum in JGT (22.3209 µg/g), Tannic acid was present in ASM, DASM, DNB2, ASMD-1 ASMD-2 and DARJ-FOP1 being maximum in DASM (1833.9346µg/g). Gallic acid was absent in all the tea samples. Ferulic acid was present in Lipton Darjeeling ten, Tea city, Tetley tea and Darjeeling tea-brake and DARJ-FOP1 and maximum in Tetley tea (148.92 µg/g). Benzoic acid was present only in Janhit green tea, Tea city and Taj Mahal and maximum in Taj Mahal tea (11087.80 µg/g), while Cinnamic acid was present only in Tetley tea, Assam dana-I and Darjeeling tea-brake. Salicylic acid was present in Assam dana-I and Darjeeling tea-brake (98.79 µg/g) (Fig. 3).

Figure 3

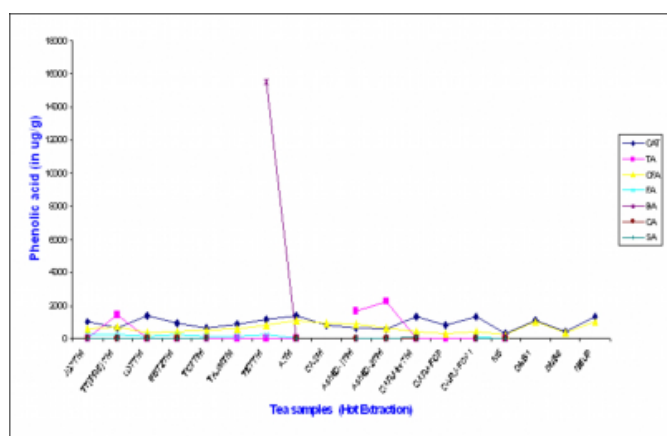
Figure 3: Phenolic acid content in samples of Indian Tea Market through HPLC Analysis (Cold Extraction) Janhit green tea \hat{A}^{TM} (JGT), Tata tea premium \hat{A}^{TM} (TT [pre]), Lipton Darjeeling Tea \hat{A}^{TM} (LDT), Brooke bond Taaza \hat{A}^{TM} (BBTZ), Tea city \hat{A}^{TM} (TCT), Taj Mahal Tea \hat{A}^{TM} (TajM), Tetley Tea \hat{A}^{TM} (TET), Assam dana (DASM), Assam dana 1 (ASMD-1), Assam dana 2 (ASMD-2), Assam Tea (ASM), North Bengal tea (NB), North Bengal dana-1 (DNB-1), North Bengal-2 (DNB-2), North Bengal Upper (NBUP), Darjeeling tea-FOP (DARJ-FOP), Darjeeling tea-FOP1 (DARJ-FOP1), Darjeeling tea-brake (DARJ-br).



In hot extraction, catechin and caffeic acid were present in all the samples. Catechin was maximum in ASMD-1 (2282.5348 µg/g) and minimum in NB (313.4076 µg/g) while caffeic acid was maximum in ASM (1069.4067µg/g) and minimum in NB (246.0799µg/g). Tannic acid was present in TT(Pre), ASM-1, ASM-2 and DARJ-FOP maximum in TT(Pre) (1430.3377 µg/g) and minimum in DARJ-FOP (34.5491µg/g). FA was present all the branded samples and DARJ-Br and DARJ-FOP and maximum in BBTZ (246.5178µg/g) and minimum in DARJ-Br (81.4652µg/g). Benzoic acid was present in TET (154.86.5346µg/g) while cinnamic acid in DARJ-br (10.2654µg/g) and salicylic acid in DARJ-br (40.6413 µg/g) (Fig. 4).

Figure 4

Figure 4: Phenolic acid content in Tea samples of Indian Tea Market through HPLC analysis (Hot Extraction) Janhit green tea \hat{A}^{TM} (JGT), Tata tea premium \hat{A}^{TM} (TT [pre]), Lipton Darjeeling Tea \hat{A}^{TM} (LDT), Brooke bond Taaza \hat{A}^{TM} (BBTZ), Tea city \hat{A}^{TM} (TCT), Taj Mahal Tea \hat{A}^{TM} (TAJM), Tetley Tea \hat{A}^{TM} (TET), Assam dana (DASM), Assam dana 1 (ASMD-1), Assam dana 2 (ASMD-2), Assam Tea (ASM), North Bengal tea (NB), North Bengal dana-1 (DNB-1), North Bengal-2 (DNB-2), North Bengal Upper (NBUP), Darjeeling tea-FOP (DARJ-FOP), Darjeeling tea-FOP1 (DARJ-FOP1), Darjeeling tea- brake (DARJ-br).



DISCUSSION

Phenolic acids constitute an important group of natural products contributing significantly to the marked pharmacological value of a number of plants, including fruits, vegetables and spices ¹². Tea is particularly rich in polyphenols, including catechin, flavone, flavonol, theaflavin, anthocyanidin, thearubigin and miniphenol formaldehyde, which are thought to contribute to the health benefits of tea ⁷. Tea polyphenols act as antioxidants *in vitro* by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions ¹³. Tea polyphenols have been proposed to have other properties such as antibiosis, anticancer, reducing plasma lipids, protecting cardiac and cerebral vessels ^{14,15, 16}, reported that tannic acid had the highest antioxidant capacity followed by gallic and caffeic acids ¹⁷. Polyphenol's Antioxidant efficiency seems to depend on the extent of hydroxylation and conjugation ¹⁸. The inhibitory activity of tea against tumorigenesis demonstrated in many animal models has generally been attributed to tea catechins ⁸. The present results indicate that caffeic acid and catechin were present in Darjeeling tea-brake and Assam dana-I and several other phenolic acids were also present in Darjeeling tea-brake and Assam dana-I. ¹⁹ reported that monomeric and polymeric

polyphenols in food are multifunctional such as antioxidants, metal chelators and inhibitors as well as strengthening the gastric mucosal barrier and immuno-stimulatory property. The total phenolic acid contents in tea were also analysed by ^{12, 9} and ¹⁰ have used phenolic acid as the biomarkers of intake for polyphenol foods.

Tea consumption has been shown to be associated with reduced risk of both cancer and cardiovascular disease in human population studies, but clinical data demonstrating bioavailability of the individual catechins and other polyphenolic components of tea are limited ²⁰. Caffeic acid besides being antioxidant also shows anti-inflammatory and anti cancer properties. It also reduces aflatoxin production ¹⁷ catechin a major constituent decreased the frequencies of sister chromatid exchanges (SCE) induced by paraquat (PQ) which is a generator of reactive oxygen species ^{16, 21} reported that green tea and food containing polyphenol might be beneficial to human health by protecting against reactive oxygen species induced genotoxicity. Since both these compounds (caffeic acid and catechin) are in maximum amount in tea the positive response for human health can be attributed to these compounds. The phenolic acids together with several other compounds are responsible for global gravitation towards tea consumption and recommendation for its use since time immemorial.

CONCLUSION

According to the present results, caffeic acid and catechin were present in Darjeeling tea-brake and Assam dana-I and several other phenolic acids were also present in Darjeeling tea-brake and Assam dana-I, so these are recommended as better tea than other tea samples for human health.

CORRESPONDENCE TO

Tel: 91-2318388; (M): 91-9452195608; E-mail: amitabh_2k@rediffmail.com

References

1. Balentine, D.A., Wiseman, S.A. and Bouwens, L.C. The chemistry of tea flavonoids. *Crit Rev Food Sci Nutr.* 1997; 37:693-704.
2. Hamilton-Millar J.M. Anti-cariogenic properties of tea (*Camellia sinesis*), *J Med Microbiol.* 2001;50:299-302.
3. Frei, R. and Higdon, J.V. Antioxidant activity of tea polyphenols *in vivo*: evidence from animal studies. *J Nutr.* 2003; 133: 32758-32848.
4. Katiyar, S.K. and Mukhtar, H. Tea consumption and cancer. *World Rev Nutr Diet.* 1996; 79:154-184.
5. Li, N., Han, C. and Chen, J. Tea preparations protect against DMBA-induced oral carcinogenesis in hamsters. *Nutr Cancer.* 1999; 35:73-79.

6. Li, N., Sun, Z., Han, C. and Chen, J: The chemopreventive effects of tea on human oral precancerous mucosa lesions. *Proc. Soc. Exp. Biol. Med.* 1999; 220:218-224.
7. Higdon, J.V. and Frei, B. Tea catechins and Polyphenols health effects, metabolism, and antioxidant functions. *Crit Rev Food Set Nutr.* 2003; 43:89-143.
8. Chung, S.Y., Laishun, C., Man, J.L., Douglas, B., May, C.K. and Stimson, P.S. Blood and Urine levels of Tea Catechins after ingestion of Different Amounts of Green Tea by Human Volunteers. *Cancer Epidemiology Biomarkers & Prevention.* 1998; 7:351-354.
9. Hodgson, J.M., Chan, S.Y., Puddey, I.B., Devine, A., Wattanapenpaiboon, N., Wahlqvist, M.L., Lukito, W., Burke, V., Ward, N.C., Prince, R.L. and Croft, K.D. Phenolic acid metabolites as biomarkers for tea- and coffee-derived polyphenol exposure in human subjects. *Br J Nutr.* 2004; 91:301-6.
10. Mennen, L.L., Sapinho, D., Ito, H., Bertrais, S., Galan, P., Hercberg, S. and Scalbert A. Urinary flavonoids and phenolic acids as biomarkers of intake for polyphenol-rich foods. *Br. J. Nutr.* 2006; 96:191-8.
11. Raquel, P., Laura, B. and Fulgeneio, S.C. Antioxidant Activity of Dietary Polyphenols as Determined by a Modified Ferric Reducing/Antioxidant Power Asssay. *J. Agric Food Chem.* 2000; 48(8): 3396-3402.
12. Mattilla, P., Hellstrom, J. and Torronen, R. Phenolic acids in berries, fruits and beverages. *J Agric Food Chem.* 2006; 54(19): 7193-9.
13. Elattar, T.M. and Virjit, A.S. Effect of tea polyphenols on growth of oral squamous carcinoma cells in vitro. *Anticancer Res.* 2000; 20: 3459-3465.
14. Yang, C.S. and Wang, Z.Y. Tea and cancer. *J Nutr Cancer Inst.* 1993; 85:1038-1049.
15. Yang, C.S., Maliakal, P. and Meng, X. Inhibition of carcinogenesis by tea. *Annu Rev Pharmacol Toxicol.* 2002; 42: 25-54.
16. Tanaka, R. Protective effects of (-)-epigallocatechin gallate and (+)-catechin on paraquat-induced genotoxicity in cultured cells. *J Toxicological Sci.* 2000;25(3): 199-204.
17. Orban, Z., Mitsiadesb, N., Burke, J.V., Tsokorb, T.R. and Chrousosa, G.P. Caffeic acid acid phenyl ester induces leucocyte apoptosis, modulates nuclear factor-kappa B and suppresses acute inflammation. *Neuro Immuno Modulation.* 2000; 7: 99-105.
18. Zhu, X.M., Zhao, Z.D. and Huang, Z.I. Experimental study on tea polyphenols in prevention of hyperlipidemia. *Zhongguo Zhong Xi Yi Yi Jie He Za Zhi* 1996; 16: 549-551.
19. Gharzouli, K., Khenouf, S., Amira, S. and Gharzouli, A. Effect of aqueous extract from *Quercus ilex* L., root bark, *Punica granatum* L. fruit peel and *Artemisia herba-alba* Asso leaves on ethanol induced gastric damage in rats. *Phytotherapy Res.* 1999; 13: 42-45.
20. Beverly, A.W., Lametta, S.S., Gary, R.B., Douglas, A.B. and Beverly, A.C. Catechins are Bioavailable in Men and Women Drinking Black Tea throughout the Day. *J. Nutr* 2001; 131:1731-1737.
21. Yang, C.S. and Landau, J.M. Effects of tea consumption on nutrition and health. *J Nutr.* 2000; 130:2409-2412.

Author Information

Rashmi Srivastava, Ph.D.

Institute of Bioengineering and Biological Sciences, Varuna Bridge

Amitabh Singh, M.Sc. (Ag.), Ph.D.

N-8/236;R-22; Ganeshdham, Newada, Sunderpur

S. Misra, Ph.D.

Institute of Bioengineering and Biological Sciences, Varuna Bridge

U.P. Singh, M.Sc. (Ag.), Ph.D.

N-8/236;R-22; Ganeshdham, Newada, Sunderpur

Aparna Tiwari, M.Sc.

Institute of Applied Medicine and Research