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Variation of antioxidant properties and phytochemical constituents of tea cultivated under various agronomic conditions at Terai region of North Bengal

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Abstract

The studies were conducted during 2007-2008 in three cultivars of tea (*Camellia sinensis*) namely, TV26, TV29 and Dangri Manipuri Jat, at tea estates of Terai, North Bengal. Commercial standard plucked tea leaves were assayed to determine antioxidants activity and related phytochemical constituents. DPPH was used to determine the antioxidant properties of bud+leaf (B+L), bud+two leaves (B+2L), bud+three leaves (B+3L) and matured leaves (L). Subsequently, total phenolics, flavonols and hydrolysable tannins were estimated using standard methods. The result showed that free-radicals scavenging potentiality and chemical composition varies significantly at different growing conditions and leaf maturation. Statistical analysis showed significant relation between antioxidant and some phytochemical composition of dry tea leaves, among which phenolics ($R^2 = 0.904$, $P < 0.001$), tannins ($R^2 = 0.567$, $P < 0.05$) and flavonols ($R^2 = 0.314$, $P < 0.05$) bear significant correlation. Soil physicochemical parameters like *p*H ($R^2 = 0.537$, $P < 0.05$), nitrogen ($R^2 = 0.618$, $P < 0.01$), K_2O ($R^2 = 0.106$, insignificant), P_2O_5 ($R^2 = 0.730$, $P < 0.01$), S ($R^2 = 0.157$, insignificant) and soil moisture ($R^2 = 0.745$, $P < 0.01$) influence free-radical scavenging activity in tea leaves. Antioxidant quality of tea varies with maturation stages of leaves and the pattern is specific for a selected varieties. From our observation it may be concluded that antioxidant quality is dependent on some fertility parameters of soil and maturation of leaf. These results suggests that appropriate agronomic practices and proper choice of plucking may help to preserve the tea antioxidant quality.

Keywords: Tea cultivars, free-radical scavenging activity, phytochemical composition, agro-climatic conditions.

Introduction

In the wake of rising global production of tea and stiff competition, the quality has gained considerable importance. The tea should not only pass the minimum quality standards prescribed by the various agencies but also meet the expected levels in antioxidants quality. The quality of tea begins in the field where the leaves accumulate the necessary chemical substances. The chemical composition of tea shoots varies with genotype, season and cultural practices. During the course of processing of plucked leaves, various biochemical changes take place in stored phytoconstituents of leaves, resulting in the degradation and formation of compounds; responsible for liquor, briskness, colour and aroma of cuppa.

Processed tea that is widely consumed now as a popular health drinks/bevarage is commercially manufactured from the young tender leaves of the tea plant (Cabrera *et al.*, 2003). Tea elixir has continued to be considered as medicine since the ancient time because of its richness in phytochemical constituents. Research on the effects of tea on human health has been fueled by the growing need to provide natural healthy diets that includes plant derived polyphenols. Research is going on to elucidate how functional components in tea cultivar could expand the role of diet in oxidative disease prevention and treatment (Misra *et al*, 2003, Hitchon *et al.*, 2004, Marian *et al.*, 2004, Pajohk *et al.*, 2006). There are evidences that tea constituents play therapeutic role in more than sixty different health conditions (Pandey *et al.*, 2005, Vanessa *et al.*, 2004, Yamamoto *et al.*, 2004, Paola *et al.*, 2005, Hang *et al.*, 2003 and Hakim *et al.*, 2004). Therefore tea appears to be an effective chemopreventive agent for toxic chemicals which are produced in the body during normal metabolic pathways or introduced from the environment. Many plant phenolics have been reported to have antioxidant properties that are even much stronger than vitamin-C and E (Karori *et al.*, 2007). In addition, currently available synthetic antioxidant like BHA, BHT and Gallic acid esters have been suspected to initiate negative side effects (Amie *et al.*, 2003, Aquil *et al.*, 2006) and hence the need to substitute them with natural antioxidants like that from tea with broad-spectrum action. Despite the upsurge of interest in the therapeutic potential of tea plants as sources of natural antioxidants, limited studies have been carried out in various growing conditions using different cultivars. Information on the tea antioxidant properties varies in tea cultivar and degree of leaf maturation, which are rare and grossly lacking. In this research, a set of 36 tea samples; from three cultivar; grown in different agro climatic conditions of Terai were analyzed for antioxidant quality.The objective of this study was to compare the antioxidants quality with some chemical constituents of three tea cultivars and correlate them with soil agronomic parameters for determining better cultivation practices to restore the antioxidant quality of tea.

Material and methods

Plant materials

Description of cultivar

Fairly drought resistance, Indochina type, Aphids susceptible tea cultivar TV26 variety is very common cultivated plant in Terai region. Leaves are semi-erect, light, medium, lanceolate to ovate shaped leaflets and petiole bearing brown pubescence hair especially at axils of leaflets. Shoot size is medium.

Indochina, triploid TV29 is also popular cultivar of Terai region due to its drought tolerant capacity and high yield index. Dark semi-erect, medium leaves are lanceolate to ovate shaped. Low pubescence hair is found in axils of leaflet (Singh *et al.*, 1994). Indochina, Jat Dangri Manipuri is a hardy plant, difficult to descript morphological character due to seed jat, wide variation found in plant to plant and very common throughout Terai region.

Collection of shoots and extraction process

Experimental three variety of tea leaves are collected from the three commercial garden of Terai regions, Darjeeling, West Bengal in November and December month, 2007. The collected sample shoots comprisede of [Bud =B, Leave = L)] B + L, B + 2L, B + 3L and mature leaves (L) were dried in hot air oven at temperature between 50 to 60° C for 12 h after which 10 g of respective plant components were chopped and ground. The ground plant materials (400 mg) was soaked in 50 ml. of methanol:water:: 4:1 and boiled for 30 min on a hot plate with glass beads. The flask containing methanol extracts were kept overnight for cold percolations. The extracts were filtered through Whatman-42 filter paper and volume was reduced by evaporation under reduced pressure in rotary evaporator. Residue was washed three times and extracts were concentrated (20 mg/ml). Methanolic extract were used for evaluation of DPPH and determination of total phenolics, flavanols and tannin contents.

Antioxidants assay

DPPH based free radical scavenging activity

The free radical scavenging activities of each fraction were assayed using a stable DPPH, as per Blois (1958). Percentage of free radical scavenging activity was expressed as percentage inhibition as per the formula given below and 50% inhibition concentration was estimated.

$$\% \text{ inhibition} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$$

Soil sampling and determination of physicochemical properties of soil

Soil samples were collected from four spots surrounding a plant, 0-45 cm depth at 1 ft apart from the collar region. Composite soil samples were prepared by mixing of four samples from each plant. Moisture content, *p*H, organic carbon (available organic nitrogen), available potassium as K_2O, available phosphorus as P_2O_5 and available sulfur were determined as per the procedure prescribed by the International Association of Soil Chemist (Baruah *et al.*, 1997).

Determination of phytochemical constituents

Estimation of total phenolics

Total phenols estimation was carried out in methanolic extracts of tea (dry mass) with Folin-Ciocalteu reagent as per Malick *et al*, 1980. The amount of total phenols was expressed as percent of extract catechol equivalent.

Estimation of flavonols

Flavonol content was determined spectrophotometrically in tea samples according to Mahadevan *et al.* (1986). The amount of flavonols was expressed as percent of extract, equivalent to phloroglucinol.

Estimation of tannins

Folin-Denis method was used for the determination of tannins as per Sadasivam *et al.* (1992) which is based on the non-stoichiometric oxidation of the molecules containing phenolics hydroxyl group, equivalent to tannic acid.

Statistical analysis

All the experiments were repeated three times and the data were represented as means and were analyzed by SPSS (ver. 11.0) for determining significance (P value < 0.001, 0.01, and 0.05 were represented as "***", "**" and "*" respectively).

Results and discussion

From the preliminary phytochemical evaluation of methanol extract of dried tea leaves (20 mg/ml concentration) in average standard plucking, it was confirmed that major groups of phytochemicals were phenols, flavonols and tannins. Fig. 1 and 3 represents the correlation between antioxidant activity with phenols and tannins and flavonols content did not execute significant level of the antioxidant activity Fig. 2.

The free-radical scavenging activity was demonstrated by DPPH, which is a stable free-radical and accept electron or hydrogen radical to become a stable diamagnetic molecules (Blois, 1958). Certain plants show antioxidant activity with DPPH S because of their phenolics constituents. Hydrolysable tannins and flavonols are a broad class of low molecular weight secondary metabolites widely distributed in plants. The beneficial effect of total phenolics and hydrolysable tannins are attributed to their antioxidant and chelating ability (Heim *et al.*, 2002). Phytochemical analysis confirm the presence of phenolics especially tannins and flavonols at varying degree in the three different cultivar viz. diploid, triploid and seed jat which represent all types of clonal varieties irrespective of genetic make up and cultural practices. Results have indicated that the antioxidant effect is related to development of reductones. Reductones are reported to be terminators of free-radical chain reaction (Dorman *et at.*, 2003).

Irrespective of cultivar and leaf maturation the antioxidant capacity of tea plant was significantly varied with soil physicochemical parameters. Fig.4 shows that DPPH (IC50 based free-radical scavenging activity was significantly correlated with soil *p*H, because soil *p*H significant affects on the availability plant nutrients and microbial activity surrounding the rhizosphere. In very low (< 4.00) and high (6.00) *p*H, the availability of major as well as micro nutrients drastically affected and immediately plant suffers from nutritional stress. Therefore soil amendment will be performed in such a way that *p*H should be in between $4.00 < pH < 6.00$. From the Fig. 4, it may predicted that antioxidant activity restoration in tea leaves is optimized when the pH range in between 4.00 -5.00. There is a correlation between available from of organic nitrogen and antioxidant quality of tea leaves (Fig. 5). Nitrogen play significant role on preservation of antioxidant potentiality up to certain limit of 0.13 % after which antioxidant value decreases. Nitrogen is a basic constituent of protein and nucleic acid, integral part of chlorophyll, imparts vigorous vegetative growth, delay in maturity of plants, related to carbohydrate utilization and enhance sink strength. Excess nitrogen is a possible cause of Zn deficiency in citrus plant (Das, 1996). Potassium show the insignificant role (Fig. 6) on preservation of antioxidant activity in tea plant, our observation clearly indicates that 80-100 ppm soil potash maintain this important property, above or below this limit may be detrimental to radical scavenging activity of tea due to catalytic nature, over activate the enzyme and disturb the osmotic pull. The effect of phosphorus on free-radical scavenging activity is presented in Fig. 7, Soil available phosphorus show the varying degree of radical scavenging action of tea leaves but significant activity was observed within 15- 45 ppm level. Harbone (1980) observed that the phosphorus deficiency increases antioxidant constituent levels; same finding was also reflected in our experiments. Sulphur is a vital part of ferredoxins which is important for light and dark reaction of photosynthesis process and important constituents of some protein, so maintenance up to certain range very much essential. In Fig. 8, insignificant action of sulphur on antioxidant was observed, above 13 and below 7.00 reduces the tea antioxidant quality. Moisture level at 15% decreases the nutrients availability of tea plants. Oxidative stress generated within the cell as a result of low water availability increases its antioxidant property so, Fig. 9 shows the significant results within 15-20 % moisture level.

For normal growth and development when nutrients are not limiting the important weather variables are solar radiation, temperature, saturation deficit of the air and rainfall (Carr *et al.*, 1992). The growth processes are manifested with the expansion of leaves, shoots, production and storage of dry matter and partitioning of secondary metabolites between the various plant organs. Maturation of tea leaf affects the accumulation pattern of antioxidant capacity as executed in Fig. 10. This may be due to higher allocation of carbon in secondary metabolites path way with up regulation of maturation. Minimum temperature 12-13° C maximum 30° C and 700 mm/ annum precipitation is ideal for tea cultivation. Below minimum temperature and above maximum temperature, storage and development of antioxidant compounds in tea leaves may be hampered. Yao *et al.* (2005) was observed the seasonal variation of phenolics compounds in Australia-grown tea. This is also another agreement of our result of agro-climatic variability of antioxidant quality of tea.

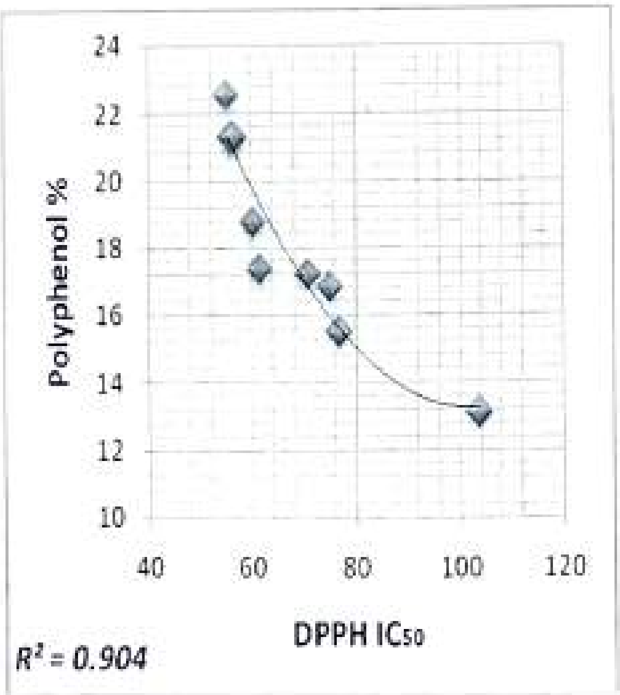

Fig 1. Relation between polyphenols and antioxidant activity (DPPH IC50)

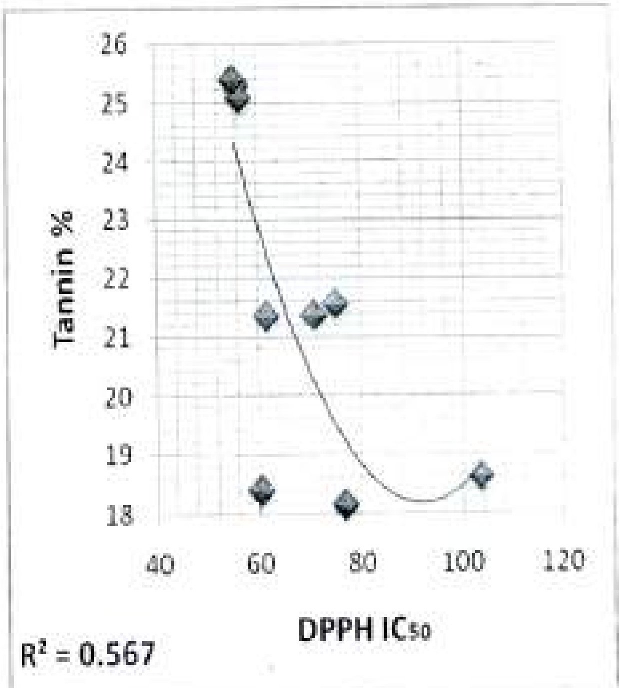

Fig 2. Relation between Tannins and antioxidant activity (DPPH IC50)

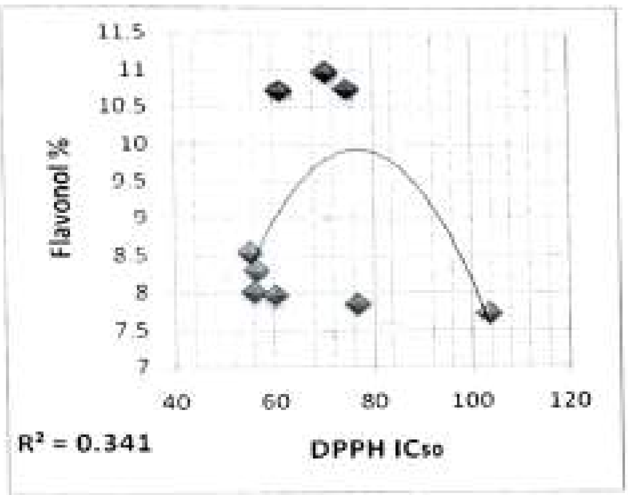

Fig 3. Relation between Flavonols and antioxidant activity (DPPH IC50)

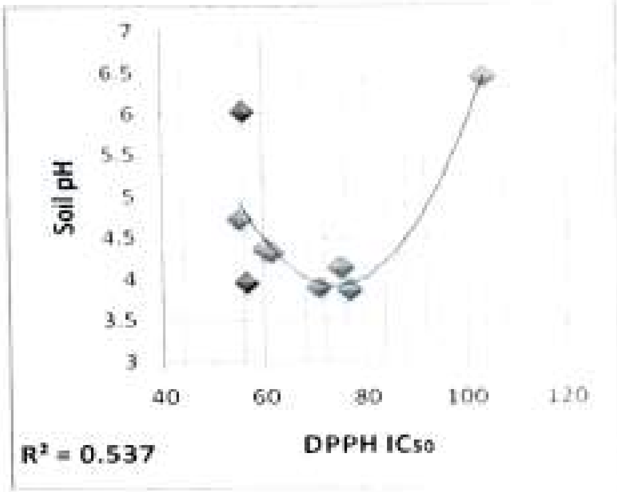

Fig 4. Relation between Soil *pH* and antioxidant activity (DPPH IC50)

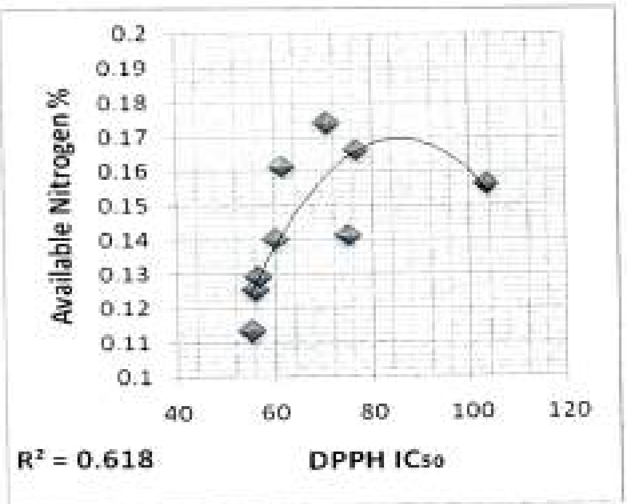

Fig 5. Relation between available Nitrogen and antioxidant activity (DPPH IC50)

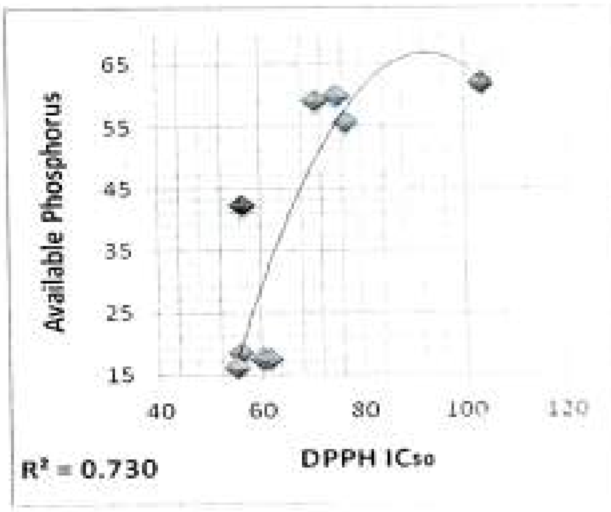

Fig 6. Relation between available Phosphorus and antioxidant activity (DPPH IC50)

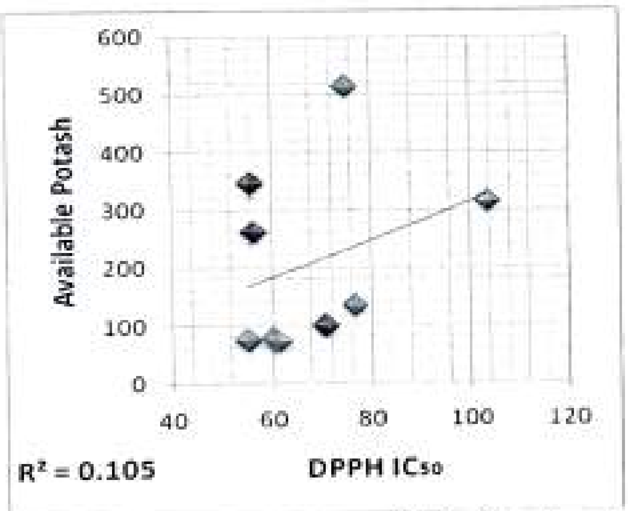

Fig 7. Relation between available Potash and antioxidant activity (DPPH IC50)

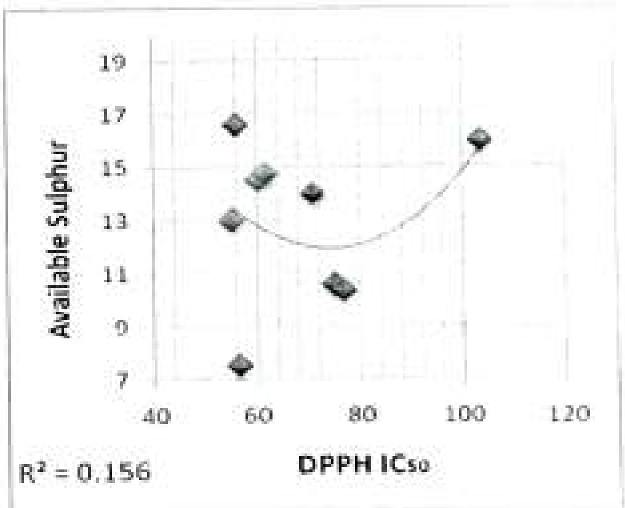

Fig 8. Relation between available Sulphur and antioxidant activity (DPPH IC50)

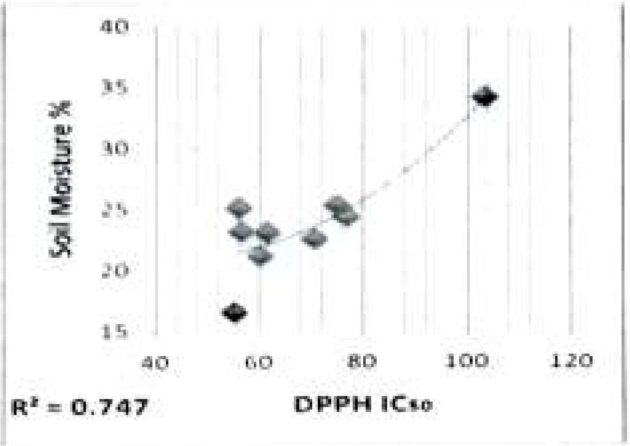

Fig 9. Relation between Soil moisture and antioxidant activity (DPPH IC50)

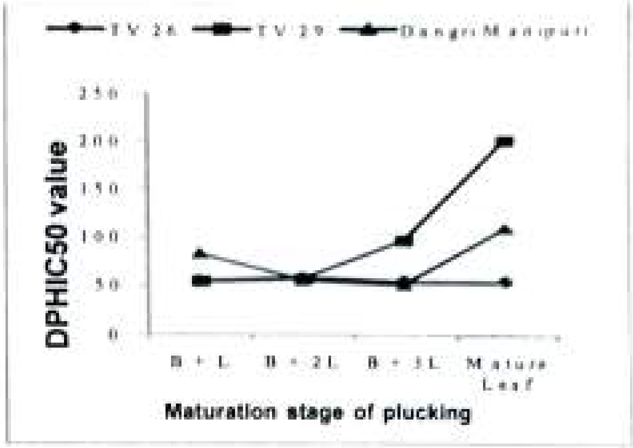

Fig 10. Pattern of antioxidant activity with leaf maturation

Conclusions

To conclude, the results have undoubtedly shown that the antioxidant quality of tea is best, if the soil *p*H is in between 4.50-5.00, nitrogen status 0.13%, available phosphorous as P_2O_5 is in between 15-45 ppm and moisture at 15-20% level. This is a preliminary report on antioxidant activity of tea under different soil agronomic practices and leaf maturation. Further studies are undertaken for the characterization of individual components of bioactive fractions of tea extracts to elucidate the mechanisms for restoring high antioxidant activity.

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