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Short Note

1, 1-diphenyl-2-picrylhydrazyl free radical scavenging activity of Kangra tea

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Plants produce secondary metabolites, organic compounds that provide natural self-defense against pathogens. In case of tea (*Camellia sinensis*) leaves, these metabolites are catechins, a group of primary polyphenols. During processing of green tea leaves to manufacture black tea, the phenolase-catalyzed oxidation transforms these catechins into dimeric theaflavins and polymeric thearubigins responsible for tea liquor characteristics. However, inactivation of polyphenol oxidase in fresh green tea leaves prevents catechins from undergoing the transformation. Fresh tea leaves have been reported to be rich in catechins, the flavanol monomers responsible for health benefits and antioxidant property of green tea (Graham, 1992; Higdon and Frei, 2003).

The polyphenolic compounds in tea are different from other plant polyphenols as most of them are found only in leaves of *Camellia sinensis* plant (Chu and Junija, 1997). Tea catechins exist as two geometrical isomers that differ in stereochemical configuration of 3', 4'-dihydroxyphenyl and hydroxyl groups at the positions 2 and 3 of the C-ring of the flavonoid structure. The principal catechins found in tea are epicatechin (EC), (-) - epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG) of which EGCG is the most abundant catechin found in tea (Nakabayashi, 1991). Other tea catechins which are present in minor quantity include (+) - gallocatechin (GC), (-) - gallocatechin gallate (GCG), (-) - catechin gallate (CG), and (+) - catechin (C). Esterification of (-) - catechin with gallic acid form (-) - catechin – 3 – gallate and (-) - gallocatechin – 3 –gallate. Catechins and their derivatives have been reported to have beneficial protective effects against cancer (Michels *et al.*, 2005), cholesterol (Maron *et al.*, 2003, Vinson *et al.*, 2004), diabetes (Vinson and Zhang 2005), cerebral damage (Suzuki *et al.*, 2004), smoking (Schwarz *et al.*, 2005) and pathogenic bacteria (Yoda *et al.*, 2004, Friedman *et al.*, 2005).

Tea has been growing on the gentle slopes of gorgeous Dhauladhar range of mighty Himalayas since 1860s when first commercial tea plantation was established by Government at Holta. The local Kangra *jat* bushes (about 150 year old) growing around Palampur (Latitude 32^0 07' 15.68" N, Longitude 76^0 31' 47.26" E, altitude 4275 feet above mean sea level, soil pH 4.8-5.8) have small, erect, hard and dark green leaves. The climate is hot and humid during summer and cold during winters. The annual potential transpiratory demand of about 1100 mm is totally met by annual rainfall of 2500 mm. Although a great deal of work pertaining to chemical composition and quality of Kangra tea has been carried out (Gulati and Ravindranath, 1996; Gulati *et al.*, 1999; Sud and Baru, 2000; Sud, 2007), however, very little work seems to have been done to explore the antioxidant potential of polyphenols from Kangra tea (Vasisht *et al.*, 2004; Sud *et al.*, 2007). Hence, the present studies were carried out to investigate antioxidant property of Kangra tea polyphenols.

The samples of commercial made black orthodox tea were collected from the Palampur Co-operative Tea Factory Ltd., Palampur and those of green tea shoots (two leaves and a bud) were collected from local tea gardens. Quantitative estimations of total polyphenols and condensed tannins were carried out in tea extracts obtained after refluxing 500mg of powdered tea sample in 25ml double distilled water for 5 minutes employing standard techniques (Makkar, 2003; Sun *et al.*, 1998). The antioxidant activity was evaluated in terms of free radical-scavenging ability of tea powders, obtained after freeze drying the aqueous tea extracts, using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay (Kordali *et al.*, 2005). The change in absorbance in 1ml 160 µmol methanolic solution of DPPH due to aqueous solutions of tea powders (concentrations:1µg,

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2μg, 3μg, 4μg and 5μg) was recorded after incubation at 30°C for 30 minutes in the dark, at 517nm using Spectronic GENESYS 5 spectrophotometer. The per cent DPPH radical scavenging was calculated by the equation:

[(control absorbance - sample absorbance) /control absorbance] x 100

These values were plotted against μg of tea extract to evaluate EC_{50} (the amount of antioxidant necessary to decrease the initial DPPH free-radical concentration by 50 per cent). The EC_{50} was calculated by the equation:

50 - y intercept / slope.

The DPPH free radical scavenging activity decreased in the order: green tea shoots whose enzymatic activity was stopped > green tea shoots whose enzymatic activity was not stopped > made black orthodox tea. The trend was found to be directly proportional to the total polyphenols and condensed tannin contents of teas. Green tea shoots, whose enzymatic activity was stopped by microwave heat treatment, had higher content of total polyphenols (177.33-188.10 g kg^{-1}) and condensed tannins (100.40-125.26 g kg^{-1}) compared to green tea shoots whose enzymatic activity was not stopped (total polyphenols: 57.60-134.70 g kg^{-1} condensed tannins: 26.93-94.13 g kg^{-1}) and made black orthodox tea (total polyphenols: 86.63- 128.37 g kg^{-1} condensed tannins: 38.23-58.37 g kg^{-1}). The DPPH free radical scavenging activity always increased with increase in the concentration of tea extract (Table 1).

The EC_{50} values in the range of 3.36-4.21 μg, 3.25-3.92 μg and 2.98-4.74 μg for made black orthodox tea and 2.83-4.20 μg, 3.29-4.60 μg, 2.70-4.94 μg for green tea shoots whose enzymatic activity was not stopped; for first (April), second (May and June) and third (July and August/September) flush seasons, respectively, were comparable. However, these values were higher compared to the EC_{50} values for green tea shoots whose enzymatic activity was stopped by microwave heating (Table 2). It was, therefore, reasonable to infer that the green tea shoots whose enzymatic activity was stopped were more potent as DPPH free radical scavenger. It was of interest to note that the first flush green tea shoots had more potent DPPH free radical scavenging activity.

The preliminary investigation on free radical scavenging property of Kangra tea seems to uphold the health claim associated with tea. However, intensive studies on therapeutic attributes of polyphenols from Kangra tea are required to be carried out.

Table 1. Mean values* of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenged by different concentrations of Kangra tea (made black orthodox, green shoots whose enzymatic activity was not stopped and fresh green shoots whose enzymatic activity was stopped) powders

Concentration of tea powder	DPPH free radical scavenged (%)		
	Made black orthodox tea	Green tea shoots (enzymatic activity not stopped)	Green tea shoots (enzymatic activity stopped)
1μg	13.93-19.77	18.67-24.70	26.13-53.00
2μg	28.74-33.48	25.23-38.00	39.20-69.25
3μg	35.60-45.35	42.06-53.69	47.20-93.25
4μg	45.75-61.70	45.70-65.79	53.69-93.75
5μg	59.77-71.16	58.47-79.33	67.78-95.00

*Based on estimations carried out in five samples of each type in triplicate

Table 2. Range of EC_{50}* of aqueous solutions of tea powders

Month	EC_{50} Value (μg of tea powder)		
	Made black orthodox tea	Green tea shoots (enzymatic activity not stopped)	Green tea shoots (enzymatic activity stopped)
April	3.36-4.21	2.83-4.20	0.82-1.43
May	3.31-3.92	3.54-3.81	1.10-3.30
June	3.25-3.52	3.29-4.60	2.78-3.87
July	2.98-3.73	3.50-4.94	2.61-2.91
August	3.80-4.58	2.76-3.21	-
September	3.72-4.74	2.70-2.96	-

*Amount of antioxidant necessary to decrease the initial DPPH free radical concentration by 50 per cent

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