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Evaluation of phenolic constituents and antioxidant activity of aqueous extracts of Kangra tea [*Camellia sinensis* (L) O Kuntze]

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Abstract
Consumption of green tea (*Camellia sinensis*) may provide protection against chronic diseases, including cancer. Green tea polyphenols are believed to be responsible for this cancer preventive effect, and the antioxidant activity of the green tea polyphenols has been implicated as a potential mechanism. The objective of this study was to determine the relationship between the plucking periods and the major constituents and the antioxidant activity in green tea. The mean values of total catechin content varied significantly in the range of 130.51 to 96.79 g kg^{-1} for Kangra Local; 111.18 to 82.09 g kg^{-1} for Kangra Asha and 136.56 to 99.26 g kg^{-1} for Kangra Jawala. The mean monthly total phenol content varied significantly in the range of 175.843 to 135.407 g $kg^{-1}$for Kangra Local; 230.360 to 109.863 g kg^{-1} for Kangra Asha and 223.036 to 184.639 g kg^{-1} for Kangra Jawala. In addition, antioxidant activity of green tea and standard catechins was investigated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. The order of antioxidant activity of standard catechins was as follows: EGCG> EGC ≥ ECG ≥ EC ≥ C. Moreover, the *cis*-catechins contents were the key factor affecting the antioxidant activity of green tea.

Keywords: Green tea; polyphenols; antioxidant activity, DPPH

1. Introduction

Tea has attracted attention of the scientific community and industries due to the health benefits associated with it during the last four decades. The associated benefits have been reported to be due to polyphenolic constituents in general and flavonoids in particular of tea. Flavonoids, a group of organic polyphenols, are the secondary metabolites that are involved in a wide range of specialized physiological functions such as growth, development and defense mechanisms in the plant kingdom (Rusak *et al*., 2008) [21]. Tea is one of the principal sources of flavonoids in human diet (Ho *et al*., 1992; Hollman & Arts, 2000) [8, 9].

Tea has been classified into three major groups on the basis of the extent of enzymatic oxidation of polyphenols in green tea shoots and degree of fermentation. The consumption of green tea in the world accounts for 20-22% (Wu & Wei 2002) [36].

Total phenolic compounds in fresh tea flush have been reported to be in the range of 25 to 35 per cent (dry weight basis) of which 20 to 24 per cent are tea catechins (Millin and Rustige1967; Sanderson 1972) [16, 22]. The major catechins (flavans-3-ols) in tea are (-) - epicatechin (EC), (-) - epigallocatechin (EGC), (-) - epicatechin gallate (ECG), (-) - epigallocatechin gallate (EGCG), (-) - gallocatechins (GC) and (-) - gallocatechin gallate (GCG). Green tea was reported to constitute 59% of the EGCG, 19% of EGC, 13.6% ECG and 6.4% EC (Cabrera *et al*., 2006) [2].

One of the most important beneficial mechanisms associated with tea consumption is the free radical scavenging ability of its polyphenol constituents responsible for antioxidant property (Frie & Higdon, 2003) [4]. Green tea polyphenols have been reported to possess antioxidant activity that provides protection from damages caused by free radical-induced oxidative stress (Katiyar *et al*., 2001; Rao *et al*., 2004) [13]. Green tea has been reported to be more potent as antioxidant compared to black tea because the latter has much lower percentage of EGCG (Katalinic *et al*., 2006) [12]. Epidemiological studies carried out during the last three decades suggested that green tea catechins have nutraceutical and therapeutic properties against cancer in humans (Vanessa & Williamson 2004) [34]. The synthesis and accumulation of polyphenolic constituents in tea were reported to be dependent on weather parameters (Bhatia & Ullah 1968; Singh *et al*., 1999) [1, 25].

Although a great deal of work on agro techniques, plant protection, nursery and quality (Vashist *et al*., 2004; Rawat & Gulati 2008) [35, 19] has been carried out, however, information pertaining to phytochemicals of nutraceutical significance from Kangra tea is scanty (Sud *et* *al*., 2007; Sud *et al*., 2007a; Sud *et al*., 2010, Ghabru 2010) [33, 31, 5, 30]. Hence, the present study was conducted to investigate the antioxidant activity of Kangra tea.

2. Materials and Methods

The present investigations "Evaluation of phenolic constituents and antioxidant activity of aqueous extracts of Kangra tea [*Camellia sinensis* (L) O Kuntze]" were carried out to explore the phenolic profile of green shoots of local cultivars, corroborate health claims associated with tea consumption and investigate correlation between phenolic profile and nutraceutical attributes of Kangra valley tea. The cultivars of Kangra tea were also assessed for their flavan-3-ols vis-à-vis (+)-catechin, (EC), (EGC), (EGCG) and (ECG) profiles. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability of aqueous extracts of green tea shoots and aqueous solutions of tea powders obtained by lyophilizing aqueous extracts of green tea shoots, was evaluated in terms of IC_{50} values.

2.1 Estimation of total polyphenols and total catechins

2.1.1 Estimation of total polyphenols

Total polyphenols were always estimated in freshly prepared tea extracts by the method of Makkar (2003) [15]. Total polyphenols were calculated using following equations:

Concentration (µg) = Absorbance at 725 nm – (– 0.05260) / 0.02982

The concentration of total polyphenols (TP) was finally expressed in terms of g $kg^{-1}$of fresh green tea shoots on dry weight basis.

2.1.2 Estimation of total catechins

Total catechins were estimated in freshly prepared tea extracts by the method of Sun *et al*., (1998). Total catechins were calculated using following equation:

Concentration = Absorbance at 500nm – (– 0.1186) / 0.003156

The concentration of total catechins (TC) was finally expressed in terms of g kg^{-1} in fresh tea shoots on dry weight basis.

2.2 Qualitative evaluation of catechin and its derivatives

Qualitative evaluations of catechin and its derivatives in tea powders were carried out using chromatographic techniques. The tea powders were subjected to size-exclusion chromatographic separation using Sephadex G-25 (Sigma, USA) as stationary phase and 50 % ethanol (AR) as mobile phase by the method of Cutler (2008) [3]. Various fractions collected were subjected to thin-layer chromatography along with standard catechins by the method of Sherma & Fried (1996) [24].

1. Size-Exclusion chromatography

Swollen Sephadex G-25 gel was degassed and packed in a glass column (49.2 x 2 cm) to a height of 42.2 cm (bed volume: 160.33 cm^3 and void volume: 53.44 cm^3). The column was equilibrated with 50% ethanol. Fresh solution prepared by dissolving 1 g tea powder into 10 mL of 50% ethanol was applied on the top of the equilibrated column. Elution at a flow rate of 0.8-1 mL per minute was done with 50 % ethanol. The eluted fractions, each of 5 mL, were collected in clean and dry test tubes. These fractions were further subjected to thin-layer chromatography and quantitative estimation of total polyphenols and total catechins by standard techniques.

2. Thin- Layer Chromatography

Thin layer chromatography was done on glass plate coated with silica gel G by the method of Stahl (1969) [28].

2.3 High performance liquid chromatography (HPLC)

Samples of tea powder were characterized for catechin profile and their quantification by HPLC techniques was done by the method of Zhu & Chen (1999) [39].

The chromatograms were monitored at 220 nm. The flow rate was 1 mL min^{-1}. The elution was done by the following gradient system:

Time duration (minutes)	ACN*	Water* (%)
0	90	10
40	86	14
50	0	100

*acidified with 0.025% orthophosphoric acid (H_3PO_4)

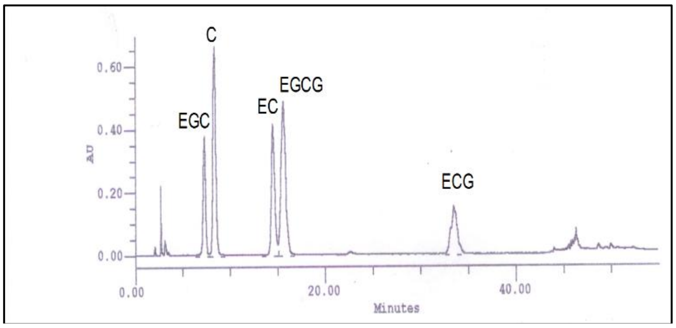

Fig 1: High performance liquid chromatogram of standards: catechin (C), epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (EGCG) and epicatechin gallate (ECG)

Figure 1 represents the HPLC profile of standard catechins: catechin (C), epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (EGCG) and epicatechin gallate (ECG). The retention times for EGC- 7.333 min, C- 8.400 min, EC- 14.533 min, EGCG-15.633 min and ECG-33.483 min (Table 1).

Table 1: HPLC profile of standard catechins monitored at 220 nm along with retention time, area and height of peak

Standard	Retention time (minutes)	Area (uV*sec)	Height (uV)
EGC	7.333	6809874	378219
C	8.400	14213958	660604
EC	14.533	10195029	414218
EGCG	15.633	16599102	486705
ECG	33.483	6958011	152399

Concentration of the standard solution injected = 0.1 mg mL^{-1}
Volume of solution injected = 20 µL

The qualitative and quantitative estimations of catechin and its derivatives in aqueous solutions of tea powders were carried out by comparing their retention time and spectrum with that of standards. The quantification was done with the help of following formula:

$$\text{Concentration of unknown} = \frac{\text{Concentration of standard}}{\text{Peak of standard}} \times \text{Peak area of unknown}$$

2.4 Evaluation of antioxidant property
Antioxidant property of Kangra tea was evaluated on the basis of free radical scavenging potential of the aqueous extracts of fresh green tea shoots and aqueous solution of tea powders using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay.

DPPH assay
The DPPH free radical scavenging activity of aqueous extracts of green tea shoots samples and aqueous solutions of tea powders was evaluated by the method of Sharma & Bhat (2009) [23].

Percent of DPPH free radical scavenging activity (% inhibition) was calculated with the help of following equation:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100 \quad \text{------- (i)}$$

3. Results and Discussion
A total of 63 samples consisting of green tea shoots (42) and tea powder (21) were analyzed for total polyphenols and total catechins during the course of present investigation. Inactivation of polyphenol oxidase in tea shoots by microwave heat treatment has been reported to yield higher levels of total phenols and total catechins compared to parching, steaming and oven heating (Gulati *et al*., 2003) [7].

Total polyphenols and total catechins
Tables 2 and 3 enlist the respective fortnightly mean values of total polyphenols (TP) and total catechins (TC), estimated in samples of green tea shoots of Kangra local, Kangra Asha and Kangra Jawala along with Critical Difference (CD) at 5% and per cent Coefficient of Variance (CV).

It was of interest to note that under similar climatic conditions, the tea shoots of Kangra Asha had higher TP content during the first flush, tea shoots of Kangra Jawala had higher TP content during summer and at the beginning rainy flush seasons whereas, Kangra Local tea shoots had higher TP content during main and winter flush seasons. Biochemical constituents of green tea shoots have been reported to be affected by the genetic aspect under different weather conditions (Yao *et al*., 2005; Obanda *et al*., 1996; Gulati *et al*., 1999) [38, 18, 6].

A perusal of Table 3 indicates that the mean values of TC content varied significantly in the range of 130.51 to 96.79 g kg^{-1} for Kangra Local; 111.18 to 82.09 g kg^{-1} for Kangra Asha and 136.56 to 99.26 g kg^{-1} for Kangra Jawala. It was of interest to note that in Kangra Asha, TC content was recorded highest during summer flush season and in Kangra Jawala, during first and summer flush seasons and in Kangra Local towards the end of rainy flush season. The factors responsible for the accumulation of TP and TC have been found to be antagonistic. This could be due to the fact that TP synthesized in tea further undergoes to metabolize TC.

Table 2: Mean values of total polyphenols (TP) in green tea shoots of Kangra Local (KL), Kangra Asha (KA) and Kangra Jawala (KJ) cultivars

Sample No.	Total polyphenols(g Kg^{-1})			
	Period	**KL**	**KA**	**KJ**
1	April 01- 14	166.68^g	245.02^a	193.98^d
2	April 15- 29	181.50^e	197.89^b	184.01^e
3	May 01- 14	185.54^e	155.58^d	233.53^a
4	May 15- 29	152.36^h	129.78^e	191.95^d
5	June 01- 14	170.86^f	118.24^e	209.24^c
6	June 15- 29	189.49^d	190.73^b	215.73^b
7	July 01- 14	147.93^h	187.06^c	211.94^c
8	July 15- 29	204.75^c	204.30^b	197.40^d
9	August 01- 14	194.61^d	171.07^c	195.45^d
10	August 15 – 29	238.25^a	161.37^d	181.06^e
11	September 01 – 14	210.58^b	209.00^b	178.49^f
12	September 15 – 29	189.74^d	176.31^c	182.13^e
13	October 01 – 14	175.18^f	176.65^c	171.47^g
14	October 15 – 29	174.64^f	172.24^c	173.46^g
Mean		184.44	178.23	194.27
CD(5%)		4.00	19.69	1.05
CV(%)		1.26	6.60	0.32

Rankings – means within each column followed by the same letter are not significantly different at $P < 0.05$.

Table 3: Mean values of total catechins (TC) in green tea shoots of Kangra Local (KL), Kangra Asha (KA) and Kangra Jawala (KJ) cultivars

Sample No.	Total catechins (g Kg^{-1})			
	Period	**KL**	**KA**	**KJ**
1	April 01- April 14	96.79^f	100.32^b	136.56^a
2	April 15- April 29	108.18^d	98.32^b	132.05^b
3	May 01- May 29	107.57^d	111.18^a	134.96^a
4	May 15- May 29	114.45^c	99.57^b	125.61^c
5	June 01- June 14	107.62^d	109.82^a	134.80^a
6	June 15- June 29	130.51^a	109.27^a	132.40^b
7	July 01- July 14	107.86^d	94.52^c	130.63^b
8	July 15- July 29	113.66^c	99.25^b	127.24^c
9	August 01- August 14	125.26^b	95.70^c	113.29^d
10	August 15 – August 29	125.61^b	97.93^c	110.99^d
11	September 01 – September 14	112.35^c	90.83^d	107.63^d
12	September 15 – September 29	125.69^b	87.09^d	109.44^d
13	October 01 – October 14	111.48^c	87.01^d	99.26^e
14	October 15 – October 29	102.34^e	82.09^e	100.73^e
Mean		104.76	97.36	121.11
CD (5%)		2.78	3.29	0.42
CV (%)		1.46	2.02	0.21

Rankings – means within each column followed by the same letter are not significantly different at $P < 0.05$.

4.2 Monthly variations in total polyphenols (TP) and total catechins (TC)

Weekly samples of dried green tea shoots of Kangra Local, Kangra Asha and Kangra Jawala were pooled to prepare representative samples for each month. These samples were also incorporated to determine catechins qualitatively and total polyphenols and total catechins quantitavely. Table 3 presents the mean monthly TP and TC contents in aqueous extracts of green tea shoots of Kangra Local, Kangra Asha and Kangra Jawala along with CD (at 5%) and per cent CV.

It was of interest to note that the samples harvested during summer flush season always had statistically the highest TP and TC contents. These observations are in agreement with the results of Return *et al*., 2010 and Yamamoto *et al*., 1997 who reported that the green teas picked in summer seasons had higher total polyphenol and catechin contents.

4.3 Tea powders

In order to evaluate Kangra tea for its antioxidant and antibacterial properties it was rather difficult to arrive at any conclusions by incorporating the aqueous extracts of samples of dried tea shoots due to lack of consistency of samples and reproducibility of empirical results.

4.3.1 Total polyphenols and total catechins

In Table 4 is given the mean monthly values of TP and TC estimated in tea powders along with CD (at 5%) and per cent CV. The TP and TC contents of tea powders of KA and KJ from tea shoots of summer flush seasons were highest whereas, tea powders obtained from tea shoots of KL during beginning of rainy flush season had highest TP and TC contents. These results corroborated earlier observations on monthly pooled samples of green tea shoots.

Table 4: Mean monthly values of TP and TC in green tea shoots of Kangra Local, Kangra Asha and Kangra Jawala

Month	Total polyphenols (g kg^{-1})			Total catechins (g kg^{-1})		
	KL	KA	KJ	KL	KA	KJ
April	141.451^{d}	189.397^{d}	198.927^{d}	71.131^{c}	99.891^{c}	110.125^{b}
May	141.969^{d}	213.362^{b}	223.036^{a}	72.697^{c}	107.009^{b}	122.339^{a}
June	175.843^{a}	230.360^{a}	190.426^{e}	98.593^{a}	120.800^{a}	113.232^{b}
July	168.027^{b}	165.147^{f}	191.250^{e}	85.244^{b}	72.792^{d}	114.533^{b}
August	145.151^{c}	176.136^{e}	214.324^{b}	77.436^{c}	64.201^{e}	117.341^{a}
September	140.895^{d}	194.102^{c}	209.534^{c}	76.651^{c}	56.180^{f}	115.556^{a}
October	135.407^{e}	109.863^{g}	184.639^{f}	73.450^{c}	50.714^{g}	87.788^{c}
Mean	149.820	182.624	201.734	79.315	81.655	111.559
CD (5%)	5.188	3.905	6.449	7.212	4.542	7.670
CV (%)	1.98	1.22	1.83	5.19	3.18	3.93

Rankings – means within each column followed by the same letter are not significantly different at $P < 0.05$.

4.4 Qualitative and quantitative evaluation of tea powders

The tea powders obtained by lyophilizing aqueous extracts of monthly pooled samples of green tea shoots of Kangra Local, Kangra Asha and Kangra Jawala were further evaluated qualitatively and quantitatively for flavan-3-ols profiles.

4.4.1 Qualitative evaluation

Figures 2, 3 and 4 represent the trends in the variations of TP and TC in terms of absorbance at 725 nm (corresponding to TP) and at 500 nm (corresponding to TC) in fractions from size exclusion chromatography in 50% ethanol from tea powders of KL, KA and KJ, respectively. A bell-shaped (normal) curve was always obtained, indicating an increasing concentration of both TP and TC up to fraction numbers 19th, 20th and 19th for KL, KA and KJ, respectively, after which there was always a steady decrease. All fractions eluted from column along with standards were further subjected to thin layer chromatography in order to visualize the presence of individual flavan-3-ol.

Thin layer chromatographs (TLC) of standard flavan-3-ols [(C), (EC), (EGC), (EGCG) and (ECG)] and fractions of tea powders from KL, KA and KJ obtained from size exclusion column chromatography are presented in Plates 1, 2, 3, 4, and 5 respectively. It is evident from a comparison of the chromatograms that the column fractions contained all the five flavan-3-ols in noticeable concentrations. The column fractions which contained significant amounts of TC were again pooled for further elucidation of the antioxidant and antibacterial potentials of Kangra teas.

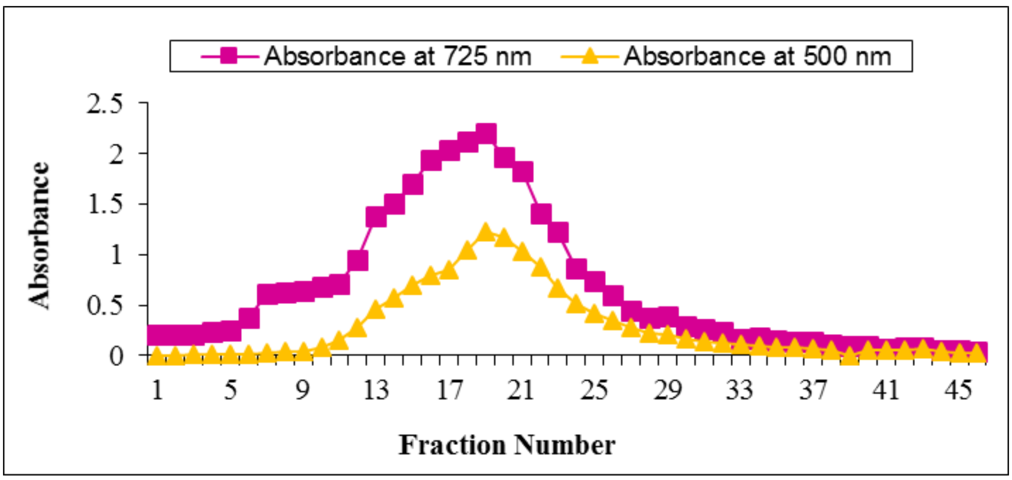

Fig 2: Total polyphenols and total catechin profile of fractions eluted with 50% ethanol from tea powder obtained by lyophilizing aqueous extract of sample of Kangra Local

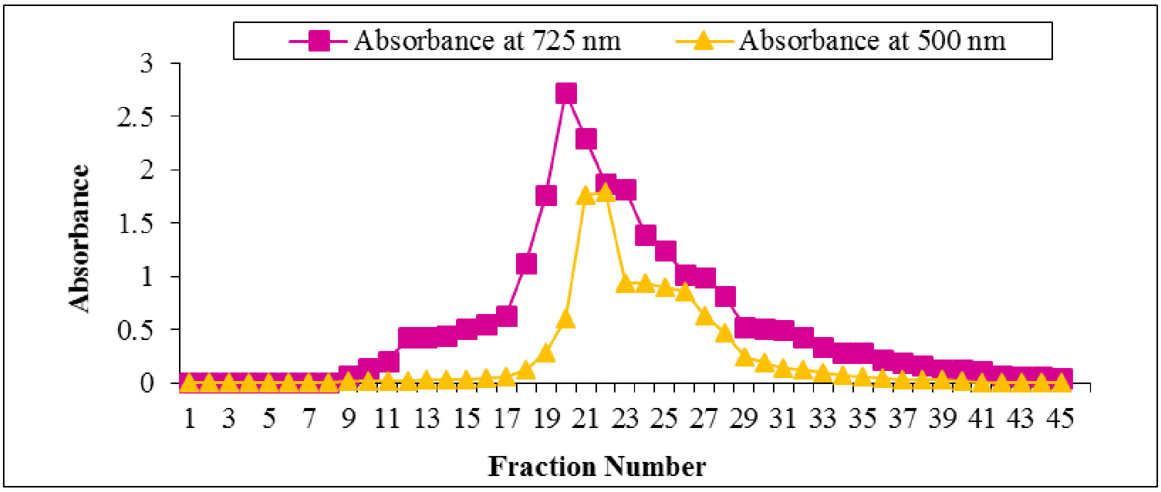

Fig 3: Total polyphenols and total catechin profile of fractions eluted with 50% ethanol from tea powder obtained by lyophilizing aqueous extract of sample of Kangra Asha

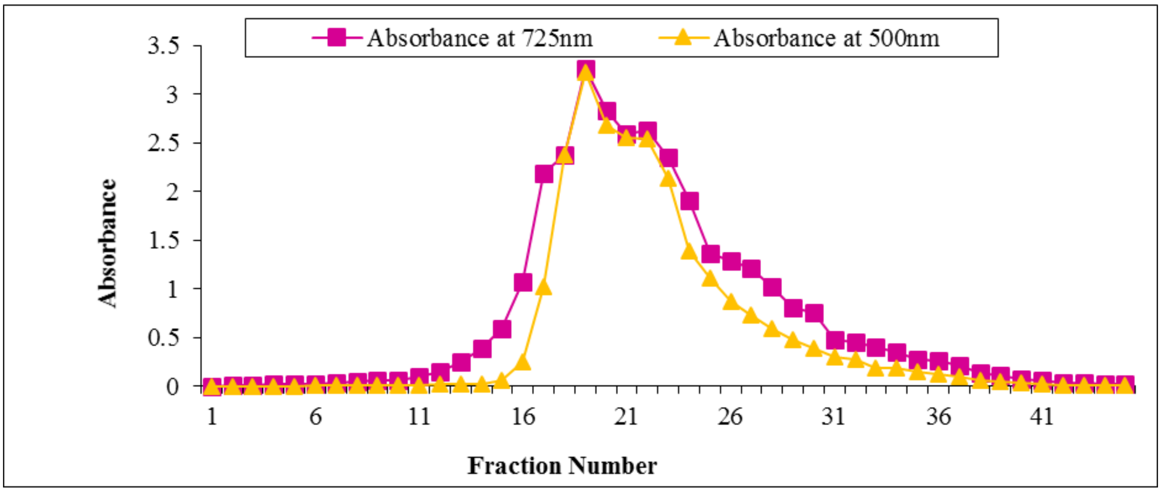

Fig 4: Total polyphenols and total catechin profile of fractions eluted with 50% ethanol from tea powder obtained by lyophilizing aqueous extract of sample of Kangra Jawala

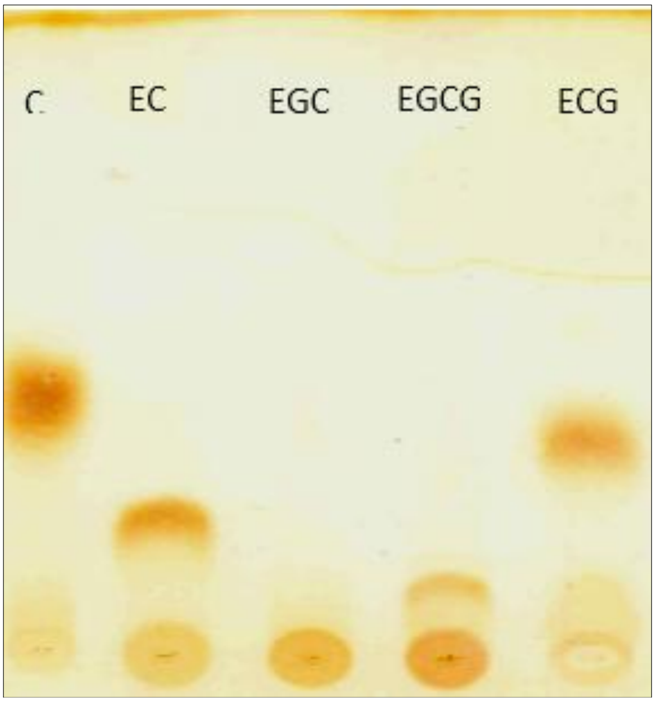

Plate 1: Thin layer chromatogram of the standard catechins: catechin (C), epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate and epicatechin gallate (ECG)

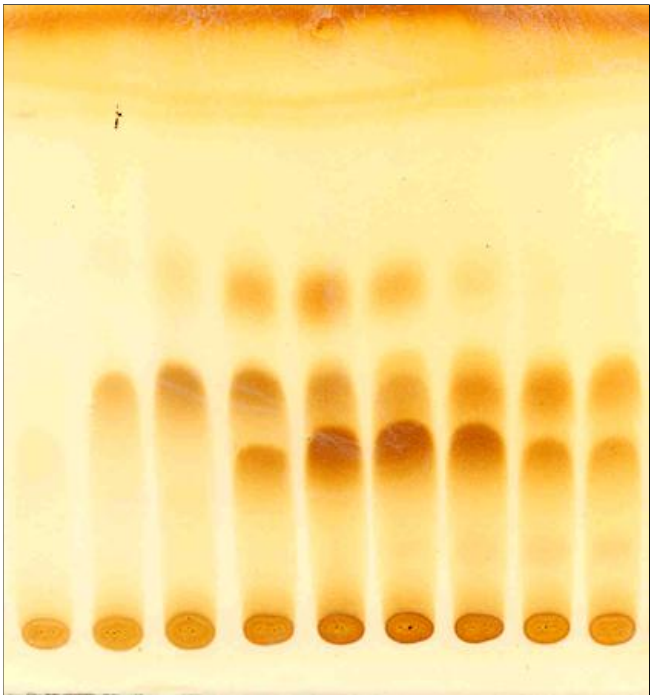

Plate 2: Thin layer chromatogram of column Fractions obtained by lyiphilizing aqueous Extracts of tea powders of Kangra (EGCG) Local

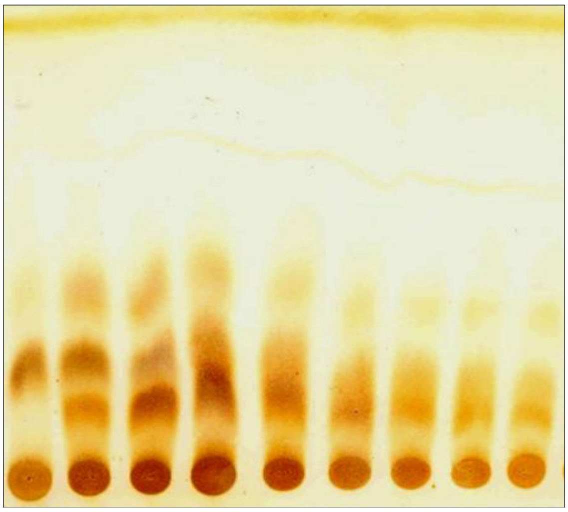

Plate 3: Thin layer chromatogram of column fraction of tea powders obtained by lyophilizing aqueous extracts of Kangra Asha

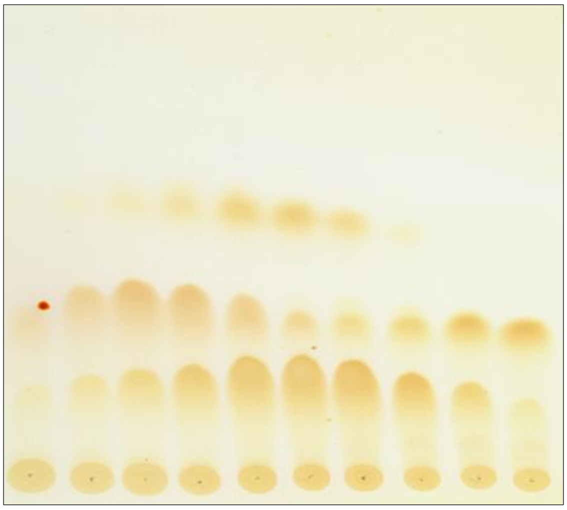

Plate 4: Thin layer chromatogram of column fraction of tea powders obtained by lyophilizing aqueous extracts of Kangra Jawala

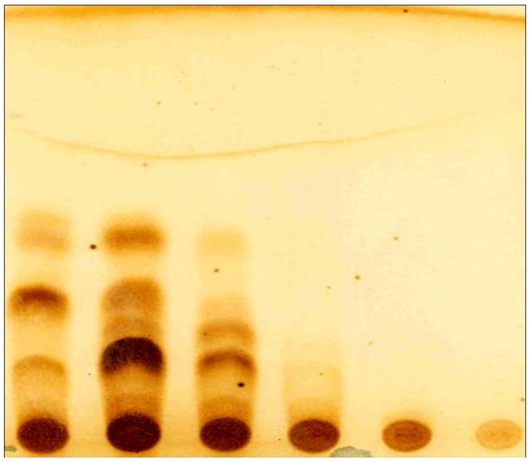

Plate 5: Thin layer chromatogram of pooled fractions of tea powders obtained by lyophilizing aqueous extracts of Kangra Tea Solvent system: Chloroform: Ethyl acetate: Acetic acid: 25:75:0.5

4.4.2 Quantitative evaluation

Tables 5, 6 and 7 depicts the values of concentrations of C, EC, EGC, EGCG and ECG estimated by high performance liquid chromatography (HPLC) in various tea powders obtained from monthly pooled samples of green tea shoots of KL, KA and KJ, respectively. The corresponding chromatograms are given in Appendix-I. Green tea has been reported to contain approximately 59% EGCG, 19% EGC, 13.6% ECG, 6.4% EC and 0.4% C of the total catechins (Cabrera *et al*., 2006) [2].

Among the local cultivars of Kangra tea, C and EC were higher in the powder from KL, EGC and ECG were higher in KJ whereas EGCG was higher in KA. These results are in corroboration with the earlier findings of Vasisht *et al*., (2004) [35] who reported that KJ contained higher amounts of catechins with galloyl moiety compared to tea shoots of KA and KL.

It was of interest to note that irrespective of the cultivars the five major flavan-3-ols always varied in order EGCG>EGC>ECG>EC>C. These observations are in accordance with the earlier results (Karori *et al*., 2007) [11].

Table 5: Mean monthly values of total polyphenols (TP) and total catechins (TC) of aqueous solutions of tea powders of Kangra Local (KL), Kangra Asha (KA) and Kangra Jawala (KJ)

Month	TP(g kg^{-1})			TC(g kg^{-1})		
	KL	KA	KJ	KL	KA	KJ
April	307.82^{f}	522.31^{c}	444.85^{d}	82.05^{c}	81.31^{b}	88.68^{c}
May	387.34^{c}	475.94^{d}	493.33^{c}	113.13^{a}	79.69^{c}	120.50^{b}
June	466.28^{a}	620.38^{a}	602.51^{a}	116.96^{a}	89.64^{a}	126.83^{a}
July	364.83^{d}	567.24^{b}	509.75^{c}	103.41^{b}	82.64^{b}	124.33^{a}
Aug	415.07^{b}	400.58^{f}	566.28^{b}	114.90^{a}	77.63^{c}	126.68^{a}
Sept	360.48^{d}	346.95^{g}	488.50^{c}	101.05^{b}	76.45^{d}	115.34^{b}
Oct	316.68^{e}	441.64^{e}	465.79^{d}	85.29^{c}	78.07^{c}	112.99^{b}
Mean	374.07	482.15	510.14	102.40	80.78	116.48
CD (5%)	9.07	11.46	19.00	8.16	1.57	1.88
CV (%)	1.39	1.36	2.13	4.55	1.11	0.92

Rankings – means within each column followed by the same letter are not significantly different at $P < 0.05$.

Table 6: Monthly variations of (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG) and (-)-epic atechingallate (ECG) in powders obtained from aqueous extracts of green tea shoots of Kangra Local

Month	C	EC	EGC	EGCG	ECG	Total
	Concentration (mg g^{-1})					
April	1.560 (1.23%)	13.338 (10.51%)	30.134 (23.75%)	62.903 (49.57%)	18.969 (14.95%)	126.904
May	2.433 (1.82%)	16.027 (12.01%)	26.515 (19.86%)	66.695 (49.96%)	21.828 (16.35%)	133.498
June	3.481 (1.73%)	21.339 (10.59%)	39.597 (19.65%)	99.306 (49.28%)	37.774 (18.75%)	201.497
July	3.661 (2.51%)	13.607 (9.34%)	34.024 (23.36%)	75.089 (51.55%)	19.283 (13.24%)	145.664
August	3.372 (1.59%)	23.705 (11.16%)	43.662 (20.56%)	102.168 (48.11%)	39.469 (18.58%)	212.376
September	3.138 (1.59%)	27.766 (14.05%)	38.933 (19.71%)	90.757 (45.94%)	36.982 (18.72%)	197.576
October	1.743 (1.25%)	15.644 (11.24%)	29.388 (21.11%)	72.245 (51.89%)	20.217 (14.52%)	139.237

Table 7: Monthly variations of (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG) and (-)-epicatechin gallate (ECG) in tea powders obtained from aqueous extracts of green tea shoots of Kangra Asha

Month	C	EC	EGC	EGCG	ECG	Total
	Concentration (mg g^{-1})					
April	1.072 (0.84%)	8.136 (6.39%)	25.047 (19.67%)	69.025 (54.22%)	24.034 (18.88%)	127.314
May	1.096 (0.81%)	8.829 (6.51%)	26.292 (19.40%)	76.662 (56.56%)	22.654 (16.71%)	135.533
June	1.339 (0.85%)	10.139 (6.42%)	28.409 (17.99%)	91.548 (57.99%)	26.443 (16.75%)	157.878
July	1.267 (0.95%)	7.741m (5.83%)	20.046 (15.10%)	86.267 (64.97%)	17.465 (13.15%)	132.786
August	1.171 (0.96%)	7.499 (6.13%)	19.569 (16.01%)	77.324 (63.20%)	16.778 (13.71%)	122.341
September	1.317 (1.05%)	8.090 (6.46%)	20.456 (16.33%)	71.397 (57.01%)	23.980 (19.15%)	125.240
October	0.232 (0.19%)	8.950 (7.52%)	28.333 (23.80%)	59.074 (49.63%)	22.676 (19.05%)	119.033

4.4. Antioxidant activity

The antioxidant potential of aqueous extracts of green tea shoots of KL, KA and KJ and aqueous solutions of tea powders was evaluated and compared with standard antioxidant vis-à-vis ascorbic acid using (DPPH) free radical assay.

Ascorbic acid has been used as a standard scavenger of DPPH free radicals in various studies (Soares *et al*., 2003; Mimica-Dukic *et al*., 2004; Sokmen *et al*., 2004; Ricci *et al*., 2005; Kano *et al*., 2005) [26, 17, 27, 20]. In the present studies, the mean IC_{50} value of (0.176 mg mL^{-1}) ascorbic acid solution is found to be 2.967µg mL^{-1} which was in agreement with the IC_{50} values reported in literature (Sharma & Bhat 2009) [23].

The mean IC_{50} values of (1 mg mL^{-1}) solutions of standard catechin and its derivatives were found to varied in the range of 1.63 to 5.82 µM. It was of interest to note that the DPPH free radical scavenging activity of flavan-3-ols decreased in the order: EGCG > ECG > EGC > EC~C > ascorbic acid. These results are in agreement with the results reported by Chen and Ho (1994), who have reported the DPPH free radical scavenging ability of tea polyphenols to vary in the order: EGCG > ECG > EGC > EC.

The mean IC_{50} values varied significantly in the range 1.060 to 0.171 µg mL^{-1} for KL, 1.220 to 0.477 µg mL^{-1} for KA, and 1.080 to 0.368 µg mL^{-1} for KJ. Hence, it was reasonable to infer that among tea catechins, the EGCG and ECG constituents might be responsible for the DPPH free radical scavenging activity. Tea extracts containing high levels of EGCG, EC, EGC, C and EC were reported to have high antioxidant activity (Karori *et al*., 2007; Su *et al*., 2007; Hu *et al*., 2009) [11, 29, 10].

Table 8: Monthly variations of (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG) and (-)-epicatechin gallate (ECG) in tea powders obtained from aqueous extracts of green tea shoots of Kangra Jawala

Month	C	EC	EGC	EGCG	ECG	Sum total
	Concentration (mg g^{-1})					
April	2.145 (1.21%)	12.681 (7.14%)	40.828 (22.99%)	86.736 (48.83%)	35.232 (19.84%)	177.622
May	2.977 (1.53%)	15.811 (8.12%)	41.664 (21.40%)	99.447 (51.08%)	34.786 (17.87%)	194.685
June	2.626 (1.27%)	16.067 (7.76%)	47.601 (22.98%)	106.256 (51.31%)	34.555 (16.68%)	207.105
July	2.129 (1.20%)	13.288 (7.50%)	36.232 (30.92%)	92.986 (52.48%)	32.563 (18.38%)	177.198
August	2.844 (1.45%)	15.268 (7.78%)	43.289 (22.05%)	90.718 (46.20%)	44.231 (22.53%)	196.350
September	3.118 (1.58%)	15.055 (7.61%)	40.701 (20.58%)	103.201 (52.19%)	35.656 (18.08%)	197.731
October	0.245 (0.13%)	14.983 (7.63%)	50.753 (25.84%)	86.135 (43.85%)	44.576 (22.69%)	196.447

Table 9: Mean IC_{50} values in terms of antioxidant activity of aqueous extracts of green tea shoots of Kangra Local (KL), Kangra Asha (KA) and Kangra Jawala (KJ) cultivars

IC_{50} (µg mL^{-1})			
Sample no.	**KL**	**KA**	**KJ**
1	1.060^a	0.503^e	0.368^h
2	0.687^c	0.530^e	0.578^f
3	0.741^b	0.426^f	0.380^h
4	0.565^d	0.506^e	0.716^d
5	0.706^b	0.477^f	0.454^g
6	0.589^d	0.479^f	0.546^f
7	0.741^b	0.627^d	0.615^e
8	0.585^d	0.528^e	0.657^e
9	0.495^e	0.617^d	0.756^d
10	0.479^e	0.610^d	0.830^c
11	0.171^g	0.769^c	0.960^b
12	0.242^f	0.814^b	0.873^c
13	0.635^c	0.836^b	1.080^a
14	0.746^b	1.220^a	0.985^b
Mean	0.603	0.639	0.700
CD (5%)	0.0175	0.148	0.0101
CV (%)	1.74	13.89	0.87

Rankings – means within each column followed by the same letter are not significantly different at $P < 0.05$.
*The IC_{50} values are based on total catechins content (µg mL^{-1}) of aqueous extracts.

Table 10: Mean IC_{50} values in terms of antioxidant activity of tea powders obtained by lyophilizing aqueous extracts of Kangra Local, Kangra Asha and Kangra Jawala

Month	IC_{50} (µg mL^{-1})		
	KL	KA	KJ
April	2.50^{a}	1.07^{d}	1.15^{a}
May	1.24^{c}	1.14^{c}	1.04^{b}
June	1.30^{c}	0.93^{e}	0.95^{c}
July	1.13^{d}	0.98^{e}	0.99^{c}
Aug	1.24^{c}	1.62^{b}	0.97^{c}
Sept	1.65^{b}	2.38^{a}	1.05^{b}
Oct	1.74^{b}	1.28^{c}	1.12^{a}
Mean	1.54	1.34	1.04
CD (5%)	0.0158	0.0161	0.0245
CV (%)	0.58	0.69	1.34

Table 11: Correlation coefficient among TP, TC, C, EC, EGC, EGCG and ECG and mean IC_{50} values of tea powders from fresh green tea shoots of Kangra Local (KL), Kangra Asha (KA) and Kangra Jawala (KJ)

	KL	KA	KJ
	IC_{50}		
TP	-0.81^{a}	-0.84^{a}	-0.92^{a}
TC	-0.89^{a}	-0.88^{a}	-0.89^{a}
C	NS	NS	NS
EC	NS	NS	NS
EGC	NS	NS	NS
EGCG	-0.79^{a}	-0.78^{a}	-0.82^{a}
ECG	NS	NS	NS

^{a} – Significant at $P < 0.05$; NS – Not significant.
TP - total polyphenols; TC - total catechins; C – catechin; EC – epicatechin; EGC- epigallocatechin; EGCG - epigallocatechin gallate and ECG - epicatechin gallate.

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