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Research Paper

Influence of phenolic compounds of Kangra tea [*Camellia sinensis* (L) O Kuntze] on bacterial pathogens and indigenous bacterial probiotics of Western Himalayas

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Abstract

Phenolic compounds of nutraceutical importance *viz.,* catechins (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epigallocatechin-3-gallate (EGCG) and (-)-epicatechin-3-gallate (ECG) were estimated in fresh green tea shoots of *Camellia sinensis* (L) O Kuntze cultivar. The total polyphenols and total catechins were in the range of 219.90 to 317.81 and 140.83 to 271.39 g/kg, respectively in monthly samples of tea. The values of C, EC, EGC, EGCG and ECG in tea powders as analyzed through high performance liquid chromatography (HPLC) were in the range of 1.560 to 3.661, 13.338 to 27.766, 26.515 to 39.597, 62.903 to 102.168 and 18.969 to 39.469 mg/g, respectively. Effect of tea extracts and standard flavanols against five pathogenic bacteria *viz., Listeria monocytogenes* (MTCC-839)*, Pseudomonas aeruginosa* (MTCC-741)*, Bacillus cereus* (MTCC-1272), *Staphylococcus aureus* (MTCC-96) and *Escherichia coli* (MTCC-443), and eleven indigenous potential bacterial probiotics belonging to genera *Enterococcus, Bacillus and Lactobacillus s*pp. obtained from fermented foods of Western Himalayas, was investigated. EGCG, ECG and EGC exhibited antibacterial activity but, C and EC did not show this activity. Tea extracts having high concentrations of EGCG and ECG were more potent in antibacterial action against bacterial pathogens. Tea extracts and standard flavan-3-ols augmented viability of potential probiotics in an order of EGCG > EGC > ECG > EC > C. Tea extracts and standard flavanols had no antibacterial activity against *Escherichia coli* (MTCC-443) but, in combination with probiotic culture supernatants, this activity was seen. The Kangra tea thus, exerts antibacterial effect on bacterial pathogens through EGCG, ECG and EGC constituents while stimulatory effect on growth of indigenous potential probiotics.

Key words: Kangra tea, Western Himalayas, probiotics, catechins, antibacterial activity.

Introduction

Green tea, produced from the leaves of the plant *Camellia sinensis*, is one of the most popular beverages consumed worldwide. It has been reported to be a rich source of dietary flavonoids in human diet (Hollman and Arts, 2000; Sharangi, 2009) and hence regarded as functional food (Wolfram, 2007). Common green tea's have five major flavonoids (flavan-3-ols) which are classified as catechins (C), (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG) and (-)-epicatechin (EC) (Cabrera *et al.*, 2006; Zaveri, 2006). These catechins have been shown to be epimerized to (-)-catechin (c), (-)-gallocatechin (GC), (-)-catechin gallate (CG), and (-)-gallocatechin gallate (GCG), after heat treatment (Ikeda *et al.*, 2005). EGCG accounts for > 50% of

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the total green tea ingredients, followed by EGC and ECG (Khokhar *et al.*, 1997). Tea and some of its polyphenolic and flavanol components have been reported to impart beneficial effects such as anticarcinogenic, antimutagenic (Cooper *et al.*, 2005; Chung *et al.*, 2003), preventing cardiovascular (Wolfram, 2007) and neurodegenerative diseases (Weinreb *et al.*, 2004), positive effects on bone metabolism (Ko *et al.*, 2009), antimicrobial (Sajilata *et al.*, 2008; Gordon and Wareham, 2010), anti-oxidant (Zhao, 2003) and hypocholesterolemic effect (Yang and Koo, 1997). (-)-Epigallocatechin gallate and (-) - epicatechin gallate in green tea have been reported to inhibit the growth of Gram-positive and Gram-negative bacteria (Gulati *et al.*, 2003; Taylor *et al.*, 2005). In addition, green tea extracts are sold at a premium price as health-promoting dietary supplements *i.e.* nutraceuticals.

Tea [*Camellia sinensis* (L) O Kuntze] is being grown on the gentle slopes of outer Western Himalayas (elevation 1290 m above mean sea level, latitude 32°27'15.68" N, longitude 76°31'42.26" E) since 1850s. Apart from tea, traditional fermented foods are regularly being consumed by the people of Western Himalayas (Kanwar *et al.*, 2011; Kanwar *et al.*, 2007) and microflora of some of these foods has already been studied (Pathania *et al.*, 2010). Eleven potential bacterial isolates obtained from these foods have been characterized and studied with respect to their probiotic attributes under *in vitro* conditions (Sourabh *et al.*, 2010). The lactic acid bacteria (LAB), generally considered as `food grade' organisms, are mostly acclaimed as the key members of probiotics and are known to be associated with various health promoting traits (Gilliland, 1990).

The interaction studies of probiotics and tea constituents of Western Himalayas are required to be carried out as both are derived from natural products of that area. With this background, the present study was aimed to explore the phytochemicals of nutraceutical significance of Kangra tea [*Camellia sinensis* (L) O Kuntze] and their interaction with indigenous potential bacterial probiotics obtained from fermented foods of Western Himalayas and standard pathogenic bacteria.

Materials and Methods

Sampling

Samples of fresh green tea shoots (two leaves and a bud) were collected from Wah Tea Estate, Rajpura, Palampur, Himachal Pradesh, India, at seven days interval during four flush seasons (first flush: April to mid May; second flush: mid May to June; rainy flush or main flush: July to mid September and winter flush: mid September to October), in the year 2009. The samples of fresh green tea shoots were always subjected to heat treatment in a microwave oven for three minutes at power 100% (P-HI) in IFB convection microwave oven (model: 30SC1, 30 L capacity, microwave 1.4 KW, frequency 2450 MHz), within twenty minutes of their plucking to inactivate polyphenol oxidase activity and finally dried in a hot air oven maintained at 45 ± 5 °C for 24 h. Inactivation of polyphenol oxidase in tea shoots was done by microwave heat treatment as it has been reported to yield higher levels of total polyphenols compared to parching, steaming and oven heating (Gulati *et al.*, 2003). The dried samples were grounded using MAC Willey Grinder (Arthur H. Thomas Type) to pass through 60 mesh sieve and finally stored in air-tight plastic containers.

Preparation of tea extracts

Dried tea sample (300 mg) was taken in a 250 mL Erlenmeyer conical flask and 100 mL of pre-boiled hot double distilled water was poured into the flask. The flask was covered with aluminum foil and kept in a water bath shaker maintained at 60 ± 5 °C for 20 min. The content of the flask was allowed to cool to room temperature and then filtered through Whatman Grade 1 filter and the final volume was made to 100 mL with double distilled water in a 100 mL volumetric flask.

Estimation of total polyphenols and total catechins

Total polyphenols: Total polyphenols were estimated in freshly prepared tea extracts (Makkar, 2003). Briefly, to 0.025 mL of freshly prepared tea extract, double-distilled water (0.975 mL), 1 N Folin-Ciocalteu's phenol reagent (0.500 mL) and 20% of sodium carbonate solution (2.500 mL) were added. The contents were mixed thoroughly by vortexing and incubated at 30 °C for 40 min. Absorbance was recorded at 725 nm with the help of Merck Spectroquant Pharo 100 spectrophotometer. The amount of total polyphenols was calculated by using standard curve prepared with tannic acid.

Total catechins: Total catechins were estimated in freshly prepared tea extracts by standard method (Sun, 1998). Standard curve was prepared by employing 50-250 µg concentrations of standard catechins.

Qualitative evaluation of flavan-3-ols

Qualitative evaluation of flavan-3-ols was carried out in monthly pooled tea powders using high performance liquid chromatography (HPLC) technique. For this, samples representing a particular month were prepared by pooling equal proportions by weight of weekly samples of dried tea shoots collected in that particular month.

Preparation of tea powders: Tea powders were prepared by lyophilizing aqueous extracts of monthly representative tea samples. For this, 7 g of tea sample was taken in a 250 mL Erlenmeyer conical flask and 100 mL of boiling hot water was added. The flask was kept at 50 ± 5 °C for 60 min on water bath shaker (RSB-12 REMI). The content of the flask was allowed to cool at room temperature; filtered through muslin cloth, centrifuged at 3000 *g* for 15 min and finally filtered through Whatman Grade 1 filter paper

to obtain absolutely clear aqueous extract. These extracts were finally dried to powder form with the help of Heto Power Dry LL 3000 freeze dryer. The powders so obtained were immediately transferred into glass tubes fitted with air-tight stoppers and were finally stored in vacuum desiccator.

High performance liquid chromatography (HPLC): Samples of tea powder were subjected to characterization and quantification of flavan-3-ols profiles by HPLC technique (Zhu and Chen, 1999). WATERS HPLC system with 510 and 515 pumps, Rheodyne injector, Novapak C-18 (4 μm, 4.6x 250 mm) column, 490E Multiwavelength detector, and Millennium 2010 data manager was employed. Mobile phase constituents used were acetonitrile (ACN), orthophosphoric acid (HPLC grade Merck, Mumbai) and double distilled water. Acetonitrile (ACN) and double distilled water were both acidified with 0.025% H_3PO_4. Standard solutions (0.5 mg/mL in 2 mL 50% methanol) of each standard flavan-3-ols [(+)-catechin, (-)-epicatechin, (-)-epigallocatechin gallate, (-)-epicatechin gallate and (-)-epigallocatechin] were also used. Sample solutions (10 mg/mL) were prepared by using 0.040 g of tea powder dissolved in 4 mL of double distilled water. An aliquot of 20 μL was used for HPLC injection. Concentration of the standard solution injected for analysis was 0.1 mg/mL. The chromatograms were then monitored at 220 nm as standard catechins (SIGMA) and their mixtures showed high resolution of peaks at this particular wavelength in the present solvent system.

Interaction of tea extracts with indigenous potential probiotic bacterial isolates and pathogenic bacteria

Bacterial cultures: Antibacterial activity of aqueous extracts of monthly pooled samples of green tea shoots was evaluated against six pathogenic bacteria *viz., Escherichia coli* (MTCC-443), *Psuedomonas aeruginosa* (MTCC-741), *Listeria monocytogenes* (MTCC-839), *Bacillus cereus* (MTCC-1272), *Staphylococcus aureus* (MTCC-96) and *Streptococcus mutans* (MTCC-890) procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India. Influence of aqueous extracts of green tea shoots was also studied on eleven well characterized potential probiotic bacteria belonging to *Enterococcus faecium* (AdF1- GU396270; AdF2- GU396271; AdF3- GU396272 and AdF11- GU396279), *Bacillus coagulans* (AdF4-GU396273), *Lactobacillus plantarum* (AdF5- GU396274; AdF6- GU396275 and AdF10- GU396278) and *Lactobacillus fermentum* (AdF7- HQ677597; AdF8-GU396276 and AdF9- GU396277) isolated from fermented foods of Western Himalayas (Sourabh *et al.*, 2010).

Agar-well diffusion method for antibacterial activity: The influence of aqueous extracts of monthly pooled samples of green tea shoots and solutions of standard flavan-3-ols, against pathogenic bacteria and probiotic bacteria was evaluated (Toda *et al.*, 1989a). Standard solutions of flavan-3-ols were prepared by dissolving 2 mg of each in 1 mL of water. For evaluating antibacterial activity, the aqueous extracts of tea samples were prepared using protocol as described above, however, with a slight modification that 10g of sample was taken for preparation of extract. The extracts/solutions were then filter sterilized and used for determination of effect on pathogenic bacteria and potential probiotic bacteria.

In another experiment, probiotic bacterial isolates were grown anaerobically in MRS broth for 20 h and then centrifuged. A hundred microlitre supernatant of each bacterium was mixed separately with 100 μL of tea extract and standard solutions of flavan-3-ols (2 mg /g in 1 mL of double-distilled water). Antibacterial activity of 100 μL of this mixture on pathogenic bacteria was evaluated by using the same protocol as described above.

Interaction of tea samples with indigenous potential probiotic bacteria: The effect of tea extracts and standard flavan-3-ols on viability of probiotic bacteria was studied by inoculating 10^6 CFU/mL of each probiotic bacteria in MRS broth tubes containing tea extract and different concentrations of standard flavan-3-ols separately. These tubes were then incubated anaerobically at 37 °C for 24 h in an anaerobic jar system (Hi-Media). After incubation, the number of surviving bacteria in terms of $\log_{10}$ CFU/mL was enumerated by standard plate technique on MRS medium. So, 1 mL of culture obtained after incubation was serially diluted in phosphate buffered saline (containing 0.20% of sodium thioglycholate to maintain anaerobic conditions), and then 100 μL sample from each dilution was immediately spread plated on MRS medium and incubated anaerobically at 37 °C for 24 h in an anaerobic jar.

Statistical analysis

Statistical analysis of the data was carried out by using WindoStat software version 8.0. The data were subjected to one way Analysis of Variance (ANOVA) at the significance level of 5%. The comparison of mean values was then done with Duncan's Multiple Range test by using the same software.

Results

The contents of total polyphenols and catechins in monthly pooled samples of tea are given in Table 1. The lowest content of total polyphenols (219.90 g/kg) was noticed in the month of October and the highest (317.81 g/kg) in the month of August and in case of total catechins, the lowest content was noticed in the month of April (140.83 g/kg) and highest in the month of August (271.4 g/kg). The qualitative and quantitative estimation of catechins and their derivatives in aqueous solutions of tea powders were also carried out by HPLC analysis. The retention times for five standard flavan-3-ols *viz.,* EGC, C, EC, EGCG and ECG were 6.78, 8.58, 14.52, 15.80 and

Table 1 - Total polyphenols, total catechins and five flavan-3-ols contents in monthly samples of fresh green tea.

Tea samples	Monthly mean total polyphenols$^{\alpha}$ (g/kg)	Monthly mean total catechins$^{\alpha}$ (g/kg)	Content (mg/g) of five flavan-3-ols in the solutions of tea powders as quantified by HPLC				
			Catechin (C)	Epicatechin (EC)	Epigallocatechin (EGC)	Epigallocatechin gallate (EGCG)	Epicatechin gallate (ECG)
April	227.63^{d}	140.83^{f}	1.56	13.34	30.13	62.90	18.97
May	252.75^{c}	197.63^{d}	2.43	16.03	26.52	66.70	21.83
June	266.28^{c}	226.02^{c}	3.48	21.34	39.60	99.31	37.77
July	283.03^{b}	249.69^{b}	3.66	13.61	34.02	75.09	19.28
August	317.81^{a}	271.40^{a}	3.37	23.71	43.66	102.17	39.47
September	288.80^{b}	251.04^{b}	3.14	27.77	38.93	90.76	36.98
October	219.90^{d}	185.19^{e}	1.74	15.64	29.39	72.25	20.22

Results are shown as mean of three replications. Mean values within the same column followed by same superscript letters do not differ significantly when compared by Duncan's Multiple Range Test. CD (5%) for monthly mean total polyphenols = 14.12. CD (5%) for monthly mean total catechins = 10.75. $^{\alpha}$Estimated in freshly prepared extracts of fresh green tea shoots.

35.80 min, respectively. The content of flavan-3-ols *viz.,* C, EC, EGC, EGCG and ECG on monthly basis in these tea powders was in the range of 1.56 to 3.66, 13.34 to 27.77, 26.52 to 43.66, 62.90 to 102.17 and 18.97 to 39.47 mg/g, respectively (Table 1).

Before evaluating the tea samples for antibacterial activity, the individual standard catechins *i.e.* C, EC, EGC, EGCG and ECG were tested for their antibacterial effect on the selected pathogens. Except catechin and epicatechin, all other standard flavanols showed antibacterial activity against all tested pathogens except *E. coli*. Minimum inhibitory concentrations (MIC) of EGC, EGCG and ECG for *L. monocytogenes* were 550, 275 and 400 µg/mL and for *P. aeruginosa* were 750, 375 and 500 µg/mL, respectively. The minimum inhibitory concentrations (MIC) of EGC, EGCG and ECG for *B. cereus* were 375, 50 and 250 µg/mL, respectively and for *S. aureus* and *S. mutans,* these respective MIC values were 500, 250 and 375 µg/mL. Except E and EC, all other standard flavanols *viz.,* EGC, EGC and EGCG exhibited antibacterial activity. Standard flavanol solutions showed no antibacterial activity against all the 11 potential probiotic bacteria.

The results of the antibacterial activity of aqueous extracts of tea samples against selected bacterial pathogens are depicted in Tables 2. Among selected pathogenic bacteria, *Bacillus cereus* was the most sensitive and *P. aeruginosa* was the least sensitive as compared to other bacterial pathogens. *Escherichia coli* was resistant to all the samples of fresh green tea shoots and standard flavanol solutions. Green tea extracts showed antibacterial activity against pathogenic bacteria but, none of potential probiotic bacteria was affected by these extracts.

Screening for antibacterial activity of combination of indigenous bacterial probiotic and tea extracts (1:1) by well diffusion assay against selected bacterial pathogens was also studied (data not shown). The inhibition noticed at lower dose of green tea extracts *i.e.* 50 µL in combination with probiotic supernatant extracts (50 µL) was comparable to the inhibition exhibited by 100 µL of green tea extracts alone. Tea extracts and standard flavanols showed no antibacterial activity against *Escherichia coli* (MTCC-443), however, in combination with probiotic culture supernatants, antibacterial activity was recorded with both tea extracts and standard flavanols.

The effect of tea extracts and standard flavan-3-ols on the viability of probiotic bacteria was also determined. Interestingly, it was found that in the presence of all the standard flavan-3-ols and tea extracts, the viable count of probiotic bacteria increased significantly as compared to control (Table 3). The general trend of increase in viability of probiotic isolates exhibited by standard flavan-3-ols was EGCG > EGC > ECG > EC > C.

Discussion

Fresh tea leaves are rich in flavanols - a group of phenolic compounds known as catechins. These catechins are the major class of biologically active ingredients of green tea which account for about 10% of the dry weight of tea (Balentine *et al.*, 1997). Local Kangra tea was evaluated for the presence of these nutraceutical compounds and their influence on indigenous potential bacterial probiotics of Western Himalayas and bacterial pathogens. Considerable variation in the total polyphenols and catechins was seen in monthly tea samples. Information available in the literature suggests that geographical origin, soil composition, differences in the composition of leaves, time of harvesting, postharvest treatments, and physical structure of the different leaves probably influence the composition of tea (Freidman, 2007). The occurrence of five major flavan-3-ols content in tea samples as determined through HPLC analysis was in the order of EGCG > EGC > ECG > EC > C which corroborate with the observations of other workers reported for tea samples of different regions (Bronner and Beecher, 1998; Karori *et al.*, 2007).

Beside catechin and epicatechin, all standard flavanols showed antibacterial activity against tested bacterial

Table 2 - Antibacterial activity of aqueous extracts of monthly samples of fresh green tea shoots against selected bacterial pathogens.

Extracts of monthly tea samples/ standard flavanols[α]	Antibacterial activity (zone of inhibition[β])				
	L. monocytogenes-MTCC 839	*P. aeruginosa*-MTCC 741	*S. aureus*-MTCC 96	*B. cereus*-MTCC 1272	*E. coli*-MTCC 443
April	6	4	8	6	NZ
May	7	3	9	7	NZ
June	9	4	12	10	NZ
July	8	5	8	6	NZ
August	10	7	14	9	NZ
September	9	4	8	7	NZ
October	4	3	6	5	NZ
EGC	14	10	14	16	-
ECG	15	11	16	17	-
EGCG	16	12	18	20	-

Results are expressed as mean of three values [α] Standard catechin (C) and epicatechin (EC) showed no inhibition zone against any of the selected pathogenic bacteria; [β] Zone of inhibition is expressed in terms of millimeter (mm). EGCG, ECG and EGC are standard flavanols. NZ, No inhibition zone.

Table 3 - Effect of green tea extracts and standard flavan-3-ols on viability (log_{10} CFU/mL) of indigenous potential probiotic bacteria.

MRS broth+extracts of monthly tea samples/ standard flavanols	log cfu/mL of potential probiotic bacteria											
	AdF1	AdF2	AdF3	AdF4	AdF5	AdF6	AdF7	AdF8	AdF9	AdF10	AdF11	MTCC 3041
Control[Y]	7.74	7.66	7.87	7.80	7.55	7.96	7.53	7.83	7.46	7.66	7.85	7.16
April	7.82 ^{f}	7.73 ^{h}	8.11 ^{d}	7.97 ^{i}	7.62 ^{e}	8.22 ^{g}	7.68 ^{f}	8.01 ^{fg}	7.59 ^{de}	7.81 $^{d e}$	8.06 ^{f}	7.21 ^{de}
May	7.86 ^{e}	7.76 $^{f g}$	8.16 ^{cd}	7.98 $^{h i}$	7.64 ^{e}	8.31 ^{f}	7.63 ^{g}	8.08 ^{ef}	7.62 ^{d}	7.83 $^{c d}$	8.10 ^{f}	7.22 ^{de}
June	7.95 ^{cd}	7.78 $^{d e}$	8.20 ^{c}	8.03 $^{g h}$	7.65 ^{e}	8.45 ^{c}	7.69 ^{f}	8.08 ^{ef}	7.65 ^{c}	7.87 ^{c}	8.18 ^{de}	7.23 ^{de}
July	7.96 ^{bcd}	7.80 $^{c d}$	8.20 ^{c}	8.07 $^{e f}$	7.74 ^{d}	8.39 $^{d e}$	7.70 ^{f}	8.12 ^{de}	7.67 ^{c}	7.81 $^{d e}$	8.18 ^{de}	7.28 ^{cd}
August	8.12 ^{a}	7.95 ^{b}	8.36 ^{b}	8.23 ^{c}	7.73 ^{d}	8.54 ^{b}	7.88 ^{c}	8.28 ^{b}	7.75 ^{a}	7.96 ^{b}	8.34 ^{c}	7.41 ^{b}
September	7.98 ^{bc}	7.80 ^{c}	8.22 ^{e}	8.09 ^{e}	7.79 ^{c}	8.40 $^{c d}$	7.82 $^{d e}$	8.14 ^{cde}	7.71 ^{b}	7.83 ^{d}	8.20 ^{d}	7.31 ^{c}
October	7.85 ^{e}	7.75 $^{g h}$	8.11 ^{d}	7.95 ^{j}	7.64 ^{e}	8.23 ^{g}	7.69 ^{f}	7.99 ^{g}	7.60 ^{de}	7.82 $^{c def}$	8.07 ^{f}	7.21 ^{de}
C	7.86 ^{e}	7.74 $^{g h}$	8.08 ^{d}	7.94 ^{f}	7.74 ^{d}	8.14 ^{h}	7.78 ^{e}	8.01 ^{eg}	7.67 ^{c}	7.75 ^{f}	7.97 ^{g}	7.23 ^{d}
EC	7.92 ^{d}	7.77 $^{e f}$	8.19 ^{c}	8.05 ^{g}	7.73 ^{d}	8.23 ^{g}	7.85 $^{c d}$	8.15 ^{cd}	7.75 ^{a}	7.78 ^{ef}	8.05 ^{f}	7.25 ^{cd}
EGCG	8.15 ^{a}	8.16 ^{a}	8.59 ^{a}	8.43 ^{a}	8.46 ^{a}	8.73 ^{a}	8.28 ^{a}	8.50 ^{a}	7.76 ^{a}	8.16 ^{a}	8.55 ^{b}	7.52 ^{a}
EGC	8.13 ^{a}	7.94 ^{b}	8.38 ^{b}	8.28 ^{b}	8.13 ^{b}	8.52 ^{b}	7.97 ^{b}	8.30 ^{b}	7.67 ^{c}	7.99 ^{b}	8.37 ^{a}	7.47 ^{ab}
ECG	8.01 ^{b}	7.82 ^{c}	8.21 ^{c}	8.18 ^{d}	7.80 ^{c}	8.35 $^{e f}$	7.87 ^{c}	8.21 ^{c}	7.65 ^{c}	7.84 $^{c d}$	8.17 ^{e}	7.27 ^{cd}

[Y]MRS broth containing no added tea extract or standard flavanol but inoculated with different probiotic bacteria. Results are shown as mean of three replications. Mean values of bacterial viability within the same column followed by different superscript letters differ significantly from control when compared by least significant difference (LSD) comparison using one way Analysis of Variance (ANOVA) at the significance level of 5%. C, EC, EGCG, ECG and EGC are standard flavanols.

pathogens except *E. coli*. Among all the selected pathogens, *B. cereus* showed highest sensitivity and *P. aeruginosa* showed least sensitivity to catechin derivatives and aqueous extracts of tea samples. It has been reported by earlier workers that Gram-positive pathogenic bacteria are more susceptible to tea catechins as compared to the Gram-negative bacteria (Friedman *et al.*, 2006; Toda *et al.*, 1989b; Toda *et al.*, 1990) probably due to differences in cell wall composition. The various catechin derivatives probably affect the lipid bilayer of cell membrane and finally result in loss of cell structure (Cox *et al.*, 2001). In the present study, standard flavanols with galloyl moiety (EGC, ECG and EGCG) exhibited antibacterial activity whereas those without galloyl moiety (C and EC) did not show this activity. Presence of galloyl group in standard flavanols has been suggested to increase its antibacterial activity (Taguri *et al.*, 2004). This fact is further strengthened by exhibition of high antibacterial activity of tea samples collected in the month of June, July, August and September where galloyl moiety containing flavanols were in higher amount.

One interesting observation that came from this study was that none of the standard flavanol solutions and green tea extracts showed any antibacterial activity against indigenous potential probiotic bacteria as noticed against pathogenic bacteria. Rather in the presence of tea samples and standard flavanols, a significant increase in viability of probiotic bacteria was observed. The general trend of increase in viability of probiotic isolates exhibited by standard flavan-3-ols was EGCG > EGC > ECG > EC > C. From these results it is evident that tea extracts and its flavan-3-ols constituents have positive effect on the growth of indigenous potential probiotic bacteria. Further studies are required to precisely understand the reasons underlying the stimulatory effect on viability of potential probiotic bacteria. In contrary to the present findings, green tea extracts have been reported to exhibit antibacterial activity against probiotic bacteria as well (Su *et al.*, 2008).

Combination of lower dose (50 μL) of green tea extracts with probiotic supernatant (50 μL) exhibited comparable inhibitory activity on pathogenic bacteria, as exhibited by double dose (100 μL) of green tea extracts. Also, tea extracts and standard flavanols which showed no antibacterial activity against *E. coli* (MTCC-443), were found to be effective against this pathogen, in combination with probiotic culture supernatants. On similar lines, more pronounced antibacterial effect of combination of green tea extract and supernatant of probiotic bacteria on *Staphylococcus aureus* and *Streptococcus pyogenes* has been reported by other workers (Su *et al.*, 2008). To our knowledge, the antibacterial potential of tea extracts in combination with standard antibiotics on pathogens has been well studied (Tiwari *et al.*, 2005; Shimamura *et al.*, 2007) whereas, same effect in combination with probiotics is very poorly investigated.

From the present results it can be concluded that the antibacterial potential of aqueous extracts of green tea shoots is mainly due to EGCG, ECG and EGC contents. Samples having high EGCG content exhibit higher antibacterial activity against pathogenic bacteria. The tested Kangra green tea extracts have shown stimulatory effect on viability of indigenous probiotic bacteria. The combinations of probiotic bacteria and tea extracts are more effective in exhibiting antibacterial activity. It seems that green tea extracts if taken along with probiotics, may have some positive effect on these bacteria and a negative effect on pathogenic bacteria. Therefore, both can be exploited as components of functional foods to augment their therapeutic value but, with the support of proper *in vivo* investigations.

References

Balentine DA, Wiseman SA, Bouwens LC (1997) The chemistry of tea flavonoids. Cri Rev Food Sci Nutr 37:693-704.

Bronner WE, Beecher GR (1998) Method for determining the content of catechins in tea infusions by high performance liquid chromatography. J Chromatogr A 805:137-142.

Cabrera C, Artacho R, Gimenez R (2006) Beneficial effects of green tea - A review. J Am Coll Nutr 25:79-99.

Chung FL, Schwartz J, Herzog CR, Yang YM (2003) Tea and cancer prevention: Studies in animals and humans. J Nutr 133:3268-3274.

Cooper R, Morre DJ, Morre DM (2005) Medicinal benefits of green tea: part II. review of anticancer properties. J Alt Compl Med 11:639-652.

Cox SD, Mann CM, Markham JL (2001) Interactions between components of the essential oil of Melaleuca alternifolia. J Appl Microbiol 91:492-497.

Freidman M (2007) Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. Mol Nutr Food Res 51:116-134.

Friedman M, Henika PR, Levin CE, Mandrell RE, Kozukue N (2006) Antimicrobial activities of tea catechins and theaflavins and tea extracts against Bacillus cereus. J Food Protect 69:354-361.

Gilliland SE (1990) Health and nutritional benefits from lactic acid bacteria. FEMS Microbiol Rev 87:175-188.

Gordon NC, Wareham DW (2010) Antimicrobial activity of the green tea polyphenol (-)-epigallocatechin-3-gallate (EGCG) against clinical isolates of *Stenotrophomonas maltophilia*. Int J Antimicrob Agents 36:129-131.

Gulati A, Rawat R, Singh B, Ravidranath SD (2003) Application of microwave energy in the manufacture of enhanced-quality green tea. J Agri Food Chem 51:4764-4768.

Hamilton-Miller JMT (1995) Antimicrobial properties of tea (Camellia sinensis L.). Antimicrob Agents Chemother 39:2375-2377.

Hollman PCH, Arts ICW (2000) Flavonols, flavones and flavanols - nature, occurrence and dietary burden. J Sci Food Agric 80:1081-1093.

Ikeda I, Tsuda K, Suzuki Y, Kobayashi M, Unno T, Tomoyori H, Goto H, Kawata Y, Imaizumi K, Nozawa A, Kakuda T (2005) Tea catechins with a galloyl moiety suppress post-

prandial hypertriacylglycerolemia by delaying lymphatic transport of dietary fat in rats. J Nutr 135:155-159.

Kanwar SS, Gupta MK, Katoch C, Kanwar P (2011) Cereal based traditional alcoholic beverages of Lahaul and Spiti area of Himachal Pradesh. Indian J Trad Knowl 10:251-257.

Kanwar SS, Gupta MK, Katoch C, Kumar R, Kanwar P (2007) Traditional fermented foods of Lahaul and Spiti area of Himachal Pradesh. Indian J Trad Knowl 6:42-45.

Karori SM, Wachira FN, Wanyoko JK, Ngure RM (2007) Antioxidant capacity of different types of tea products. Afr J Biotechnol 6:2287-2296.

Khokhar S, Venema D, Hollman PC, Dekker M, Jongen W (1997) A RP-HPLC method for the determination of tea catechins. Cancer Lett 114:171-172.

Ko CH, Lau KM, Choy WY, Leung PC (2009) Effects of tea catechins, epigallocatechin, gallocatechin, and gallocatechin gallate, on bone metabolism. J Agric Food Chem 57:7293-7297.

Makkar HPS, (2003) Quantification of tannins in tree and shrub foliage- A Laboratory manual. Kluwer Academic Publishers, Sordrecht, Netherlands, pp 43-54.

Pathania N, Kanwar SS, Jhang T, Koundal KR, Sharma TR (2010) Application of different molecular techniques for deciphering genetic diversity among yeast isolates of traditional fermented food products of Western Himalayas. World J Microbiol Biotechnol 26:1539-1547.

Sajilata MG, Bajaj PR, Singhal RS (2008) Tea polyphenols as nutraceuticals. Comp Rev Food Sci Food Safety 7:229-254.

Sharangi AB (2009) Medicinal and therapeutic potentialities of tea (Camellia sinensis L.) - A review. Food Res Int 42:529-535.

Shimamura T, Zhao WH, Hu ZQ (2007) Mechanism of action and potential for use of tea catechin as an anti-infective agent. Anti-Infect Agents Med Chem 6:57-62.

Sourabh A, Kanwar SS, Sharma PN (2010) Diversity of bacterial probiotics in traditional fermented foods of Western Himalayas. Int J Probio Prebio 5:193-202.

Su P, Henriksson A, Nilsson C, Mitchell H (2008) Synergistic effect of green tea extract and probiotics on the pathogenic bacteria, *Staphylococcus aureus* and *Streptococcus pyogenes*. World J Microbiol Biotechnol 24:1837-1842.

Sun B, Ricardo-da silva JM, Spranger I (1998) Critical factors of vanillin assay for catechins and proanthocyanidins. J Agric Food Chem 46:4267-4274.

Taguri T, Tanaka T, Kouno I (2004) Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. Biol Pharm Bull 27:1965-1969.

Taylor PW, Hamilton-Miller JMT, Stapleton PD (2005) Antimicrobial properties of green tea catechins. Food Sci Technol Bull 2:71-81.

Tiwari RP, Bharti SK, Kaur HD, Dikshit RP, Hoondal GS (2005) Synergistic antimicrobial activity of tea & antibiotics. Indian J Med Res 122:80-84.

Toda M, Okubo S, Hiyoshi R, Shimamura T (1989a) Antibacterial and bactericidal activities of Japanese green tea. Jap J Bacteriol 44:669-672.

Toda M, Okubo S, Hiyoshi R, Shimamura T (1989b) The bactericidal activity of tea and coffee. Lett Appl Microbiol 8:123-125.

Toda M, Okubo S, Ikigai H, Shimamura T (1990) Antibacterial and anti-hemolysin activities of tea catechins and their structural relatives. Jap J Bacteriol 45:561-566.

Weinreb O, Mandel S, Amit T, Youdim MB (2004) Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. J Nutr Biochem 15:506-516.

Wolfram S (2007) Effects of green tea and EGCG on cardiovascular and metabolic health. J Am Coll Nutr 26:373S-388S.

Wu CD, Wei, GX (2002) Tea as a functional food for oral health. Nutrition18:443-444.

Yang TTC, Koo MWL (1997) Hypocholesterolemic effects of Chinese tea. Pharmacology Res 35:505-12.

Zaveri NT (2006) Green tea and its polyphenolic catechins: Medicinal uses in cancer and non cancer applications. Life Sci 78:2073-2080.

Zhao B (2003) Antioxidant effects of green tea polyphenols. Chin Sci Bull 48:315- 319.

Zhu QH, Chen ZY (1999) Isolation and analysis of green tea polyphenols by HPLC. Anal Lab 18:70-72.