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Comparative analysis of antibacterial activity of leaf aqueous extracts of Kangra tea [*Camellia sinensis* (L) O Kuntze] on pathogens

Richa Thakur and Rajni Devi

Abstract
Extracts of leaves from the tea plant *Camellia sinensis* contain polyphenolic components with activity against a wide spectrum of microbes. Studies conducted over the last 20 years have shown that the green tea polyphenolic catechins, in particular (−)-epigallocatechin gallate (EGCg) and (−)-epicatechin gallate (ECg), can inhibit the growth of a wide range of Gram-positive and Gram-negative bacterial species with moderate potency. Evidence is emerging that these molecules may be useful in the control of common oral infections, such as dental caries and periodontal disease. Though catechins have been found in other plants derivatives such as grapes, pomegranates, those found in tea have proven to be the most effective antioxidants known. The catechins epigallocatechin gallate (EGCG) is found in the greatest concentration and most studied for its health benefits. In the present study, the aqueous extracts of green tea shoots and tea powder solutions which had higher concentrations of EGCG and ECG were potent growth inhibitors of selected bacterial pathogens: *L. monocytogenes* (MTCC-839), *P. auroginosa* (MTCC-741), *B. cereus* (MTCC-1272), *S. aureus* (MTCC-96) and *E. coli* (MTCC-443). Teas of summer and rainy flush seasons exhibited superior biological activity compared to first and winter flush.

Keywords: Green tea, polyphonic catechins, antibacterial activity

1. Introduction
The discovery of tea and origin of tea drinking are generally ascribed to China where its history dates back to 2737 B.C. (Ukers 1935) [23]. Since its discovery tea drinking has always been claimed to be beneficial to human health. In the account of the "Herbal Canon of Shen Nong", the Chinese Emperor claimed that tea was able to detoxify 72 kinds of poisons 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) [15].

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 (Figure 1.1) viz. (-) - epicatechin (EC), (-) - epigallocatechin (EGC), (-) - epicatechingallate (ECG), (-) - epigallocatechingallate (EGCG), (-) - gallocatechins (GC) and (-) - gallocatechingallate (GCG) (Sanderson 1972; Millin *et al*.1969) [17, 12] (Figure 1.2). 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]. Epidemiological studies carried out during the last three decades suggested that green tea catechins have nutraceutical and therapeutic properties against cancer in humans (Vanessa and Williamson 2004) [24]. Tea catechins have also been reported to possess antiallergic (Yamamoto *et al.* 2004) [26], antimicrobial (Paola *et al*. 2005) [14] and antioxidant activities (Karori *et al*. 2007) [10]. Tea polyphenols have also been reported to possess antimicrobial property against some bacteria responsible for causing food-borne diseases in addition to their inhibitory effects on exotoxins (Ikigai *et al*. 1990) [7].

Tea cultivating regions and processing units in Himachal Pradesh (Latitude 30º 22' 40" N to 33º 12' 20" N, Longitude 75º 45' 55" E to 79º 04' 20" E and altitude ranging between 350 meters to 6,975 meters above mean sea level), India are located around Palampur (32º 06' 23.44" N 76º 32' 36.53" E), Dharamshala (32º 13' 14.38" N 76º 19' 07.82" E) of district Kangra and Jogindernagar (31º 59' 24.96" N 76º 47' 25.77" N) of district Mandi between an

altitude of 800 to 1600 meters above mean sea level. Kangra valley became synonymous to tea, popularly known as Kangra Tea or Kangra Valley Tea towards the end of nineteenth century.

1.1 Antibacterial property

Tea polyphenols have been reported to be the most potent nutraceutical phytochemicals (Dufresne and Farnworth, 2001) [4]. The results of the studies conducted over last 20 years have exhibited the potential of different types of teas in the inhibition of growth of a wide range of microorganisms (Toda *et al*. 1989, Sakanaka *et al*. 2000; Mbata *et al*. 2008) [20, 16, 11]. Tea catechins have been reported to exhibit activity against phytopathogens: *Erwinia*spp. and *Pseudomonas* spp., pathogens: *Staphylococcus*, *Salmonella*, *Shigella*, *Vibrio*, *Helicobacter pylori*,*Clostridium* and food-borne bacteria: *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Serratiamarcescens*, *Streptococcus mutans*and *Bacillus cereus* (Ahn *et al*. 1991; Yam *et al.* 1997; Friedman 2007) [1, 25, 5]. There is a good evidence that the catechin components of green tea are responsible for the observed antibacterial activity (Hara 2001) [6]. Nakayama *et al.* (1993) [13] and Song *et al*. (2005) [18] have also reported that the tea catechins inhibit influenza virus through virucidal effect.

Hence, the present study was conducted to investigate the nutraceutical attributes of Kangra tea [*Camellia sinensis* (L) O Kuntze] with the objective to determine the antimicrobial activity of Kangra tea.

2. Material & method

2.1 Sampling

Samples of green tea shoots (two leaves and a bud) representing various flush seasons incorporated in the present studies were procured from the depository in the Department of Chemistry and Biochemistry, College of Basic Sciences, CSKHPKV, Palampur (Table 1). The dried samples were ground using MAC Willey Grinder (Arthur H. Thomas Type) to pass through 20 mesh sieve and finally stored in air–tight plastic containers. The containers were opened only at the time of analysis.

2.2 Moisture Content

The stored samples were always dried completely prior to aqueous extractions for analytical estimations. Sample (1 g) of dried green tea shoots stored in air-tight container was taken in a pre-weighed Petri dish and placed in a hot air oven maintained at 60±5 ^{0}C. The Petri dish was taken out in a dessicator after regular intervals of 2 hours till a constant weight is reached. The difference between initial and final weight was taken as the moisture content of the sample.

2.3 Extraction

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 minutes. The contents of the flask were allowed to cool to room temperature and then filtered through Whatman Grade 1 filter paper in a 100 mL measuring flask. The final volume was made with the help of double distilled water.

2.4 Antibacterial activity

Growth inhibitory activity of column pooled fractions of tea powders and lyophilized powders were examined against five selected pathogenic bacteria i.e. *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 741), *Listeria monocytogenes* (MTCC 839), *Bacilluscereus* (MTCC 1272) and *Staphylococcus aureus* (MTCC 96) procured from the Institute of Microbial Technology (IMTECH), Chandigarh. The bacterial cultures were maintained on freshly prepared nutrient agar (NA) medium and were stored at 4 °C for further use.

2.5 Antibiotic sensitivity

Antibiotic sensitivity of five bacterial pathogens was tested against 5 standard antibiotics (Amoxycillin-AM 30 mcg/disc, Norfloxacin-Nx 10 mcg/disc, Streptomycin-S 300 mcg/disc, Tetracycline-T 30 mcg/disc, Penicillin-P 10 mcg/disc) using paper disc method and the zone of clearance around the disc was measured.

2.6 Agar-well diffusion method for antibacterial activity

Antibacterial activity of aqueous solutions of tea powders was evaluated by the methodof Toda *et al.* (1989a) [21]. The antibacterial activity was always tested in freshly prepared solutions. Minimum Inhibitory concentrations (the lowest concentration of the extract that did not permit turbidity or growth of the test organism) of the standard catechins and aqueous solutions of tea powders were determined by addition of increased concentration of solutions against pathogenic bacteria.

2.7 Statistical analysis

All statistical estimations were carried out in triplicate to minimize the experimental error. The data were pooled and analyzed statistically. The analysis was carried out using ANOVA (Analysis of variance) followed by analysis of least significant difference ($p \leq 0.05$).

3. Results & discussion

In order to evaluate Kangra tea for its biological attributes, antibacterial potential of aqueous solutions of tea powders obtained by lyophilizing aqueous extracts of green tea shoots of Kangra Local, Kangra Asha and Kangra Jawala, were studied against five standard bacterial pathogens i.e. *Pseudomonas aeruginosa* (MTCC-741), *Listeria monocytogenes* (MTCC-839), *Escherichia coli* (MTCC-443), *Bacillus cereus* (MTCC-1272) and *Staphylococcus aureus* (MTCC-96). Antibacterial activity was also evaluated in column pooled fractions.

3.1 Sensitivity pattern of bacterial pathogens for standard antibiotics

All the five selected bacterial pathogensviz.*P. aeruginosa*, *L. monocytogenes*, *E. coli*, *B. cereus* and *S. aureus*were first tested for their inherent resistance against five broadly used antibiotics (Am - Amoxycillin; S – Streptomycin; T - Tetracyclin; Nx- Norfloxacin; P - Penicillin G). Table 2 depicts the sensitivity pattern of selected bacterial pathogens against different antibiotics (Plate 1). It was observed that *L. monocytogenes*and *S. aureus* were relatively more sensitive towards these antibiotics as compared to *B. cereus*, *P. aeruginosa* and *E. coli*.

3.2 Sensitivity of selected bacterial pathogens for standard catechins

Before evaluating the tea samples for antibacterial activity, the individual catechins i.e. C, EC, EGC, EGCG and ECG were tested for their antibacterial effect on the selected pathogens (Plate 2). Minimum inhibitory concentrations (MICs) of EGC, EGCG and ECG were 0.75, 0.36 and 0.50 mg mL^{-1} for *L. monocytogenes* and were 0.75, 0.36 and 0.50 mg mL^{-1} for *P. aeruginosa*, respectively. The MICs of EGC, EGCG and ECG were 0.36, 0.05 and 0.25 mg mL^{-1} for *B. cereus*, whereas for *S. aureus* the MIC of EGC, EGCG and ECG were 0.50, 0.25 and 0.36 mg mL^{-1}, respectively. *E. coli* was found to be resistant against all the standard catechin derivatives. All selected bacterial pathogens were resistant against catechin and epicatechin (Table 3). Among the selected bacterial pathogens, *S. aureus* showed the highest sensitivity to catechin derivatives and *E. coli* was the least sensitive. A comparison of EGC, EGCG and ECG indicated that EGCG had highest effect in terms of MIC against all the selected bacterial pathogens followed by ECG and EGC. These results are in agreement with Taguri *et al*. (2004) [19] who reported higher antibacterial effect of EGCG against all bacterial groups: *S. aureus* (20 strains), *Salmonella* (26 strains), *E. coli* (23 strains) and genus *Vibrio* (27 strains) as compared to EGC suggesting that this could probably be due to the presence of galloyl group in EGCG which increases its antibacterial activity.

3.3 Antibacterial activity of Kangra tea

Antibacterial activity of aqueous solutions of tea powders obtained by lyophilizing aqueous extracts of fresh green tea shoots of Kangra Local, Kangra Asha and Kangra Jawala were evaluated in terms of MIC (Plate 3). The values of mean monthly MIC of aqueous solutions of tea powders obtained by lyophilizing aqueous extracts of green tea shoots of Kangra Local, Kangra Asha and Kangra Jawala are given in Tables 4, 5 and 6, respectively.

It is evident from the tables that MIC of aqueous solutions of tea powders obtained by lyophilizing aqueous extracts of fresh green tea shoots of Kangra Jawala was lowest followed by Kangra Asha and Kangra Local. These observations are in accordance with the results of Cox *et al.* (2001) [3], who reported that enhanced antibacterial activity with Kangra Jawala is probably due to higher content of flavan-3-ols (30-40%) along with some oil and water soluble fractions.

In Tables 7, 8 and 9 are given the zone of inhibition of aqueous solutions of tea powders obtained by lyophilizing aqueous extracts of green tea shoots of Kangra Local, Kangra Asha and Kangra Jawala. As evident from the tables, different zones of inhibition were noticed for equivalent TC concentration against the bacterial pathogens e.g, *S. aureus* exhibited highest zone of inhibition followed by *L. monocytogenes* and *P. aeruginosa* whereas, *E. coli* and *B. cereus* were found to be resistant for all the samples of tea powders. Among the bacteria used in the present study, *S. aureus* is gram-positive and showed a higher sensitivity to polyphenols. This was consistent with the results of the previous reports, which suggested higher susceptibility of food-borne pathogenic gram-positive bacteria to tea catechins compared with gram-negative bacteria (Toda *et al.* 1990) [22]. Ikigai *et al.* (1993, 1998) [8, 9] proposed that this difference is caused by repulsion between catechins and the surfaces of gram-negative bacteria, which is coated with lipopolysaccharide. However, *E. coli* was found to be resistant against all the samples of tea powders. *Escherichia coli* is one of the major constituents of gastrointestinal tract of humans, therefore, its resistance against tea catechins seems to be useful particularly in reference to maintaining the normal microflora of intestinal tract.

A linear correlation among zone of inhibition (mm) and TC content in aqueous solutions of tea powders obtained by lyophilizing aqueous extracts of fresh green tea shoots of Kangra Local, Kangra Asha and Kangra Jawala was evaluated. The TC content in samples was significantly and positively (p<0.05) correlated with zone of inhibition (mm) of all the selected bacterial pathogens except in *B. cereus* and *E. coli*, where no inhibition was noticed. A linear correlation was also established among EGCG, ECG, C, EGC and zone of inhibition (mm) for all the bacterial pathogens in Kangra Local, Kangra Asha and Kangra Jawala (Table 10). It was found that in case of Kangra Local and Kangra Asha the TP, TC and EGCG contents were significantly and positively (p<0.05) correlated with zone of inhibition (mm) of all the selected bacterial pathogens except in *B. cereus* and *E. coli* whereas, incase of Kangra Jawala the TP, TC, EGCG and ECG were positively correlated with zone of inhibition (mm) of the selected bacterial pathogens except in *B. cereus* and *E. coli*, where no inhibition was noticed.

3.4 Antibacterial activity of column pooled fractions

Column pooled fractions were also evaluated for antibacterial activity in terms of MIC against selected bacterial pathogens. Tables 11, 12 and 13 represent the contents of total catechins, IC_{50} values and zone of inhibition obtained with Kangra Local, Kangra Asha and Kangra Jawala. A correlation among total catechins, IC_{50} and zone of inhibition was also evaluated for the column pooled fractions. The TC content was significantly and negatively (p<0.05) correlated with IC_{50} values and zone of inhibition for all the selected bacterial pathogens except in *B. cereus* and *E. coli* (Table 14).

Table 1: Sample number, period and treatment of fresh green tea shoots

Sample number	Sampling period	Treatment
1	April 01 – April 14	The samples of fresh green tea shoots were subjected to heat treatment for 3 minutes in a microwave oven within 20 minutes of their plucking and finally dried in a hot air oven maintained at 45±5 ºC
2	April 15 – April 29	
3	May 01 – May 14	
4	May 15 – May 29	
5	June 01 – June 14	
6	June 15 – June 29	
7	July 01 – July 14	
8	July 15 – July 29	
9	August 01- August 14	
10	August 15 – August 29	
11	September 01 – September 14	
12	September 15 – September 29	
13	October 01 – October 14	
14	October 15 – October 29	

Table 2: Antibiotic sensitivity pattern of standard bacterial pathogens included in the study

Antibiotics Codes	Pathogens				
	L. monocytogenes	*P. aeruginosa*	*B. cereus*	*S. aureus*	*E. coli*
	Zone of Inhibition (mm)				
AM	30	7	7	36	15
S	28	7	14	13	14
T	20	8	14	23	13
Nx	23	17	19	14	20
P	23	R	R	27	R

Am - Amoxycillin; *S* – *Streptomycin*; *T* - Tetracyclin; *Nx*- Norfloxacin; *P* - Penicillin G; R – Resistant (No inhibition).

Table 3: Mean MIC of standard tea catechins

Standard catechins	MIC(mg mL^{-1})				
	L. monocytogenes	***P. aeruginosa***	***B. cereus***	***S. aureus***	***E. coli***
EGC	0.75	0.75	0.36	0.50	NZ NZ
C	NZ	NZ	NZ	NZ	NZ
EC	NZ	NZ	NZ	NZ	NZ
EGCG	0.36	0.36	0.05	0.25	NZ NZ
ECG	0.50	0.50	0.25	0.36	NZ

NZ- No inhibition zone detected

Table 4: Mean Minimum Inhibitory Concentration (MIC) of aqueous solutions of tea powders obtained by lyophilizing aqueous extracts of green tea shoots of Kangra Local (KL) against selected bacterial pathogens

Pathogens	***L. monocytogenes***	***P. aeruginosa***	***B. cereus***	***S. aureus***	***E. coli***
Months	**MIC (mg mL^{-1})**				
April	200	200	NZ	200	NZ
May	200	200	NZ	200	NZ
June	200	200	NZ	200	NZ
July	200	200	NZ	200	NZ
August	200	200	NZ	200	NZ
September	200	200	NZ	200	NZ
October	200	200	NZ	200	NZ

NZ – No inhibition zone detected.

Table 5: Mean Minimum Inhibitory Concentration (MIC) of aqueous solutions of tea powders obtained by lyophilizing aqueous extracts of green tea shoots of KangraAsha (KA) against selected bacterial pathogens

Pathogen	***L. monocytogenes***	***P. aeruginosa***	***B. cereus***	***S. aureus***	***E. coli***
Months	**MIC (mg mL^{-1})**				
April	180	180	NZ	180	NZ
May	180	180	NZ	180	NZ
June	180	180	NZ	180	NZ
July	180	180	NZ	180	NZ
August	180	180	NZ	180	NZ
September	180	180	NZ	180	NZ
October	180	180	NZ	180	NZ

NZ – No inhibition zone detected.

Table 6: Mean Minimum Inhibitory Concentration (MIC) of aqueous solutions of tea powders obtained by lyophilizing aqueous extracts of green tea shoots of KangraJawala (KJ) against selected bacterial pathogens

Pathogens	***L. monocytogenes***	***P. aeruginosa***	***B. cereus***	***S. aureus***	***E. coli***
Months	**MIC (mg mL^{-1})**				
April	50	50	NZ	50	NZ
May	50	50	NZ	50	NZ
June	50	50	NZ	50	NZ
July	50	50	NZ	50	NZ
August	50	50	NZ	50	NZ
September	50	50	NZ	50	NZ
October	50	50	NZ	50	NZ

NZ- No inhibition detected.

Table 7: Zone of Inhibition of aqueous solutions of tea powders obtained by lyophilizing aqueous extracts of green tea shoots of Kangra Local (KL) against selected bacterial pathogens

Pathogens	***L. monocytogenes***	***P. aeruginosa***	***B. cereus***	***S. aureus***	***E. coli***
Months	**Zone of Inhibition (mm)**				
April	3	6	NZ	11	NZ
May	5	6	NZ	12	NZ
June	6	8	NZ	15	NZ
July	7	8	NZ	17	NZ
August	4	5	NZ	9	NZ
September	3	4	NZ	9	NZ
October	3	4	NZ	7	NZ

NZ – No inhibition zone detected.

Table 8: Zone of inhibition of aqueous solutions of tea powders obtained by lyophilizing aqueous extracts of green tea shoots of Kangra Asha (KA) against selected bacterial pathogens

Pathogens	***L. monocytogenes***	***P. aeruginosa***	***B. cereus***	***S. aureus***	***E. coli***
Months	**Zone of inhibition (mm)**				
April	7	7	NZ	13	NZ
May	7	7	NZ	12	NZ
June	11	15	NZ	17	NZ
July	9	8	NZ	15	NZ
August	6	7	NZ	11	NZ
September	6	6	NZ	11	NZ
October	7	6	NZ	9	NZ

NZ – No inhibition zone detected.

Table 9: Zone of inhibition of aqueous solutions of tea powderobtained by lyophilizing aqueous extracts of green tea shoots of KangraJawala (KJ) against selected bacterial pathogens

Pathogens	***L. monocytogenes***	***P. aeruginosa***	***B. cereus***	***S. aureus***	***E. coli***
Months	**Zone of inhibition (mm)**				
April	11	8	NZ	10	NZ
May	11	9	NZ	11	NZ
June	15	15	NZ	16	NZ
July	12	9	NZ	11	NZ
August	15	16	NZ	17	NZ
September	8	14	NZ	12	NZ
October	8	12	NZ	12	NZ

NZ – No inhibition zone detected.

Table 10: Correlation coefficient among TC, TP, EGCG, ECG, C, and EGC and zone of inhibition (mm) for selected bacterial pathogens for Kangra Local, Kangra Asha and Kangra Jawala

	KL				
	L. monocytogenes	***P. aeruginosa***	***B. cereus***	***S. aureus***	***E. coli***
TC	0.79^a	0.89^a	NS	0.77^a	NS
TP	0.76^a	NS	NS	NS	NS
EGCG	0.83^a	0.81^a	NS	0.80^a	NS
ECG	NS	NS	NS	NS	NS
C	NS	NS	NS	NS	NS
EC	NS	NS	NS	NS	NS
EGC	NS	NS	NS	NS	NS
	KA				
TC	0.96^a	0.94^a	NS	0.91^a	NS
TP	0.91^a	0.76^a	NS	0.86^a	NS
EGCG	0.78^a	0.76^a	NS	0.89^a	NS
ECG	NS	NS	NS	NS	NS
C	NS	NS	NS	NS	NS
EC	NS	NS	NS	NS	NS
EGC	NS	NS	NS	NS	NS
	KJ				
TC	0.75^a	0.86^a	NS	0.83^a	NS
TP	0.82^a	0.71^a	NS	0.89^a	NS
EGCG	0.85^a	0.82^a	NS	0.79^a	NS
ECG	0.77^a	0.80^a	NS	0.95^a	NS
C	NS	NS	NS	NS	NS
EC	NS	NS	NS	NS	NS
EGC	NS	NS	NS	NS	NS

^{a} – Significant at $P<0.05$; NS – Not significant.

Table 11: Total catechins, IC_{50} and zone of Inhibition (mm) of column pooled fractions (PF) of Kangra Local (KL)

Sample no.	**Fraction no.**	**TC (µg mL^{-1})**	**IC_{50} (µg mL^{-1})**	**Pathogens**				
				L. monocytogenes	***P. aeruginosa***	***B. cereus***	***S. aureus***	***E. coli***
PF-1	1-8	169.26^f	1.024^b	NZ	NZ	NZ	NZ	NZ
PF-2	9-12	328.34^e	1.012^b	NZ	NZ	NZ	NZ	NZ
PF-3	13-14	805.18^c	0.997^c	NZ	NZ	NZ	NZ	NZ
PF-4	15-18	1225.10^b	0.897^d	8	10	NZ	7	NZ
PF-5	19-20	1667.67^a	0.587^f	10	15	NZ	10	NZ
PF-6	21-22	1357.56^b	0.690^e	9	11	NZ	8	NZ
PF-7	23-26	764.79^c	0.988^c	NZ	NZ	NZ	NZ	NZ
PF-8	27-39	545.50^d	1.002^b	NZ	NZ	NZ	NZ	NZ

PF-9	40-46	140.99^{f}	1.432^{a}	NZ	NZ	NZ	NZ	NZ
Mean		778.27	0.959					
CD(5%)		3.38	0.00268					
CV(%)		0.25	0.16					

NZ – No inhibition zone detected

Table 12: Total catechins, IC_{50}andzone of inhibition of column pooled fraction (PF) of Kangra Asha

Sample no.	Fraction no.	TC (µg mL^{-1})	IC_{50} (µg mL^{-1})	Pathogens				
				L. monocytogenes	*P. aeruginosa*	*B. cereus*	*S. aureus*	*E. coli*
PF-1	1-18	176.00^{g}	1.487^{a}	NZ	NZ	NZ	NZ	NZ
PF-2	19-21	1842.55^{a}	0.114^{g}	7	12	NZ	14	NZ
PF-3	22-23	1710.44^{b}	0.272^{f}	6	9	NZ	10	NZ
PF-4	24-27	1550.30^{c}	0.587^{e}	6	9	NZ	10	NZ
PF-5	28-29	1396.03^{d}	0.868^{d}	NZ	NZ	NZ	NZ	NZ
PF-6	30-33	463.41^{e}	1.057^{c}	NZ	NZ	NZ	NZ	NZ
PF-7	34-47	212.49^{f}	1.113^{b}	NZ	NZ	NZ	NZ	NZ
Mean		1050.17	0.785					
CD(5%)		3.92	0.00310					
CV(%)		0.21	0.23					

NZ – No inhibition zone detected

Table 13: Total catechins, IC_{50} and zone of Inhibition of column pooled fraction (PF) of Kangra Jawala

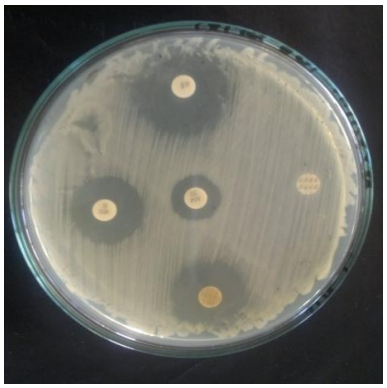
Sample no.	Fraction no.	TC (µg mL^{-1})	IC_{50} (µg mL^{-1})	Pathogens				
				L. monocytogenes	*P. aeruginosa*	*B. cereus*	*S. aureus*	*E. coli*
PF-1	1-16	176.27^{f}	1.741^{a}	NZ	NZ	NZ	NZ	NZ
PF-2	17	1706.67^{c}	0.307^{e}	NZ	NZ	NZ	NZ	NZ
PF-3	18	1522.41^{c}	0.396^{d}	NZ	NZ	NZ	NZ	NZ
PF-4	19	2015.42^{b}	0.146^{f}	8	10	NZ	7	NZ
PF-5	20-21	2307.21^{a}	0.095^{g}	10	15	NZ	10	NZ
PF-6	22-24	1929.87^{b}	0.167f	NZ	NZ	NZ	NZ	NZ
PF-7	25-27	1227.23^{d}	0.404^{d}	NZ	NZ	NZ	NZ	NZ
PF-8	28-32	392.99^{e}	0.554^{c}	NZ	NZ	NZ	NZ	NZ
PF-9	33-45	344.80^{e}	1.477^{b}	NZ	NZ	NZ	NZ	NZ
Mean		1291.43	0.587					
CD (5%)		5.48	0.00639					
CV (%)		0.25	0.63					

NZ – No inhibition zone detected

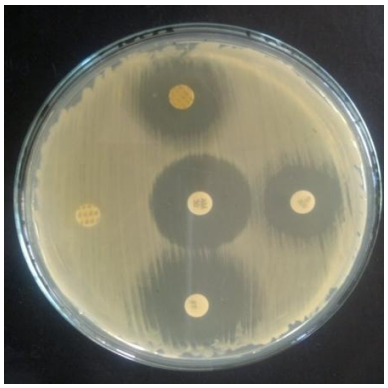
Table 14: Correlation coefficient among total catechins (TC), IC_{50} values and zone of inhibition (mm) of selected bacterial pathogens in column pooled fractions of Kangra Local (KL), Kangra Asha (KA) and Kangra Jawala (KJ) cultivars

		IC_{50}	*L. monocytogenes*	*P. aeruginosa*	*B. cereus*	*S. aureus*	*E. coli*
KL	TC	-0.89	0.88^{a}	0.87^{a}	NS	0.88^{a}	NS
KA	TC	-0.92	0.83^{a}	0.82^{a}	NS	0.82^{a}	NS
KJ	TC	-0.87	0.82^{a}	0.95^{a}	NS	0.87^{a}	NS

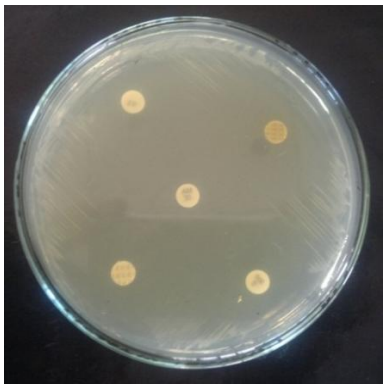
^{a} – Significant at P<0.05; NS – Not significant.



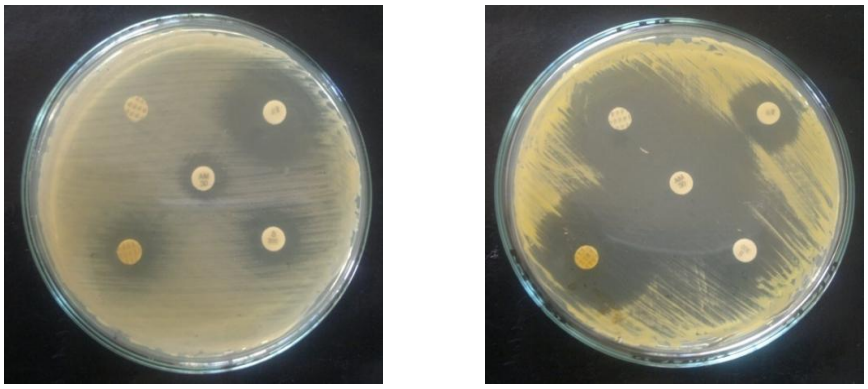
Bacillus cereus (MTCC-1272)



Escherichia coli (MTCC-443)

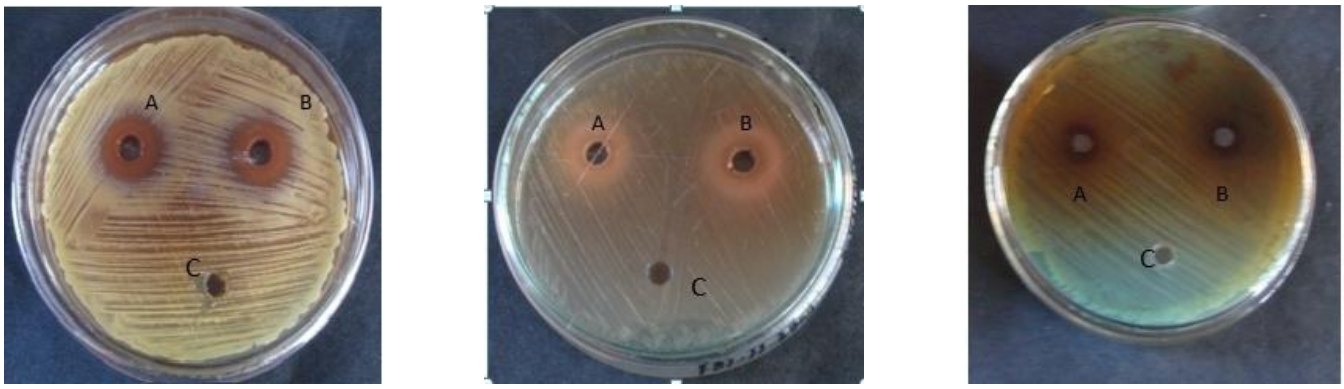


Listeria monocytogenes (MTCC-389)



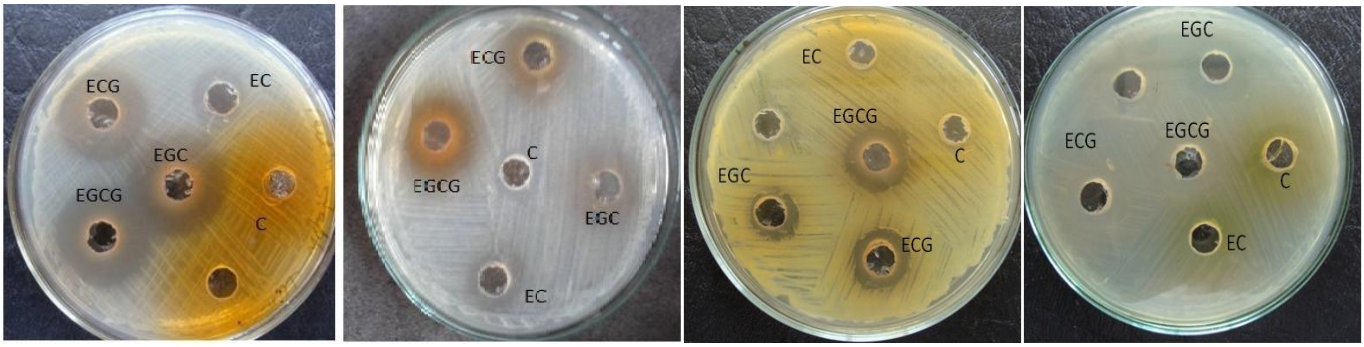
Pseudomonos aeruginosa (MTCC-741) *Staphylococcus aureus* (MTCC-96)

Plate 1: Sensitivity of *L. monocytogenes, P. aureginosa, E. coli, B. cereus* and *S. aureus* against standard antibiotics



Staphylococcus aureus (MTCC-96) *Listeria monocytogenes* (MTCC-839) *Pseudomonas aeruginosa* (MTCC-741)

Plate 2: Sensitivity of *L. monocytogenes, P. aeruginosa and S. aureus* for aqueous solutions of tea powders obtained by lyophilizing aqueous extracts of green tea shoots: A- June, B- August, C- Control



Bacillus cereus (MTCC-1272) *Listeria monocytogenes* (MTCC-839) *Staphylococcus aureus* (MTCC-96) *Pseudomonas aeruginosa* (MTCC-741)

Plate 3: Sensitivity of *L. monocytogenes, P. aeruginosa, B. cereus and S. aureus* against standard catechins

4. Conclusion

The local cultivars of Kangra tea have reasonable potential to act as free radical scavengers and bactericidal against pathogens. Among the cultivars, Kangra Jawala due to its higher TC and EGCG contents has better potential as compared to Kangra Local and Kangra Asha. Thus, it is evident that the genetic make-up of these cultivars and climatic conditions of flush seasons seem to have influence on the synthesis and accumulation of TP, TC and flavan-3-ol content of green tea shoots.

5. References

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