5. SUMMARY AND CONCLUSIONS

Health benefits of tea drinking have been attributed to tea catechins (flavan-3-ols). The present investigations: “Studies on flavan-3-ols and biological activity of Kangra tea [Camellia sinensis (L) O Kuntze]” were carried out to investigate seasonal profile of polyphenols with special reference to flavan-3-ols and antioxidant and antibacterial activities of Kangra tea. Seasonal variations of total polyphenols and total catechins in fresh green tea shoots of Kangra tea, as affected by the changes in weather conditions during four flush seasons for three consecutive years have been evaluated. Total polyphenols and total catechins contents in leaves of increasing age vis-a-vis bud, first leaf and second leaf were also evaluated. A correlation among polyphenolic constituents of tea shoots and weather parameters was sought. Seasonal profile of flavan-3-ols was also elucidated. The antioxidant potential of Kangra tea in terms of 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging and metal ions chelation was determined. Antibacterial activity of Kangra tea against six selected bacterial pathogens was investigated. A correlation between biological activity and individual flavan-3-ols was sought.

A total of 270 samples comprising of two leaves and a bud (93), bud (31), first leaf (31), second leaf (31), pooled on monthly basis (42) and tea powder (42) were incorporated during the course of present investigations. Fresh shoots of Kangra local tea [Camellia sinensis (L) O Kuntze] cultivar harvested at seven day intervals throughout the plucking seasons (April to October/November) during three consecutive years (2007, 2008 and 2009) were always subjected to microwave heat treatment to cease polyphenol oxidase enzyme activity prior to drying. Total polyphenols (TP) and total catechins (TC) contents were estimated in weekly dried samples of fresh green tea shoots, bud, first leaf and second leaf. The data were pooled and analyzed statistically using Duncan’s Multiple Range Test (D.M.R.T).

The mean weekly TP and TC contents in green tea shoots varied significantly (p<0.05) over the flush seasons and the cropping years. During 2007
the mean weekly TP content varied significantly from 205.518 g kg\(^{-1}\) in sample harvested on June 5 to 113.719 g kg\(^{-1}\) in October 23 sample; and mean weekly TC content varied significantly from 110.209 g kg\(^{-1}\) in sample of May 29 to 40.904 g kg\(^{-1}\) in green tea shoots harvested on October 2. During 2008 the mean weekly TP content varied significantly between 269.529 g kg\(^{-1}\) and 140.210 g kg\(^{-1}\) in samples harvested on September 4 and October 30, respectively; whereas mean weekly TC content varied significantly between 174.774 g kg\(^{-1}\) and 37.185 g kg\(^{-1}\) in samples harvested on June 12 and August 21, respectively. During 2009 the mean weekly TP content varied in the range 263.223 g kg\(^{-1}\) in sample harvested on May 18 to 104.759 g kg\(^{-1}\) in July 20 sample; and mean weekly TC content varied between 162.619 g kg\(^{-1}\) and 82.673 g kg\(^{-1}\) in samples harvested on August 24 and July 20, respectively (Tables 4.1 and 4.2). These intra and inter cropping seasonal variations of TP and TC in fresh green tea shoots could be due to the weather conditions prevailed in the region.

During 2007 when weather was warm and dry statistically the highest TP and TC contents were recorded in summer months and lowest during October. During 2008 when weather was cold with high accumulated seasonal rainfall statistically the highest TP content was recorded during September and lowest during October, whereas statistically the TC content was recorded highest during June and lowest during August. During warm and humid 2009 statistically the highest TP content was recorded during May and TC content during August, however, both TP and TC contents during July were statistically lowest.

In case of tea leaves with increasing age the mean weekly TP content also varied significantly (p<0.05) in the range of 258.025 to 98.391 g kg\(^{-1}\) for bud, 283.011 to 125.101 g kg\(^{-1}\) for first leaf and 252.562 to 107.818 g kg\(^{-1}\) for second leaf (Table 4.3). Statistically the highest mean weekly TP contents were always recorded in the samples harvested on May 18, whereas the samples harvested on July 20 recorded statistically the lowest mean weekly TP contents in leaves with increasing age. The mean weekly TC content also varied significantly in the range of 173.399 to 76.645 g kg\(^{-1}\) for bud, 183.058 to 88.111 g kg\(^{-1}\) for first leaf and 159.179 to 85.875 g kg\(^{-1}\) for second leaf (Table 4.4). In case of bud and first
leaf the samples harvested on June 8 recorded statistically the highest mean weekly TC contents; whereas in second leaf it was in August 24 sample; however, statistically the lowest mean weekly TC contents were always recorded in samples harvested on July 20. Among the leaves with increasing age the mean weekly TP and TC contents were always higher in samples of first leaf followed by second leaf and bud, except during summer flush season when the samples of bud recorded higher TP and TC contents compared to samples of second leaf.

The phenolic constituents also varied significantly (p<0.05) in monthly pooled samples of green tea shoots, bud, first leaf and second leaf. In fresh green tea shoots the mean monthly TP content varied significantly in the range of 177.543 to 150.212 g kg\(^{-1}\) during 2007, 263.100 to 185.194 g kg\(^{-1}\) during 2008 and 250.194 to 173.791 g kg\(^{-1}\) during 2009; whereas the mean monthly TC content varied significantly in the range of 109.021 to 68.866 g kg\(^{-1}\) during 2007, 105.878 to 87.125 g kg\(^{-1}\) during 2008 and 143.242 to 120.930 g kg\(^{-1}\) during 2009 (Table 4.5). In case of leaf with increasing age the mean monthly TP and TC contents also varied significantly (p<0.05) in the range of 231.865 to 150.178 g kg\(^{-1}\) in bud, 253.408 to 174.016 g kg\(^{-1}\) in first leaf, 211.069 to 173.134 g kg\(^{-1}\) in second leaf and 154.568 to 115.976 g kg\(^{-1}\) in bud, 156.313 to 123.877 g kg\(^{-1}\) in first leaf, 154.314 to 110.665 g kg\(^{-1}\) in second leaf, respectively (Table 4.6). The samples harvested during summer months always had statistically the highest TP content during all the cropping years. However, the TC content was statistically the highest either during summer or rainy months.

Significant variations in the levels of phenolic constituents of fresh tea shoots and leaves of increasing age of Kangra tea were attributed to the changes in weather conditions that varied significantly during different flush seasons and cropping years. Linear correlations among mean weekly TP, TC contents and five weather parameters (maximum and minimum temperatures, relative humidity, bright sunshine hours, rainfall and evaporation) were detailed in Tables 4.8, 4.9, 4.10 and 4.11. Both the TP and TC contents exhibited a significant positive correlation with temperature and evaporation during warm and dry 2007.
During cold with high accumulated seasonal rainfall 2008 a significant positive correlation of TP with temperature and TC with evaporation was observed. However, during warm and humid 2009, TP in fresh green tea shoots and leaves with increasing age exhibited a significant positive correlation with evaporation. Lack of any overt correlations in the present study could be due to the natural interdependency among weather parameters.

Stepwise regression analysis was carried out in order to obtain a set of critical variables associated with synthesis and accumulation of phenolic constituents in tea shoots. During 2007 when weather was warm and dry the TP content was affected by mean weekly maximum temperature, mean weekly bright sunshine hours, accumulated weekly rainfall and TC content was affected by mean weekly maximum temperature only. During 2008 when the weather was cold with high accumulated seasonal rainfall the TP content was affected by mean weekly minimum temperature, mean weekly bright sunshine hours and mean weekly evaporation, whereas the TC content was affected by mean weekly relative humidity and mean weekly bright sunshine hours. During 2009 when weather was warm and humid the TP content was affected by mean weekly minimum temperature, mean weekly relative humidity, mean weekly bright sunshine hours, accumulated weekly rainfall and mean weekly evaporation, however, the TC content was affected by mean weekly minimum temperature, mean weekly bright sunshine hours and accumulated weekly rainfall (Table 4.12). In bud, first leaf and second leaf the TP content was affected by mean weekly relative humidity, mean weekly bright sunshine hours and accumulated weekly rainfall. In addition it was also affected by mean weekly minimum temperature in bud and second leaf. The TC content was always affected by accumulated weekly rainfall and in case of first leaf and second leaf it was also affected by mean weekly minimum temperature (Table 4.13).

It was reasonable to conclude that the mechanisms that induced seasonal variations in the levels of phenolic constituents of tea shoots may include one or all the weather parameters which varied markedly across harvesting seasons over the years under investigation.
Flavan-3-ols profile of Kangra tea was elucidated incorporating tea powders obtained by lyophilizing aqueous extracts of pooled samples. Significant mean monthly variations in the levels of TP and TC in tea powders were almost similar to those of in fresh green tea shoots (Tables 4.14 and 4.15). Qualitative evaluation of tea powders and fractions eluted from Sephadex G-25 size exclusion chromatography indicated the presence of five major flavan-3-ols: (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG) and (-)-epicatechin gallate (ECG) in Kangra tea (Plates 4.2, 4.3, 4.4 and 4.5).

The flavan-3-ols contents varied in the range of C: 1.43 to 1.07%, EC: 5.26 to 3.64%, EGC: 33.28 to 23.78%, EGCG: 49.52 to 39.67% and ECG: 24.17 to 20.00% during 2007 when weather was warm and dry; in the range of C: 1.46 to 0.69%, EC: 5.95 to 3.54%, EGC: 36.01 to 26.71%, EGCG: 44.64 to 38.08% and ECG: 24.38 to 15.66% during 2008 when weather was cold with high accumulated seasonal rainfall; and in the range of C: 2.51 to 1.23%, EC: 14.05 to 9.34%, EGC: 23.75 to 19.65%, EGCG: 51.88 to 45.93% and ECG: 18.75 to 13.23% during 2009 when weather was warm and humid (Tables 4.18, 4.19 and 4.20). The levels of flavan-3-ols in tea powders obtained by lyophilizing aqueous extracts of tea leaves of increasing age varied in the range of C: 2.84 to 2.09%, EC: 12.38 to 10.47%, EGC: 22.11 to 18.06%, EGCG: 51.27 to 46.05% and ECG: 19.02 to 14.15% for bud; C: 2.18 to 1.23%, EC: 12.06 to 9.44%, EGC: 23.08 to 17.62%, EGCG: 59.94 to 47.68% and ECG: 18.80 to 13.73% for first leaf; and C: 1.98 to 0.71%, EC: 13.78 to 7.69%, EGC: 29.34 to 19.78%, EGCG: 53.57 to 47.84% and ECG: 15.46 to 10.96% for second leaf (Tables 4.21, 4.22 and 4.23). The flavan-3-ols contents in tea powders of summer and rainy flush seasons were invariably high. Those tea powders which were obtained by lyophilizing aqueous extracts of (i) buds harvested during summer, (ii) first leaf harvested during rainy and (iii) second leaf harvested during summer and rainy flush seasons had high flavan-3-ols contents. Flavan-3-ols always varied in the order (-)-epigallocatechin gallate > (-)-epigallocatechin > (-)-epicatechin gallate > (-)-epicatechin > (+)-catechin in Kangra tea.
The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability of standard catechin, its derivatives and standard antioxidants (ascorbic acid and butylated hydroxytoluene) decreased in the order: EGCG>ECG>EGC>EC~C>ascorbic acid> butylated hydroxytoluene.

Aqueous extracts of fresh green tea shoots, first leaf and second leaf harvested during August, 2009 and that of bud harvested during the month of June, 2009 had lower IC$_{50}$ values and high antioxidant potency. Among the aqueous extracts of bud, first leaf and second leaf, the IC$_{50}$ values of all the extracts of first leaf; except those of bud harvested during May and June, were always lower (Table 4.24). The IC$_{50}$ values of 1 mg mL$^{-1}$ aqueous solutions of tea powders obtained from aqueous extracts of bud, first leaf and second leaf during 2009 varied significantly in the range 2.867 to 1.433 µg mL$^{-1}$, 2.611 to 1.215 µg mL$^{-1}$ and 3.364 to 1.266 µg mL$^{-1}$, respectively. The IC$_{50}$ values of 1 mg mL$^{-1}$ aqueous solutions of tea powders obtained from fresh green tea shoots varied significantly in the range 3.215 to 1.106 µg mL$^{-1}$ during 2007; 3.519 to 1.296 µg mL$^{-1}$ during 2008 and 3.841 to 1.669 µg mL$^{-1}$ during 2009 (Table 4.25).

The aqueous solutions of tea powders obtained by lyophilizing aqueous extracts; of fresh green tea shoots, first leaf and second leaf harvested in August and bud harvested during June recorded lower IC$_{50}$ values. Among the leaves with increasing age the aqueous solutions of tea powders obtained from samples of first leaf always had low IC$_{50}$ values and hence high DPPH free radical scavenging potency; except for the solutions of tea powders obtained from bud harvested during June and September which had IC$_{50}$ values lower than the tea powders from first leaf. It was of interest to note that 1 mg ml$^{-1}$ of solutions of tea powders from fresh green tea shoots, bud, first leaf and second leaf having high TC contents recorded lower IC$_{50}$ values and more antioxidant potency.

The initial significant negative correlation observed between IC$_{50}$ values with TC content was further established to be between IC$_{50}$ values and EGCG, ECG and EGCG+ECG contents of aqueous solutions of tea powders (Table 4.26). Hence it was reasonable to conclude that the EGCG and ECG contents were mainly responsible for DPPH free radical scavenging activity of Kangra tea.
The IC$_{50}$($Fe^{2+}$) values of 1 mM ethylenediaminetetra-acetic acid (EDTA) and 25 mM citric acid (versatile metal chelators) were 1.589 µg mL$^{-1}$ and 1455.895 µg mL$^{-1}$, respectively. The IC$_{50}$($Fe^{2+}$) values of 5 mg mL$^{-1}$ aqueous solutions of tea powders varied significantly in the range 1243.002 to 724.953 µg mL$^{-1}$ for fresh green tea shoots, 1294.715 to 606.372 µg mL$^{-1}$ for bud, 1165.471 to 658.681 µg mL$^{-1}$ for first leaf and 1450.102 to 1003.294 µg mL$^{-1}$ for second leaf (Table 4.27). Ferrous ions chelation potential of tea powders (in terms of IC$_{50}$($Fe^{2+}$) values) was in between the IC$_{50}$($Fe^{2+}$) values of EDTA and citric acid. The IC$_{50}$($Fe^{2+}$) exhibited a correlation with TC and flavan-3-ols which was analogous to that observed with IC$_{50}$ values of DPPH free radical scavenging ability of aqueous solutions of tea powders (Table 4.28).

The antibacterial activity of standard catechin derivatives against selected bacterial pathogens viz. Psuedomonas aeruginosa (MTCC-741), Listeria monocytogenes (MTCC-839), Escherichia coli (MTCC-443), Bacillus cereus (MTCC-1272), Staphylococcus aureus (MTCC-96) and Streptococcus mutans (MTCC-890) reported in terms of minimum inhibitory concentrations (MIC) was found to be decreased in the order: EGCG>ECG>EGC. All selected bacterial pathogens were resistant against catechin and epicatechin.

The MIC of aqueous extracts (10 g sample extracted in 100 mL preboiled hot double distilled water for 20 minutes at 60±5°C) of monthly samples of fresh green tea shoots varied significantly in the range of: 2.253 to 1.541 mg mL$^{-1}$ for L. monocytogenes, P. aeruginosa, S. aureus and S. mutans and 2.253 to 1.073 mg mL$^{-1}$ for B. cereus (Table 4.30). For the samples of bud, the MIC varied significantly in the range of: 2.437 to 1.735 mg mL$^{-1}$ for all the selected bacterial pathogens (Table 4.31). In case of samples of first leaf, the MIC varied significantly in the range of: 2.130 to 1.233 mg mL$^{-1}$ for L. monocytogenes; 2.414 to 2.130 mg mL$^{-1}$ for P. aeruginosa; 2.122 to 1.192 mg mL$^{-1}$ for B. cereus and 2.179 to 1.233 mg mL$^{-1}$ for S. aureus and S. mutans (Table 4.32); and for the samples of second leaf it varied from: 2.265 to 1.551 mg mL$^{-1}$ for L. monocytogenes, S. aureus and S. mutans; 2.265 to 2.121 mg mL$^{-1}$ for P. aeruginosa and 2.259 to 1.250 mg mL$^{-1}$ for B. cereus (Table 4.33). Escherichia
coli was found to be resistant for all the standard catechins and samples. The aqueous extracts of samples which had high TC content were found to be more effective growth inhibitors of selected bacterial pathogens. Thus exhibiting similar pattern to antioxidant nature of tea extracts. The correlation of TC contents of aqueous extracts was positively significant with zones of inhibition and negatively significant with MIC.

A comparative TLC (Thin layer chromatography) bioautograph study between aqueous solutions of standard catechins (0.5 mg mL\(^{-1}\)) and tea powder (1 mg mL\(^{-1}\)) indicated that EGCG was one of the active components present in tea solution which might be responsible for antibacterial activity of Kangra tea (Plate 4.13). Growth inhibition of bacterial pathogens \( (B. \ cereus \ and \ S. \ aureus) \) by aqueous solution of tea powder was found to be influenced by the addition of ferrous ions which suggest that the antibacterial activity of tea catechins could be due to their ability to chelate ferrous ions, indicating that this activity is bacteriostatic rather than bactericidal in nature (Plate 4.14).

**Conclusions**

- Significant seasonal variations of total polyphenols, total catechins and flavan-3-ols in fresh green tea shoots and leaves with increase age (bud, first leaf and second leaf) were due to weather parameters which varied significantly across the harvesting flush seasons over the years. Fresh green tea shoots and leaves with increasing age of summer and rainy flush seasons invariably had higher contents of total catechins and flavan-3-ols.

- The per cent composition of flavan-3-ols: \((+)-\)catechin = 2.51 to 0.69%, \((-)-\)epicatechin = 14.05 to 3.54%, \((-)-\)epigallocatechin = 36.01 to 19.65%, \((-)-\)epigallocatechin gallate = 51.88 to 38.08% and \((-)-\)epicatechin gallate = 24.38 to 13.23% in fresh green tea shoots of Kangra local were lower than the reported values. The order of variations of flavan-3-ols was \((-)-\)epigallocatechin gallate > \((-)-\)epigallocatechin > \((-)-\)epicatechin gallate > epicatechin > \((+)-\)catechin.
The antioxidant, ferrous ions chelation and antibacterial potency of Kangra tea were directly proportional to total catechins contents of tea in general and (−)-epigallocatechin gallate and (−)-epicatechin gallate contents in particular.

The antibacterial effect of Kangra tea was bacteriostatic instead of bactericidal; however, more work is required to be carried out to establish this property.

The summer and main flush seasons green shoots of Kangra local tea are suitable for the manufacture of green tea.