2. REVIEW OF LITERATURE

Tea is probably one of the most widely consumed non-alcoholic beverages in the world. The discovery and origin of tea drinking was ascribed to Chinese emperor Shen Nung, a renowned herbalist, who also claimed that tea infusion was able to detoxify 72 kinds of poisons (Ukers 1935; Longzai 1986). Today tea is being cultivated as a commercial crop in wide range of soil types under tropical, sub-tropical and temperate climatic conditions all over the World from 45°N (Russia) to 30°S (South Africa) and from 150°E (New Guinea) to 60°W (Argentina) (Harler 1971). In India the Singhpo tribe of North-East grew a variety of tea plant since time immemorial unknown to the rest of the world (Taknet 2002).

Tea plant belongs to Theaceae family and the genus *Camellia*. Sealy (1958) proposed the classification of tea cultivars based on leaf characteristics. Wight (1962) revised the classification on the basis of morphological characters such as leaf size, leaf shape, length of pistil and flower sizes. Tea cultivated all over the world consists of following three distinct taxa each with specific plant types:

1. China type: *Camellia sinensis* (L) O Kuntze
2. Assam type: *Camellia assamica* (Mast.) Wight ssp. assamica
3. Cambod type: *Camellia assamica* ssp. *lasiocalyx* Planch ex Watt

However, most of the cultivated tea is heterogeneous as a result of large scale dispersal of the tea plant; it’s out breeding nature and the free hybridization between geographical races (Kingdon-Ward 1950). The tea plant is an evergreen shrub that is kept at manageable height of about one meter by periodical pruning operations. The commercial tea is manufactured from green tea shoots (two leaves and a terminal bud) which constitute 10-18% of the total biomass produced by the tea plant. Tea bush has been reported to yield 1 to 4 t ha⁻¹ y⁻¹ of dry biomass which is much less than other vegetative crops growing under similar conditions; due to small harvest index of tea that restricts tea biomass production (Magambo and Cannell 1981).

2.1 Classification
There are six types of processed teas: green, yellow, dark, white, oolong and black. These have been further classified into three major groups: non-fermented, semi-fermented and fully fermented; on the basis of degree of fermentation and extent of oxidation of polyphenols. The polyphenols present in fresh tea shoots are hardly oxidized during the processing of green tea, but these are non-enzymatically oxidized in yellow and dark teas, whereas white, oolong and black teas are enzymatically fermented to varied extent; with white having the least and the black having the most fermentation. The steps involved in the processing of fresh tea shoots to manufacture different types of teas, summarized in Figure 2.1, have been well documented (Hara et al. 1995). Globally the production and consumption of black tea (78 per cent) is highest followed by green (20 per cent) and oolong (2 per cent) (Mitscher and Dolby 1998).

![Figure 2.1 Process of manufacture of different types of teas](image)

**Figure 2.1 Process of manufacture of different types of teas**

Green, yellow and dark teas are unfermented. Polyphenols are hardly oxidized in green tea, but they are non-enzymatically oxidized in yellow and dark teas. White, oolong and black teas are fermented with white having the least fermentation and black the most. All the six types of teas have distinct flavours.
and differ in quality characteristics of their infusions corresponding to the degree of enzymatic or non-enzymatic oxidation of the polyphenols. In the processing of white tea the buds and young leaves are withered, fired and air-dried. Rolling or crushing of tea leaves is avoided in order to conserve most of the polyphenols in their natural state. The processing of green, yellow and dark teas involve heating the fresh tea shoots at elevated temperature, thereby avoiding the enzymatic oxidation of polyphenols present in fresh green tea leaves. The yellow and dark teas differ from green tea for in the manufacture of yellow and dark teas the inactivation of polyphenol oxidase enzyme (EC. 1.10.3.1) is followed by stacking of tea leaves to affect thermal oxidation of polyphenols. The infusion of white tea is light orange-yellow with plain taste; green tea infusion has a distinct aroma with deep mellow taste; yellow and dark teas are yellow and brownish-yellow, respectively, in infusion and have a mellow taste.

In the manufacture of oolong tea, the fresh green tea leaves are subjected to partial fermentation. The catechins, theaflavins and thearubigins levels in oolong tea have been reported to be between unfermented green and white teas and fully fermented black tea (Peterson et al. 2005). During processing of fresh tea shoots to manufacture black tea, the polyphenol oxidase enzyme present in chloroplast, comes in contact with catechins. The enzymatic oxidation transforms the catechins into enzymatic oxidation products - theaflavins and thearubigins (Figure 2.2) responsible for black tea liquor characteristics (Roberts 1958; Roberts and Williams 1958; Roberts 1962; Bhatia 1964; Sanderson and Gonzales 1971). The infusion of oolong tea is golden yellow with rich mellow taste and that of black tea is golden brown with strong and brisk taste.

White tea has been reported to contain highest level of antioxidants and lowest level of caffeine compared to any other tea (Sharangi 2009).
2.2 Polyphenolic constituents

Fresh tea leaves have been reported to contain moisture (75%-80%), polyphenols (25-30%), caffeine (2-4%), proteins (15-20%), amino acids such as theanine or 5-N-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, lysine (1-4%), carbohydrates such as cellulose, pectins, glucose, fructose, sucrose (5-7%), minerals and trace elements such as Ca, Mg, Cr, Mn, Fe, Cu, Zn, Mo, Se, Na, P, Co, Sr, Ni, K, F and Al (4-5%) (Wickremasinghe 1978; Hara et al. 1995; Natesan and Ranganathan 1990; Fernandez et al. 2002). Caffeine and polyphenols present in tea are fairly important for their stimulating effect and health benefits, respectively. The mild stimulating effect of tea has been attributed to caffeine and two other isomeric dimethyl xanthines, theophylline and theobromine (Cloughley 1982; Graham 1992).

Green tea has been reported to be a rich source of dietary flavonoids (Balentine and Paetau-Robinson 2000; Dufresne and Farnworth 2001; Sharangi 2009). The five major flavonoids (flavan-3-ols) in fresh green tea shoots, classified as catechins, which have been reported to exhibit bioactive properties (Gramaza et al. 2005; Zaveri 2006) are: (−) - epigallocatechin-3-gallate (EGCG),
(−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), (−)-epicatechin (EC) and (+)-catechin (C) (Figure 2.3). Nanjo et al. (1996) and Karori et al. (2007) on the basis of free radical scavenging effects of tea catechins and their derivatives on 1, 1-diphenyl-2-picrylhydrazyl radical and antioxidant capacity of different types of tea products reported EGCG, EGC and ECG to be potent antioxidants in tea. Being water soluble tea catechins have been reported to impart astringency to tea infusions (Wang et al. 1998). Among different types of teas the green tea was reported to contain highest amount of catechins (26.7%) followed by oolong (23.2%) and black (4.3%) teas (Cabrera et al. 2003; Peterson et al. 2005). Fresh green tea shoots have also been reported to contain gallic acid (GA), chlorogenic acid and caffeic acid, kaempferol, myricetin, quercetin and leucoanthocyanins (Gripenberg 1962; Ulyanova 1963; Sakamoto 1970; Stagg and Swaine 1971; Eden 1976). Variations in weather conditions during different harvesting seasons in the region of its cultivation have been reported to affect the synthesis and accumulation of polyphenols in tea shoots (Caffin et al. 2004; Yao et al. 2005; Chen et al. 2010).

Tea catechins are synthesized by malonic and shikimic acid pathway (Haslam 1992). Chalcone synthase (CHS) is pivotal for biosynthesis of flavonoids in plants. The chalcone synthase/chalcone isomerase (CHI) and flavonone-3-hydroxylase (F3H) catalyses coumaroyl-CoA and caffeoyl-CoA, into dihydroflavonol which are the immediate precursor of tea flavanols. The delineation of dihydroflavonol into characteristic flavan-3-ols is attributed to enzymatic activity of anthocynidin synthase (ANS) and lucoanthocynidin reductase (LAR) (Punyasiri et al. 2004). The biosynthesis pathway of flavonoids is presented in Figure 2.4 (Winkel-Shirley 2001).
Figure 2.3 Structures of tea catechins (flavan-3-ols)
Figure 2.4 Pathway for biosynthesis of flavan-3-ols
2.3 Tea and Human Health

Tea has been regarded a medicinal herb since its discovery in China and the validation of concoction of dried tea leaves in water has attracted many scientific endeavors during the recent past (Cabrera et al. 2006; Sharma et al. 2007). Two groups of natural compounds; namely the xanthine bases (caffeine and theophylline) and catechins present in fresh green tea leaves have been of special interest. Whereas the caffeine content has been reported to be responsible for stimulating wakefulness and decreasing the sensation of fatigue; the catechins of tea have been advocated to possess health benefits (Stagg and Millin 1975; Weisburger 1997; McKay and Blumberg 2002; Pham-Huy et al. 2008). Epidemiological studies during the recent years have suggested that green tea reduces risks of several chronic diseases including cancer and cardiovascular disorders by increasing plasma antioxidant capacity in humans (Hara 1992; Nakagawa et al. 1999; Duthie et al. 2000). Numerous studies have also been reported to demonstrate green tea catechins’ anti-mutagenic and anticarcinogenic (Chung et al. 2003), insulin activity (Anderson and Polansky 2002), antimicrobial (Taylor et al. 2005), anti-oxidant (Zhao 2003) and hypocholesterolemic properties (Yang and Koo 1997). Tea catechins have also been used as additives in many food matrices such as meats, poultries, fishes and vegetable oils to impart and augment the antioxidant characteristics of these edibles (Yilmaz 2006). Antimicrobial activity of tea against a large number of pathogenic bacteria has been reported in literature (Hamilton-Miller 1995; Yam et al. 1997; Chou et al. 1999). The antioxidant and antibacterial activities of tea have been well reviewed and documented (Frei and Higdon 2003; Friedman 2007; Sajilata et al. 2008).

2.3.1 Antioxidant Attributes

Polyphenols have been reported to possess antioxidant property that provides protection from damages caused by free radical-induced oxidative stress (Chung et al. 1998; Katiyar et al. 2001; Rao et al. 2004). The antioxidant attributes of tea have been ascribed to flavan-3-ols vis-à-vis (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epigallocatechin gallate and (−)-epicatechin gallate present in fresh tea leaves (Salah et al. 1995; Cabrera et al. 2003; Anesini et al. 2008; Hu et al. 2009). Flavan-3-ols have been reported to remove endogenously generated superoxide, hydrogen peroxide, hydroxyl radicals and nitric oxide (NO) produced by various metabolic processes (Unno et al. 2000; Nakagawa and Yokozawa 2002; Tsai et al. 2007). Various mechanisms vis-à-vis depolarization of electrons, formation of intramolecular hydrogen bonds (Van Acker et al. 1996) and rearrangement of the molecular structure (Jovanovic et al. 1994; Salah et al. 1995; Sharma et al. 2007) have been proposed for antioxidant activity of tea. The higher free radical scavenging potency of green tea compared
to black tea has been attributed to the higher flavan-3-ols content of green tea (Lee et al. 2002; Karori et al. 2007). The order of free radical scavenging power has been reported to be (−)-epigallocatechin gallate > (−)-epicatechin gallate > (−)-epicatechin > (−)-epigallocatechin suggesting that catechins with galloyl moiety were more potent (Shen et al. 1993; Chen and Ho 1994). Catechin gallate esters have been reported to be more effective antioxidants than vitamin C (Rice-Evans et al. 1997). Another significant property of flavan-3-ols that has contributed to the antioxidant trait of tea is bonding between flavan-3-ols and metal ions through phenolic hydroxyl group. Flavan-3-ols have been reported to prevent oxidative reactions by chelating free ferrous ions (Figure 2.5) responsible for catalyzing the formation of reactive oxygen species (Tang et al. 2002).

![Figure 2.5 Catechin as chelator](image)

### 2.3.2 Antibacterial activity

The results of the studies conducted over last 20 years have exhibited the potential of different types of teas in the inhibiting of growth of a wide range of microorganisms (Toda et al. 1989, 1989a; Sakanaka et al. 2000; Mbata et al. 2008). 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, Serratia marcescens, Streptococcus mutans* and *Bacillus cereus* (Ahn et al. 1991; Fukai et al. 1991; Hara and Ishigami 1989; Ishigami and Hara 1993; Yam et al. 1997; Friedman 2007).

(−)-Epigallocatechin gallate and (−)-epicatechin gallate in green tea have been reported to inhibit the growth of Gram-positive and Gram-negative bacteria (Hamilton-Miller 1995; Taylor et al. 2005). Sakanaka et al. (1989) and Rasheed and Haider (1998) reported anti-cariogenic activity of tea catechins against *Streptococcus mutans* and *Streptococcus sobrinus*. Hamilton-Miller and
Shah (1999) reported that aqueous extracts of green tea (*Camellia sinensis*) caused extensive morphological changes in methicillin-resistant *Staphylococcus aureus*. (−)-Epicatechin gallate and (−)-epigallocatechin gallate were reported to be powerful antagonists of human immunodeficiency virus (HIV) (Nakane and Ono 1990). Nakayama *et al.* (1993) and Song *et al.* (2005) also reported the catechins: (−)-epigallocatechin gallate, (−)-epicatechin gallate and (−)-epigallocatechin from green tea to inhibit influenza virus through virucidal effect.

Although studies on chemical composition and quality (Sud and Bhattarcharjee 1992; Gulati and Ravindranath 1996; Sud and Baru 2000; Gulati *et al.* 2003), nutrient status (Sud *et al.* 1995; Sud and Koundal 2006) and characterization of volatile components (Rawat *et al.* 2007; Rawat and Gulati 2008) have been reported in literature, however, little work seems to have been carried out on the attributes of polyphenols from Kangra tea of Himachal Pradesh (Pal 2007, Kumar 2008; Sharma 2009; Sud *et al.* 2010). Vashist *et al.* (2004) reported that Kangra Jawala and TRI- 2026 grown under the climatic conditions of Kangra valley of Himachal Pradesh contained high levels of epigallocatechin gallate compared to North-East Indian tea. A preliminary study on antioxidant activity of Kangra tea has been carried out (Sud *et al.* 2007). The green tea shoots whose enzymatic activity had been ceased was reported to have lower IC\(_{50}\) value compared to the green tea shoots whose enzymatic activity was not ceased and made black orthodox tea. The aqueous extracts of fresh tea shoots of Kangra local jat were reported to inhibit the growth of *Escherichia coli* NCTC 10418, *Salmonella typhi*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Shigella dysenteriae*, *Acinetobacter* spp., *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* NCTC 10662, *Staphylococcus albus* and *Proteus* spp. (Sud *et al.* 2007a).

In view of the foregoing review of literature, the present study was aimed to explore the occurrence and nature of phytochemicals of nutraceutical significance from Kangra tea [*Camellia sinensis* (L) O Kuntze] of Himachal Pradesh. Variations in polyphenolic constituents of green tea shoots (two leaves and a bud, bud, first and second leaf), as affected by the changes in weather conditions during different seasons, have been investigated. Flavan-3-ols profiles of two leaves and a bud, bud, first and second leaf have been elucidated. Biological activity of Kangra tea in relation to 2,2-diphenyl-1-picrylhydrazyl free radical scavenging, ferrous ions chelating and antibacterial potential against bacterial pathogens have been explored. A correlation between the polyphenolic contents and biological activity has been established.