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# Flavan-3-Ols and biological implication of *Camellia sinensis* (L) O Kuntze

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**Abstract**
Tea, throughout its history, has been one of the most popular non-alcoholic beverages in the world. All cultivated tea is generally assigned to one species *Camellia sinensis* (L) O Kuntze, most of the tea grown in the world today is highly heterogeneous as a result of the large-scale dispersal of the tea plant during the long history of its cultivation, its out-breeding nature, and the free hybridization between geographical races. Based on the extent of fermentation the tea shoots undergo during processing, teas divided into three major groups: unfermented tea (green tea), semi-fermented tea (oolong tea) and fully fermented tea (black tea). Fresh tea leaves are rich in flavonoids - a group of phenolic compounds known as catechins. Tea is being advocated to be a functional food and an important source of dietary flavonoids. Epidemiological studies carried out during the last three decades suggested that green tea catechins have nutraceutical and therapeutic attributes. Green tea catechins have been reported to possess antimicrobial, antiallergic, anti-carcinogenic and antioxidant activities. EGCG was reported to be the most active compound with 32% potency.

**Keywords:** green tea, *Camellia sinensis*, epigallocatechin gallate, catechins

## Introduction
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) [43, 85]. 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) [34]. 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) [78].
Tea plant belongs to Theaceae family and the genus *Camellia*. Sealy (1958) proposed the classification of tea cultivars based on leaf characteristics. Wight (1962) [92] 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) [41]. 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) [44].

## 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 1, have been well documented (Hara *et al.* 1995) [33]. 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) [47].

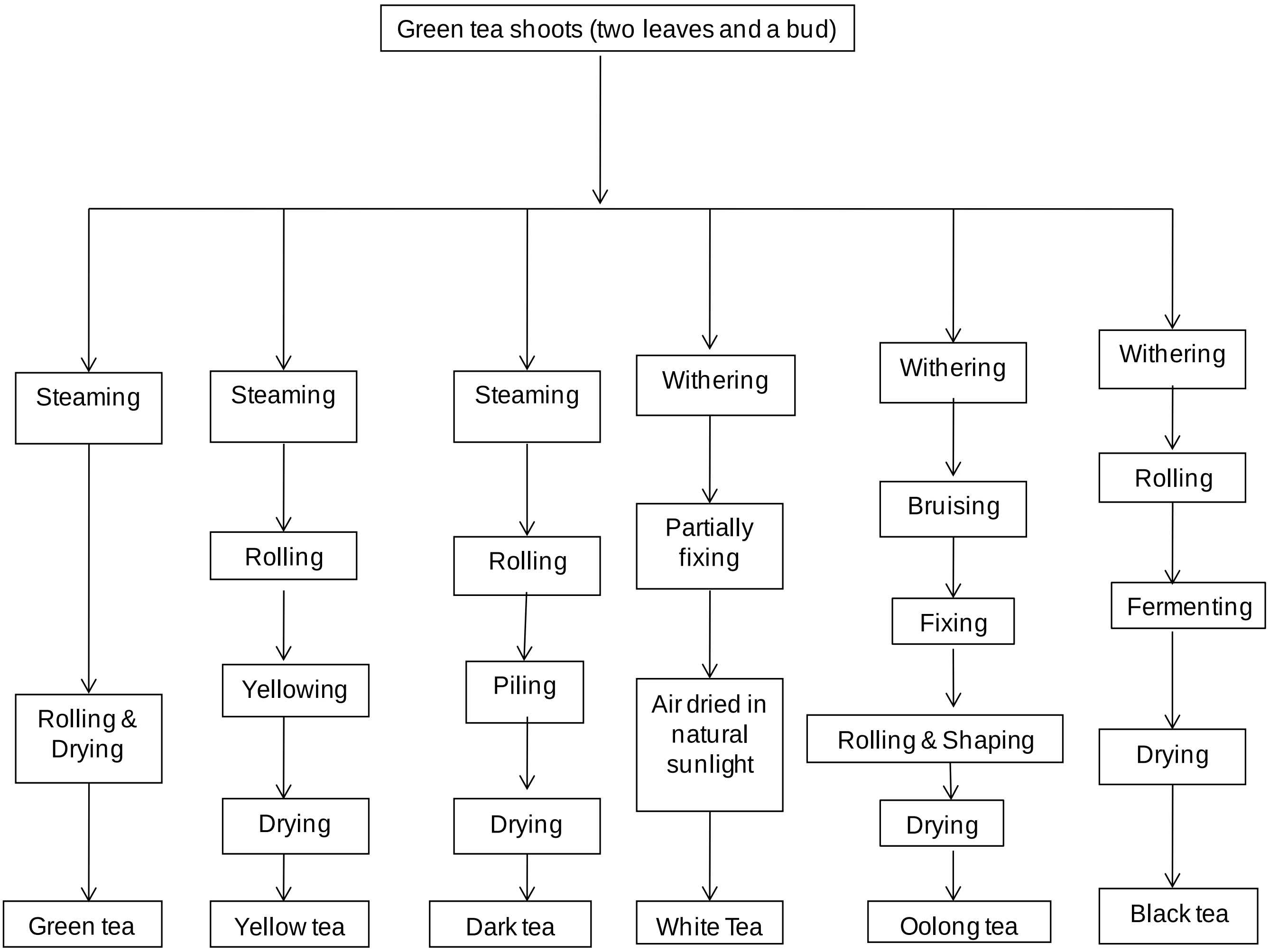



**Fig 1** Process of manufacture of different types of teas

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 theurubigins levels in oolong tea have been reported to be between unfermented green and white teas and fully fermented black tea (Peterson *et al*. 2005) [55]. 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) responsible for black tea liquor characteristics (Roberts 1958 [62]; Roberts and Williams 1958 [62]; Roberts 1962 [63]; Bhatia 1964 [5]; Sanderson and Gonzales 1971) [69]. 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) [71].

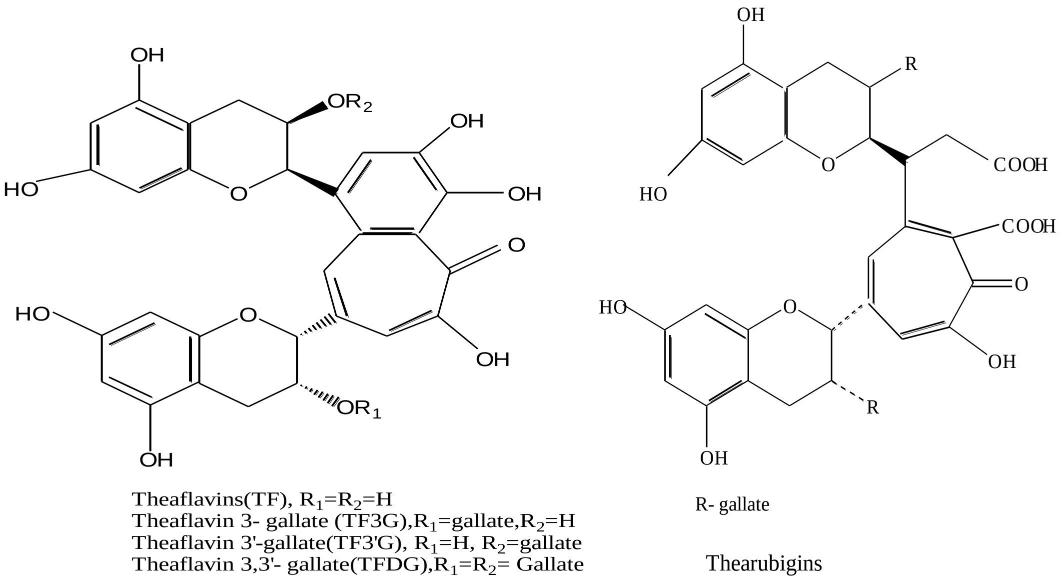


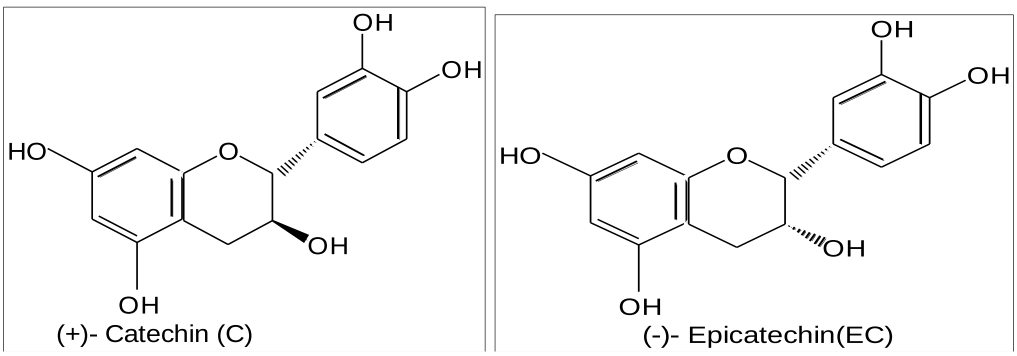

**Fig 2:** Molecular structures of theaflavins and thearubigins

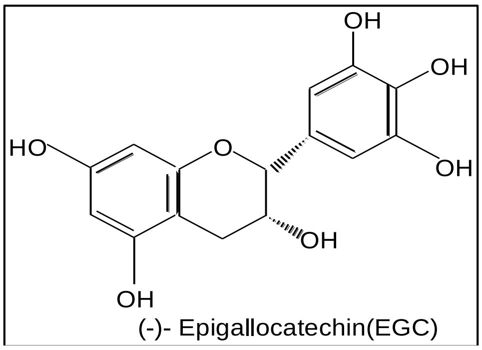
**Polyphenolic constituents**

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 [33]; Natesan and Ranganathan 1990; Fernandez *et al.* 2002) [54, 21]. 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) [15, 26].

Green tea has been reported to be a rich source of dietary flavonoids (Balentine and Paetau-Robinson 2000 [4]; Dufresne and Farnworth 2001 [18]; Sharangi 2009) [71].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) [98] are: (–) -epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), (–)-epicatechin (EC) and (+)-catechin (C) (Figure 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; Sharma *et al*., 2011) [55]. 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; Tariq and Reyal 2012). 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) [10, 11].

Tea catechins are synthesized by malonic and shikimic acid pathway (Haslam 1992) [35]. Chalcone synthase (CHS) is pivotal for biosynthesis of flavonoids in plants. The chalcone synthase/chalcone isomerase (CHI) and flavonone-3-hydrolase (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) [57]. The biosynthesis pathway of flavonoids is presented in Figure 4 (Winkel-Shirley 2001) [93].





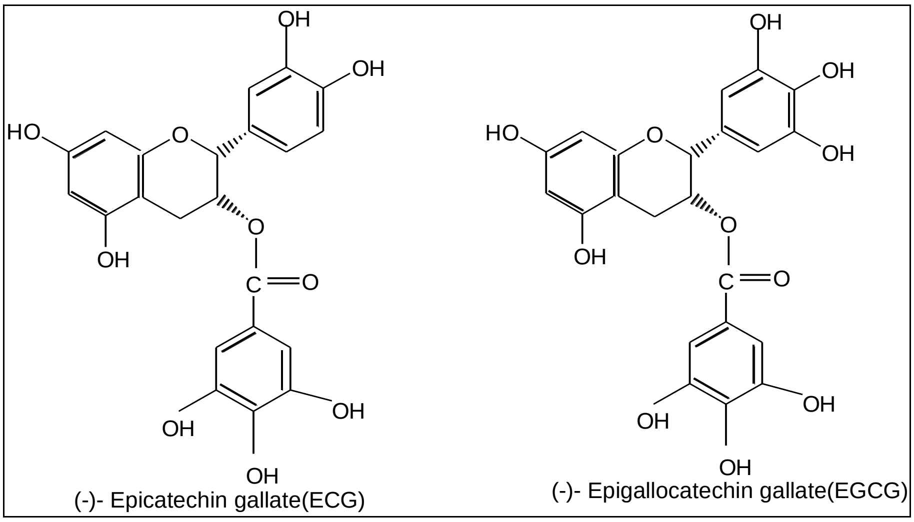




**Fig 3:** Structures of tea catechins (flavan-3-ols)

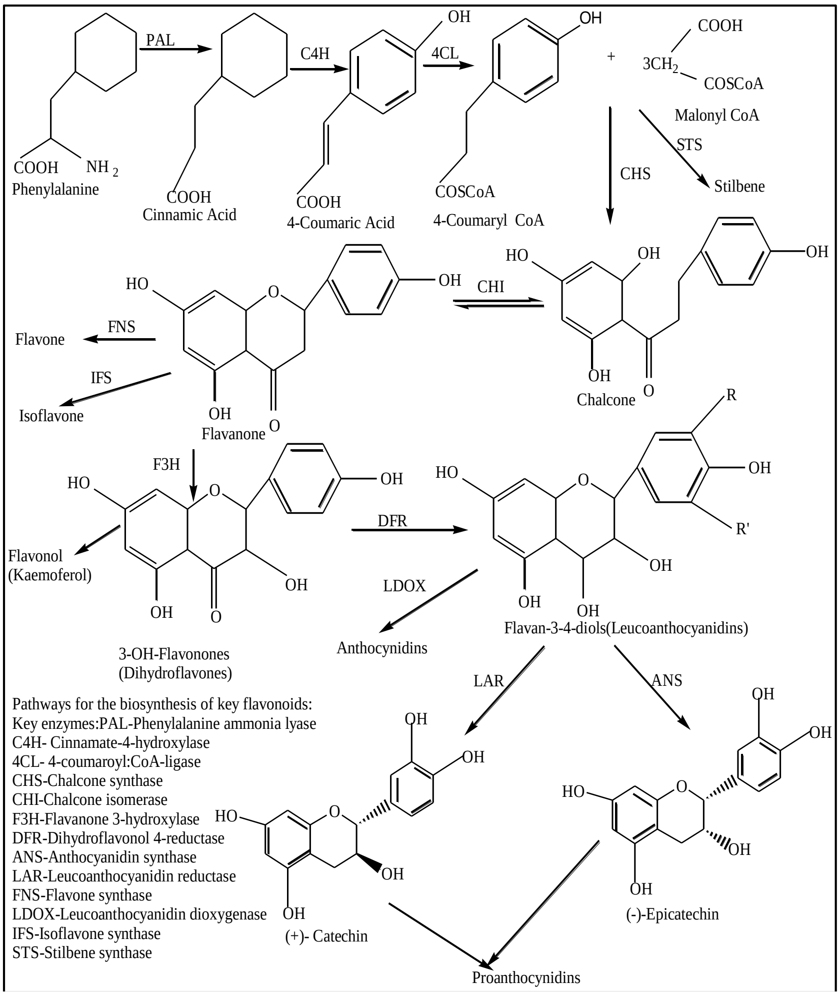



**Fig 4:** Pathway for biosynthesis of flavan-3-ols

**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) [7, 72]. 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 [76]; Weisburger 1997 [90]; McKay and Blumberg 2002 [46]; Pham-Huy *et al*. 2008 [56]; Del Rio *et al*., 2013) [17]. 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 [32]; Nakagawa *et al*. 1999 [49]; Duthie *et al*. 2000) [19]. Numerous studies have also been reported to demonstrate green tea catechins' anti-mutagenic and anticarcinogenic (Chung *et al*. 2003 [14]; Crew *et al*., 2015) [16], insulin activity (Anderson and Polansky 2002), antimicrobial (Taylor *et al*. 2005) [81], anti-oxidant (Zhao 2003) [99] and hypocholesterolemic properties (Yang and Koo 1997), cardiovascular disease (Menezes *et al*., 2017) [48]. 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) [97]. Antimicrobial activity of tea against a large number of pathogenic bacteria has been reported in literature (Hamilton-Miller 1995 [29]; Yam *et al.* 1997; Chou *et al*. 1999) [12]. The antioxidant and antibacterial activities of tea have been well reviewed and documented (Frei and Higdon 2003 [22]; Friedman 2007 [23]; Sajilata *et al.* 2008) [64].

**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) [40, 58]. 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 [29]; Cabrera *et al*. 2003 [6]; Anesini *et al*. 2008 [3]; Hu *et al.* 2009) [36]. 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 [87]; Nakagawa and Yokozawa 2002 [50]; Tsai *et al.* 2007) [84]. Various mechanisms vis-à-vis depolarization of electrons, formation of intramolecular hydrogen bonds (Van Acker *et al*. 1996*)* [88] and rearrangement of the molecular structure (Jovanovic *et al.* 1994 [38]*;* Salah *et al.* 1995 [68]; Sharma *et al.* 2007) [72] 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 [42]; Karori *et al.* 2007) [39]. 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 [74]; Chen and Ho 1994) [10]. 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 5) responsible for catalyzing the formation of reactive oxygen species (Tang *et al*. 2002).

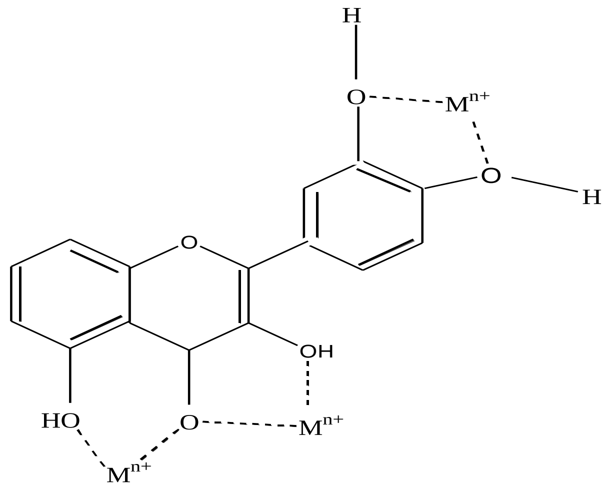



**Fig 5:** Catechin as chelator

**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 [82], 1989a [83]; Sakanaka *et al*. 2000; Mbata *et al*. 2008; Chan *et al*., 2011). 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) [23].

(−)-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 [29]; Taylor *et al*. 2005) [81]. Sakanaka *et al.* (1989) [66] and Rasheed and Haider (1998) [59] reported anti-cariogenic activity of tea catechins against *Streptococcus mutans* and *Streptococcus sobrinus*. Hamilton-Miller and Shah (1999) [30] 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) [51]. Nakayama *et al*. (1993) [52] and Song *et al.* (2005) [75] also reported the catechins: (-)-epigallocatechin gallate, (−)-epicatechin gallate and (−)-epigallocatechin from green tea to inhibit influenza virus through virucidal effect.

**Conclusion**

Tea has been consumed in India and other Asian countries since ancient times in order to maintain and improve health. Health benefits of tea drinking have been attributed to tea catechins (flavan-3-ols). It possess antioxidant, antimutagenic, antidiabetic, anti-inflammatory, antibacterial and antiviral, and above all, cancer-preventive properties. Flavan-3-ols also acts positively on neurodegenerative diseases such as Parkinson and Alzheimer disease. Tasty and inexpensive beverage as an interesting alternative to other drinks, which do not only show the beneficial effects of green tea, but are also more energetic. Taking all this into account, it would be advisable to consider the regular consumption of green tea in our daily diets.

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