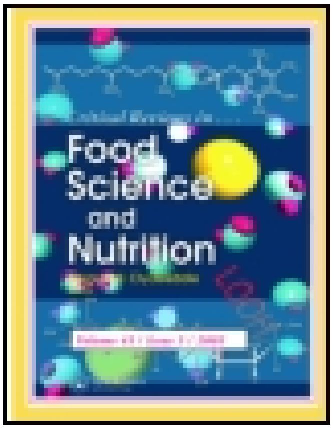

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Antimicrobial Properties of Teas and Their Extracts in vitro

Md. Wasim Siddiqui[a], A. B. Sharangi[b], J. P. Singh[a], Pran K. Thakur[c], J. F. Ayala-Zavala[d], Archana Singh[e] & R. S. Dhua[c]

[a] Department of Food Science and Technology, Bihar Agricultural University, Sabour, Bhagalpur, Bihar (813210) India

[b] Department of Spices and Plantation Crops, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal (741252) India

[c] Department of Post Harvest Technology of Horticultural Crops, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal (741252) India

[d] Centro de Investigación en Alimentación y Desarrollo, A.C. (CIAD, AC). Carretera a la Victoria Km 0.6, La Victoria. Hermosillo, Sonora (83000) México

[e] Post Gradúate Department of Botany, Government M. S. J. Post Gradúate College, Bharatpur, Rajasthan (321001) India

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Antimicrobial properties of teas and their extracts *in vitro*

Md. Wasim Siddiqui[1]*, A. B. Sharangi[2], J. P. Singh[1], Pran K. Thakur[3], J. F. Ayala-Zavala[4], Archana Singh[5], R. S. Dhua[3]

[1] Department of Food Science and Technology

Bihar Agricultural University, Sabour, Bhagalpur, Bihar (813210) India

[2] Department of Spices and Plantation Crops, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal (741252) India

[3] Department of Post Harvest Technology of Horticultural Crops, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal (741252) India

[4] Centro de Investigación en Alimentación y Desarrollo, A.C. (CIAD, AC). Carretera a la Victoria Km 0.6, La Victoria. Hermosillo, Sonora (83000) México

[5] Post Gradúate Department of Botany, Government M. S. J. Post Gradúate College, Bharatpur, Rajasthan (321001) India

*** To whom correspondence should be addressed:**

Md. Wasim Siddiqui

 ACCEPTED MANUSCRIPT

Department of Food Science and Technology

Bihar Agricultural University, Sabour, Bhagalpur, Bihar (813210) India

Email- **wasim_serene@yahoo.com**

ABSTRACT

Tea has recently received the attention of the pharmaceutical and scientific communities due to the plethora of natural therapeutic compounds. As a result, numerous researches published in a bid to validate their biological activity. Moreover, major attention has been drawn to antimicrobial activities of tea. Being rich in phenolic compounds, tea has the preventive potential of colon, esophageal, and lung cancers, as well as urinary infections and dental caries, amongst others. The venture of this review was to illustrate the emerging findings on the antimicrobial properties of different teas and tea extracts, which have been obtained from the several *in vitro* studies investigating the effects of these extracts against different microorganisms. Resistance to antimicrobial agents has become an increasingly important and urgent global problem. The extracts of tea origin as antimicrobial agents with new mechanisms of resistance would serve an alternative way of antimicrobial chemotherapy targeting the inhibition of microbial growth and the spread of antibiotic resistance with potential use in the pharmaceutical, cosmetic and food industries.

KEYWORDS: Tea, *Camellia sinensis*, antimicrobial properties, phenolic compounds, food borne pathogen.

Introduction

Produced from young leaves of *Camellia sinensis* L. (Kuntz), tea is one of the most widely consumed fluids after water. Beside the attractive flavours of teas, their popularities come from their primary and secondary antioxidant properties therefore designated as ñhealth drinkò (Owuor and Kwach, 2012; Taylerson, 2012). Tea is a native of China, spread to India and Japan, then to Europe and Russia, arriving in the New World in the late 17th century. It is cultivated in more than 30 countries worldwide. Of the total amount of tea produced and consumed in the world, 78% are black, 20% is green, and 2% are Oolong (Chan et al., 2011; Muktar and Ahmad, 2000). The finest quality green teas, in terms of flavour, are made from the bud and the next two leaves, and sometimes the bud alone, of actively growing plants (Lee et al., 2011; Song et al., 2012).

Commercially, there are three major varieties of *C. sinensis*: the China type (*C. sinensis* var. *sinensis*), the Assam types (*C. sinensis* var. *assamica*), and the hybrid type (*C. sinensis* var. *assamica* ssp *lasiocalyx*) (Owuor and Kwach, 2012). Leaf buds and young leaves are used in making tea, the age of the leaves determining the taste and the name of the particular commercial variety. On the basis of methods of processing, tea is designated as black (fermented), Oolong (semi-fermented), green (unfermented), or white (unfermented) depending on the process used, though all come from the same plant (http://www.dreamship.com/tea_origin.htm; http://www.boulderteahouse.com/abouttea.html). White tea is processed from the buds whereas the green and black teas are processed from the leaves. Oolong tea is partially fired and then steamed, thus being intermediate between green and black teas. Due to the different processing, each tea sample has a unique character, taste, and chemical profile (Rio et al., 2004; Sharangi, 2009; Wu et al., 2012; Owuor and Kwach, 2012). Black tea is consumed primarily in Western countries and in South Asian countries such as India and Sri Lanka, whereas green and Oolong teas are consumed mainly in East Asian countries such as China, Japan, and Taiwan (Taylerson, 2012).

The World Health Organization (WHO) has catalogued more than 20,000 plant species with medicinal properties providing treatments for pneumonia, ulcers, diarrhoea, bronchitis, colds, and diseases of the respiratory tract (Gonçalves et al., 2008). Medicinal and therapeutic potentialities of tea reviewed by Sharangi (2009) concluded that among the many faceted beneficial roles of teas, several major ones like antioxidant activity, antiulcer effect, anti-inflammatory effect, antimicrobial properties, anticancer properties, antimutagenic activity along with the attenuating and/or reducing effects on blood pressure, coronary heart disease (CHD) and cardiovascular disease, atherosclerosis, oxidative damage are important. Moreover, teas have been proved to enhance insulin activity, helps in treating asthma, retard cataract, maintains fluid balance, bone health and dental health, improves mean body mass index and body weight, prevents cellular DNA damage, inhibits HIV, lowers stress hormone levels etc. (Sharangi, 2009). Utilization of plant extracts as an alternative to chemical or synthetic antimicrobials and antioxidants to combat the food borne pathogens, inhibiting lipid oxidation and thus extending the shelf life is an increasing trend in the food industry (Perumalla and Hettiarachchy, 2011; Seow et al., 2013). Tea leaves contain various health promoting constituents, especially the phenolics such as catechin, epigallocatechin and epigallocatechin gallate (EGCG), have been shown to have antioxidant properties with epigallocatechin (EGC) being the strongest (Song et al., 2012; Owuor and Kwach, 2012). The phenolic compounds of tea leaves may be involved in the defence of the plants against invading pathogens including insects, bacteria, fungi, and viruses. Tea extracts have many bioactive actions such as antioxidants (Chan et al., 2011), antimutagenic (Wu et al., 2007), antibacterial (Chan et al., 2011), antiviral, antitumor (Friedman, 2007) and antifungal (Kim et al., 2011) activities. Friedman (2007) published an overview of antibacterial, antitoxin, antiviral, and antifungal activities of teas and tea flavonoids. These metabolites include phenolic compounds, the six so-called catechins, and the methyl-xanthine alkaloids caffeine, theobromine, and theophylline (Friedman, 2007). These phenolics of tea are also known for their antibacterial activity. Sakanaka et al. (2000) reported that the inhibitory action of tea phenolics towards the development and growth of spores of *Bacillus stearothermophilus*, which is a thermophilic spore-forming bacterium. The heat resistance of *B. stearother-mophilus* spores was reduced by the addition of tea phenols.

In general, antibacterial activity decreases when the extent of tea fermentation is increasing, implying stronger activity in green tea than black tea (Chan et al., 2011; Bancirova, 2010). Green tea catechins, particularly EGCG and EGC, have antibacterial activity against both Gram-positive and Gram-negative bacteria (Bancirova, 2010; Hamilton-Miller, 1995). Green tea can prevent tooth decay by inhibiting oral bacteria (Wang et al., 2000). To the best of our knowledge there is only one review published on antimicrobial properties of teas by Hamilton-Miller (1995). This review illustrates the emerging and/or updated findings on the antimicrobial properties of different teas and tea extracts, which have been obtained from the several *in vitro* studies investigating the effects of these extracts against different microorganisms.

Chemical composition of tea

C. sinensis is mainly composed of phenolic compounds (catechins and flavonoids), specially alkaloids such as caffeine and theophylline, volatile oils, polysaccharides, amino acids, lipids, vitamins, inorganic elements for instance aluminium, fluorine, manganese, among others (Sharangi, 2009; Padmini et al., 2010; Enzveiler et al., 2011). Caffeine is the main alkaloid present in tea that accounts for approximately 2% of the plant composition (Ashihara et al., 2008; Enzveiler et al. 2011). Figure 1 illustrates some of the phenolic compounds found in tea extracts.

Tea contains a group of compounds, particularly catechins, known for their antioxidant-related effects (Gradisar et al., 2007; Morley et al., 2005). The major tea catechins are (ī)-epicatechin (EC), (ī)-epigallocatechin (EGC), and (ī)-epigallocatechin gallate (EGCG). Among these, EGCG is the most luxuriant components in tea extract and the most potent chemical tested for biological activity. These phenolics may account for as much as 30% of the dry weight of fresh tea leaves (Archana and Abraham, 2011). Many compounds of tea have been identified, such as flown-3-ols, flavonols, Gallic acid, theaflavins, purine alkaloids, and amino acids (Friedman et al., 2009; Wang et al., 2010). The main flavan-3-ols in tea are (ī)-epicatechin and its gallate derivatives, which will be partly replaced by the theaflavins (theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin-3-3'-digallate) and thearubigins after enzymatic oxidation (called tea fermentation) (Wu et al., 2012; Balentine et al., 1997). Major flavonols are quercetin, kaempferol and myricetin, which not only affect the astringent taste of tea but also its colour (Hollman and Arts, 2000; Lee et al., 2008). Flavonols are usually found in tea bound to sugars as

O-glycosides, such as quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-rutinoside, and kaempferol-3-*O*-rutinoside (Aherne and OöBrien, 2002). A standardized profiling method based on high performance liquid chromatography combined with ultraviolet (UV) and mass spectrometric detection (MS) was established by Wu et al. (2012) to analyse the phenolic compounds of selected tea varieties used for manufacturing of green, black and Oolong teas. The composition and content include flavonol, flavones glycosides, phenolic acids, and purine alkaloids. Each tea variety had a unique chemical profile. They concluded that tea varieties with a high content of total catechins seem to be more suitable for fermented tea like Oolong or black tea (Wu et al., 2012). Although, both black and green teas contain similar amounts of flavonoids, their chemical structures are different. Green tea contains more catechins (simple flavonoids). Leaf oxidation during black tea processing converts these simple flavonoids to the aflavins and the arubigins (Naderi et al., 2011).

Factors affecting the chemical composition of tea

The major quality parameters that are tested in made tea include theaflavins (TF), thearubigins (TR), high polymerized substances (HPS), total liquor colour (TLC) and total soluble solids (water extract). TF has a direct correlation with the quality and price realization. Tea quality is dependent on agronomic, cultural, and processing practices. The quality affecting variables are variety, soil type, altitude, weather, and growing surroundings. The premium quality teas grow at higher elevations where low temperatures allow the leaves and buds to develop and mature at a slower rate (Owuor and Kwach, 2012). Numerous studies have been conducted to determine how levels of the chemical composition in tea vary according to variety, agricultural conditions, and processing (Khokhar and Magnusdottir, 2002; Lee and Ong, 2000; Singh et al., 1999). The biochemical pathways within the plant are climate dependent and variation in the climatic factors can change the chemical composition, which determine the quality and price of tea. Kottur et al. (2010) studied the influence of season (summer, pre-monsoon, monsoon and winter) on biochemical parameters of green tea shoots and quality parameters of black teas under Indian conditions. Accumulation of phenolic compounds was the highest during summer while amino acids and chlorophylls were higher in monsoon periods. Theaflavins, caffeine, water extract and flavour index were the highest in dry season teas while the crude fibre content was low in dry season teas. Finally, they opined that teas manufactured during dry seasons fetch higher prices.

Recently, Song et al. (2012) showed the relationship between tea leaf age, bud and first two leaves and shade levels. They estimated the relative concentrations of six major compounds of tea leaf such as L-theanine, caffeine, and the major tea catechins that are EGCG, EGC, EC, and ECG, as well as at the ferric reducing antioxidant power of bud and leaf extracts. The concentration of L-theanine and caffeine decreased as leaf age increased moving from bud to first and then second leaf, while the concentration of the four catechins increased from the bud to first and second leaves.

Generally, cooler months produced higher catechins and carotenoid compounds (Borthakur et al. 2008). These seasonal differences have been attributed to the influence of weather aspect of development and growth of individual shoots (Owuor and Kwach, 2012). Ashihara et al. (2008) verified that caffeine synthesis was more pronounced in leaves harvested during spring, due to an increased expression of the TSC1 gene that codifies caffeine synthesis.

Suteerapataranon et al. (2009) showed that the tea variety and type did not affect the caffeine content in Chiang Rai tea infusions.

The amount of processing significantly affects the concentration of bioactive molecules in the final tea. Recently, Wu et al. (2012) investigated that the compositions of catechins were lower in the tea varieties for green tea manufacturing, while the content of myricetin glycosides was the lowest in the tea variety for Oolong tea manufacturing. The content of individual phenolic compounds in the selected tea varieties is highly variable. However, the content of total catechins is proposed to be helpful to classify tea according to the future application as non-fermented green and fermented Oolong or black tea (Wu et al., 2012).

For the processing of high quality black tea, it is critical to determine and use correct fermentation time. Consequently, different methods have been evaluated to assess optimal fermentation time. The effects of variations in fermentation time and temperature with the changes in theaflavins (TF), thearubigins (TR) as well as sensory characteristics of colour liquor brightness, briskness and astringency of two black tea cultivars (Promising 100 and Chinese) were investigated by Asil et al. (2012). They concluded that TR content and total colour in black tea varied with cultivars showing higher levels in cultivar ñPromising 100ò than in cultivar ñChineseò, while TF, brightness and organoleptic properties did not differ in the two cultivars. The processing of black tea at low fermentation temperatures improved black tea quality, because low fermentation temperatures caused higher TF levels. Moreover, higher levels of TF can also be produced by reduction in fermentation time (Asil et al., 2012).

In addition, some prior studies have shown that higher shade levels seemed to increase the L- theanine and caffeine levels in tea leaves (Hirai et al., 2008; Ohta and Harada, 1996), while causing a decrease in the catechin (phenol) levels (Saijo, 1980). Natural shade not only influences the amount of sunlight received by tea leaves, but also other environmental conditions, such as ground and air temperature, humidity, and soil chemistry. These factors are also important for the actively growing tea plant and could influence leaf chemistry in different ways. In addition, shade levels may increase the L- theanine and caffeine concentration in tea leaves (Song et al., 2012; Hirai et al., 2008). Owuor and Kwach (2012) recently carried out a review on quality and yields of black tea in responses to harvesting which gives some important issues on the harvesting and other factors affecting tea quality in Kenya. The determination of the concentration of chemical composition in teas will predict the ranking of teas and indicate the geographical origins. As reviewed the chemical composition of tea can vary for pre- and postharvest factors, and these chemical constituents are responsible for the antimicrobial activity of tea extracts

Antimicrobial activities of tea extracts

In search of potent plant extracts, food grade additives that can effectively inhibit food borne pathogens and/or having antioxidant properties is a continuing challenge and an opportunity (Perumalla and Hettiarachchy, 2011). Researchers are increasingly recognizing plant remedies as a very important low-cost alternative to industrially produced antibiotics that are not available to all who need them because of their high price. Microbes like *Staphylococcus aureus, Salmonella typhi,* and *Pseudomonas aeruginosa* are the frequent pathogens of human infection.

S. aureus is an opportunistic pathogen of human skin. *S. typhi* is an enteric pathogen involved in typhoid and enteric fever pathogenicity. *P. aeruginosa* is a pathogen associated with pyogenic infection and urinary tract infection. These microorganisms are highly pathogenic and the rate of prevalence of infection caused by these microorganisms considerably increases in recent years (Archana and Abraham, 2011; Perumalla and Hettiarachchy, 2011). The antimicrobial activity of tea and tea extract is greatly affected by the nature of the test medium/ microbial strains, and this is probably the main reason for the variability of results from different centres (Taylor et al., 2009).

Treatment of the disease with modern medicine is often and generally associated with the development of side effects. Hence, the use of plant products has been increasing worldwide to lower side effects (Padmini et al. 2010). The antimicrobial properties of plant extracts are enviable tools for controlling undesirable microorganisms especially in the treatment of many infections. Previous works by Toda et al. (1989) showed that moderate daily consumption of green tea killed *Staphylococcus aureus, Vibrio parahemolyticus, Clostridium perfringens, Bacillus cereus, Pleisomonas shigelloides, etc.* Green tea contains between 30 and 40 percent of water extractable phenols, while black tea contains between 3 and 10 percent (Archana and Abraham, 2011). Recently, Chan et al. (2011) investigated minimum inhibitory dose (MID) of green teas (Sea Dyke, Ito En, and Boh), black teas (Boh Cameron Highlands and Boh Bukit Cheeding) and Herbal tea (Ho Yan Hor) extracts against gram-positive *Micrococcus luteus*, *Staphylococcus aureus*, and *Bacillus cereus*, and gram-negative. *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa* using the disc-diffusion method. They stated that extracts and fractions of all six teas showed no activity against the three gram-negative bacteria. Green

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teas inhibited all three gram-positive bacteria with *S. aureus* being the least susceptible. Black and herbal teas inhibited the growth of *M. luteus* and *B. cereus,* but not *S. aureus* (Chan et al., 2011). Gram-negative bacteria are less susceptible to antibiotics as their outer membrane of lipoproteins and lipopolysaccharides is able to regulate the access of antibacterial agents into the underlying structures (Unten et al., 1997). Outer cell membranes of gram-negative organisms have several lipid compounds that protect the cells from antimicrobial agents. Varying degrees of sensitivity of the different microorganisms may be due to both the intrinsic tolerance of microorganisms and the nature and combinations of phytochemical compounds present in the extract of the teas. Table 1 and 2 divulge the antibacterial and antifungal activities of different tea extracts, respectively.

Chosa et al. (1992) examined the antimicrobial and microbiocidal activities of tea extracts, (-) epigallocatechin gallate (EGCG) and theaflavin digallate (TF3) against Mycoplasma. Green tea and black tea presented antimicrobial activities against *M. pneumoniae*. At a concentration of 0.2%, green tea and black tea confirmed microbicidal activities against *M. pneumoniae* and *M. orale* but not against *M. salivarium*. Extracts of Pu-erh tea showed a slight microbicidal activity against *M. pneumoniae* and *M. orale*. EGCG purified from green tea and TF3 from black tea markedly showed microbicidal activities against *M. pneumonia*, *M. orale,* and *M. salivarium*. As reviewed the antimicrobial activities of tea extracts depend on the variety of evaluated tea.

Antibacterial activity of different tea extracts

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Black tea extracts. Kim et al. (2001) appraised the antibacterial potential of the methanolic extracts of *Camellia japonica* L. against food borne pathogens. The extract showed good bactericidal response against the pathogens *Salmonella typhimurium, Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*. Mughal et al. (2010) while observing antibacterial activity against *Streptococcus mutans,* demonstrated that the crude methanolic extract of black tea showed full growth of bacteria between the concentrations 0.1- 0.5 mg/ml while the moderate activity was recorded at the concentrations of 0.6- 1.0mg/ml. The crude methanolic extract of black tea, which was diluted in ethanol, showed good bactericidal activity nearly at all concentrations. Padmini et al. (2010) evaluated the antibacterial activity of the extracts of mint, tea, and mint tea (1:1) against the microorganisms such as *S. aureus, S. typhi,* and *P. aeruginosa.* Tea extract showed strong antibacterial activity against *S. aureus* with minimum inhibitory concentration at 150 L where the zone of inhibition was 27-mm. 200 L and 250 L concentration of the tea extract produced a significant antibacterial effect *S. typhi* and *P. aeruginosa* and the zone of inhibition was observed at 24.1 and 18.1 mm, respectively.

The methanolic extracts of green and black tea were examined on *Streptococcus mutans* (a dental caries causing bacteria) by Naderi et al. (2011). Five different concentrations (50, 100, 200, 300, and 400 mg/mL) were used. The black tea had an antibacterial effect on 100 to 400 mg/mL concentrations. The minimum inhibitory concentration of black tea was 50 mg/mL. The mean diameter of inhibition zone was 10.9 mm for methanolic extract of black tea. Typhoid and cholera caused due to the infection of *Salmonella enterica serovar* Typhi and *Vibrio cholerae*, respectively, are endemic in many developing countries like India. Tea extracts have an influence on these microorganisms. Tiwari et al. (2005) reported antibacterial activity of *C. sinensis* against different bacterial genera including *S. typhi*.

Turkmen et al. (2007) studied the inhibitory effect of different solvent extracts of black tea on the growth of different bacterial strains (*Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Hafnia alvei, Yersinia enterocolitica, and Bacillus cereus*). Extraction solvents (acetone, *N, N*-dimethyl- formamide (DMF), ethanol, and methanol) used as controls had no inhibitory effects on the six bacteria. All black tea extracts except for the 50 % methanol one showed varying antibacterial activity against *S. aureus*, *H. alvei* and *B. cereus*, depending on the solvent used, but no activity was shown against the other bacteria. Gram-positive bacteria were more sensitive to tea extracts than gram-negative ones.

Recently, Mandal et al. (2011) determined the zone diameter of inhibition (ZDI) and minimum inhibitory concentration (MIC) of black tea extracts against *Salmonella enterica serovar* Typhi and *Vibrio cholerae*. They reported the *S.* Typhi isolates had ZDIs of 12- 17mm, and the ZDI ranged 13-21mm for *V. cholerae* Ogawa isolates, and thus all the bacterial isolates were sensitive to tea extracts. All the *S. typhi* isolates (12; 100%) showed extract MICs 400-600µg/mL, and most of them (7; 58.33%) had MIC of 500µg/mL. Among nine *V. cholerae* Ogawa isolates, 6 (66.67%) had MICs of 400-600µg/mL, and the rest 3 (33.33%) isolates had 200-300µg/mL. Mandal et al. (2011) also demonstrated that the extract at 32 εg/mL, started to exhibit killing effect on *S. typhi*, while the extract had a similar effect on *V. cholerae* Ogawa at 64 εg/mL. The extract showed bactericidal activity at concentrations of 512 εg/mL and 256 εg/mL, respectively, against *S. typhi* and *V. cholerae* Ogawa, leaving 2.19 $\log_{10}$ cfu/mL and 2.58

log_{10} cfu/mL, respectively, after 24 hours. Michalczyk and Zawislak (2008) reported that the black tea extract had strongest inhibition activity than any other (Pu-erh and green) to against human intestinal bacteria, and the *S. enteritidis* was inhibited largely by black tea than by green tea.

Green tea extracts. The effectiveness of green teas as an antimicrobial agent is attributed to its low degree of fermentation. During the fermentation process, catechins such as EGCG are destroyed, reducing the teaœs antimicrobial properties. Phenolics of green tea are considered to scavenge free radical and to chelate metal ions, being used as natural antioxidants, antimicrobial and antiviral agents (Yilmaz, 2006). Gadang et al. (2008) demonstrated that phenolics extracted from green tea extract have inhibitory effects on gram-positive as well as gram-negative bacteria. Green tea catechins reduce the heat-resistance of the spore-forming thermophilic spoilage bacteria *Bacillus stearothermophilus* and *Clostridium hermoaceticum* that proliferate in vending machines, causing sour spoilage in milk and other drinks (Sakanaka et al., 2000). The use of phenolics or other natural antimicrobials may result in a reduction of temperatures used in thermal processing of foods. Because widely consumed well-done meat products exposed to high cooking temperatures employed to kill pathogens also induce the formation of potentially carcinogenic heterocyclic amines (Friedman, 2007).

Almajano et al. (2008) appraised antimicrobial activity of green tea and attributed such action to the presence of catechins, at 1.5% concentrations. Wu et al. (2007) reported that water extracts of different teas including green tea had antibacterial activity against *S. aureus* and *B.*

subtilis at final concentration of 2 mg/ mL but no activity against *E. coli*. Chou et al. (1999) demonstrated that dry tea flush and green tea, in particular, are capable of killing bacteria. Dry tea proved most effective against *B. subtilis, Proteus vulgaris* and *Staphylococcus aureus*, but green tea demonstrated better performance against *Escherichia coli* and *Salmonella*. Turkmen et al. (2009) demonstrated the antioxidant and antibacterial activities of methanol, ethanol and water extracts and their crude, ethyl acetate and water fractions derived from fresh tea leaves (FTL) and green tea (GT) against a number of bacteria species. In both FTL and GT, ethyl acetate fractions and the crude extracts were found to have activity against only two bacteria, namely *S. aureus* and *B. cereus*, depending on the extract type. In FTL, *S. aureus* was inhibited by both ethyl acetate fractions and crude extracts but *B. cereus* was inhibited by only ethyl acetate fractions. The stronger antibacterial activity of FTL extracts (as compared to GT extracts) is related to the phenolic contents (Türkmen et al., 2009).

Chakraborty and Chakraborti (2010) assigned antibacterial activity by measuring the ZDI and MIC at four different concentrations (10, 25, 25, and 100 mg/ mL) of the methanolic extract of green tea leaves. Fifty percent methanolic extract of leaves showed greater antibacterial activity against *B. cereus* followed by *P. aeruginosa.* The ZDI was found to increase as the concentration of the extract increased. The MIC was 100 mg/ mL for *E. coli* and 10 mg/ mL for *B. cereus.* In assessing the antibacterial capacity of EGCG against various strains of *Staphylococcus* (gram-positive cocci) and gram-negative rods including *E. coli*, *Klebsiella pneumoniae* and *Salmonella*, it was revealed that 50ï 100 εg/mL was required to inhibit growth of *Staphylococcus* growth whereas the concentrations higher than 800 εg/mL was required to inhibit gram-negative rods (Yoda et al., 2004).

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Tahir and Moeen (2011) appraised the antibacterial activity of aqueous and ethanolic extract of green tea using paper diffusion test and minimum inhibitory concentration. Water extract of *C. sinensis* produce zones of inhibition 6 to 18 mm and 8 to 27 mm, respectively against *L. acidophilus* and *S. mutans*. Ethanolic extracts of *C. sinensis* produced larger zones of inhibition 15 to 33mm and 19 to 35mm against *L. acidophilus* and *S. mutans*, respectively. The MIC for the aqueous extract against *L. acidophilus* and *S. mutans* extract was 0.9 and 0.8 mg/mL, respectively whilst for ethanolic extract it was 0.7 and 0.7 mg/mL against *L. acidophilus* and *S. mutans*, respectively.

Archana and Abraham (2011) found greater antibacterial activity in fresh green tea extracts (methanolic and aqueous) against E*. coli, Enterococcus faecalis, Staphylococcus aureus,* and *Pseudomonas aeruginosa.* The fresh green tea extract with methanol was found to have high antimicrobial activity followed by commercial green tea leaves and the least activity was found in dust tea. The Minimum inhibitory concentration of black tea is effective for *Staphylococcus aureus, Salmonella typhi and E. coli* that were found to be at higher concentrations when compared to fresh green tea leaves.

The antimicrobial activity of Iranian green and black tea tested against *Streptococcus mutans* revealed that green tea had an antibacterial effect on 100 to 400 mg/ml concentrations. The minimum inhibitory concentration of green was 150 mg/ml, respectively. The mean diameter of inhibition zone was 9.5 mm for methanolic extract of green tea (Naderi et al., 2011). Mbata et al. (2008) observed the ABA of methanolic extract of green tea against *Listeria monocytogenes* and showed that the methanolic extract had greater antibacterial property (ZDI 20.1mm) in relation to the hydrophilic extract (ZDI 10 mm). The water extract did not show inhibitory effect, while the methanolic extract had ZDI of 15mm. The MIC for the methanol and aqueous leaf extract was 0.26 and 0.68 mg/ mL, respectively.

White tea extracts. As compared to black and green teas, only few studies have been carried out on antimicrobial activity of white tea. White tea differs from others regarding the processing methods. This tea is obtained from the young leaves, even before the buds are completely opened. Recently, Enzveiler et al. (2011) investigated the antimicrobial activity of aqueous extract of white tea against different bacteria. Antimicrobial activity was verified only in samples at 5 to 20 mg/ mL concentrations, in which ZDIs were observed against *S. aureus*, with no significant differences among them. Similarly, the MIC was observed 10 mg/ mL for this type of microorganism.

Antifungal Activity of Tea extracts

Different tea compounds and tea extract exhibited variable time- and concentration-dependent fungicidal activities against several fungi (Friedman, 2007). Chakraborty and Chakraborti (2010) assigned antifungal activity against two *Aspergillus* spp. by measuring the ZDI and MIC at four different concentrations (10, 25, 25, and 100 mg/ mL) of the methanolic extract of green tea leaves. They reported no activity of both the species at any concentration. Archana and Abraham (2011) reported AFA of different tea extracts (fresh leaves, green, and black teas) against fungi such as *Fusarium sp. Aspergillus fumigates, Aspergillus niger,* and *Candida albicans.* With black tea extract, *Aspergillus niger* did not show any positive results.

Candida albicans found to be sensitive to fresh green tea extract. The fresh green tea extract with methanol had higher antimicrobial activity than aqueous extract. Hirasawa, and Takada (2004) also reported the effectiveness of green tea extracts against *Candida albicans*, but only when the pH was adjusted to 6.5.

Preparation of tea extracts

Several solvents were reported for the extraction of tea and their efficacy varies according to their nature. Mughal et al. (2010) observed that different extracts (methanolic and ethanolic) were different in their antimicrobial effectiveness depending on the extracting solvent used. Methanol was the best solvent for extracting antimicrobial substances from tea samples based on the number of organisms inhibited and the diameter of inhibitory zones produced. Archana and Abraham (2011) assessed antimicrobial (bacteria and fungus) of different teas extracted using water and methanol. The methanolic extract of fresh green tea exhibited greater antimicrobial activity. The methanol extracts of the tea produced larger ZDI against the bacteria. These observations may be attributed to green tea catechin compounds and phenolics. These compounds have been found to possess antibacterial action (Mbata et al., 2008). Tahir and Moeen (2011) appraised the antibacterial activity of water and ethanol extract of green tea against *Lactobacillus acidophilus* and *Streptococcus mutans*. They found that ethanol extracts of green tea exhibited greater antibacterial activities against *L. acidophilus* and *S. mutans* than water extracts. Ethanol is also classified as a polar solvent, although not as polar as the water. This means it is miscible in water and it will extract mostly the ionic compounds of tea. Ethanol has better dissolving capabilities than water because it has a slightly low dipole and dielectric constant than water, thus it is polar (Ayala-Zavala et al., 2011).

An interesting study by Turkmen et al. (2007) demonstrated the effect of different solvents on phenolic contents, antioxidant and antibacterial activities of black tea. The samples were extracted for 2, 8, and 18 h with absolute acetone, N, N-dimethyl- formamide (DMF), ethanol, and methanol and their 50% aqueous solutions. All of the different solvent extraction methods showed a wide range of phenol contents (0.4-114 GAE/g dry weight), depending on the solvent used and the extraction times. All the 50% solvent extracts contained higher amounts of phenolics, compared to their corresponding neat ones, after 2, 8, and 18 h of extraction. In general, increasing the extraction time from 2 to 18 h significantly increased the antibacterial activity of the extracts depending on the bacterium tested and the solvent used. Among the solvents experimented, the highest levels of phenolics after 2 h of extraction were achieved using 50% acetone, followed by 50% DMF > 50 % ethanol > 50 % methanol > DMF > methanol > ethanol å acetone, respectively (Turkmen et al., 2007).

Kim et al. (2008) evaluated the antibacterial and antifungal activities of green tea (GT) water extract and various solvent fractions [methylene chloride (MC), water, and ethyl acetate (EtOAc)]. They stated that most of the catechins were in a layer of EtOAc of the aqueous extract from the GT leaves; the caffeine shifted to the MC layer. A portion of the catechins and caffeine remained in the H_2O fraction. There are four types of catechin present in GT. Of these four catechins, EGCG has the most potent physiologic activity, accounting for 30.43 mg/g of the GTE, 114.73 mg/g of the EtOAc fraction, and 11.32 mg/g of the H_2O fraction. The caffeine was concentrated at 936.72 mg/g in the MC fraction. The anti-microbial tests showed that the GTE and EtOAc fraction of the GT exerted strong growth inhibition of *P. mirabilis* (Gram-negative) and *S. pyogenes* (Gram-positive bacteria); the H_2O fraction was much less effective. On the other hand, the MC fraction of the GT had no anti-microbial activity against these pathogens compared with other fractions (Kim et al., 2008).

Hot water has long been used for extraction since it is the traditional way of brewing tea and some studies have shown it to be an efficient way of extracting tea. In the extraction of green tea, the yield of hot water extracts was significantly higher than methanol and ethyl acetate extracts (Farhoosh et al., 2007; Nkubana and He, 2008). Chan et al. (2011) extracted black, green, and herbal tea samples using hot water and reported that the yield of green tea extract obtained by the hot water extraction (42%) was higher than methanol (41%) and ethyl acetate (25%) extractions. Hassas-Roudsari et al. (2009) reported that higher temperatures reduce the polarity of water, thus increasing its extraction efficiency and capability to dissolve less polar compounds. Raising the temperature of the water also reduces its surface tension and viscosity, which increases the diffusion rate and the rate of mass transfer during extraction (Chan et al., 2011).

Antimicrobial mechanisms of tea extracts

Antimicrobial mode of action of plant extract is attributable to their phenolic compounds present. As a defensive function in response to microbial infection, plants synthesize phenolic compounds (Ayala-Zavala et al. 2011). It is therefore possible that they can act as effective antimicrobial substances against a wide array of microorganisms. The biologically active molecules of plant extract are pondered as antimicrobial agents. However, the antimicrobial activity of plant extracts depends on not only phenolic compounds, but also the property is donated by the presence of different secondary metabolites like hydroxyl groups of the active constituents (Siddiqui et al., 2012; Ayala-Zavala et al. 2011). Phenols as catechin act on different bacterial strains belonging to different species (*Escherichia coli*, *Bordetella bronchiseptica*, *Serratia marcescens*, *Klebsiella pneumonie*, *Salmonella choleraesis*, *Pseudomonas aeruginosa*, *Staphilococcus aureus*, and *Bacillus subtilis*) by generating hydrogen peroxide and by altering the permeability of the microbial membrane (Ferrazzano et al., 2011). This may also be attributable to the ability of these substances to bind to bacterial adhesions and by doing so they disturb the availability of receptors on the surface (Padmini et al., 2010). Some reports indicated that active constituents might attack the cell wall and cell membrane, thereby destroying their permeability barrier and causing the release of intracellular constituents like ribose and sodium glutamate. In addition, they interfere with electron transport, nutrient uptake, protein, and nucleic acid synthesis and enzyme activity leading to the inhibition of bacterial growth (Zhang et al., 2006).

The inhibitory effects of tea as other plant extracts against microorganism might be related to the role of mineral contents, active and effective phytochemical constituents. The presence of micronutrients and biologically active constituents in plant extract usually interfere with growth and metabolism as well as destruction of microorganisms (Padmini et al. 2010). Phenolic compounds also enhance the antimicrobial activity by increasing the retention of certain minerals like Cu, Mg, Zn, and Fe (Padmini et al. 2010).

Schelz et al. (2010) opined that the antimicrobial effect of tea is based on the denaturation of membrane proteins, resulting in the outer membrane disruption, subsequent K^+ leakage, respiration inhibition, and cell lysis. The phenolic extracts are also able to inhibit the synthesis of DNA, RNA, proteins, and polysaccharides in fungal and bacterial cells. Microorganisms stressed by exposure to phenols up regulate proteins related to defensive mechanisms, which look after cells as simultaneously down regulating various metabolic and biosynthetic proteins involved, for example, in amino acid and protein synthesis as well as phospholipid, carbon, and energy metabolism (Hu et al., 2010; Ferrazzano et al., 2011). Furthermore, phenolics interfere with bacterial quorum sensing, *i.e.*, the fabrication of small signal molecules by bacterial cells of *Escherichia coli*, *Pseudomonas putida* and *Burkholderia cepacia*, which set off the exponential growth of a bacterial population (Hubert et al., 2003).

Synergistic antimicrobial activities amongst tea extracts and other antibiotics

The synergistic antimicrobial activity of tea and antibiotics against enteropathogens are effective. The combined use of tea and antibiotics could be useful in fighting emerging drug-resistance problem especially among enteropathogens (Archana and Abraham, 2011). Yanagawa et al. (2003) observed additive effects of the antibiotic amoxicillin and EGCG against nonresistant and anti- biotic-resistant clinical isolates of *Helicobacter pylori*. The combined activity of tea leaves and antibiotic chlorophenicol was found to be effective in green tea leaves followed by commercial green tea leaves and dust tea. Combinations of green tea with butylated hydroxyanisole were more efficient against bacteria and fungi than green tea alone (Simonetti et al., 2004). Synergistic activity is effective for specific organisms like *E. coli, E. faecalis, Aspergillus fumigatus* (Archana and Abraham, 2011). Su et al. (2008) reported that green tea extract in combination with probiotics significantly reduced the viable count of both pathogens at 4 h and 24 h intervals that had completely abolished the recovery of viable *Staphylococcus aureus* and *Streptococcus pyogenes*.

Tiwari et al. (2005) studied that the synergistic antimicrobial activity of tea and antibiotics against enteropathogens. Antimicrobial activity of boiled water tea extracts and organic solvent extract were studied against *Salmonella typhimurium*, *S. typhi*, *S. typhi Ty2a, Shigella dysenteriae, Yersinia enterocolitica*, and *Escherichia coli* determining minimum inhibitory concentration, minimum bactericidal concentration and death rate kinetics at MBC of tea extract in the presence of sub inhibitory concentration of antibiotic. Black Tea extracts effectively inhibited the growth of *S. typhimurium, S. typhi, S. typhi Ty2a, S. dysenteriae, Y. enterocolitica, and E. coli.* Based on death rate kinetics results, *S. typhi Ty2a* appeared to be highly sensitive and *Y. enterocolitica* the most resistant. Chloramphenicol and tea extract in combination inhibited the growth of *S. dysenteriae* at 2.5 εg/mL chloramphenicol (MIC 5 εg/mL) and 5.094 mg/mL Black Tea extract (MIC 9.089 mg/mL). Tea extract showed synergistic activity with chloramphenicol and other antibiotics like gentamycin, methicillin and nalidixic acid against test strains.

Mughal et al. (2010) observed synergistic activity of black tea extract against bacterial strain (*Streptococcus mutans*) by taking equal amounts (0.1mg/ml) of antibiotics (chloramphenicol, tetracycline, levofloxacin, and gentamycin). The crude methanolic extract when mixed with tetracycline showed moderate results in the concentrations of 0.5-1.0 mg/mL but it gave poor results in other concentrations. The synergistic activity of extract and levofloxcin diluted in ethanol showed the best activity against *S.mutans* at all concentrations. The synergistic combination showed better result as compared to the black tea alone.

Conclusions and future directions

Different *in vitro* studies provide evidence that the tea extracts are potentially a rich source of antimicrobial agent against several microorganisms with the bioactivity attributed by their phytochemical constituents and mineral contents. The antimicrobial activity may also be attributed to a synergistic action of phytotherapeutic composition or secondary metabolites. All the extracts (black, white, and green) of tea showed significant antimicrobial effects along with other beneficiary effects like free radical scavenging property and anti-atherogenic property. The current review furnishes credence to their ethnic-pharmacological use as a remedy to treat infections and diseases caused by the microorganism. Many of us would agree with the ancient Chinese saying: ñBetter to be deprived of food for three days, than of tea for one".

The analysed information suggests that the combined use of catechins and antifungal drugs may be useful in the treatment of super-infections of the oral cavities, intestine, and vagina resulting from over consumption of antibiotics. The available information provides a basis for future studies on the potential of the tea compounds to inactivate bacteria in liquid and solid foods and to protect humans against infectious diseases. Precise screening of the compounds may

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lead to the identification of contents that are sufficiently potent to be useful as antibacterial, antifungal, or antiviral chemotherapeutic. In addition to the structural alteration of weak and moderately active antimicrobial flavonoids, investigation into the mechanisms of action of these compounds is likely to be a productive area of research. Such information may assist in the optimization of a lead compoundôs activity, provide a focus for toxicological attention, and aid in the anticipation of resistance.

As future directions, tea extracts must be explored as a natural source of antimicrobial compounds with potential use as food additives. The applications of tea extracts may be contemplated to extend the shelf life of food decayed by microorganisms, however, the collateral effects that its addition may cause on the quality of the treated food must also be considered. Some of the collateral effects could be noticed in the flavour, colour, and therefore on the consumer acceptability of the treated food. Moreover, the possible generation of functional food when the addition of teat extracts occurs to food matrices could be an option. For instance, green tea infusion could be a potential natural preservative that could be used to extend the shelf life of fruit juices by improving their microbiological safety. The applied aspects of tea, extracts treatments could be considered to optimize the antimicrobial efficacy of the extracts and offer to consumer products treated with natural preservatives.

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http://www.dreamship.com/tea_origin.htm

http://www.boulderteahouse.com/abouttea.html

Table 1. Antibacterial activity of various extracts of tea

Country of study	Type of tea	Effective against	Test method	Extraction/ solvent used	Reference
Pakistan	Black Green	*Streptococcus mutans*	Agar well diffusion method	Methanol Ethanol	Mughal et al. (2010)
India	Black	*Staphylococcus aureus* *Salmonella typhi* *Pseudomonas aeruginosa*	Agar-gel diffusion inhibition test	Water	Padmini et al. (2010)
India	Black	*Salmonella enterica* *Vibrio cholera*	Agar diffusion technique Agar dilution technique	Ethanol	Mandal et al. (2011)
China	Green	*Listeria monocytogenes*	Disc diffusion method Agar diffusion method Microbroth dilution techniques	Methanol Water	Mbata et al. (2008)
Brazil	White	*Staphylococcus aureus* *Escherichia coli* *Pseudomonas aeruginosa*	Disk-agar diffusion assay	Water	Enzveiler et al. (2011)
Turkey	Fresh leaves Green	*Staphylococcus aureus* *Listeria monocytogenes* *Hafnia alvei* *Salmonella enteric* *Escherichia coli* *Bacillus cereus*	Disc diffusion method	Methanol Ethanol Water	Türkmen et al. (2009)
India	Green tea leaves	*Staphylococcus aureus* *Escherichia coli* *Bacillus cereus* *Pseudomonas aeruginosa*	Agar well diffusion method Paper disc diffusion method	Methanol	Chakraborty and Chakraborti (2010)
Korea	Green	*Proteus mirabilis* *Streptococcus pyogenes*	Paper disc method	Water	Kim et al. (2008)
Taiwan	pu-erh tea	*Staphylcoccus aureus,*	--	Water	Wu et al. (2007)

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Malaysia	Black Green Herbal	*Bacillus subtilis* *Escherchia coli* *Micrococcus luteus* *Staphy lococcus aureus* *Bacillus cereus* *Escherichia coli* *Salmonella typhi* *Pseudomonas aeruginosa*	Disc-diffusion method	Hot water	Chan et al. (2011)
India	Fresh tea leaves Green tea Black tea	*Escherichia coli* *Enterococcus faecalis* *Salmonella typhi* *Streptococcus aureus* *Pseudomonas aeruginosa* *Vibrio cholerae*	Agar-gel diffusion inhibition test paper disk diffusion inhibition test	Water Methanol	Archana and Abraham (2011)
Iran	Green Black	*Streptococcus mutans*	Agar dilution method	Methanol	Naderi et al. (2011)
Turkey	Black	*Staphylococcus aureus* *Listeria monocytogenes* *Escherichia coli* *Hafnia alvei* *Yersinia enterocolitica* *Bacillus cereus*	Disc-diffusion method	Methanol Ethanol *N, N*-dimethylformamide Acetone	Turkmen et al. (2007)
Pakistan	Green	*Lactobacillus acidophilus* *Streptococcus mutans*	Paper disc diffusion inhibition test	Water Ethanol	Tahir and Moeen (2011)

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Table 2. Antifungal activity of various extracts of tea

Country of study	Type of tea	Effective against	Test method	Extraction/ solvent used	Reference
Korea	Green	*Candida albicans*	Paper disc method	Water	Kim et al. (2008)
India	Green tea leaves	*Aspergillus parasiticus* *Aspergillus flavus*	Agar well diffusion method Paper disc diffusion method	Methanol	Chakraborty and Chakraborti (2010)
India	Fresh tea leaves Green tea Black tea	*Fusarium sp.* *Aspergillus fumigates* *Aspergillus niger* *Candida albicans*	Agar-gel diffusion inhibition test Paper disk diffusion inhibition test	Water Methanol	Archana and Abraham (2011)

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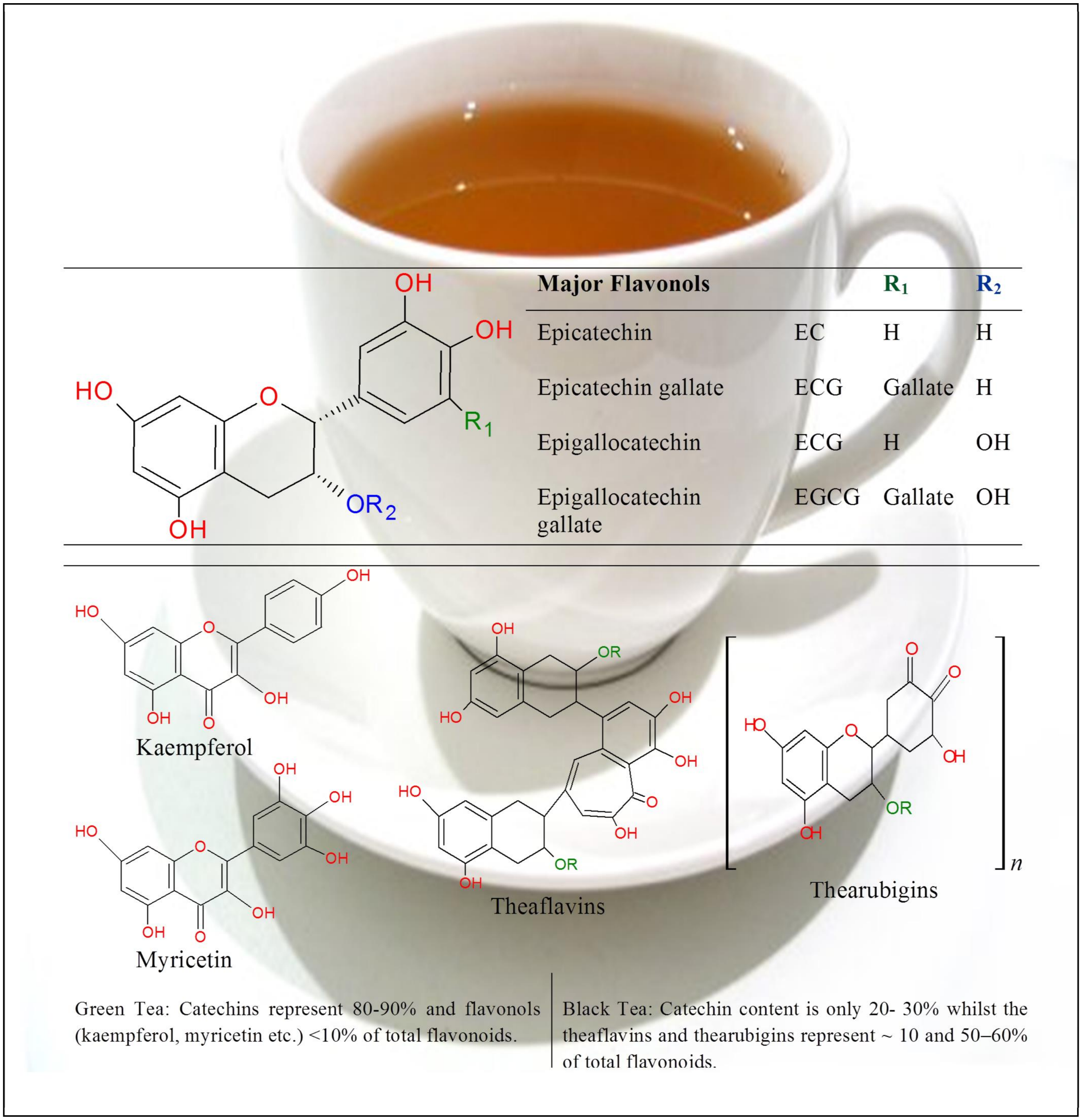
Figure 1. Polyphenolic compounds found in green and black tea extracts



Major Flavonols		R_1	R_2
Epicatechin	EC	H	H
Epicatechin gallate	ECG	Gallate	H
Epigallocatechin	ECG	H	OH
Epigallocatechin gallate	EGCG	Gallate	OH

Green Tea: Catechins represent 80-90% and flavonols (kaempferol, myricetin etc.) <10% of total flavonoids.

Black Tea: Catechin content is only 20- 30% whilst the theaflavins and thearubigins represent ~ 10 and 50–60% of total flavonoids.