TEA POLYPHENOLS – THEIR ANTIOXIDANT PROPERTIES AND BIOLOGICAL ACTIVITY – A REVIEW

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Tea is the most widely consumed beverage in the world. Tea leaves are a source of polyphenols, especially catechins, a decisive group for their antioxidative activity. This paper reviews what is known of green tea species, its leaves processing and changes occurring in tea components. The main green tea polyphenols are catechins: (±)-catechin C, (-)-epicatechin EC, (+)-gallocatechin GC, (-)-epigallocatechin EGC, (-)-epicatechin gallate ECG, and (-)-epigallocatechin gallate EGCG. Antioxidant and antimicrobial activity of these compounds and crude extracts of teas are described. The review highlights also the potentials of green tea, its health benefits in terms of antimutagenic and anticarcinogenic properties as well as protective agents against cardiovascular diseases.

INTRODUCTION

Chemical compounds that are naturally occurring dietary components and their influence on human body are more and more interesting for scientists. Polyphenols, biologically active compounds of plant origin, are classified as non–nutrients. An average daily intake of polyphenols with the diet is approximately 1 gram, which can be important in fighting free radicals activity in human body [Scalbert & Williamson, 2000]. Recent research have shown the antioxidant potential of food constituents. Numerous epidemiological studies have indicated that food and beverages rich in polyphenols are an important factor in preventing diseases, decreasing mortality from cardiovascular and neoplastic diseases, and slowing down the aging processes [Di Carlo et al., 1999]. Green tea contains relatively large amounts of polyphenols, mainly catechins and their derivatives, considered to exert a protective effect against cancer and cardiovascular diseases. As tea is one of the most popular beverages, it could be a tremendously important source of polyphenolic constituents.

TEA’S PROFILE

Tea is an evergreen shrub or tree from Theaceae family, species Camellia. Its leaves are dark green and shiny, opposite and round, flowers are large, white, pink or red and fruits small and brown. Two basic botanical varieties include: Chinese tea’s shrub (Camellia sinensis) as well as Indian tea tree (Camelia assamica) [Balentine et al., 1997; Chu & Juneja, 1997]. Tea is grown in a number of countries, however mainly in China, India, Japan and on Ceylon [Fernandez et al., 2002]. Harvesting lasts throughout the year yet its time influences the quality of tea leaves. The best teas are from the spring collection as then they are delicate and the most aromatic, top grade teas, however, are gained from undeveloped leaves and young leaflets of top twigs, which, further prepared, show suitable gustatory features and special aroma [Chu & Juneja, 1997]. According to Chinese mythology, a tea plant was discovered thousands years ago in South-East Asia. Initially tea leaf infusion has been considered for medicine, later on it became the most popular beverage all over the world. Tea market is very rich. Over three hundred tea species are produced. The basic classification includes three categories of teas: green, oolong and black [Harbowy & Balentine, 1997]. According to The Tea Council, the world production of tea in 1998 was 2.96×10⁶ tones, and US Food and Drug Administration claims that in 1999 it was 2.87×10⁶ tones. In the annual output about 20% is constituted by green tea, and over 70% – by black tea. According to Mukhtar and Ahmad [1999], at present the average tea consumption reaches about 120 mL/day/person. The preferences in tea consumption are different in various regions of the world. Most Americans and Europeans prefer black tea; the Japanese and Northern China inhabitants prefer green tea. Oolong tea is mainly consumed in Taiwan and Southern China.

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TECHNOLOGICAL PROCESSING OF TEA LEAVES – THE TEA TYPES

Processed tea leaves were classified into three types, in respect of different fermentation degree: non-fermented (green), semi-fermented (oolong) as well as entirely fermented (black) [Chu & Juneja, 1997]. The process of tea production depending on its kind consists of four basic stages. Collected shrub or tree tea leaves undergo partial withering. Then the leaves are roasted to inactivate oxidative enzymes, rolled up, dried and sorted. The colour of the final product tea is green, its taste is gentle and constraining. In China, the taste of green tea is improved by the addition of jasmine flowers. The process of black tea production is more complicated. The leaves are also subjected to withering during which the first fermentation processes occur. Next, they are rolled up and dried. Fermented leaves are roasted, until the dark-brown or black colour, thereafter a suitable aroma appears. Blocking the activity of enzymes (polyphenol oxidase and glycosidase) as well as stopping the fermentation is the aim of roasting [Chu & Juneja, 1997; Lin et al., 1998a]. Semi fermented tea – oolong – is partially fermented tea with considerably shorter fermentation time than the black one [Balentine et al., 1997].

TEA POLYPHENOLS

Tea leaves are, apart from wine, fruit and vegetables, a very good source of polyphenolic compounds [Ho et al., 1997]. Green tea leaves consist of flavonoids as well as phenolic acids which can make up to 30% of fresh leaves dry weight and only 10% of dry weight of black tea [Wang et al., 2000]. Those constituents are referred to as bioflavonoids or vitamin P, as they show a wide spectrum of biological effects in living organisms [Dreosti, 1996]. The most important polyphenol groups tea leaves are substances whose nomenclature is still a divergence. These compounds are often referred to as flavan-3-ols or catechins [Balentine, 1997]. Tea leaves have been reported to be the only food product containing epigallocatechin gallate (EGCG) [Chu & Juneja, 1997].

In tea leaves three basic polyphenol groups can be distinguished: catechin, theaflavins and thearubigens [Yanishlieva-Maslarowa & Heinonen, 2001]. Both green and black tea contains a similar quantity of flavonoids, differing in respect of their chemical structures. Green tea is characterised by a higher content of simple flavonoids – catechins which become transformed to more complex compounds (theaflavins and thearubigens) during the fermentation of tea leaves [Balentine et al., 1997]. Other products of polyphenol oxidation are tannins. Tannins were divided into two groups: hydrolysable, water-soluble, including simple phenolic acids, gallic acid esterified into the polyols, and nonhydrolysable tannins (condensed), the polymers of elementary flavonoids particles [Chung et al., 1998].

Catechins are flavan derivatives and in a group of flavonoids they are distinguished by the highest oxidation degree of heterocyclic ring and by good solubility in water. In tea leaves, they appear mainly in the form of gallic acid esters. The main catechins of green tea are: (+)-catechin (C), (-)-epicatechin (EC), (-)-gallocatechin (GC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG) [Ninomiya et al., 1997]. The structural formula of catechin was introduced in Figure 1.

The presence of methylated catechins was reported by Saio et al. [1982] and Sano et al. [1999]. According to mass spectrometry and nuclear magnetic resonance two catechins derivatives were identified as (-)-epigallocatechin-3-(3-O-methylgallate) and as (-)-epigallocatechin-3-(4-O-methylgallate) [Amarowicz & Shahidi, 2003]. The process of black tea containing oolong is mainly an addition of jasmine flowers. The process of black tea production is more complicated. The leaves are also subjected to withering during which the first fermentation processes occur. Next, they are rolled up and dried. Fermented leaves are roasted, until the dark-brown or black colour, thereafter a suitable aroma appears. Blocking the activity of enzymes (polyphenol oxidase and glycosidase) as well as stopping the fermentation is the aim of roasting [Chu & Juneja, 1997; Lin et al., 1998a]. Semi fermented tea – oolong – is partially fermented tea with considerably shorter fermentation time than the black one [Balentine et al., 1997].

The content of proanthocyanidins in green tea is at least 10 times lower than catechins [Hashimoto et al., 1992]. Four dimeric proanthocyanidin gallates (prodelphinidin B-2 3′-O-gallate, procyanidins B-2 3,3′-di-O-gallate, B-2 3′-O-gallate, and B-4 3′-O-gallate) and two dimeric flavan-3-ol gallates in which two flavanol units are linked at the B-ring have been reported by Nonaka et al. [1984]. At least 12 proanthocyanidins were identified in green tea samples by means of HPLC-MS and HPLC-DAD [Kiehne et al., 1997]. Among them EGC-EGCG, EC-EC-EC, EC-EGC, EGC-EGC, EGCG-EGCG, EC-EGC, EGCG-ECG, ECG-EGCG, ECG-ECG were present.

EGCG is an extremely active compound with eight -OH groups determining its high antioxidant activity. Investigations have shown that green tea contains a larger quantity of catechins than the black one [Khokhar & Magnusdottir, 2002]. In contrast, black tea contains a larger quantity of gallic acid, which is likely to be released during fermentation from catechin gallates [Halder et al., 1998]. The content of catechins in tea leaves is also a result of multiple factors, leaf age, because tea produced from young leaves contains considerably larger quantities of these compounds. The content of EC, EGC, EGCG, and ECG in eight commercial green teas determined by Price and Spitzer [1993] reached 0.87–2.0%, 2.75–5.44%, 2.19–3.95%, 0.56–0.91%, respectively (results in % w/w). Mukhtar and Ahmad [1999] studied the polyphenolic composition of tea leaves infusion. They showed that the cup of green tea infusion contained about 400 mg of polyphenolic antioxidants, of which 200 mg was EGCG. On the contrary, while Graham [1992] reported, that a cup of green tea infusion might contain about 90 mg of EGCG. On the grounds of the above-mentioned data it is possible to believe that many factors influence the composition of tea leaves polyphenols as well as prepared infusion.

It was observed that the content of catechins in tea leaves is closely correlated with the quality of infusion. The highest content of catechins was noted in teas produced from young tea leaves [Thanaraj & Seshardi, 1990].

CHANGES OF TEA POLYPHENOLS DURING PROCESSING

During the fermentation of tea leaves the monomeric catechins are subject to the action of polyphenol oxidase [Halder et al., 1998]. The activity of this enzyme causes oxidation of catechins to quinones which further undergo poly-
Tea polyphenols activity

merization to bisflavans and to more complex structures of theaflavins, thearubigens and higher molecular mass compounds [Lin et al., 1998a; Bailey & Nursten, 1993]. Theaflavins and their gallates, appear in black tea due to oxidative condensation between (-)-epicatechin and (-)-epigallocatechin [Tanaka et al., 2002]. Gradual lowering of flavanol content is another consequence of tea leaves fermentation process [Sava et al., 2001]. Different investigations

FIGURE 1. Chemical structure of green tea catechin.
have indicated that the process of fermentation influences the level of catechins in tea leaves, however the content of alkaloids becomes altered to a small extent [Schulz et al., 1999]. The degree of tea fermentation was assumed as 0 to 85% (0% – green, 85% – black). On the grounds of the conducted analyses it was found that a catechin level (EGCG, EGC, ECG, EC, C and GCG) lowers gradually during fermentation, however, the level of gallic acid grows. The levels of theaflavins and thearubigens also decrease gradually, while the level of caffeine increases in the process of fermentation from 8.69 to 16.03 mg/100 mg of leaf dry weight [Lin et al., 1998a]. Transformations of polyphenols can be due to physical and chemical factors (temperature, oxygenation) or they can be the result of specific hydrolyzing enzymes affecting the phenolic compounds (β-glycosidase) and oxidizing these compounds (polyphenol oxidase) [Halder et al., 1998]. In fermented leaves of tea, the activity of oxidative enzymes leads to the transformation of tannins and catechins to brown-black products. Some of these products show affinity to melanin [Sava et al., 2001]. After obtaining suitable qualitative features tea leaves are dried to inhibit further oxygenation reactions. The inactivation of enzymes is the indispensable factor for product’s stability during storage [Dougan et al., 1979; Temple et al., 2001].

FACTORS INFLUENCING CATECHIN CONTENTS IN TEA LEAVES EXTRACTS

Chen et al. [1996a] studied the influence of temperature and time of extraction on the content of polyphenols in oolong tea. They stated that the rise of catechin content was proportional to an increase in temperature and time. The highest quantity of catechins was extracted at a temperature of 77–80°C [Khoskhar & Magnusdottir, 2002]. In other investigations it was found that high temperature of steam and tea leaves infusion (120°C, 30 min) causes epimerization of C, EGCG, ECG, EGC and EC which undergo conversion to suitable epimers: (+)-gallocatechin gallate (GCG), (+)-catechin gallate (CG), (+)-gallocatechin (GC) as well as (-)-catechin [Chen et al., 2001]. During investigations on catechin migration to infusion it was noticed that the smaller flavonoids molecules: EC and EGC were extracted more quickly than EGCG and ECG. The efficiency of a hot aqueous extraction for catechins from green tea was not dissimilar to that for major flavour and colour components from black tea [Price & Spitzer, 1994]. The results of investigations show that with an increase in the pH of tea leaves infusion, its chemical composition changes considerably. A high decrease in theaflavins content was noted (from 30.7% in pH 4.9 to undetectable at pH 9.45). Secondly, the EGCG, ECG and EGC were reduced by about 97; 45.5 and 3.0%, respectively. However, the content of caffeine, gallic acid and epicatechin was observed to increase. The increase in epicatechin content may result from disintegration or separation from ECG and EGCG. The rise in caffeine content is linked with disintegration of the caffeine-theaflavins aggregate, as the theaflavins are degraded in alkaline medium [Sprio et al., 1987; Liang & Xu, 2001]. Chen et al. [1998, 2001] analysed the influence of pH on the quantity of catechins in green tea infusion. The results suggest that the rise in medium pH causes the increase in catechins degradation. In alkaline medium catechins were unstable and almost totally disintegrated in several minutes, while at pH<4 they showed large stability [Zhu et al., 1997; Friedman & Jurgens, 2000]. Their results are consistent with investigations of catechin stability in canned tea drinks. Moreover, ascorbic acid was shown to display better protective properties than citric acid. This is possibly due to the reducing properties of ascorbic acid protecting catechins and also lowering the concentration of dissolved oxygen in a solution, which can contribute to the rate of catechins decrease by the oxygenation process. It is believed that green tea catechins undergo degradation during the production and storage of drinks. Heating these drinks at a temperature of 98°C for 15 min, caused a decrease in catechin content by about 1–15%, the subsequent 6 h of heating caused a 5% decrease. Heating at 120°C for 20 min caused 23% degradation of catechins. Catechin content was also studied in the tea leaves infusions prepared several times. It decreased with every next infusion of tea leaves. Flavonols, however, were considerably more slowly released from tea leaves and their content decrease was lower [Wang et al., 2000].

ANTIOXIDANT ACTIVITY OF TEA POLYPHENOLS

Results of numerous investigations have confirmed that flavonols isolated from green, black and red tea leaves possess very strong antioxidant proprieties (Table 1). Vastag [1998] found that a cup of tea leaves infusion is “the injection of antioxidants” showing a higher capability for scavenging free radicals than vitamin C and E.

Results of the early Japanese investigations on the antioxidant activity of catechins in oil systems were often contradictory. Matsuzaki and Hara [1985] have shown that EGCG demonstrated the strongest activity, whereas Tanizawa et al. [1983] reported that EC showed the strongest antioxidative activity among catechins. According to Kajimoto [1963], EGC was the strongest natural antioxidant of green tea.

In a β-carotene-linoleate model system EGCG possessed the strongest antioxidative activity and EGC showed the weakest effect [Amarowicz & Shahidi, 1995]. The antioxidative activity of a reconstituted catechin mixture in the proportions present in the crude extract was lower than that of the crude mixture itself. Roedig-Penman and Gordon [1997] reported that the green tea water extract was highly effective as a natural antioxidant for an oil-in-water emulsion storage (pH 5.5, 40 days at 30°C).

The DPPH radical scavenging ability of catechins was EGCG>ECG>EGC>EC and that of theaflavins was TF₃>TF₁>TF [Chen & Ho, 1995]. The authors reported also that EGCG, EGC, ECG showed higher lipid oxidation-inhibition activity, as measured by the Rancimat assay, compared to BHT and theaflavins. The green and black teas had much higher antioxidant activities against peroxyl radicals than 22 common vegetables [Cao et al., 1996]. In experiments of Gadov et al. [1997], the tea extracts (of rooibos, green, oolong, black teas) were strong inhibitors of β-carotene bleaching and active scavengers of DPPH radical.
Aim/Models/Methods

### TABLE 1. Antioxidant activity of green tea polyphenols.

<table>
<thead>
<tr>
<th>Material tested</th>
<th>Aim/Models/Methods</th>
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<tbody>
<tr>
<td>Aqueous extracts of rooibos, green, oolong and black teas</td>
<td>DPPH• scavenging and β-carotene bleaching</td>
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<td>123 batches of tea (most of them were green tea)</td>
<td>Principal component regression (PCR) was used to model the relation between the total antioxidant capacity and the NIR spectra of green tea</td>
<td>Zhang et al. [2004]</td>
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<tr>
<td>Catechins</td>
<td>The antioxidative mechanisms of catechins were studied by investigation products generated by AAPH-induced radical oxidation</td>
<td>Kondo et al. [1998]</td>
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<td>Catechins and their epimered, acylated, and glucosylated derivatives</td>
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<td>Green tea extracts, EGCG, ECG</td>
<td>Model food emulsion; PV, conjugated dienes</td>
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<tr>
<td>Green tea catechins</td>
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<tr>
<td>Green tea catechins</td>
<td>Inhibition of peroxynitrite formation by 3-morpholinosydnonimine and scavenging of peroxynitrite itself</td>
<td>Chung et al. [1998b]</td>
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<td>ECG, EC, C</td>
<td>Antiradical activity against alkyl peroxyl radical</td>
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<td>Green tea extract, individual catechins, reconstituted catechin mixture</td>
<td>β-Carotene bleaching</td>
<td>Amarowicz &amp; Shahidi [1995]</td>
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<tr>
<td>Green tea catechins and theaflavins</td>
<td>DPPH• and superoxide scavenging, Rancimat method</td>
<td>Chen &amp; Ho [1995]</td>
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<td>Various tea extracts</td>
<td>DPPH• scavenging, reduction power, scavenging of superoxide, hydroxyl radical scavenging activity by EPR spectrometry</td>
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<td>Catechin</td>
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<td>Green and black tea extracts</td>
<td>Different brewing conditions; TEAC, LDL oxidation</td>
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<td>Ethanol extracts of black, dark-green and ginseng teas</td>
<td>Canola oil; oxygen consumption</td>
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<td>Tea theaflavins and methylated catechins</td>
<td>Canola oil; oxygen consumption</td>
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<td>Green tea catechins</td>
<td>Meat model system; TBARS, hexanal and pentanal content</td>
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<td>Green tea extract</td>
<td>Raw minced red meat, poultry and fish muscle; TBARS</td>
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<tr>
<td>Green tea extracts</td>
<td>Meat model system; TBARS</td>
<td>Amarowicz et al. [2005]</td>
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<tr>
<td>Green tea catechins</td>
<td>NMR analytical approach to clarify the antioxidative molecular mechanism of catechins using DPPH•</td>
<td>Sawai &amp; Sakata [1998]</td>
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<td>Green tea catechins</td>
<td>Formation of radicals and chemiluminescence during the autoxidation of tea catechins</td>
<td>Yoshioka et al. [1991]</td>
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<td>Green tea catechins</td>
<td>Relative antioxidative activities of catechins against radicals generated in the aqueous phase and against propagating lipid peroxyl radicals</td>
<td>Salah et al. [1995]</td>
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<td>EC and ECG</td>
<td>Protective effect of EC and ECG on lipid peroxidation in phospholipid bilayers</td>
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<td>Green and black tea extracts</td>
<td>Antioxidant activities against peroxyl and hydroxyl radicals</td>
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<td>Tea catechin oxypolymers</td>
<td>Antioxidant and pro-oxidant effects tested using a deoxyribose assay, a photoreduction of NBT assay, a lipoxygenase assay, PV assay, and animal test</td>
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<td>C and ECG</td>
<td>Recovery of growth arrest of <em>Escherichia coli</em> under oxidative conditions</td>
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<td>Green tea extract</td>
<td>Degradation and oxidation products of stigmasterol in TAG of sunflower oil</td>
<td>Rudzińska et al. [2004]</td>
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<tr>
<td>Green and black tea extracts</td>
<td>DPPH• and ABTS•• scavenging</td>
<td>Gramza et al. [2005]</td>
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</table>
The antiradical activity of green and black tea ethanol and water extracts was measured with the use of two different methods of scavenging: the stable free radicals ABTS•⁺ and DPPH⁺ [Gramza et al., 2005]. The extracts examined showed different antiradical activity. The best activity in scavenging ABTS•⁺ expressed as TAA (total antioxidant activity) was reported for black tea aqueous and ethanol extracts. Green tea extracts were four times less effective. Aqueous extracts showed 50% lower activity than equivalent ethanol extracts. Research proved that the antiradical activity of plant extracts is dependent on the mechanisms of oxidative activity of free radicals used and the chemical structure of antioxidants.

In the study by Rudzińska et al. [2004], a green tea ethanol extract inhibited, to some extent, degradation of stigmasterol in a model system containing TAG of sunflower oil and sterol standard over 9 days of incubation at 60°C. In a meat model system at a concentration of 200 mg/kg, the antioxidant activity of individual catechins evaluated by TBA values was EGCG>EGC>EGC>EC. The results of hexanal and pentanal contents in the headspace volatiles of meat treated with catechins showed the same order of catechins as antioxidants [Shahidi & Alexander, 1998]. In an experiment with raw minced red meat, poultry and fish muscle, the antioxidant potential of green tea catechins (40% of EGCG, 24% of EGC, 12% of EGC, and 10% of EC) was two to fourfold greater than that of α-tocopherol at the same concentration [Tang et al., 2001]. The percent inhibition of TBARS development by several crude green tea extracts using a meat model system varied from 27.9 to 55.7% (i.e. based on extract addition of 25 ppm), 62.8 to 88.7% (i.e. 50 ppm), 78.7 to 100% (i.e. 100 ppm), and 94.3 to 100% (i.e. 200 ppm). Proanthocyanidin fraction was less active compared to the crude extract from which it was derived [Amarowicz et al., 2005b].

The results of research by Nanjo et al. [1996] with acelated and glucosylated catechin derivatives suggested that the gallolyl moiety attached to flavan-3-ol at 3 position has a strong capability for scavenging the DPPH radical as well as the gallolyl moiety attached to flavan-3-ol at 3 position has been reported to reduced mutation induction by UV in Escherichia coli [Ahmad et al., 1997; Katiyar & Mukhtar, 1997]. Table 2 compiles some results dealing with the anticancerogenic properties of green tea polyphenolics obtained in animal models.

Kim and Masuda [1997] claimed that it is highly likely that green tea shows a beneficial effect to reduce cancer risk. To elucidate the inhibitory effects of catechins on carcinogenesis the authors listed such mechanisms as: antioxidation activities of green tea polyphenols; trapping of carcinogens; inhibition of nitrosation reactions; inhibition of the growth of intestinal clostridia; inhibition of biochemical signals of tumor initiation; and inhibition of biochemical signals of tumor promotion. According to Kuroda and Hara [1999], tea polyphenols demonstrate their antimutagenic and anticarcinogenic activity due to such various mechanisms as: inhibition of mutagens and carcinogens acting extracellularly (desmuta-
TABLE 2. Green tea polyphenols as anticarcinogenic compounds in animal models.

<table>
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<th>Compound tested</th>
<th>Organ/chemicals used for tumorgenesis/animals</th>
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<td>Green tea infusion (2% oral administration)</td>
<td>Esophageal; N-nitrosomethylbenzylamine (NMBzA); rats</td>
<td>Han &amp; Xu [1990]</td>
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<td>Green tea infusion (1.2% oral administration)</td>
<td>Forestomach; benzo(a)pyrene (BP); mice</td>
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<td>EGCG (0.05% solution)</td>
<td>Glandular stomach; N-methyl-N’-nitro-nitrosoguanidine (MNNG); rats</td>
<td>Yamane et al. [1995]</td>
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<td>EGCG (0.05% solution)</td>
<td>Duodenum; MNNG; mice</td>
<td>Fujita et al. [1989]</td>
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<td>EGCG (topic application)</td>
<td>Skin; 7,12-dimethyl benzanthracene (DMBA) and benzopyrene dioloxide (BPDE); mice</td>
<td>Khan et al. [1988]</td>
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<tr>
<td>EGCG (topic application)</td>
<td>Skin; ultraviolet radiation; mice</td>
<td>Katiyar &amp; Mukhtar [2001]</td>
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<td>EGCG (oral application)</td>
<td>Spontaneous skin tumor; mice</td>
<td>Paul et al. [2005]</td>
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<tr>
<td>Tea infusion (0.65% or 1.25%)</td>
<td>Lung; 4-methyl nitrosoamoioino-3-pyridyl-1-butanone (NNK); mice</td>
<td>Xu et al. [1992]</td>
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<tr>
<td>Green tea solution (0.1, 0.2, 0.4, 0.6%)</td>
<td>Lung; 4-methyl nitrosoamoioino-3-pyridyl-1-butanone (NNK), mice</td>
<td>Liao et al. [2004]</td>
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<td>Green tea leaves (2.5% in diet)</td>
<td>Liver; NDEA; rats</td>
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<td>Green tea polyphenols (500 mg/kg/day)</td>
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<td>Green tea polyphenols</td>
<td>Colon; azoxymethane; rats</td>
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<td>Polyphenolic fraction of green tea (oral infusion)</td>
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Sesso et al., 1999; Geleijnse et al., 1999; Davies et al., 2003]. On the grounds of the results obtained Imai and Nakachi [1995] suggested that subjects consuming more than 10 cups of green tea infusion daily, were characterized by a decreased cholesterol level of blood. Other investigations indicated that after consumption of 300 and 450 mL of green tea infusion, the antioxidant activity of blood serum increased [Sung et al., 2000]. Five servings of tea per day over a 3-week treatment reduced total cholesterol (6.5%), LDL cholesterol (11.1%), apolipoprotein B (5%), and lipoprotein(a) (16.4%) in mildly hyper-cholesterolemic adults, compared with the placebo with added caffeine [Davies et al., 2003]. There were significant differences in plasma LDL and adiponectin levels, and plasma LDL particle size of the patients with the coronary artery disease before and after 1-month intake of oolong tea [Shimada et al., 2004]. Kuhn et al. [2005] reported that LDL receptor expression was increased dramatically in HepG2 and HeLa cells treated with (-)-EGCG. This identified molecular mechanism may be related to the previously reported cholesterol-lowering and heart disease-preventative effects of green tea. The theaflavin-enriched green tea extract was studied as an effective adjunct to a low-saturated-fat diet to reduce LDL-C in hypercholesterolemic adults (a total of 240 men and women aged 18 years or older [Maron et al., 2003].

Tea flavonoids protect low density lipoproteins LDL fraction against oxidation by activating synthesis of the prostaglandins and possess antiplatelet and metal chelating proprieties [Acker et al., 1998]. A comparative analysis of the antioxidant activities of green and black tea water extracts showed the same rate of oxidation inhibition in blood serum lipoproteins and protection of blood vessels from blocking [Hodgson et al., 1999, 2002]. Green tea cate-

<table>
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<td>Lung; benzo(a)pyrene (BP); mice; increase of apoptotic cells.</td>
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<td>Ehrlich's ascites carcinoma (EAC); initiation of mitochondrial death cascade in EAC cells.</td>
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<td>EGCG</td>
<td>Human pancreatic cancer cells; caspase-3 activation, poly-ADP ribose polymerase cleavage; EGCG-invoked Bax oligomerization and depolarization of mitochondrial membranes to facilitate cytochrome c release into cytosol.</td>
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<td>EGCG</td>
<td>Two human melanoma cell lines; upregulation of proapoptotic Bax and activation caspase-7, and -9.</td>
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<td>EGCG (oral administration)</td>
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<td>Catechins</td>
<td>Colon cancer cells; ECG is the strongest NAG-1 (non-steroid anti-inflammatory drug activated gene) inducer among the catechins tested.</td>
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<td>Tea leaves (oral administration)</td>
<td>Lung carcinogenesis; the apoptosis index was significantly higher in lung adenomas from 0.6% tea-treated mice.</td>
<td>Liao et al. [2004]</td>
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<td>EGCG</td>
<td>Human cancerous lines (cervix carcinoma, chronic myelogenous leukemia, multiple myeloma); a combined treatment of radiation and EGCG resulted in a significant enhancement of apoptosis.</td>
<td>Baatout et al. [2004]</td>
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<td>Black tea</td>
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<td>Bhattacharyya et al. [2004]</td>
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<td>Theaflavins (TF-1, TF-2A, TF-2B, TF-3) and EGCG</td>
<td>Murine myeloid leukemia cells; the four tested theaflavins and EGCG were found to be potent in inducing apoptosis.</td>
<td>Lung et al. [2004]</td>
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<td>EGCG</td>
<td>Human endocervical cells (HEN); DNA ladder assay; induction of apoptosis.</td>
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<td>EGCG</td>
<td>Hep-2 laryngeal, LoVo colon, HeLa cervical carcinoma cells; induction of apoptosis in cells of the examined neoplastic line in a dose-related manner.</td>
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Tea polyphenols activity

chins can regenerate \( \alpha \)-tocopherol, the main antioxidant protecting the LDL fraction against oxidation [Kono et al., 1992; Vinson & Dabbagh, 1998; Zhu et al., 1999].

Investigations conducted on rats fed green tea catechins showed a decrease in cholesterol and triacylglycerol levels in blood and their non-toxicity in relation to liver and kidneys. Additional interesting aspect of the research was that in spite of the same quantity of diet consumed, a decrease of about 10–18% body weight was observed in relation to the control group non-feed with tea catechins [Lin et al., 1998b].

On the basis of the experiments of Kuo et al. [2005], it seemed that the fully fermented pu-erh and black tea leaves and partially fermented oolong tea leaves were more effective on their growth suppressive and hypolipidemic effects as compared to the non-fermented green tea leaves. Pu-erh tea leaves administered orally (4% of diet) to male Sprague-Dawley rats for 30 weeks could increase the level of HDL-C and decrease the level of LDL-C. The results of experiments with mice suggest that daily intraperitoneal injections of EGCG (10 mg/kg) reduces evolving atherosclerotic lesions to a different extent [Chyu et al., 2004]. There were concentration-dependent decreases in the total cholesterol content in cultured rat mesangial cells when treated with 40 ng/L to 40 mg/L of tea polyphenolics [Zhang & Gan, 2003]. EGCG treatment in the apolipoprotein E-null mice resulted in an increase in the antioxidant capacity in local vascular tissue and systemic circulation and reduced vascular smooth muscle cell proliferation and redox-sensitive gene activation in vitro. EGCG reduced cuff-induced evolving atherosclerotic plaque size [Chyu et al., 2004].

The effect of purified catechins on micellar solubility of cholesterol was examined in an in vitro study by Ikeda et al. [2003]. The addition of gallate esters of catechins reduced micellar solubility of cholesterol by precipitating cholesterol from bile salt micelles [Zhang & Gan, 2003]. EGCG treatment in the apolipoprotein E-null mice resulted in an increase in the antioxidant capacity in local vascular tissue and systemic circulation and reduced vascular smooth muscle cell proliferation and redox-sensitive gene activation in vitro. EGCG reduced cuff-induced evolving atherosclerotic plaque size [Chyu et al., 2004].

The effect of purified catechins on micellar solubility of cholesterol was examined in an in vitro study by Ikeda et al. [2003]. The addition of gallate esters of catechins reduced micellar solubility of cholesterol by precipitating cholesterol from bile salt micelles. (-)-Catechin gallate and (-)-gallocatechin gallate were more effective to precipitate cholesterol than (-)-epicatechin gallate and (-)-epigallocatechin gallate, respectively. These observations strongly suggest that heat-epimerized catechins may be more hypocholesterolemic than tea catechins. In experiments of Raederstorff et al. [2003], after 4 weeks of treatment, the total cholesterol and low density lipoprotein plasma levels were significantly reduced in the group of rats fed 1% EGCG when compared to the non-treated group. In an in vitro biliary micelle model, the addition of 55 \( \mu \)mol to 1300 \( \mu \)mol of EGCG not only decreased cholesterol solubility in a dose-dependent manner in these micelles but also altered the size of the mixed lecithin/taurocholate/cholesterol micelles as demonstrated by light scattering. This study provided evidence suggesting that the cholesterol-lowering effect of green tea is mainly elicited by EGCG. It was suggested that one of the underlying mechanisms by which EGCG affects lipid metabolism is by interfering with the micellar solubilization of cholesterol in the digestive tract, which then in turn decreased cholesterol absorption. The findings of Loest et al. [2002] provided direct evidence that green tea has a profound inhibitory effect on the intestinal absorption of cholesterol and in ovariectomized (OX) rats. The supplement-

diet decreased lipid levels in rats and increased the fecal excretion of total lipids and cholesterol [Matsumoto et al., 1998].

**ANTIBACTERIAL AND ANTIVIRAL ACTIVITY**

There are numerous studies on the antimicrobial activity of tea extracts, catechins and other polyphenols (Table 4). ECG reduced oxacillin resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) at concentrations below the MIC [Stapleton et al., 2004a]. Substitution of the gallate group of ECG with 3-O-acyl chains of varying lengths \( C_2-C_8 \) led to enhanced anti-staphylococcal activity with chain lengths of \( C_2 \) and \( C_8 \). 3-O-octanoyl catechin was bacteri
cidal against MRSA as a result of membrane damage. ECG was found also as an agent to combat beta-lactam resistance in *S. aureus*. Modulation of beta-lactam resistance by ECG significantly enhanced the activities of flu
cloxacillin and the carbapenem antibiotics imipenem and meropenem against 40 MRSA isolates, with MIC (90) values for the antibiotics reduced to the susceptibility breakpoint or below [Stapleton et al., 2004b].

The proanthocyanidin fraction separated from the crude tea extract had pronounced effect on *Escherichia coli* K12, as noted by the *ca.* two log reduction [Amarowicz et al., 2000].

Green tea catechins can act as inhibitors of some enzymes important for microorganisms. EGCG was effective in inhibiting protein tyrosine phosphatase (PTPase) activity in *Prokera intermedia* at 0.5 \( \mu \)mol, and related species at 5 \( \mu \)mol [Okamoto et al., 2003]. The catechin oligomers isolated from oolong tea extract markedly inhibited glucosyltransferase (Gtase) from *Streptococcus sobrinus* 6715 [Hamada et al., 1996]. Inhibitory effect of green tea catechins on cytotoxicity of *S. aureus* at concentrations below 1% EGCG was observed by Okamoto et al. [2004].

Arakawa et al. [2004] observed a reactive oxygen species that was generated from the catechins as the bactericidal mechanism. EGCG reacted with the dissolved oxygen in aqueous solution, resulting in the generation of hydrogen peroxide. The results of Yoda et al. [2004] indicate that the structure of the bacterial cell wall and different affinities of EGCG with various cell wall components are responsible for different susceptibilities of *Staphylococcus* and Gram-negative rods to EGCG. The results suggested that the inhibitory effect observed is due to the presence of galloyl moiety in the structure. In the presence of a non-lethal concentration of Cu(II), washed *Escherichia coli* cells were killed by (-)-epigallocatechin (EGC) and (-)-epicatechin (EC) [Hoshino et al., 1999]. These authors suggested that the recycling redox reactions between Cu(II) and Cu(I), involving catechins and hydrogen peroxide on the cell surface, must be of significance to the mechanism of killing. As the degree of polymerization of catechin increased, GTase was inhibited more effectively. The experiments of Ikigai et al. [1993] demonstrated that bactericidal catechins primarily act on and damage bacterial membranes. The observation that the Gram-negative bacteria are more resistant to bactericidal catechins than Gram-positive bacteria can be
TABLE 4. Antimicrobial activity of green tea polyphenols.

<table>
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<th>Compound tested</th>
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<td>Green tea catechins</td>
<td><em>Helicobacter pylori</em></td>
<td>Weak inhibition of <em>Helicobacter pylori</em> growth.</td>
<td>Shin et al. [2005]</td>
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<td>EGCG</td>
<td><em>Legionella pneumophila</em></td>
<td>EGCG enhanced the <em>in vitro</em> resistance of alveolar macrophages to <em>Legionella pneumophila</em> infection by selective immunomodulatory effects on cytokine formation.</td>
<td>Yamamoto et al. [2004]</td>
</tr>
<tr>
<td>EGC</td>
<td><em>Staphylococcus aureus</em></td>
<td>EGCG showed a weak activity against <em>Staphylococcus aureus</em>; substitution of the gallate group of ECG with 3-O-acyl chains of varying lengths led to enhanced antistaphylococcal activity.</td>
<td>Stapleton et al. [2004]</td>
</tr>
<tr>
<td>EGCG</td>
<td><em>S. aureus, S. epidermidis, S. hominis, S. haemolyticus</em></td>
<td>Minimum Inhibitory Concentrations (MICs): 50–100 µg/mL.</td>
<td>Yoda et al. [2004]</td>
</tr>
<tr>
<td>Lung Chen tea, ECG, EC</td>
<td><em>Helicobacter pylori</em></td>
<td>The MIC90 for Lung Chen was 0.25–0.5% (w/w) and these of ECG and EC were 50–100 and 800–1600 µg/mL, respectively.</td>
<td>Yee &amp; Koo [2000]</td>
</tr>
<tr>
<td>Aquous extracts of tea</td>
<td>30 bacterial species</td>
<td>Tea extracts were bactericidal to staphylococci and <em>Yersinia enterocolitica</em> at well below “cup of tea” concentration. In black tea extracts, theaflavin and its gallates are the antibacterially active components.</td>
<td>Yam et al. [1997]</td>
</tr>
<tr>
<td>Green and black tea</td>
<td><em>Mycoplasma</em></td>
<td>At a concentration of 0.2%, green tea and black tea showed microbicidal activities against <em>M. pneumoniae</em> and <em>M. orale</em> but not against <em>M. salivarium</em>.</td>
<td>Chose et al. [1992]</td>
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<tr>
<td>Green tea, black tea, and EGCG</td>
<td><em>Bordetella pertussis</em></td>
<td>Green tea, black tea, and EGCG might act as prophylactic agents against <em>Bordetella pertussis</em> infection.</td>
<td>Horiuchi et al. [1992]</td>
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<tr>
<td>Green tea extract, EGCG, and theaflavin digallate (TF3)</td>
<td>Methicillin resistant <em>Staphylococcus aureus</em> (MRSA)</td>
<td>20% tea extract, EGCG (63 µg) and TF3 (125 µg) added to one mL of culture medium each inhibited the growth of all strains of MRSA and food with poisoning <em>S. aureus</em> tested.</td>
<td>Toda et al. [1991]</td>
</tr>
<tr>
<td>Green tea catechins and theaflavins</td>
<td>21 phytopathogenic bacterial species</td>
<td>Tea catechins as well theaflavins showed a marked inhibitory effect against phytopathogenic bacteria.</td>
<td>Fukai et al. [1991]</td>
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<tr>
<td>Green tea catechins</td>
<td><em>Staphylococcus aureus, Vibrio cholerae</em></td>
<td>EGC, ECG, EGCG inhibited the growth of <em>Staphylococcus aureus</em> and <em>Vibrio cholerae</em>.</td>
<td>Toda et al. [1990]</td>
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</table>

explained, to some extent, by the presence of negatively charged lipopolysaccharide.

Tea leaves flavonoids show also germicidal and antiviral properties. It was stated that both green and black tea leaves components damage bacteria’s cellular membrane, which was used in the treatment of diarrhea, cholera and typhus infections [Shetty et al., 1994]. Its anticasirous properties as well as antiviral and against HIV virus were also shown [Otak et al., 1991; Gupta et al., 2002; Nakane & Ono, 1989]. It was proved that epicatechin and epigallocatechin gallates from tea leaves inhibit the RNA opposite transcriptase, playing an essential part in HIV viruses replication. Moreover, it was stated that free epicatechin and epigallocatechin did not show similar influence.

**OTHER BIOLOGICAL PROPERTIES**

Investigations into thermogenic tea properties showed synergistic action of caffeine and catechins, possibly stimulating the thermogenesis. It was stated that individuals, consuming tea extract containing 90 mg EGCG, three times daily, burned 266 kcal/day more than the group without the addition of catechins. It enabled the presumption that this property might be helpful in overweight and obesity control [Dullo et al., 2000]. Tea polyphenols showed also strong inhibition of lipases *in vitro*, causing reduced triacylglycerols lipolysis [Juhel et al., 2000].

Catechins can also inhibit the release of some allergic reactions factors such as: leucotrienes, prostaglandins, by modifying activities of multiple enzymes taking part in inflammatory states of the human body [Middelton et al., 1998].

Green tea catechins can influence the endocrine system [Kao et al., 2000]. EGCG considerably lowered food consumption level, body mass, estradiol, testosterone and leptine levels in the rats studied. It was also found that catechins can modulate steroid hormones concentration in the body, which is possibly an essential element in anticancerogenic prophylaxis [Strom et al., 1999]. Black tea polyphenols – theaflavins – also show beneficial influence on living organism. They are strong antioxidants protecting healthy cells of rat liver against oxidative stress, and preventing DNA damages [Feng et al., 2002].

The studies of Yamamoto et al. [2004] showed that EGCG enhanced the *in vitro* resistance of alveolar macrophages to *Legionella pneumophila* infection by selective immunomodulatory effects on cytokine formation. Furthermore, the tobacco smoking-induced impairment of alveolar macrophages regarding antibacterial as well as immune activity was also recovered by EGCG treatment. These results indicate that EGCG may be a potential immunotherapeutic agent against respiratory infections in immunocompromised patients, such as heavy smokers.

Green tea polyphenols are known to prevent dental caries. The effects of these compounds on cariogenicity were
observed in in vitro tests. Catechins inhibited the growth of cariogenic bacteria Streptococcus mutans and S. sobrinus [Sakanaka et al., 1989]. The addition of tea polyphenols to a diet or to drinking water reduced the appearance of caries in rats [Sakanaka et al., 1992]. Chewing gums containing tea polyphenols was found effective in decreasing dental plaque formation in humans [Sakanaka et al., 1996].

REFERENCES

Tea polyphenols activity


Tea polyphenols activity


Herbata jest naj чаще spożywanym napojem na świecie. Liście herbat są źródłem polifenoli, szczególnie katechin. Składniki te są źródłem polifenoli [katechiny (C), epikatechiny (EC), galokatechiny (GC), epigalokatechiny (EGC), galusanu epikatechiny (ECG), galusanu epigalokatechiny (EGCG)] podczas procesu fermentacji herbaty oraz aktywności przeciwutleniającej, właściwości antymutagenne, antynowotworowe i anti–miażdżycowe ekstraktów związków polifenolowych herbaty i czystych katechin.

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POLIFENOLE HERBATY – ICH WŁAŚCIWOŚCI PRZECIWUTLENIAJĄCE I AKTYWNOŚĆ BIOLOGICZNA – ARTYKUŁ PRZEGŁĄDOWY

Herbata jest najczęściej spożywanym napojem na świecie. Liście herbat są źródłem polifenoli, szczególnie katechin. Artkuł omawia zmiany chemiczne polifenoli [katechiny (C), epikatechiny (EC), galokatechiny (GC), epigalokatechiny (EGC), galusanu epikatechiny (ECG), galusanu epigalokatechiny (EGCG)] podczas procesu fermentacji herbaty oraz aktywność przeciwutleniającą, właściwości antymutagenne, antynowotworowe i anty–miażdżycowe ekstraktów związków polifenolowych herbaty i czystych katechin.