Chapter 3.

Green Tea

---- *Camellia sinensis (L.)Kuntze*

General Description

Literature References

The Green Tea Preparation

The Extraction of Green Tea

Product Specifications

Attachment

Literature References:

- (-)-Epigallocatechin gallate 989-51-5. in Summary of data for chemical selection, prepared by Technical resources international, Inc. for NCI.
- Chung F, Schwartz J, Herzog C.R., and Yang Y.M. Tea and Cancer prevention: studies in animals and humans. J. Nutr. 133:3268S-3274S, 2003.
- Pisters KMW, Newman RA, Coldman B, Shin DM, Khuri FR, Hong WK, Glisson BS, and Lee JS. Phase I trail of oral tea extract in adult patients with solid tumors. J Clin Oncology, 19 (6): 1830-1838; 2001.
- Chow HH, Cai Y, Alberts DS, Hakim I, Dorr R, Shahi F, Crowell JA, Yang CS, Hara Y.
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- Brown MD. Green tea (Camellia sinensis) extract and its possible role in the prevention of cancer. Altern Med Rev. 1999 Oct;4(5):360-70.

Green Tea

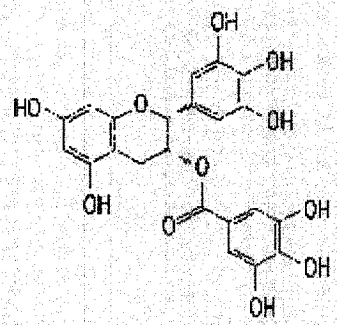
--- Camellia sinensis (L.)Kuntze

Genral Description

Tea is generally in the form of black, oolong, and *green tea,* all originating from the tea plant, Camellia sinensis. Tea is cultivated in approximately thirty countries worldwide, and is consumed globally. Although the level of tea consumption varies around the world, it is believed that tea consumption is second only to water (Ahmad et al., 1998, Nutrition and Chemical Toxicity, John Wiley and Sons, Sussex, England, pp. 301-343). Black tea is consumed predominantly in Western and some Asian countries and *green tea* is consumed predominantly in China, Japan, India, and a few countries in North Africa and the Middle East (Ahmad et al., 1998, Nutrition and Chemical Toxicity, John Wiley and Sons, Sussex, England, pp. 301-343). *Green tea* has been prized as a traditional tonic and has been widely consumed in East Asia. Recent studies have attempted to link *green tea* to antioxidant benefits including protection against the damage caused by cigarette smoke, pollution, stress, and other toxins (for an overview, see e.g., Mitscher, 1998, The *Green Tea* Book, Avery Publishing Group, Garden City Park, New York and Weisburger, 1997, Can. Lett. 114:315-317).

Scientists have now identified many of the natural substances in *green tea* that may provide the majority of its health benefits. One class of chemicals that has attracted much study is the polyphenols, also known as catechins. The polyphenols describe a class of substituted phenolic compounds that are known as flavanols or catechins. The polyphenols in *green tea* that have been identified are catechin (C), epicatechin (EC), gallocatechin (GC), gallocatechin gallate (GCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCg) (FIG-1). In addition, caffeine, theobromine, theophylline, and phenolic acids, such as gallic acid, are also present as constituents of *green tea* in smaller quantities than the polyphenols (Ahmad et al., 1998, Nutrition and Chemical Toxicity, John Wiley and Sons, Sussex, England, pp. 301-343).

Epigallocatechin gallate (EGCg), the major catechin in *green tea,* has been the focus of many studies to determine if it is responsible for the antioxidant and anti-carcinogenic effects of *green tea,* as reviewed by Ahmad and Mukhtar, 1999, Nutr. Rev. 57:78-83.



$C_{22}H_{18}O_{10}$

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FIG. 1 Chemical Structure of Epigallocatechin gallate (EGCg)

Literature References

- (-)-Epigallocatechin gallate 989-51-5. in Summary of data for chemical selection, prepared by Technical resources international, Inc. for NCI.
- Chung F, Schwartz J, Herzog C.R., and Yang Y.M. Tea and Cancer prevention: studies in animals and humans. J. Nutr. 133:3268S-3274S, 2003.
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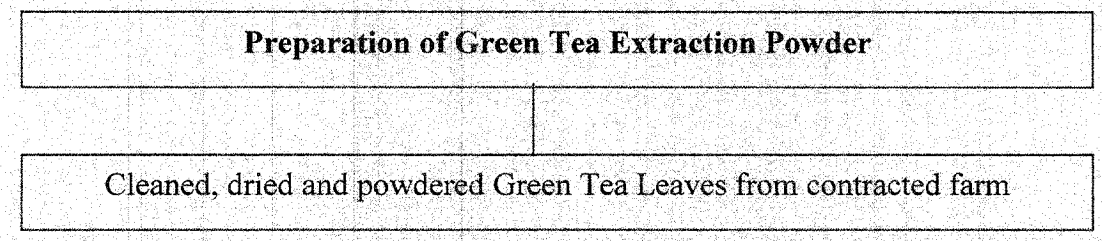
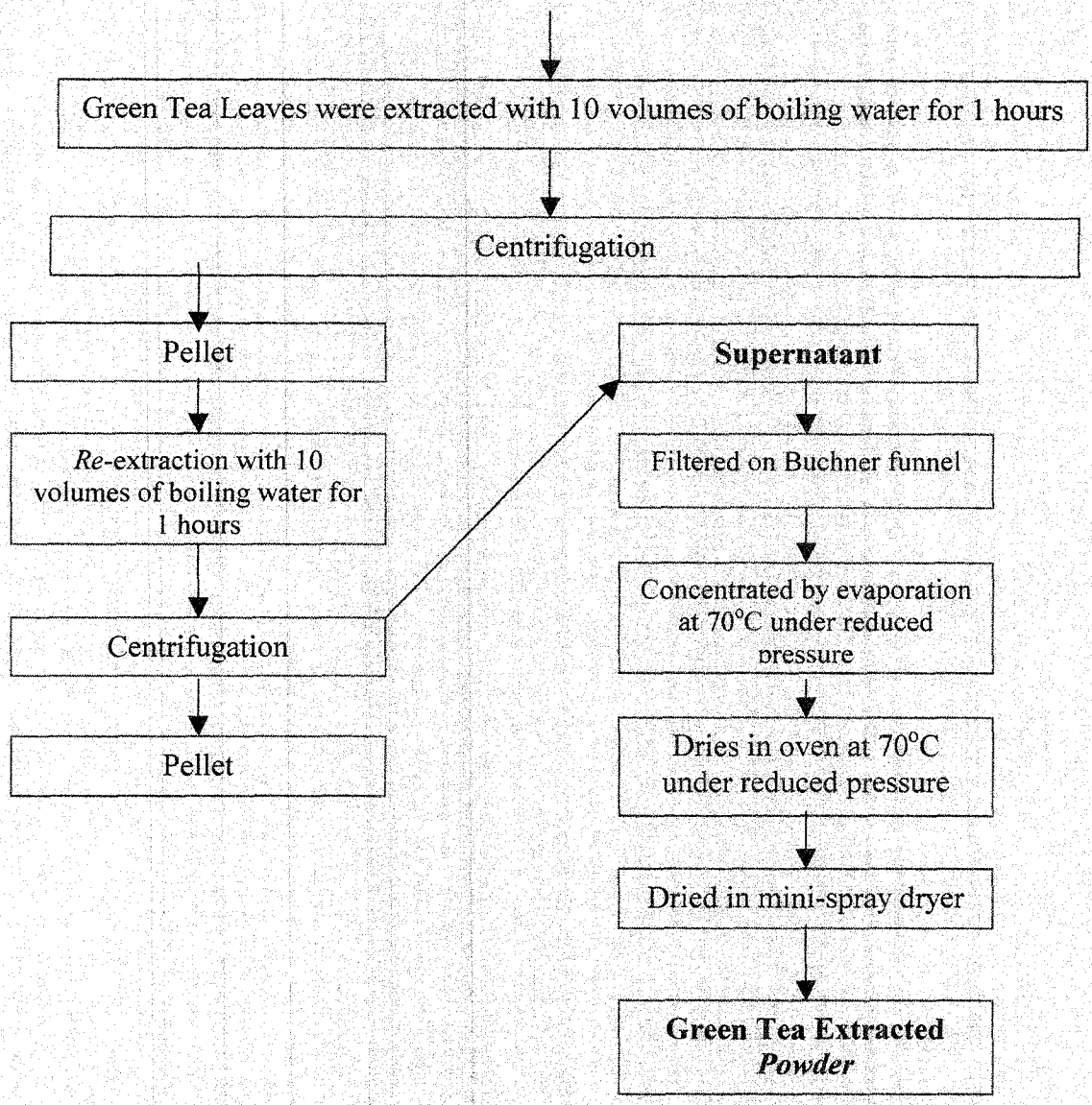
The Green Tea Preparation

Through laboratory screening based on the potent for inhibition of cancer cell growth, one strain of green tea has been selected from twenty strains of green teas as raw material source of our product. This tea strain is called Long Jin (or Dragon Well) produced in Hong Zhou of China. The contract between the farmer and North China Pharmaceutical Corporation showed that the plant of the green tea didn't administrated any chemical fertilizer, and pesticide. The tealeaves collected were not processed through fermentation, which is a traditional method for commercial tea products. The tealeaves will be washed, dried and powdered for extraction process.

The Extraction of Green Tea

EGCg is a marker for extraction of green tea in our products. The concentration of our green tea material contains 40% EGCg. The extraction method used in NCPC manufacture is hot water extraction procedure see below chart.





Product Specifications (translated version from Chinese version by NCPC manufacture attached below)

Product Name: Green tea extracted powder (100%)
Content: 40% EGCg, see following Figs 2-4.
Manufacturer: North China Pharmaceutical Corporation (NCPC)
380# East Heping Rd., Shijiazhuang, Hebei Provice, China.
PC050015

Microorganisms:

Total plate count	0/g (Less than 10 CPU/g)
E. coli	*0/100g (*Less than 30 CFU/100g)
Yeast	Not detected
Pathogenic bacteria	Not detected

Heavy Metals:

Arsenic	< 1.0 ppm
Cadmium	< 1.0 ppm
Mercury	< 0.5 ppm
Lead	< 1.0 ppm
Copper	< 20 ppm

Pesticide Contamination:

Hexachlorobenzene	Not Detected
Quintozene	
Pentachloronitrobenzene (PCNB)	Not Detected
Pentachloroaniline	Not Detected
Pentachlorothioanisol	Not Detected
Lindane	Not Detected

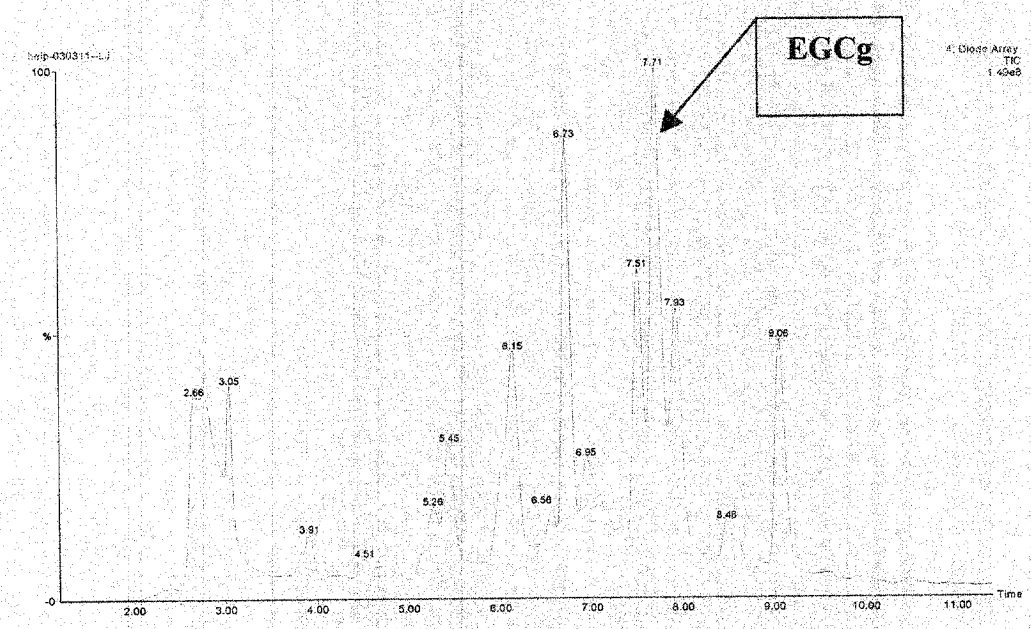


FIG. 2 Epigallocatechin gallate (EGCg)

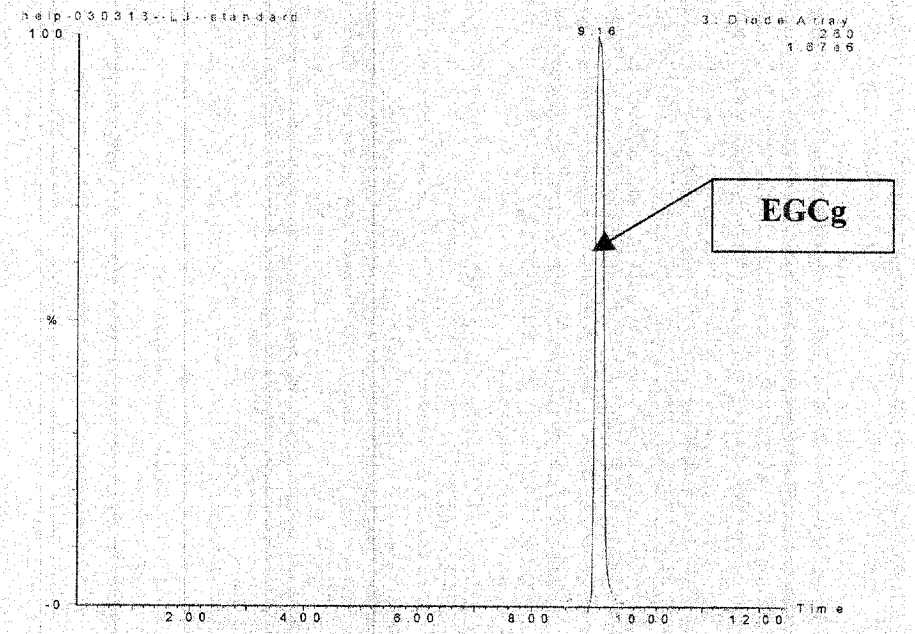


FIG. 3 Epigallocatechin gallate (EGCg)

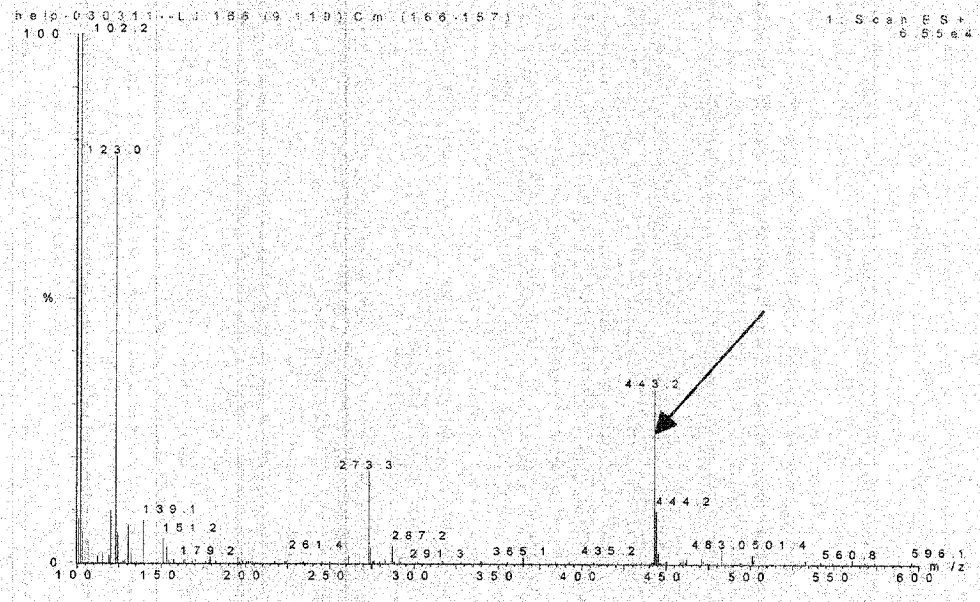


FIG. 4 Epigallocatechin gallate (EGCg)

华北制药集团新药研究开发有限责任公司
检验报告

样品名称：绿茶提取物	报告日期：2004 年 08 月 10 日
批号：20040802	产品来源:华北制药集团新药研究开发有限责任公司

检测项目	检测结果
茶多酚含量	53.69 %
重金属	
砷	<0.1 mg/kg
铅	<0.2 mg/kg
汞	<0.01 mg/kg
农药残留	
六六六	<0.005 mg/kg
滴滴涕	<0.005 mg/kg
卫生学检查	
菌落总数	0 个/g
霉菌	2 个/g
酵母菌	0 个/g
致病菌	未检出

质检部负责人：贺[illegible]

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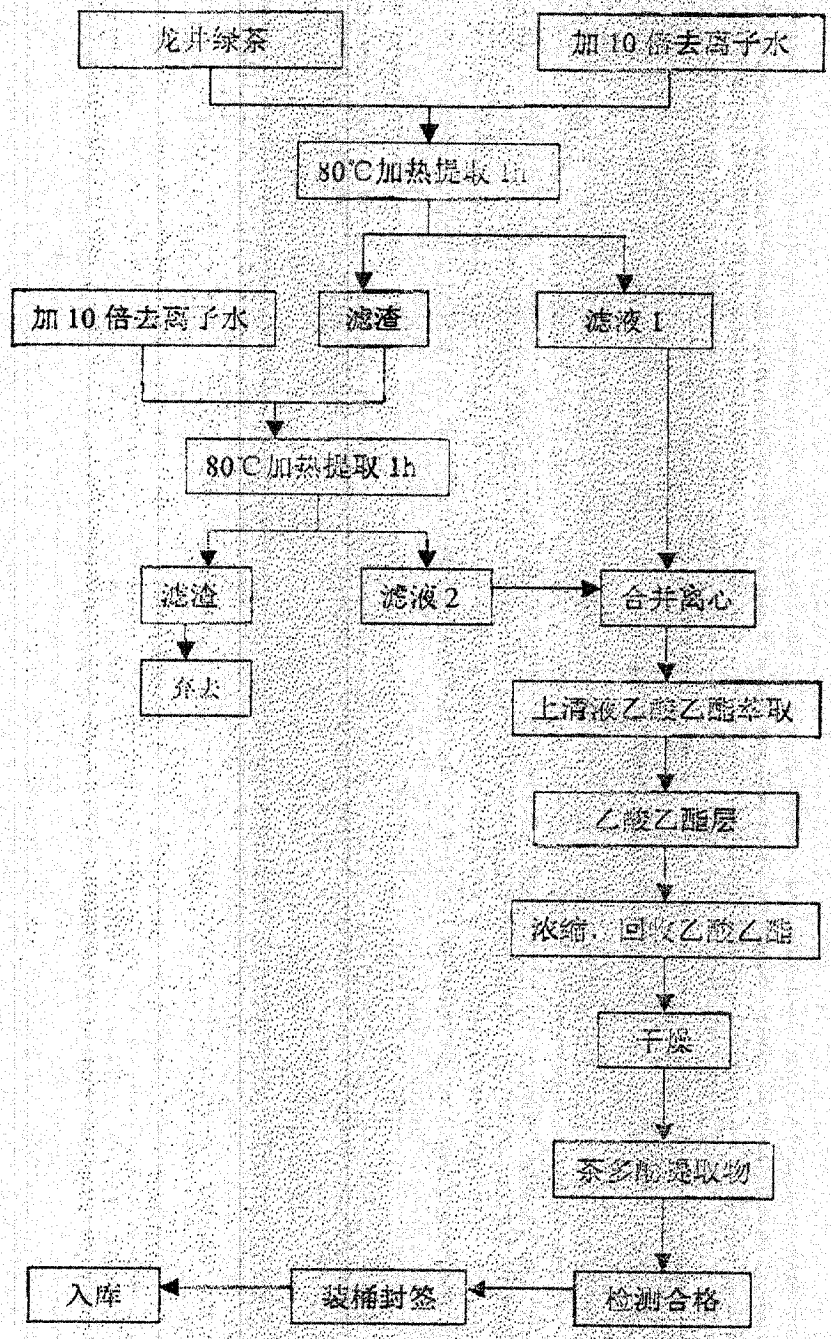
绿茶提取物工艺流程



生产环境：

药材提取、浓缩、精制、干燥、分装全部生产工艺在符合 GMP 要求的车间进行。

Green Tea

Literature References

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SUMMARY OF DATA FOR CHEMICAL SELECTION

(–)-Epigallocatechin gallate

989-51-5

BASIS OF NOMINATION TO THE CSWG

(–)-Epigallocatechin gallate (EGCG) is brought to the attention of the CSWG by the Division of Cancer Biology, National Cancer Institute (NCI). EGCG is being considered as a potential cancer chemopreventive agent. As such, it requires evaluation with regard to its toxicity.

EGCG is the major component of the polyphenolic fraction of green tea. Along with other tea catechins, and polyphenols in general, it is an antioxidant that is thought to prevent tumorigenesis by protecting cellular components from oxidative damage via free radical scavenging. Indeed, a number of studies have demonstrated the free radical scavenging activites of EGCG, as well as its antimutagenic, antitumorigenic, anti-angiogenic, antiproliferative, and/or pro-apoptotic effects on mammalian cells both *in vitro* and *in vivo*. Deregulation brought about by the 1994 Dietary Supplement Health and Education Act allows unrestricted marketing of green tea extract, thus leading to its widespread production and, therefore, increased exposure of US consumers to EGCG.

Because EGCG is a component of a dietary supplement, its manufacturers cannot be compelled by the government to test the safety of this substance. The information on the possible adverse effects associated with EGCG consumption, including its potential carcinogenicity, remains scarce. EGCG consideration as a potential chemopreventive agent warrants an independent investigation into its safety.

INPUT FROM GOVERNMENT AGENCIES/INDUSTRY:

Dr. Harold Seifried of the Division of Cancer Prevention (DCP) at the NCI provided information on evaluation of EGCG by the DCP as a potential chemopreventive agent. A Lipton Tea customer service representative provided information on distribution of imported tea in the US. A US Tea Council spokesperson provided general information on the health benefits of tea.

SELECTION STATUS

ACTION BY CSWG: 12/12/00

Studies requested:

Subchronic (90-day) toxicity study

Standard genotoxicity battery in bacteria and mammalian cells

Rationale/remarks:

A suspected active ingredient of green tea possibly responsible for its anticarcinogenic effects

Widespread consumer exposure to tea, green tea, and dietary supplements containing green tea extracts

Of significant scientific interest as a possible chemopreventive agent; NCI Division of Cancer Prevention is performing substantial work on the possible benefits of green tea extract and its components

Need for NTP to coordinate its efforts with NCI and other parts of NIEHS

Consider for 2-year cancer bioassay if warranted by results of the subchronic study

NCI will conduct Ames and mouse lymphoma assays

CHEMICAL IDENTIFICATION

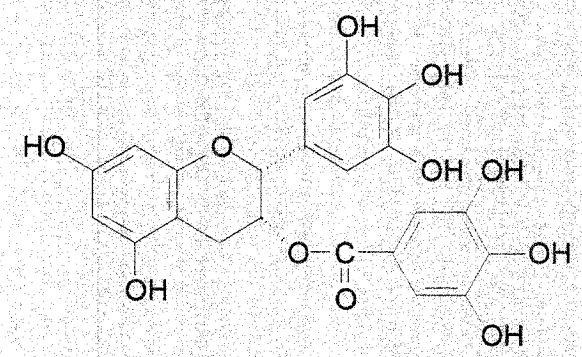
CAS Registry Number: 989-51-5

CAS Name: (–)-Epigallocatechin gallate, EGCG (9CI)

Synonyms and Trade Names: Epigallocatechin gallate; epigallocatechin 3-gallate; (–)-epigallocatechin-3-*O*-gallate; 3,4,5-trihydroxybenzoic acid, (2*R*-*cis*)-3,4-dihydro-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-2*H*-1-benzopyran-3-yl ester; (2*R*, 3*R*)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-1(2*H*)-benzopyran-3,5,7-triol 3-(3,4,5-trihydroxybenzoate)

Structural Class: Flavanol (catechin) (Balentine, 1997)

Structure, Molecular Formula, and Molecular Weight:



$C_{22}H_{18}O_{11}$ Mol. wt.: 458.40

Chemical and Physical Properties:

Description: EGCG is a major component of green tea extract (GTE). In its pure form, it is an odorless white, faint pink, or cream-colored powder or crystals (Alexis Corporation, 2000; Merck, 1997; Sigma-Aldrich, 2000).

Melting Point: 218 °C (Merck, 1997)

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Solubility: Sol. in water (clear, colorless solution at 5 mg/ml), acetone, ethanol, methanol, pyridine, and tetrahydrofuran (Sigma-Aldrich, 2000).

Technical Products and Impurities: EGCG is available in research quantities from Sigma-Aldrich as two different preparations containing no less than 80% or no less than 95% of the compound, respectively, as determined by HPLC (Sigma-Aldrich, 1999). It is also available from Alexis Corporation at a purity of no less than 98% (Alexis Corporation, 2000; Fisher Scientific, 2000).

GTE (CAS RN 84650-60-2, NLM, 2000), standardized to 55% of EGCG is available from Nature's Way Products, Inc. in the form of 100 – 500 mg capsules or tablets. It is distributed through a number of retail outlets, as well as via online merchants such as Drugstore.com, MotherNature.com, Vitamin Discount Connection, VitaminShoppe.com, Sweettree.com, and Willner Chemists. It is also sold in various combination with other dietary supplements (Drugstore.com, 2000; MotherNature.com, 2000; Nature's Way, 1999; VitaminShoppe.com, 1999).

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EXPOSURE INFORMATION

Production and Producers:

Tea (*Camellia sinensis*) is native to the East Asia region. Its first recorded use dates from the fourth century A.D. in China. The modern tea industry has its origins in the spread of cultivated tea from China into Japan around the seventh century and into Europe in the sixteenth century. Development of tea plantations in India, Africa, South America, Russia (Georgia), Australia, and the Pacific islands led to a variety of localized practices and tea products (Balentine, 1997).

Manufacturing Process. Tea Production. Tea has become an important agricultural product throughout the world, particularly in equatorial regions. Areas which receive annual rainfall of at least 50 inches/yr and have an average temperature of 30 °C with slightly acidic soil are the most favorable for cultivation of *Camellia sinensis*. Tea is generally pruned and maintained as a shrub-like bush of 1 – 1.5 m in height (Balentine, 1997).

Harvesting worldwide is generally done by hand using a small knife, although mechanized harvesting is utilized in Japan, Georgia, Australia, and Argentina. The consumable product is prepared in tea factories, which are usually located near large plantations (Balentine, 1997).

Tea comes in black, green, and oolong varieties, all produced from the leaves of *Camellia sinensis*. The traditional method of black tea production involves placing the plucked leaves on withering racks where excess moisture is removed, followed by rolling, fermentation, and drying. Thus, black tea is a product of fermentation, a process during which the macerated leaves are oxidized leading to a change in color and the development of the characteristic aroma (Balentine, 1997; Segal, 1996).

In contrast, green tea is made by steaming or otherwise heating the leaves immediately after plucking, thus preventing fermentation. Oolong tea is fermented only partially; the leaves are then rolled and dried (Segal, 1996).

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EGCG Extraction. EGCG is present in all types of tea. However, its content in black and oolong tea is usually less than half of that in unfermented (green) tea (Anon., 2000).

EGCG is extracted from unfermented or half-fermented tea leaves by treatment with hot water (80 – 100 °C), or a 40 – 75% aqueous solution of alcohol, or a 30 – 80% aqueous solution of acetone. The extract is washed with chloroform and transferred into an organic solvent, such as ethyl acetate, *n*-butanol, methyl isobutyl ketone, or acetone. The organic solvent is then removed by distillation, and the residual component is freeze-dried or spray-dried. The tea catechins are separated by reverse-phase high-performance liquid chromatography (HPLC) using an eluting solution containing 0 – 25% acetone, 0 – 35% tetrahydrofuran, and 65 – 85% water (by volume). The resulting EGCG product can then be concentrated, dried, and powdered, or purified by recrystallization from water (Hara, 1986).

Production/import level. EGCG is the principal active ingredient in green tea which is sold by most major food retailers. Green tea is available from approximately 70 raw material manufacturers (Natural Product Industry Center, 2000).

In 1998 world production levels of tea reached nearly three million tons (UK Tea Council, 2000). In 1991 supermarket sales of tea surpassed the $1 billion level, and consumer purchases increased steadily for two consecutive years (through 1993). The US tea industry expects continued strong growth, particularly from increases in the ready-to-drink segment, food service category, and gourmet teas (Stash Tea, 1999a).

The US imports of green tea for consumption totaled $1.3 million in 1999 (ITA, 1999). According to the Tea Council of the USA, more than 200 million pounds of tea, including more than 65% in tea bags, are packaged for consumption in the US annually (Stash Tea, 1999a). According to a spokesperson for Lipton Tea (one of the major suppliers of tea in the US), all Lipton-brand tea bags are packaged in the US (Lipton Tea, 2000).

Chemical Sources International lists twelve suppliers of EGCG, including eight in the US, and seven suppliers of GTE, including two in the US (Chemical Sources International, 2000). The OPD chemical buyers directory lists three suppliers of green

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tea (Aceto Corp.; Flavine International, Inc.; and Infinity Marketing Group, Inc.) and 31 suppliers of GTE. There is no listing of EGCG manufacturers (Tilton, 2000). EGCG is available from Sigma-Aldrich and Alexis Corporation (Sigma-Aldrich, 2000; Fisher Scientific, 2000).

Neither EGCG nor GTE is listed in the EPA's Toxic Substances Control Act (TSCA) Inventory (NLM, 1999).

Use pattern: Prior to World War II the amount of black and green tea consumed in the US was comparable, each type of tea accounting for approximately 40% of the market with oolong constituting the rest. Due to the war-related exclusion of Japan and China from the US market, the pattern of tea consumption in the US became skewed toward black tea. Immediately after the war, nearly 99% of all tea consumed in the US was black. Since then, the share of green tea consumed in the US has been rising steadily, reaching 4% in 1996 (Segal, 1996). In 1999 green tea accounted for approximately 10% of all tea imported by the US (ITA, 1999).

The most popular green tea products are the dried herb for making tea (loose or in tea bags) and encapsulated extracts. Green tea has also become a popular ingredient in sunblocks, cream rinses, and other body care products (MotherNature.com, 2000). Some examples of various green tea-containing products are presented in Table 1.

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Table 1. Some green tea products sold to consumers

Product Name	Company	Description
Green Tea Extract	Natural Brand	315-mg capsules, containing 90% polyphenols, including 55% EGCG. The product also contains red clover blossoms.
Standardized Green Tea Extract	Nature's Way	175-mg capsules, containing 44 mg of polyphenols. Other ingredients: dicalcium phosphate and gelatin.
Green Tea Extract	NOW	400-mg capsules, containing 40% catechins (60% polyphenols). The product also contains magnesium stearate.
Green Tea Extract	Natural Connections	100-mg capsules, containing 50 mg of catechins. Ingredients include rice protein, gelatin, cellulose, silica, and magnesium stearate.
Stash Matcha Green Tea	Stash Tea	Powdered green tea leaves, 1 oz. The product is dissolved in hot water (1/2 teaspoon per 8 oz. of water) and consumed as a beverage.
Sencha	O-Cha	Green tea leaves, 100 g. The product is brewed and consumed as a beverage.
Green Tea Facial Cleansing Lotion	Aubrey Organics	Skin lotion, 4-oz. bottle. Ingredients include aloe vera fillet, coconut fatty acid cream base, corn oil and coconut oil soap, green tea (matcha), lemon peel, blue camomile, citrus seed extract, vitamins A, C and E.
Green Tea Sunblock for Children SPF 25	Aubrey Organics	Sunblock lotion, 4-oz. bottle. Ingredients include green tea (matcha), *p*-aminobenzoic acid, coconut fatty acid cream base, aloe vera, shea butter, Rosa Mosqueta® rose hip seed oil, white camellia oil, titanium dioxide, lemon peel oil, blue camomile, citrus seed extract, vitamins A, C and E.

Sources: Aubrey Organics, 1999a; Aubrey Organics, 1999b; Drugstrore.com, 2000; Natural Connections, 1999; NatureRX.com, 1998; Nature's Way, 1999; O-Cha, 2000; Stash Tea, 1999b.

Human Exposure:

Occupational Exposure. No reports of occupational exposure to EGCG were found in the available literature. No listing was found for EGCG in the National Occupational Exposure Survey (NOES), which was conducted by the National Institute of Occupational Safety and Health (NIOSH) between 1981 and 1983.

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Environmental Exposure. Environmental exposure to EGCG appears to be limited to the cultivation and processing of tea. No specific information on environmental exposure to EGCG was found in the available literature.

Consumer Exposure. Human exposure to EGCG occurs primarily via consumption of tea as a beverage, as well as through the use of dietary supplements and skin care products containing GTE.

Tea is the most widely consumed beverage in the world next to water and can be found in almost 80% of all US households. According to the Tea Council of the USA, nearly 127 million people – half of the US population – drink tea daily (Stash Tea, 1999a).

No figures on the number of GTE consumers were found in the available literature. The manufacturer-recommended dosage of GTE consumed as a dietary supplement varies greatly and ranges from one 170-mg capsule (93.5 mg EGCG) 1 – 3 times per day to six 100-mg capsules (50 mg catechins) twice daily (Drugstore.com, 2000; MotherNature.com, 2000; Natural Connections, 1999; Nature's Way, 1999; Nutrimart, 2000).

Regulatory Status: Since 1994, dietary supplements have been regulated under the Dietary Supplement Health and Education Act (DSHEA). For dietary supplements on the market prior to October 15, 1994, the DSHEA requires no proof of safety in order for them to remain on the market. The labeling requirements for supplements allow warnings and dosage recommendations as well as substantiated "structure or function" claims. All claims must prominently note that they have not been evaluated by the FDA, and they must bear the statement "This product is not intended to diagnose, treat, cure, or prevent any disease" (FDA, 1995).

Tea is subject to the Federal Food, Drug, and Cosmetic Act, as well as the Tea Importation Act. Under the latter law, tea offered for entry must meet the standards of purity, quality, and fitness for consumption prescribed under 21 CFR 1220 (FDA, 1997).

No standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace allowable levels of EGCG. EGCG is not on the American

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Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a threshold limit value (TLV) or biological exposure index (BEI) have been made.

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EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY:

Human Data: No epidemiological studies or case reports investigating the association of exposure to EGCG and cancer risks in humans were identified in the available literature.

In a Hong Kong retrospective study of 200 female lung cancer patients and 200 matched controls, subjects were interviewed about eating habits, smoking histories, and lifetime exposures to environmental pollutants. The results demonstrated a statistically significant 2.7-fold increase in lung cancer risk among green tea-drinkers (Tewes *et al.*, 1990).

EGCG has been implicated in causing green tea-induced asthma. The study conducted by Shirai and co-workers examined three groups of subjects: five nonatopic nonasthmatic volunteers, five asthmatics with no previous exposure to green tea dust, and three patients with green tea-induced asthma. All three patients exhibited an immediate skin and bronchial response to EGCG, while none of the asthmatic and healthy controls showed a positive reaction. EGCG also caused a dose-dependent histamine release in five out of seven green tea-sensitive patients, but not in asthmatic and normal controls (Shirai *et al.*, 1994; Shirai *et al.*, 1997).

GTE was cited in 28 Adverse Event Reports made to the FDA Office of Special Nutritionals as of October 20, 1998, out of a total of 2621 adverse events involving 3451 products. None of the reported adverse events involved consumption of GTE alone without any additional dietary supplements. According to FDA, there is no certainty that a reported adverse event can be attributed to a particular product or ingredient (FDA, 1998).

Kamijo and co-workers have reported on a 36-yr-old patient with schizophrenia, who, upon consumption of gradually increasing quantities of oolong tea (up to fifteen liters a day), was hospitalized with delirium (which resolved after abstinence from oolong tea), acute renal failure with hyponatremia, and severe rhabdomyolysis. However, the clinical course suggested that caffeine, which is present in oolong tea, was primarily responsible for the rhabdomyolysis and delirium (Kamijo *et al.*, 1999).

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Animal Data:

Acute Studies. The oral LD_{50} of EGCG in mice was reported as 2170 mg/kg bw (NLM, 1999).

Subacute Studies. Stratton and co-workers report a study in which female BALB/c mice were dehaired by shaving or with a topical depilatory and treated daily with 1 – 10% of EGCG in Hydrophilic Ointment U.S.P. for 30 d. In chemically dehaired mice, EGCG induced dermal toxicity (manifested as erythema and papular lesions) by day 5 at the highest concentration. A 7% reduction in body weight was also observed. No toxicity was observed at the two lower concentrations or when mice were dehaired by shaving, leading to the conclusion that topical depilatories may potentiate the dermal toxicity of EGCG. No toxicity was observed in a similar experiment involving female SKH1 hairless mice (Stratton *et al.*, 2000).

Carcinogenicity Studies. In NIH Black rats, subcutaneous administration of the tannin fraction from *Camellia sinensis* for 78 wk, induced tumors at the injection site in 67 and 73% or males and females, respectively ($P < 0.005$) (Kapadia *et al.*, 1976). However, in 6 – 8-wk-old male Swiss mice, observed until death, various tea fractions did not produce tumors when injected subcutaneously at 1 – 2 mg/animal/d for 5 d/wk for an unspecified period of time (probably fifteen months). In the same study, the tea fractions administered by gavage 5 d/wk for fifteen months did not increase the tumor incidence (Nagabhushan *et al.*, 1991).

Hirose and co-workers examined the effects of dietary administration of green tea catechins to F344 male rats treated with carcinogens in a multi-organ carcinogenesis model. Carcinogenesis of the small intestine was inhibited by catechins. However, they increased hepatocarcinogenesis when applied both during (0.1 or 1% catechins) and after (1% catechins) carcinogen exposure, as was evident by a significant increase in the numbers of glutathione S-transferase placental form-positive liver foci per cm^2. Incidences and/or multiplicity of lung tumors increased in groups of animals treated with catechins, although the values were not significantly different from the controls (Hirose *et al.*, 1993).

In a 567-day study, male and female CD-1 mice were painted once with benzo[*a*]pyrene (BP), then three times a day with black tea infusion. Treatment

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with tea (but not gallic or tannic acid) significantly shortened the latency of skin tumors, especially squamous cell carcinomas, as compared to the tea-untreated controls (Bogovski & Day, 1977).

Short-Term Tests: Review of the available information on EGCG and GTE mutagenicity, reported in the available literature, has yielded equivocal data. The results of EGCG, green tea, and black tea genotoxicity studies are summarized in Table 2.

Metabolism: *Human Studies*. Some pharmacokinetic studies of orally administered tea polyphenols have been conducted. In a 56-day study with ten healthy adult subjects, green tea consumption resulted in the highest fecal and urinary excretions, as well as highest retention and blood concentrations of polyphenols, as compared to black tea, decaffeinated black tea, and no tea treatment. The results of this study demonstrate that green tea polyphenols are at least partially absorbable (Brown, 1999).

Nakagawa and co-workers showed a dose-dependent increase in plasma concentration of EGCG administered to healthy volunteers (Nakagawa *et al.*, 1997)

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Table 2. Genotoxicity studies of EGCG and tea.

Preparation tested	Test system/strain or cell line	Dose; study details (activation, solvent, schedule)	Result	References
Endpoint: mutation				
EGCG	*S. typhimurium* TA100	Ames test w S-9, dose not reported	High dose – positive; low dose – negative	Weisburger, 1996
Green tea extract	*S. typhimurium* TA98, TA100	Ames test w/wo S-9, 0 – 5000 mg/plate	Negative	Yamane *et al.*, 1996
Green and black tea infusions	*S. typhimurium* TA98, TA100	Ames test w/wo S-9, 0 – 1.0 ml/plate infusion of 4 g/150 ml water	Positive only with cell-free extract of cecal bacteria w/wo S-9	Tewes *et al.*, 1990
Black tea	*S. typhimurium* TA98, TA100, TA1535, TA1538	Ames test w/wo S-9, 0 – 550 mg/plate	Negative	Nagabhushan *et al.*, 1991
Black tea	*D. melanogaster* wing	Somatic mutation and recombination test (SMART), 0 – 40% (w/v)	Positive	Graf *et al.*, 1994
Endpoint: sister chromatid exchange (SCE)				
EGCG	Chinese hamster cells	20 mg/ml	Potentiated mitomycin C-induced SCE	Imanishi *et al.*, 1991
Endpoint: chromosomal aberrations (CA)				
EGCG	Chinese hamster cells	20 mg/ml	Potentiated mitomycin C-induced CA	Imanishi *et al.*, 1991
Black tea infusion	Swiss mouse bone marrow cells	1.756 mg/0.16 ml distilled water, by gavage, 2X/d, 7 d	Mildly clastogenic	Mukherjee *et al.*, 1997
Endpoint: DNA damage				
EGCG	pBR322plasmid	0 – 0.1 mM	Potentiated DNA single strand breaks by diethanolamine NONOate	Ohshima et al., 1998
Endpoint: microsomal degranulation				
Green tea infusion	Isol. Wistar rat liver microsomes	40 mg/ml *in vitro*	Positive	Minocha *et al.*, 1986
Green tea infusion	Wistar rats (*in vivo* study)	160 mg/kg bw 1X sc, rats sacrificed after 10 hr and microsomes prepared	Positive	Minocha *et al.*, 1986

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In a study with eighteen individuals given 1.5, 3.0, or 4.5 g of decaffeinated green tea in 500 ml of water, the time-dependent maximum plasma concentrations (C_{max}) and urinary excretions of tea catechins were measured. Plasma concentration values reached C_{max} at 1.4 – 2.4 hr after ingestion in a dose-dependent manner, approaching a plateau at the 3.0 g dose. The half-life of EGCG was approximately 5 – 5.5 hr, almost twice that of epigallocatechin (EGC) and epicatechin, both of which, unlike EGCG, were excreted in the urine (Brown, 1999).

When an infusion of green tea containing approximately 400 mg of catechins was given to healthy volunteers, EGCG was detected in plasma samples, reaching C_{max} at two hours post-administration. Urine samples collected at 6 – 48 hr contained detectable amounts of catechin metabolites totaling 60 mg (Brown, 1999).

When eighteen healthy adults consumed eight cups per day of green tea, black tea, black tea with milk, or water, for three days, a gradual increase in plasma catechin levels in the mornings and evenings was observed. The levels declined overnight when no tea was consumed. Green tea catechins were mainly found in the protein-rich fraction of plasma (60%) and in high-density lipoproteins (23%). Addition of milk to black tea did not affect any of the parameters measured (van het Hof *et al.*, 1999).

In another study with six healthy volunteers, peak saliva levels of EGCG (4.8 – 22.0 μg/ml) were observed within six minutes following oral consumption of green tea infusion. These levels were two orders of magnitude higher than those in the plasma, although the half-life of salivary catechins was only 10 – 20 min. Interestingly, holding an EGCG solution in the mouth for a few minutes resulted in the presence of EGCG and EGC in the saliva and subsequently of EGC in urine, suggesting that EGCG was converted to EGC in the oral cavity and that both catechins were absorbed through the oral mucosa. A catechin esterase, which converts EGCG to EGC, was found in the saliva (Brown, 1999).

Animal Studies. A study on bioavailability of ^{3}H-EGCG in CD-1 mice revealed a wide distribution of radioactivity in multiple organs. Specifically, radioactivity was found in all reported EGCG target organs (digestive tract, liver, lung, pancreas, mammary

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gland, and skin), as well as in the brain, kidney, uterus, ovary, and testes. Significant amounts of radioactivity were found in various organs one hour after administration, and the levels gradually increased up to 24 hr. Within 24 hr, 6.6% of total administered radioactivity had been excreted in urine, 37.1% in feces (Suganuma *et al.*, 1999).

Nakagawa and Miyazawa showed that EGCG administered via a stomach tube to male Sprague-Dawley rats was absorbed from the digestive tract, reaching detectable levels in the plasma, liver, brain, small intestinal mucosa, and colon mucosa, with the small intestinal mucosa constituting the most enriched tissue (Nakagawa and Miyazawa, 1997).

Other Biological Effects:

Teratogenicity. Neural tube defects, such as anencephaly and spina bifida, which are usually associated with folic acid and/or vitamin B_{12}-deficiency (DiGiuseppi *et al.*, 2000), have been linked to the maternal tea consumption during the periconceptional period (Correa *et al.*, 2000). A study of 464 mothers of anencephalics and 1785 controls showed a significant ($P<0.001$) correlation between daily consumption of three or more cups of tea and the incidence of anencephaly (Fedrick, 1974). EGCG appeared to inhibit rat embryo limb bud cell differentiation in the *in vitro* micromass test (Flint & MacLean, 1994).

Neuromuscular Effects. Both black and green tea extracts have been reported to exhibit proconvulsive properties in mice, manifested in acceleration of the onset of convulsions, their prolongation, and increased mortality (Gomes *et al.*, 1999). GTE facilitated skeletomotor function of innervated rat diaphragm at lower concentrations and exerted a paralytic effect at higher concentrations. GTE did not have any effect on denervated rat diaphragm. It was suggested that the polyphenol content of GTE was the active constituent responsible for the GTE effect on neuromuscular junctions (Das *et al.*, 1997).

Tumor Inhibition. A vast amount of information on the chemopreventive effects of EGCG and other tea catechins has been accumulated over the past decade. EGCG is a subject of ongoing Phase IIb clinical trials for prevention of non-melanoma skin cancer, aimed at demonstrating that specific histopathologic and morphometric

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abnormalities, genetic alterations, and immunohistochemical biomarker changes can be safely modulated by chemoprevention agents (Levine *et al.*, 2000). Selected tumor inhibition data on EGCG are presented in Table 3.

Antimutagenic Properties. EGCG exhibited significant dose-dependent antimutagenic activity against *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), *N*-nitroso-*N*-methylurea (MNU), folpet, captan, 9-aminoacridine (9AA), 4-nitroquinoline-*N*-oxide (4-NQNO), and BP, as assessed by Ames test (Hour *et al.*, 1999). EGCG also suppressed aflatoxin B_1-induced chromosomal aberrations in rat bone marrow cells *in vivo* when administered 24 hr prior to the carcinogen injection (Ito and Fujie, 1991).

It has been suggested that EGCG and other tea catechins suppress tumor promotion by inhibiting the release of tumor necrosis factor-*a*, which is believed to stimulate tumor promotion and progression of initiated cells as well as premalignant cells (Fujiki *et al.*, 2000). Furthermore, EGCG was shown to reduce specific binding of both the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-type and the okadaic acid-type tumor promoters (the two major classes of tumor-promoting agents) to their receptors. This "sealing" effect of EGCG is achieved by its interaction with the phospholipid bilayer of the cell membrane (Fujiki *et al.*, 1999).

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Table 3. Tumor inhibition studies of EGCG

Species	Strain/ sex	Dose/route of administration/duration	Carcinogen/route/ dose	Results	Ref.
Mouse	C57BL/male	0.15 mg/mouse/d in drinking water for 12 wk	*N*-Ethyl-*N'*-nitro-*N*-nitrosoguanidine (ENNG), 100 mg/L for 4 wk in drinking water	Inhibited incidence of duodenal tumors	NLM, 2000
Mouse	C57BL/male	0.005% in diet for 12 wk	ENNG, 100 mg/L for 4 wk in drinking water	Inhibited incidence of duodenal tumors	NLM, 2000
Mouse	Strain A/ female	560 ppm in drinking water for 13 wk	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone, 11.65 mg/kg 3X/wk for 10 wk, gavage	Inhibited multiplicity of lung tumors	NLM, 2000
Mouse	SENCAR/ female	5 µmol/0.2 ml acetone 1X/d for 7d prior to carcinogen, dermal	Initiator: 7,12-dimethylbenz-[*a*]-anthracene (DMBA), 10 nmol/0.2 ml acetone 1X, dermal Promoter: 12-*O*-tetradecanoylphorbol-13-acetate (TPA), 3.2 nmol/0.2 ml acetone 2X/wk, dermal	Inhibited multiplicity of skin tumors	NLM, 2000
Mouse	C3H/HENCR J male & female	0.05% in drinking water for 58 wk	None	Inhibited incidence and multiplicity of spontaneous tumors	NLM, 2000
Rat	Wistar/male	0.05% in drinking water for 15 wk	MNNG, 80 mg/L for 28 wk in drinking water	Inhibited incidence and multiplicity of stomach tumors	Yamane *et al.*, 1996
Rat	Wistar/male	0.05% in diet for 16 wk	ENNG, 80 mg/L for 28 wk in drinking water	Inhibited incidence of stomach tumors	Yamane *et al.*, 1996
Rat	Sprague-Dawley/ female	0.5% (58.4 – 81% EGCG) in diet for 23 wk	DMBA, 50 mg/kg 1X, gavage	Promotion of or no effect on mammary gland tumors	NLM, 2000

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Antiangiogenic Effects. Green tea consumption by animals inhibited vascular endothelial growth factor (VEGF)-induced angiogenesis. EGCG was able to inhibit endothelial cell growth *in vitro* and the angiogenesis process *in vivo* (L'Allemain, 1999). It has been suggested that the mechanism of anti-angiogenic action of EGCG involves urokinase inhibition (Swiercz *et al.*, 1999).

Antihepatotoxic Effects. Green tea has been shown to inhibit chemically induced hepatic tissue damage in rodents. A 2% green tea infusion, given to male rats or mice as drinking water prior to the chemical treatment, inhibited the hepatotoxicity induced by intraperitoneal administration of 2-nitropropane, galactosamine, and penta-chlorophenol (Hasegawa *et al.*, 1998).

Antiproliferative/Apoptotic Effects. EGCG has been shown to induce growth arrest and/or apoptosis in various cell lines. Treatment of prostate cancer cell lines DU145 and LNCaP with EGCG resulted in a dose-dependent apoptosis (Gupta *et al.*, 2000). DNA flow cytometric analysis indicated that 30 μM of EGCG blocked cell cycle progression at the G1 phase in MCF-7 human breast carcinoma cells, perhaps via inhibition of cyclin-dependent kinases 2 and 4 or induction of their inhibitors p21 and p27 (Liang *et al.*, 1999).

Peripheral blood T lymphocytes from adult T-cell leukemia patients and a transformed T cell line KODV, but not normal human peripheral blood lymphocytes, treated with 27 μg/ml of either GTE or EGCG, exhibited DNA fragmentation characteristic of apoptosis (Li *et al.*, 2000). EGCG also inhibited the proliferation and viability of HTB-94 human chondrosarcoma cells (in a dose-dependent manner) and induced apoptosis. These effects were suggested to be mediated by caspase-3, thus providing a possible mechanistic explanation for the antitumor properties of EGCG (Islam *et al.*, 2000).

Antioxidant/Free Radical Scavenging Effects. Most of the potentially beneficial effects of tea catechins are attributed to their antioxidant properties, i.e. their ability to scavenge free radicals to produce a phenoxyl radical, as shown in Figure 1 (adapted from Pietta *et al.*, 1996).

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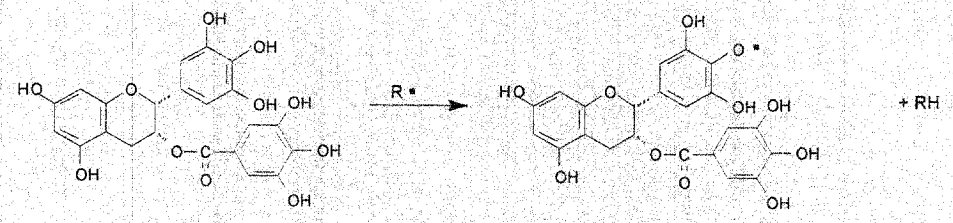

Figure 1. Free radical (R•) scavenging by flavonoids.

The free radical scavenging properties of catechins have been well studied, particularly during the last decade. The early evidence of antioxidative properties of EGCG came from the experimental data that showed EGCG-induced inhibition of soybean lipoxygenase (IC_{50} = 10 – 20 μM) (Ho *et al.*, 1992). Later, it was reported that EGCG inhibited TPA-induced oxidative DNA base modification in HeLa cells, inhibited Cu^{2+}-mediated oxidation of low density lipoprotein (LDL), reduced *tert*-butyl hydroperoxide-induced lipid peroxidation, and blocked the production of reactive oxygen species derived from NADPH-cytochrome P450-mediated oxidation of the cooked meat carcinogen, 2-amino-3methylimidazo[4,5-*f*]quinoline (Surh, 1999).

Interestingly, while low concentrations of EGCG inhibited Jurkat T cell DNA damage caused by hydrogen peroxide or 3-morpholinosydnonimine (a peroxynitrite generator), at high concentrations EGCG itself induced cellular DNA damage (Johnson & Loo, 2000).

Cardiovascular Effects. Several flavonoids and related phenolics have been reported to inhibit either enzymatic or non-enzymatic lipid peroxidation, an oxidative process implicated in several pathologic conditions, including atherosclerosis (Pietta *et al.*, 1996). In particular, tea polyphenols have been suggested to play a role in lowering the oxidation of LDL-cholesterol, with a consequent decreased risk of heart disease (Weisburger, 1999). In a cross-cultural correlation study of sixteen cohorts, known as the Seven Countries Study, the average flavanol intake was inversely correlated with mortality rates of coronary heart disease after 25 years of follow-up (Hertog *et al.*, 1995; Hollman *et al.*, 1999).

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Adding support to the observations in humans are the findings that in rats rendered hypercholesterolemic by excessive dietary fat, green tea polyphenols lowered blood cholesterol levels and reduced blood pressure in spontaneously hypertensive animals (Dreosti, 1996).

Antibacterial and Immunomodulatory Effects. A number of reports have alluded to the bactericidal, bacteriostatic, and/or antitoxic activity of EGCG and other tea components against *Bordetella pertussis* (Horiuchi *et al.*, 1992), *Helicobacter pylori* (Mabe *et al.*, 1999), enterohemorrhagic *Escherichia coli* (Okubo *et al.*, 1998), *Yersinia enterocolitica* (Yam *et al.*, 1997), the oral bacterium *Porphyromonas gingivalis* (Sakanaka *et al.*, 1996), several species of *Mycoplasma* (Chosa *et al.*, 1992), and even methicillin-resistant *Staphylococcus aureus* (Kono *et al.*, 1994).

The data regarding immunomodulatory properties of EGCG remain somewhat contradictory. Some observations indicate that EGCG exerts strong anti-inflammatory effects on the host. For example, EGCG has been implicated in reducing ultraviolet radiation-induced inflammatory responses and infiltration of leukocytes in human skin (Katiyar *et al.*, 1999) and blocking lipopolysaccharide (endotoxin)-induced tumor necrosis factor production and lethality in BALB/c mice (Yang *et al.*, 1998). Other reports, however, point to pro-inflammatory characteristics of EGCG, such as stimulation of human monocyte and polymorphonuclear cell iodination and interleukin-1 production (Sakagami *et al.*, 1992), as well as erythrocyte-dependent B cell mitogenicity (Zenda *et al.*, 1997).

Structure-Activity Relationships: EGCG is structurally related to other tea flavanols, catechin, epicatechin, epicatechin gallate, and epigallocatechin, as well as to gallic acid. Catechin (CAS No. 154-23-4) has been tested for carcinogenicity in a two-year bioassay and was found to induce stomach tumors in F344 rats (NLM, 2000). Flavanols (except epigallocatechin) and gallic acid have been found genotoxic (NLM, 1999; NLM, 2000). In addition, investigations into the flavanol-induced tumor inhibition produced an extensive amount of data on the antitumorigenic properties of flavanols (NLM, 2000). Selected carcinogenicity, mutagenicity, and tumor modification data found in the available literature are presented in Table 4.

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Table 4. Carcinogenicity, mutagenicity, and tumor modification studies of EGCG and related compounds

Compound	Carcinogenicity Data	Mutagenicity Data	Tumor Inhibition/Promotion/ Antimutagenicity Data
Epigallocatechin gallate [989-51-5]	No data found in the available literature	Positive results in Ames test at high doses (Weisburger, 1996) Potentiated mitomycin C-induced chromosomal aberrations and sister chromatid exchanges in Chinese hamster cells (Imanishi *et al.*, 1991)	Inhibited duodenal, lung, and skin tumors in mice and stomach tumors in rats; did not inhibit mammary gland tumors in rats (see Table 3)
Catechin [154-23-4]	Induced glandular stomach adenocarcinomas in male and female F344 rats given 2% in diet for 104 wk (NLM, 2000) Promoted forestomach and glandular stomach cancers in F344 rats initiated with MNNG (NLM, 2000)	DNA repair in *E. coli* (NLM, 1999) Sister chromatid exchanges, unscheduled DNA synthesis, DNA inhibition, sex chromosome loss and nondisjunction in human lymphocytes, and positive results in mouse and hamster micronucleus test (NLM, 1999) Positive results in Ames test (NLM, 1999)	Inhibited DMBA-initiated mammary gland tumors in Wistar rats, DMBA-initiated and croton oil promoted skin papillomas in Swiss mice & BP-initiated forestomach tumors in Swiss mice (NLM, 2000) Ineffective vs EHEN-initiated liver or kidney tumors in Wistar rats, vs NHA-initiated pancreatic adenocarcinomas, & DMBA-initiated and TPA-promoted dermal tumors in CF-1 mice (NLM, 2000) Slightly reduced mammary gland tumor

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			incidence in C3H mice given 2.5 mg/d in drinking water for 15 months [8/20 vs 12/20] (NLM, 2000)
Epicatechin [490-46-0]	No information found in the available literature	Sister chromatid exchanges in human lymphocytes (NLM, 1999)	Inhibited multiplicity of DMBA-initiated and croton oil-promoted dermal tumors in Swiss mice and 3-MC-initiated sarcomas in Swiss mice (NLM, 2000) No effect on mutagenicity of BP or IQ in *S. typhimurium* w/wo S-9 (Catterall *et al.*, 2000)
Epigallocatechin [970-74-1]	No information found in the available literature	No information found in the available literature	Inhibited multiplicity of 4-methylnitrosamino-1-(3-pyridyl)-1-butanone-initiated lung adenomas in A/J mice (NLM, 2000) Inhibited incidence and multiplicity of ENNG-initiated duodenal tumors in C57BL mice (NLM, 2000) Inhibited incidence and multiplicity of MNNG-initiated stomach tumors in Wistar rats (NLM, 2000)

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Gallic acid [149-91-7]	No information found in the available literature	Positive results in Ames test at 100 µg/plate (NLM, 1999) Gene conversion and mitotic recombination in *S. cerevisiae* at 100 mg/L (NLM, 1999) Positive results in cytogenetic analysis in mouse (at 100 µmol/kg ip) and CHO cells (at 50 mg/L) (NLM, 1999)	Inhibited incidence and/or multiplicity of lung and skin tumors in Strain A, CD-1, CF-1 and SENCAR mice (NLM, 2000)

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