

GREENSELECT™

A CAFFEINE-FREE, STANDARDIZED EXTRACT
FROM GREEN TEA LEAVES





BOTANY

The tea plant is *Camellia sinensis* (L.) O. Kuntze (synonymous: *Thea sinensis* L.), belonging to the Theaceae family (Fig. 1). *Camellia sinensis* is an evergreen shrub or tree which in its natural state grows to a height of 10-15 m. The leaves are alternate, evergreen, oval, acuminate, with serrate edges. Mature leaves are bright green in colour, leathery and smooth, varying in length from 5 to 20 cm. The flowers may be solitary or may occur in clusters of 2-4. They are fragrant, 2.5-3 cm wide, with white petals and yellow anthers. The

tea plant is thought to have originated in southern Yunnan (var. *sinensis*) and in the Assam province in India (var. *assamica*). In China, tea has been cultivated since ancient times; the development of a flourishing trade led to the introduction of tea cultivations in Japan and since the 19th century in India, Ceylon, Java, Sumatra, Russia (Georgia) and Africa (Kenya). In plantations the shrubs are maintained by pruning to a height of 1 to 1.5 m. This plant grows well at an altitude of 500 to 2000 m, in tropical and semitropical regions with adequate rainfall (not less than 2000 mm) and well drained and slightly acid soil.

According to Chinese tradition tea drinking was introduced to China with the reign of the legendary Emperor Shen Nong, in the third millennium B.C..

Tea is mentioned in the ancient Chinese dictionary "Erya" and in a commentary of the philologist Guo Pu (276-324 A.D.) a plant similar to tea is described.

But the most reliable and ancient mention of tea, rather than wine, drinking is in a biography of the historian Wei Yao (second half of 3rd century). The origin of the tea plant is described in an old legend about a Buddhist holyman, named Daruma who, to punish himself for falling asleep when he was praying, cut off his eyelids.

Where the eyelids fell a bush grew, the leaves of which, dried and infused in hot water, produced a beverage that kept one awake. Many guidebooks concerning the use and sophisticated ways of preparing tea have been written, but the most ancient and important in the world is the "Chajing", by the Chinese scholar and poet Lu Yu (around 760 A.D.).

The Chajing is considered a sort of Bible of tea cultivation, harvesting, processing and tasting. The process of obtaining tea from the leaves of the tea plant described in this book is similar to the methods used today. Buddhist monks encouraged the cultivation of tea and the habit of tea drinking, as a remedy for intemperance, throughout China and Japan. Tea reached Europe in the 16th century and Russian Mongolia, in the 17th. In the second half of the 17th century tea was known in France, Germany and Holland and became a popular beverage in London, where it was served in numerous tea houses.

Fig. 1 Tea, *Camellia sinensis*.



From Chinese Pharmacopeia "Bencao Pin Hui Jing Yao" written by Liu Wentai, Wang Pan and Gao Tinghe, 1505.

TYPES OF TEA

More than 300 types of tea are produced in China but the most widely used can be divided into three classes: black tea, green tea and oolong tea.

● Black tea is obtained from leaves allowed to ferment and then dried. During the fermentation there is an extensive enzymatic oxidation of the catechin polyphenols, producing related compounds called thea-flavins.

● **GREEN TEA IS PRODUCED BY STEAMING OR DRYING THE FRESH LEAVES AT ELEVATED TEMPERATURES. THIS PROCESS LEAVES THE POLYPHENOLS INTACT.**

● Oolong tea is obtained from partially fermented leaves.

Tea, after water, is the most diffuse beverage in the world: green tea is widely used in China, Japan and other eastern countries while black tea is more diffuse in western countries.

Nevertheless, green tea is also used in North America and Europe.¹⁻³

CHEMISTRY

Green tea infusion contains flavanols (catechin polyphenols), flavonols, and phenolic acids.³⁻⁵ Several flavanols have been isolated and identified from

green tea and two of these, (-)-epigallocatechin 3-O-gallate and (-)-epigallocatechin, exhibited a marked antioxidant activity.⁶

MEDICINAL PROPERTIES

In addition to its pleasant flavor and special aroma, green tea possesses medicinal properties that have been known since antiquity.

The beneficial effects of tea were first reported in the textbook of Chinese herbal medicine "Shen Nong Ben Cao Jing" (100-200 A.D.) and later its pharmacological and therapeutic effects were described in various books and monographs, and in the ancient Chinese Pharmacopeia "Ben Cao Gang Mo", written by Li Shizheng during the Ming dynasty (16th century).¹ According to traditional medicine, tea possesses stimulant, digestive, diuretic, analgesic and antitoxic properties.⁷ Since 1970 the health effects of green tea have been the object of numerous scientific studies. The results of these investigations have confirmed the traditional medicine indications and highlighted other pharmacological activities such as antiatherosclerotic, hypocholesterolemic, antihepatotoxic, radioprotective, antimicrobial, anticariogenic and antitumor effects.

EPIDEMIOLOGICAL STUDIES

Case-control studies in Japan indicated that the consumption of green tea lowered the risk of stomach and colon cancer.^{8,9} In the Shizuoka Prefecture, where green tea is one of the staple commodities and the main beverage, the death rate from cancer, especially stomach cancer, in both sexes, is significantly lower compared to the average of Japanese people.¹⁰

A cross-sectional study on Japanese men in Northern Kyushu, Kono *et al.*¹¹ found that serum total cholesterol levels decreased with increasing consumption of green tea, while triglycerides and high-density lipoprotein cholesterol did not show significant changes.

Another interesting study concerning the inhibitory effect of tea infusions on the formation of mutagenic nitrosamine in human volunteers is reported by Stich.¹² A recent critical evaluation of epidemiological literature confirms the favorable effect of tea and indicates green tea in particular as a source of protective polyphenols against proliferative diseases.¹³

BIOLOGICAL STUDIES

At the National Symposium on the Health Effects of Chinese Tea in Beijing (1989), many studies on the antimutagenic and anticarcinogenic effect of tea were presented.¹

Recent investigations have demonstrated that green tea possesses free radical scavenging, antioxidant and antiinflammatory properties.^{14,15}

Free radicals are known to have an important role in the mechanism of pathological processes such as carcinogenesis and atherosclerosis. Compounds that exhibit antioxidant and/or antiinflammatory activities can inhibit chemically-induced tumor promotion in mouse skin.¹⁶

Recent studies have demonstrated that constituents of green tea, specifically the polyphenolic fraction, are able to inhibit chemically-induced tumor promotion on the skin of mice and other organs such as the lung, forestomach, esophagus, duodenum and small intestine, colon, liver, pancreas and mammary gland.⁵

BIOCHEMICAL MECHANISMS

The biochemical mechanisms that can be involved in the pharmacological activity of green tea polyphenols are summarized here:^{5,17}

1 Enhancement of the antioxidant defence systems (stimulation of glutathione peroxidase and catalase) and of the activity of phase II enzymes (glutathione-S-transferase and quinone reductase).

2 Interaction with electrophilic carcinogen species to form flavanol-carcinogen adducts, due to their strong free radical scavenging activity.

3 Inhibition of enzymatic pathways involved in cancer initiation, such as the cytochrome P-450 system, responsible for the activation of procarcinogens to highly reactive molecules and formation of carcinogen-DNA adducts.

4 Inhibition of enzymatic activities involved in tumor promotion as epidermal ODC (ornithine decarboxylase), TPA-caused stimulation of cyclooxygenase, lipoxygenase and protein kinase-C activities.

5 Inhibition of a proteolytic enzyme, named urokinase, involved in the increase of tumour size and in the process of metastatic dissemination.

GREENSELECT™

The active constituents of green tea leaves are a group of polyphenols belonging to the flavanol class.¹⁸ GREENSELECT™ is a caffeine-free extract from green tea leaves, characterized by a high content (not less than 60%) of polyphenols, the main constituent (-)-epigallocatechin 3-O-gallate accounting for not less than 40% of the extract (Patent Appl. WO 96/28178).

The structures and HPLC profile of GREENSELECT™ polyphenols are shown in Fig. 2.

The identification of the polyphenol constituents has been performed by HPLC-MS analysis and comparison with the retention times of standard compounds.

GREENSELECT™ has been submitted to toxicological¹⁹ and pharmacological investigations demonstrating its safety and *in vitro* and *in vivo* antioxidant properties (Fig. 3-5, Table 1).^{20,21} These results constitute the most relevant biochemical feature at the basis of the protective effect exerted by GREENSELECT™ against free radical-induced tissue damage.

Fig. 2 Polyphenol constituents and HPLC profile of GREENSELECT™.

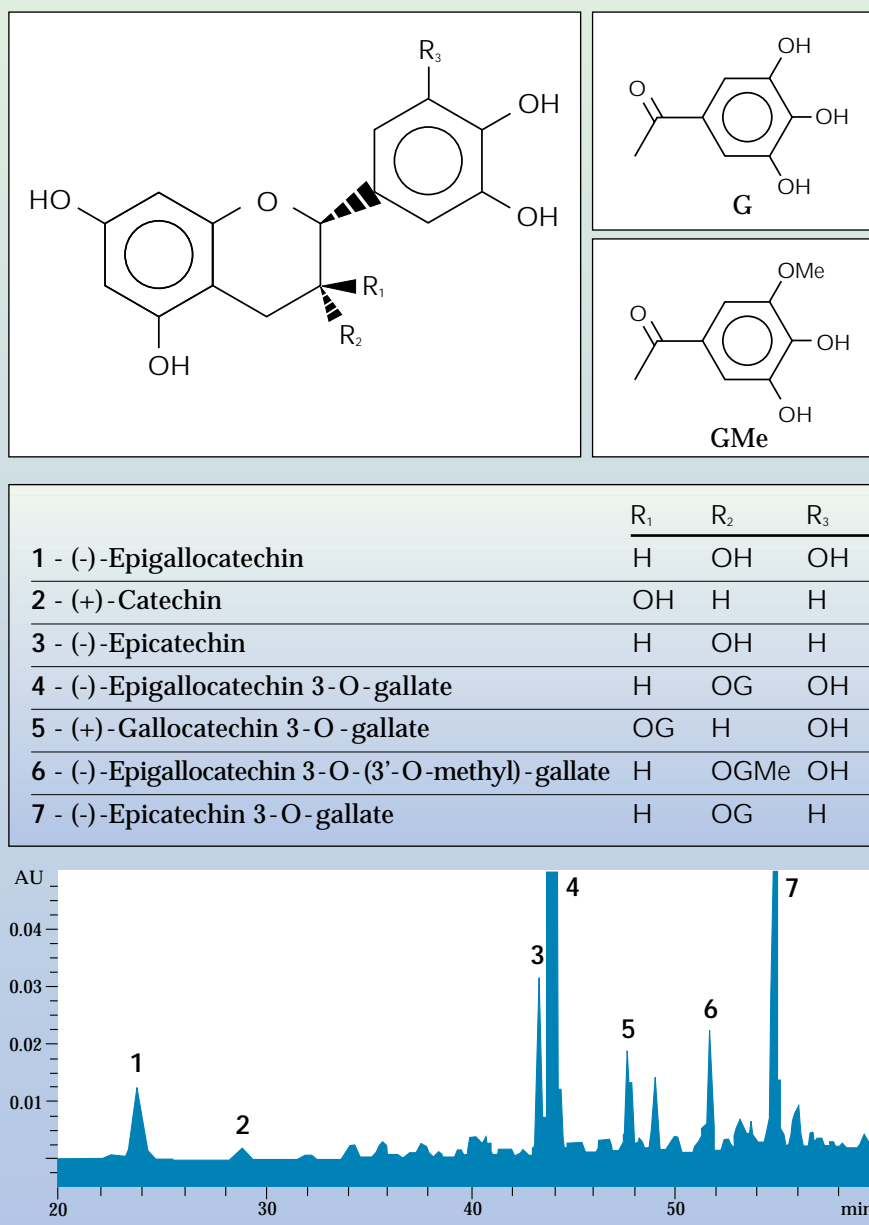


Fig. 3 *In vitro* activity of GREENSELECT™ on lipid peroxidation induced by Fe²⁺/ascorbic acid on rat liver microsomes (IC₅₀ = 3.6 μM).

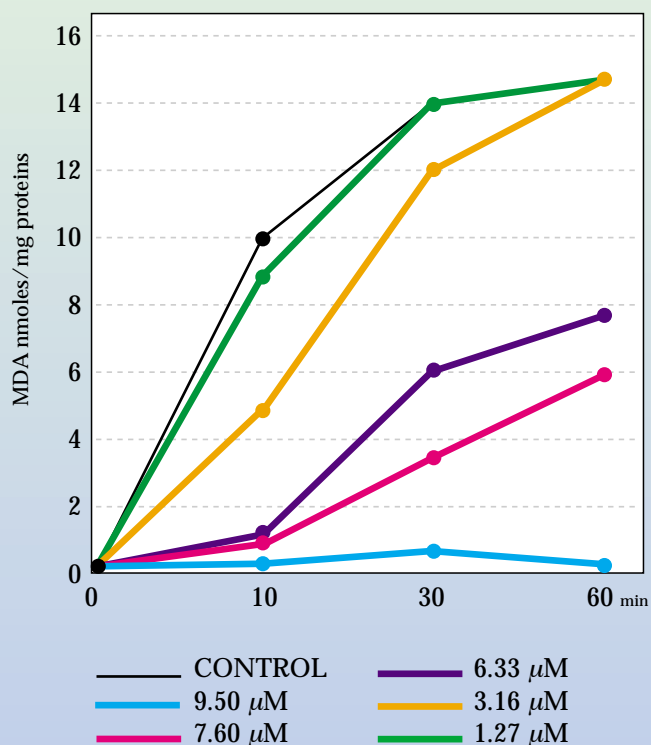


Fig. 4 *In vitro* activity of GREENSELECT™ on the propagation phase in ultrasound-induced lipid peroxidation on liposomes (IC₅₀ = 0.44 μM).

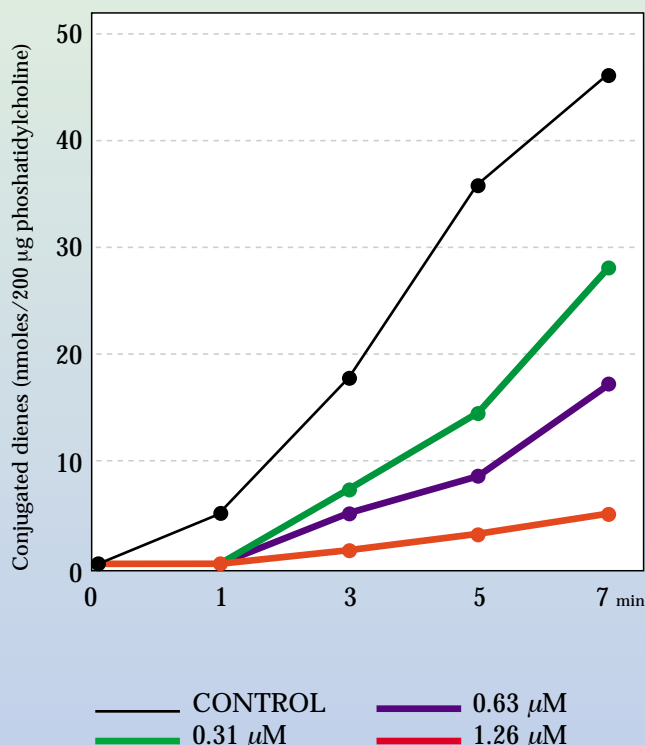
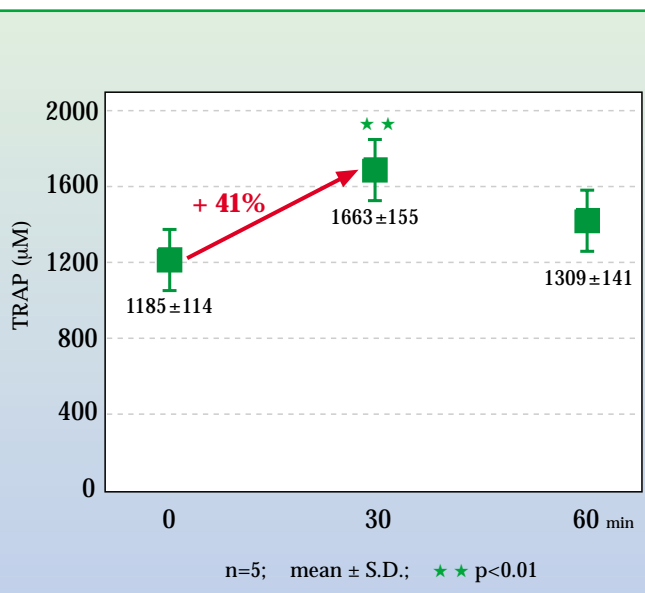


Table 1 Effect of subchronic administration of GREENSELECT™ (100 mg/die) on plasma and red blood cells (RBC) lipophilic antioxidants in healthy volunteers.

| PLASMA AND RBC ANTIOXIDANTS | BASELINE (Mean ± S.E.) | 4 WEEKS TREATMENT (Mean ± S.E.) |
|-----------------------------|------------------------|---------------------------------|
| Plasma retinol (μg/dL) | 48.2 ± 11.9 | 47.8 ± 12.2 |
| Plasma α-tocopherol (μg/mL) | 10.6 ± 1.3 | 9.6 ± 1.6* |
| Plasma β-carotene (μg/dL) | 32.0 ± 21.1 | 37.9 ± 20.4* |
| RBC α-tocopherol (μg/g) | 1.89 ± 0.37 | 2.00 ± 0.26* |

n=15; age=20-40 years
*p<0.05 vs baseline

Fig. 5 Acute effect of GREENSELECT™ (300 mg) on total plasma antioxidant capacity (TRAP) in healthy volunteers.



USAGE

100 - 200 mg daily of GREENSELECT™ are recommended for counteracting damage due to over production of free radicals at tissue level.

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