P H Y T O S O M E

GREENSELECT® PHYTOSOME®

indena®

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

During the Tang dynasty (618-906 A.D.) the habit of tea drinking spread from Southern China, place of origin of the tea plant, to the whole of China.

Many guidebooks have been written regarding the cultivation, use and sophisticated ways of preparing tea, but the oldest and most important in the world is “Chajing”, by the Chinese scholar and poet Lu Yu (around 760 A.D.).[1]

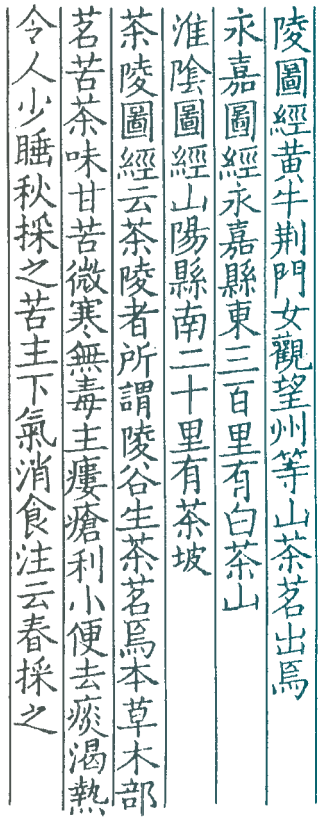
At first tea was regarded as a medicine (Fig. 1) and its stimulant and invigorating properties were much appreciated by the Buddhist monks, which contributed to its diffusion throughout China and Japan.

Tea reached Russia and Europe in the first half of the17th century and in the second half it was known in Holland, France, Germany and became a popular beverage in England and in the American colonies.[2]

BOTANY

The tea plant is called *Camellia sinensis* (L). O. Kuntze (synonymous: *Camellia thea* Link. or *Thea sinensis* L.), of the Theaceae family (Fig. 2).
C. sinensis is an evergreen shrub or tree that in its natural state grows to a height of 10-15 m. In plantations the shrubs are maintained by pruning to a height of 1 to 1.5 m.
The leaves are alternate, elliptic obovate or lanceolate, with serrate margin. The young leaves are more or less pubescent. Mature leaves are bright green in colour, leathery and smooth, varying in length from 5 to 25 cm.
The flowers, solitary or 2-4 together on short branchlets in the leaf axils, are fragrant, 2.5-3 cm wide, with 5-6 white petals and several yellow stamens.
The fruits are capsules, brownish-green in colour, 1-4 lobed, each lobe bearing 1-3 seeds, brown in colour, spherical or flattened.[3,4] This plant grows well at an altitude of 500 to 2000 m, in tropical and subtropical regions with adequate rainfall (not less than 2000 mm) and well-drained and slightly acid soil.[3]
The tea plant is thought to be a native of Yunnan, southern China (var. *sinensis*) or of Assam province in India (var. *assamica*).[3,5] 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).[6,7]

Fig. 1 A page from "Chajing" (Tea Bible).


陵圖經黃牛荊門女觀望州等山茶茗出焉
永嘉圖經永嘉縣東三百里有白茶山
淮陰圖經山陽縣南二十里有茶坡
茶陵圖經云茶陵者所謂陵谷生茶茗焉本草木部
茗苦茶味甘苦微寒無毒主瘻瘡利小便去痰渴熱
令人少睡秋採之苦主下氣消食注云春採之

Bencao, "trees" section.
Míng tea is *kûchá*, "bitter tea".
It has a sweet-bitter taste.
It is naturally rather cooling.
It is nontoxic.
Principle use: it makes abscesses maturate, it has diuretic and expectorant functions, it calms cough; it has a cooling effect and keeps awake.
Tea picked in autumn, has a bitter taste, it is carminative and digestive.
The commentary says: it has to be collected in spring.

Fig. 2 Tea, *Camellia sinensis*.

TYPES OF TEA

There are three main groups of teas: black tea, oolong tea and green tea.[6,7]

- 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.
- Oolong tea is obtained from partially fermented leaves.

- STEAMING OR DRYING THE FRESH LEAVES AT ELEVATED TEMPERATURES PRODUCES **GREEN TEA**.
  THIS PROCESS LEAVES THE POLYPHENOLS INTACT.

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.

CHEMISTRY

Green tea infusion contains flavanols (catechin polyphenols), flavonols, and phenolic acids.[6,7]
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.

PHARMACOLOGY

According to the folk Chinese medicine, tea possesses stimulant, digestive, diuretic, analgesic and antitoxic properties.[8] Since the second half of 1980, the health effects of green tea and of green tea polyphenols have been the subject of numerous scientific studies.[7] The results of these investigations highlighted interesting pharmacological activities such as cancer-preventing, antimutagenic, antiatherosclerotic, hypocholesterolemic, cardioprotective, antibacterial and anticariogenic effects.[7,9]

CANCER-PREVENTING ACTIVITY

- Green tea polyphenols are able to inhibit growth and cause apoptosis of human lung cancer cell line[10] and other cells, such as human prostate carcinoma cells.[11]
- Green tea and green tea polyphenols are endowed with a protective activity against skin tumours induced by chemicals and ultraviolet radiation in mice.[12]
- Green tea and green tea polyphenols proved to inhibit chemically induced carcinogenesis in different organs such as lung, esophagus, stomach, duodenum, colon, mammary gland and liver.[7,9,10,13,14]

Fujiki has reported that green tea polyphenols showed to be easily distributed from the digestive tract to various target organs.[10]

ANTIMUTAGENIC ACTIVITY

- Green tea polyphenols exert a protective activity against mutagenic substances.[15,16]
- Tea and tea polyphenols antagonize the formation of carcinogens during the cooking of meats and fish[17,18] and the aflatoxin B_1-induced chromosome aberration in rat bone marrows cells.[19]

ANTINFLAMMATORY ACTIVITY

- Green tea polyphenols reduce UVB-induced inflammatory response in mice and in human skin.[20,21]

ANTIATHEROSCLEROTIC, HYPOCHOLESTEROLEMIC, CARDIOPROTECTIVE ACTIVITIES

Low-density lipoprotein (LDL) oxidation is reported to be involved in the atherosclerosis and coronary heart disease development.

Dietary antioxidants through a protective effect against LDL oxidation may reduce risk of heart disease.[7,22]

Green tea polyphenols are able to inhibit copper-induced or cell-mediated oxidation of LDL *in vitro*.[23]

Green tea is found to decrease serum and liver cholesterol in rats fed on hypercholesterolemic diet.[24]

It has been also reported that green tea polyphenols reduce hypertension in mice and rats.[7]

ANTIBACTERIAL AND ANTICARIOGENIC EFFECTS

Green tea and green tea polyphenols are endowed with a broad range of antibacterial activity as well as with an anticariogenic effect.[7,25]

EPIDEMIOLOGICAL STUDIES AND CLINICAL DATA

Case-control studies in Japan, published between 1988-1997, indicated that the consumption of green tea lowered the risk of stomach and colon cancer.[26-28]

In 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.[29]

In a cross-sectional study on Japanese men in Northern Kyushu, Kono *et al.*[30] 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.

In 1997 Kohlmeier *et al.* evaluated the epidemiological studies on stomach, colon and lung cancer and reported that a protective effect of green tea against gastric and colon cancer is linked with high intakes in high-risk populations.[31]

At the 2nd International Science Symposium on Tea and Human Health (1998)[32], the efficacy and the role of tea and its components in reducing cancer risk and the incidence of cardiovascular disease have been discussed, taking account of both the experimental works and the epidemiological data. Tea and in particular green tea have been recognized to play an important role, as a dietary source of antioxidants, in preventing some pathological processes mediated by oxidative damage.

Recently Tsubono *et al.*[33] in a population-based prospective cohort study did not find any association between green tea consumption and the risk of gastric cancer.

The results of epidemiological studies are not always in agreement. This fact can be due to various factors such as different methodologies, inadequate control and different amounts of tea.[31,34]

Recently it has been reported that green tea polyphenols, topically applied on skin of human volunteers, are able to prevent UVB-induced erythema and DNA damage.[35] Thermogenic properties and promotion of fat oxidation in healthy men were also reported for a green tea extract rich in caffeine and polyphenols.[36]

In addition, in a small double-blind placebo-controlled study, green tea chew candy reduced gum inflammation in subjects with periodontal disease.[37]

TOLERABILITY

Green tea and related preparations, up to a dose equivalent to approximately ten cups of infusion daily, are on the whole well tolerated. The caffeine content can however induce some side effects such as insomnia and gastrointestinal complaints.[38]

BIOCHEMICAL MECHANISMS

The biochemical mechanisms that can be involved in the pharmacological activity of green tea polyphenols are summarized hereunder:[12,39-42]

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 urokinase, one of hydrolases involved in the increase of tumour size and in the process of metastatic dissemination;

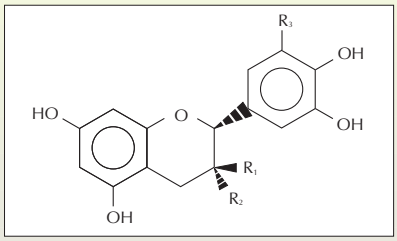
6 Inhibition of gelatinases MMP-2 and MMP-9, two of the proteases that play a key role in cancer development and in angiogenesis;

7 Inhibition of type 1 5α-reductase, an enzyme involved in the development of androgen-dependent abnormalities in different organs, such as prostate and skin.

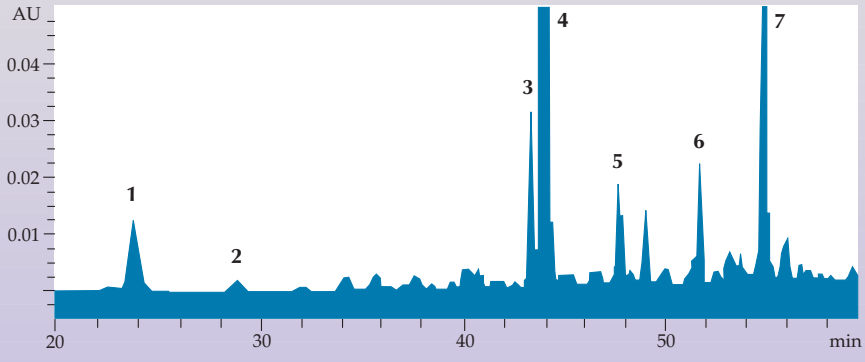
GREENSELECT® PHYTOSOME®

The active constituents of green tea leaves are a group of polyphenols belonging to the flavanol class.[43] GREENSELECT® is a caffeine-free extract from green tea leaves, characterized by a high content (not less than 66.5%) of polyphenols, the main constituent (-)-epigallocatechin 3-O-gallate (EGCG) accounting for 50-65% of the extract (Patent US 5,989,557; US 6,096,359).
The structures and HPLC profile of GREENSELECT® polyphenols are shown in Fig. 3.
Polyphenols are reported to be poorly absorbed from the gastrointestinal tract.
Our previous experience demonstrated that the complexation with phospholipids improves the bioavailability of these natural compounds.[44,45]

Fig. 3 Polyphenol constituents and HPLC profile of GREENSELECT®.



	R_1	R_2	R_3
1 (-)-Epigallocatechin	H	OH	OH
2 (+)-Catechin	OH	H	H
3 (-)-Epicatechin	H	OH	H
4 (-)-Epigallocatechin 3-O-gallate	H	OG	OH
5 (+)-Gallocatechin 3-O-gallate	OG	H	OH
6 (-)-Epigallocatechin 3-O-(3′-O-methyl)-gallate	H	OGMe	OH
7 (-)-Epicatechin 3-O-gallate	H	OG	H



In order to increase the bioavailability of tea polyphenols a complex with soy phospholipids (GREENSELECT® PHYTOSOME®) was prepared.
After oral administration to healthy volunteers of a single dose of GREENSELECT® PHYTOSOME® (400 mg as total flavanols), the peak concentrations of EGCG is more than doubled in respect of the non complexed form (Fig. 4).[46]
Consequently a 20% increase of total radical antioxidant parameter (TRAP) was observed (Fig. 5).
In a subacute (2 weeks) treatment with a daily dosage of 135 mg (as total flavanols), GREENSELECT® PHYTOSOME® showed to protect vitamin E and PUFAs (polyunsaturated fatty acids) of red blood cell (RBC) membrane (Fig. 6).[47]
GREENSELECT® PHYTOSOME® in toxicological studies (including 4 weeks treatment in rats) demonstrated to be safe and well tolerated.[48]

Fig. 4 Time course of plasma EGCG after ingestion of GREENSELECT® and GREENSELECT® PHYTOSOME®.

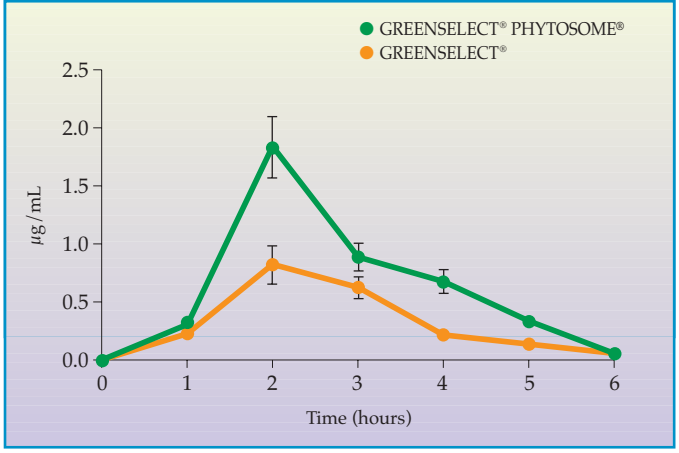


Fig. 5 TRAP modification (means ± SE, μmol/L TROLOX) and percent variation after intake of GREENSELECT® and GREENSELECT® PHYTOSOME®.

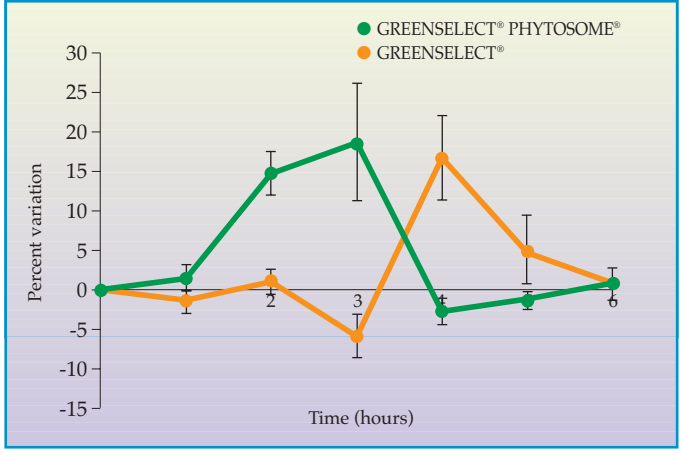
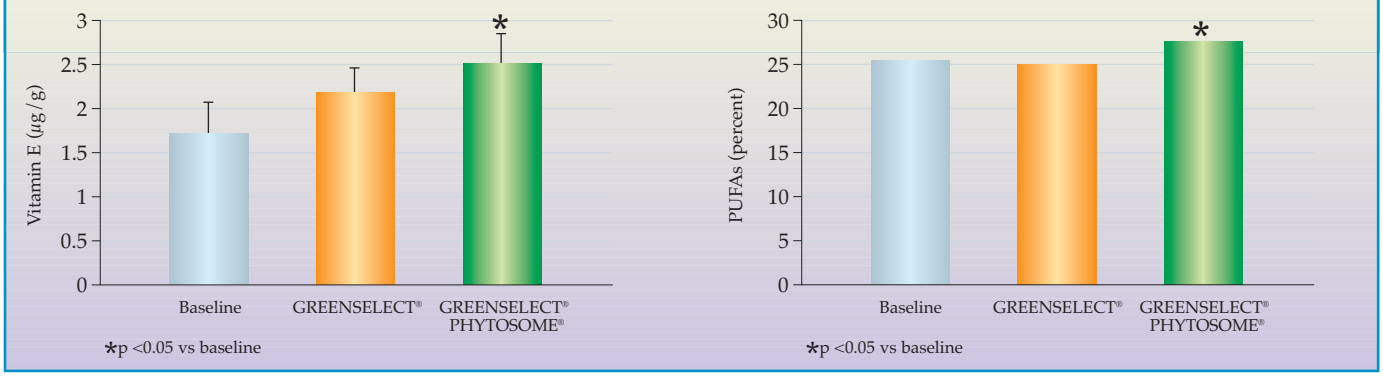


Fig. 6 Levels of vitamin E and PUFAs of RBC membranes after intake of GREENSELECT® and GREENSELECT PHYTOSOME®.



CONCLUSIVE REMARKS

The complexed form of green tea catechins proved to be better absorbed than the uncomplexed form.
An increase of the plasmatic levels corresponded to a marked improvement of the plasma antioxidant and membrane protective activities.
GREENSELECT® PHYTOSOME® is therefore a bioavailable and safe preparation, that can be used for prevention of cancer, heart and liver diseases, atherosclerosis, and for protection against sun damage to skin.

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