

*Study to Promote the
Industrial Exploitation of
Green Tea Polyphenols
in India*



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Coverpage insets:

3D image of (-)- epigallocatechin-3-gallate
pictures of tea bud and flower

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Earth, Environmental and Marine Sciences and Technologies
ICS-UNIDO, AREA Science Park
Padriciano 99, 34012 Trieste, Italy
Tel.: +39-040-9228108 Fax: +39-040-9228136
E-mail: elisa.roa@ics.trieste.it

**Study to Promote the Industrial
Exploitation of Green Tea Polyphenols
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Contributors

The study was carried out by the following team at the University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India:

Karan Vasisht
M. Pharm., Ph.D.

Pritam Dev Sharma
M. Pharm., Ph.D.

Maninder Karan
M. Pharm., Ph.D.

Dev Dutt Rakesh
M.Sc., Ph.D.

Sandeep Vyas
M.Sc.

Shalini Sethi
M. Pharm.

Ritu Manktala
M.Sc.

Preface

In the recent years, there has been an increased interest in tea with a focus on its polyphenols, which have been demonstrated to prevent cancer development and metastasis. The tea polyphenols possess potent antioxidant properties, which safeguard the human body against a number of diseases including cardiovascular. The consumption of the required volume of tea beverage to obtain beneficial effects is not so feasible in those parts of the world where tea is not a common beverage. Developing non-conventional tea products with concentrated doses of polyphenols could be an option to overcome this problem which could benefit the society and the tea industry.

India is the largest producer of tea and it chiefly manufactures black tea. During the manufacture of black tea, the natural polyphenols of the tea are oxidized unlike in green tea. As a result, black tea possesses less health benefits than green tea.

India, traditionally home to black tea, has ignored the potential to produce and propagate green tea. With the increasing interest in green tea polyphenols all over the world, there is a potential for the Indian tea industry to enhance the green tea production and develop newer products based on natural tea polyphenols. To achieve this objective, it is important to identify the Indian tea cultivars with optimal content of tea polyphenols for industrial exploitation.

ICS took this initiative for the benefit of the tea industry in India and analyzed the samples of various cultivars with a view to identify the one containing the highest content of epigallocatechin gallate, the most active polyphenol in tea leaves. A large number of tea samples from different parts of the country were analyzed for their content of epigallocatechin gallate to provide a ready source of information to the Indian tea industry.

The results of the present study should help the Indian tea industry to focus on the production of green tea and develop non-conventional products to take advantage of the growing public interest in green tea polyphenols for health benefits.

Gennaro Longo
Area Director
Earth, Environmental and Marine
Sciences and Technologies

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Contents

1.	Introduction	1
2.	Chemical Constituents of Tea	7
3.	Green Tea Polyphenols–Extraction and Estimation	11
4.	Biological Significance of Green Tea	15
5.	Experimental	23
	5.1 Materials and Methods	23
	5.2 Development of TLC Fingerprint Profile of Green Tea	25
	5.3 Preparation of Standard Plots of EGCG and ECG	26
	5.4 Optimization of Extraction Procedure for Tea Polyphenols	28
	5.5 Studies on Stability of Tea Polyphenols (EGCG)	31
	5.6 Polyphenol Content in Cultivated Varieties of Indian Tea	33
6.	Survey Data	35
	6.1 Cultivated Varieties	35
	6.2 Tea Production and Area Under Tea Cultivation in India	38
	6.3 Production Trends of Different Types of Indian Tea	43
	6.4 Green Tea Production in India	45
	6.5 Problems and Prospects of Green Tea Production	47
7.	Conclusions	49
8.	Summary	55
	References	i - vii
	Appendices	i - xxii

1. Introduction

Throughout the history of human civilization, man has selected three important non-alcoholic beverages from nature's resources, namely tea, coffee and cocoa. Among these, tea is the most widely consumed beverage. It is consumed by half of the world's population for its attractive aroma, taste and health benefits. It is a safe and easily affordable drink for all sections of society throughout the world and there is considerable evidence that consumption of tea is one of the most important ways to prevent a number of human ailments. It has evoked great interest in the medical community in the past few decades. Scientific research has validated the positive effects of tea on health, especially green tea, and shifted its reputation from being "the cup that cheers" to "the cup that heals".

Technically, tea is made from the top three leaves, a leaf bud and two tender leaves of the tea plant *Camellia sinensis* (L.) O. Kuntze (syn. *Thea sinensis* L.) family Theaceae (Figures 1-3).

The tea plant is native to Southeast Asia and is believed to have been discovered accidentally by the Chinese Emperor Shen Nung around 2737 B.C. Since then, its popularity has grown steadily to become China's most popular drink and its fame has spread to other parts of the world. In 1610 tea was taken by Dutch traders to Europe. In 1835 the East India Company established experimental tea plantations in the State of Assam in India. The first consignment of eight chests of Indian tea was auctioned in London in 1839, beginning the advent of Indian tea on the world market. Its cultivation then spread to Sri Lanka in 1857. Encouraged by this worldwide trend, Russia started tea cultivation with plantations in the Caucasus region in 1905. Tea cultivation in Indonesia started in 1910 and soon after the cultivation of tea was started in Kenya (1925) and other parts of Africa.¹

Types of Commercial Tea

Tea is commercially available in three types: green, black and the lesser-known oolong. These types vary in their method of manufacture and thereby in their chemical content. Black tea is widely used in India and green tea is popular in China, Japan and Taiwan. Oolong tea is mainly produced in China and Taiwan. Small quantities of green

and oolong tea are also produced in India and Sri Lanka. Worldwide, 80% of total production is of black tea, 20% green and only 2% oolong tea.

Green Tea

Green tea is unfermented and is the least processed of the three types of tea. In the manufacture of green tea, the freshly harvested leaves are steamed immediately to inactivate the enzymes, especially polyphenol oxidase (PPO), to prevent oxidation and polymerization of primary polyphenols. In the cells primary polyphenols are located in the vacuoles and are kept separate from the enzymes that are present in the chloroplast. If the leaf is allowed to ferment the enzymes and polyphenols come in contact with each other resulting in oxidation and polymerization of polyphenols to form complex compounds.² Enzyme deactivation during the manufacturing process prevents polymerization of primary polyphenols, which pass as such into green tea. Green tea infusion has a leafy taste, a smell of fresh vegetables, low caffeine content and no calories.³



Figure 1: Field view of a tea plantation

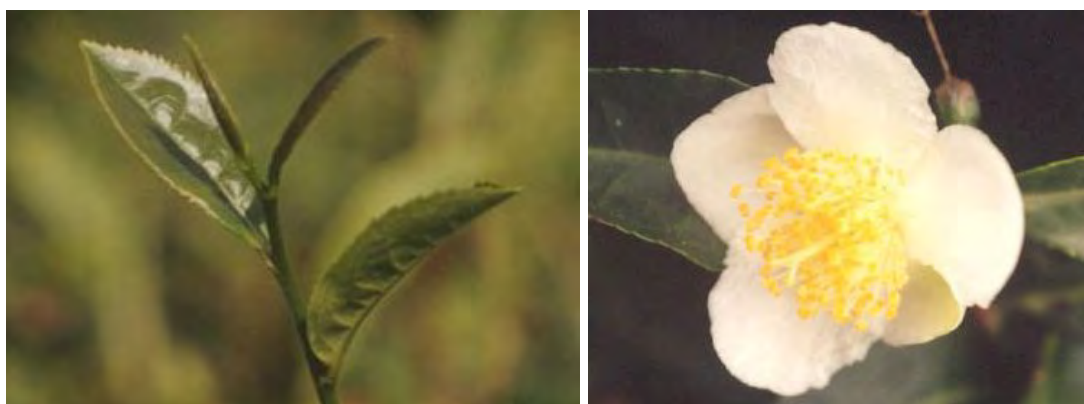


Figure 2: Growing tip of a tea plant

Figure 3: Flower of a tea plant

Black Tea

Black tea is the most popular of the three varieties. It is a completely fermented type, prepared by keeping the leaves for fermentation after plucking. This process results in oxidation and polymerization of polyphenols, changing the nature of the chemical constituents of tea leaves and forming two groups of colouring polyphenols; the yellow group (theaflavins) and the red group (thearubigins).³ These polyphenols are responsible for the briskness, strength, colour, taste, aroma and pungency associated with black tea. The infusion of black tea has a bright red or copper colour, astringent taste and characteristic aroma.²

Oolong Tea

Oolong is a semi-fermented tea where primary polyphenols are allowed to partly oxidize. Oolong tea is not common and is intermediate in characteristics between green and black tea. Immediately after plucking, the tea leaves are partially fermented for about half the time of black tea. It has the colour of black tea and flavour of green tea.

Cultivated Varieties of Tea

There are three cultivated varieties of tea: Cambodian, Chinese and Indian.³ The different tea varieties hybridize freely. Varieties and their important characters are given in Table 1.

Table 1: Common cultivated varieties of tea

Variety name	Taxonomic name	Important characteristics
Cambodian	<i>Camellia sinensis</i> var. <i>assamica</i> subsp. <i>lasiocalyx</i>	Grows to about 5 m in height. Used for hybridization with other varieties.
Chinese	<i>C. sinensis</i> var. <i>sinensis</i>	Grows up to 3 m. Small leaved, hardy plant able to withstand cold winters.
Indian	<i>C. sinensis</i> var. <i>assamica</i>	Grows from 3 to 18 m. Cultivated in India, mainly in Assam, Darjeeling and Nilgiris.

Tea Plucking Seasons

The flavour and the quality of tea are affected by the plucking season. During the first and second flush (Table 2), fine plucking of two leaves and a bud is made resulting in a high quality tea, whereas at other times three or even four leaves are plucked giving a lower quality. The tea plucking cycle is maintained at about 7 day intervals. The leaves are generally collected in bamboo baskets, taking particular care not to crush them. The plucking season of tea begins with the sprouting of plants towards the end of February and ceases in November as the plants are dormant in the winter period.

Table 2: Tea plucking periods

Flush name	Period of year	Description
Easter or first flush	Late February–mid April	The leaves are tender and very light green in appearance. The infusion is light, clear, and bright, with a pleasant brisk flavour.
Spring or second flush	May–June	The spring flush is prominent in its quality. The leaves have a purplish bloom. The taste of the tea is full, with a fruity note.
Summer flush	July–September	Summer flush tea is stronger and bright.
Autumn flush	October–November	The leaves are large and give a round taste and coppery infusion.

Grading of Black Tea

The most distinguished tea comes from the first bud of the year, while twigs and other leaves down the stem tend to yield a poorer quality product. The best tea is hand-harvested.⁴ The freshly gathered shoots are collected and subjected to withering, rolling, fermentation and drying. Black tea is sorted into various grades, mostly according to their leaf or particle size. The harvesting and manufacturing of tea has a considerable impact on the finished size of the leaf and tea grade. In the final sorting or grading, tea acquires the colourful names that are used in the tea trade. This nomenclature does not refer to the quality but to the size and appearance of the tea.

There are two main types of black tea, orthodox and CTC (crush, tear, curl), which fall into several grades. The manufacturing of orthodox black tea requires hand-plucking. This method yields all the possible leaf sizes and grades. CTC tea can be hand-plucked or machine-harvested. The leaves always break during manufacture. There are two broad categories of black tea grades: leaf (unbroken-leaf) and broken-leaf. The leaf grades have larger and intact leaves, greater visual appeal and fetch a higher price. Broken-leaf grades fetch a lower price, however they possess an excellent flavour and aroma, and give a stronger tea than the leaf grades.⁵ The tea grades used in the tea trade are listed below in descending order of quality:⁶

- ❑ Supreme Finest Tippy Golden Flowery Orange Pekoe (SFTGFOP): the highest quality of FOP with plenty of golden tips. “S” stands for the supreme light coloured infusion of Darjeeling teas;
- ❑ Finest Tippy Golden Flowery Orange Pekoe (FTGFOP): high quality FOP;
- ❑ Tippy Golden Flowery Orange Pekoe (TGFOP): FOP with a greater proportion of golden tips than GFOP;
- ❑ Flowery Orange Pekoe (FOP): term used to describe the largest tea leaves;

- ❑ Orange Pekoe (OP): term often used to describe the largest leaf grade teas of Sri Lanka and occasionally of South India. The term "orange" is believed to have originated from the orange colour of tea leaves when they are plucked or from the Dutch House of Orange – the first importer of tea to Europe. The term "pekoe" is derived from a Chinese word referring to the tips of young tea buds;
- ❑ Broken Orange Pekoe (BOP): it is the highest of the broken-leaf grades;
- ❑ Broken Orange Pekoe Fannings (BOPF): tea leaves are smaller than BOP leaves and commonly used in tea bags;
- ❑ Golden Flowery Orange Pekoe (GFOP): similar to FOP, but with golden tips that are more delicate;
- ❑ Fannings: broken grade;
- ❑ Dust: broken grade and the lowest in quality.

These grades apply to black teas from India, Sri Lanka, Java, Sumatra, Africa and a few Chinese teas.

The grading of green and oolong teas is different. Green tea grades, unlike black tea, are related to quality and flavour. Green teas are priced according to variety, the province and estate where they are grown, and the flush or picking used.

2. Chemical Constituents of Tea

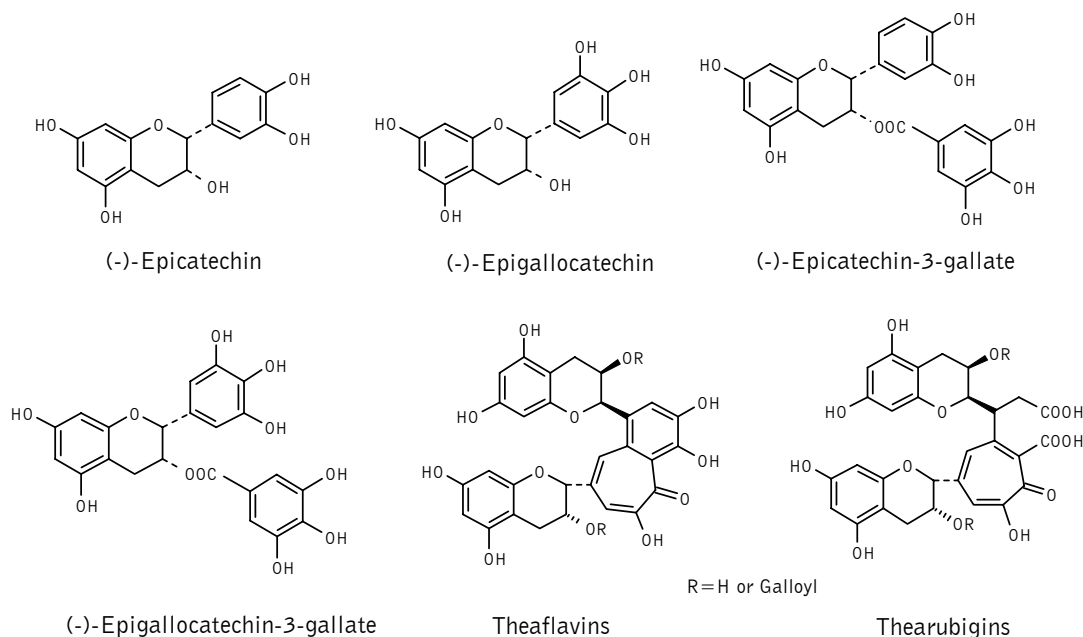
The constituents of green tea leaf include carbohydrates, proteins, polyphenols, caffeine, theanine, vitamins and minerals. Both commercially and biologically, polyphenols and caffeine are more important than the other constituents.

Polyphenolic Constituents

Green tea contains 30 to 42% polyphenols on a dry weight basis.⁴ It contains predominantly flavanols, flavandiols and phenolic acids, such as gallic, coumaric or caffeic acid. The polyphenols are the derivatives of catechin and gallic acid.⁷ Catechin is synthesized in tea leaves through mixed pathways of malonic and shikimic acid. Gallic acid is derived through shikimic acid pathway. The primary polyphenols of green tea are devoid of tanning properties and lack colour in tea infusions. They are bitter in taste and unique, as most of them are not found in other plants.⁸

Primary polyphenols are catechin derivatives and were first reported by Roberts and Woods in 1951 using paper chromatography.⁹ The natural polyphenols in tea include (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and epicatechin (EC). The highest concentration is of EGCG, followed by EGC, ECG and EC in decreasing order.¹⁰⁻¹³ Other catechins including (+)-gallocatechin (GC), (-)-gallocatechin gallate (GCG), (-)-catechin gallate (CG) and (+)-catechin (C) are present in minor quantities.² A cup of green tea contains about 300 to 400 mg of polyphenols.³

Primary polyphenols oxidize during the fermentation process of black tea and transform to compounds with tanning properties. Oxidized polyphenols are present up to 6%. Black tea polyphenols are divided into two groups: thearubigin and theaflavin (20 and 2 to 6%, respectively on a dry weight basis). Thearubigins have higher molecular weights and are chemically poorly characterized. They are partly polymeric proanthocyanidins, and impart colour to the tea infusions.^{3, 14} Theaflavins in black tea are theaflavin 3-*O*-gallate, theaflavin 3'-*O*-gallate and theaflavin 3,3'-di-*O*-gallate.³ They contribute to the briskness of the tea infusion. A good tea must contain a balanced quantity of these compounds.^{4, 15}



A large number of dissimilar chemical reactions initiated by enzymes during fermentation are of practical use in the commercial manufacturing of black tea. The sequence of reactions in the process of tea fermentation is shown in Figure 4.¹⁵

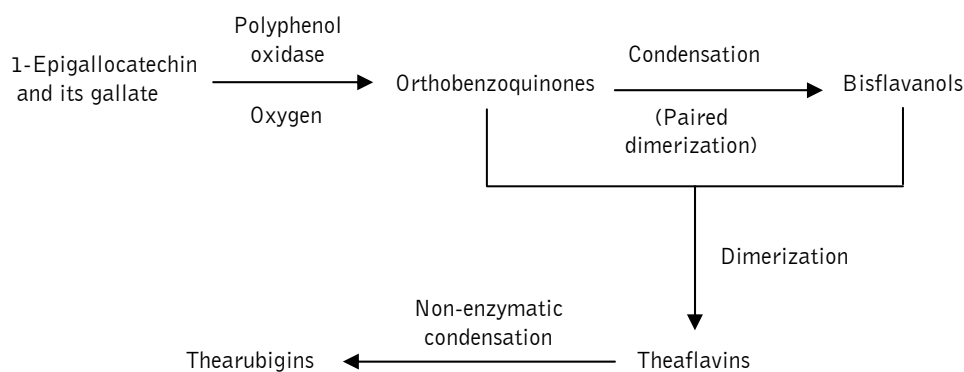


Figure 4: Reactions involved in the process of tea fermentation

The polyphenol oxidase of tea leaf works best at 28.3°C and the reaction slows down with an increase or decrease in temperature. Oxidation takes place more quickly in fresh leaf than in withered leaf.¹⁶ The action of the oxidase converts 1-epigallocatechin and its gallate to corresponding orthoquinones, after which the enzyme action ceases. The changes that follow are non-enzymatic and the rate of reaction increases steadily with the rise in temperature. The four monomers of fresh tea leaves are epigallocatechin (EGC), epicatechin (EC) and their gallate esters namely epigallocatechin gallate (EGCG) and epicatechin gallate (ECG).^{4, 16}

Non-polyphenolic Constituents

Caffeine

The presence of caffeine was first observed in tea in 1820.⁸ A similar compound from tea was isolated and named theine, which was later identified to be the same as caffeine.¹⁰ Caffeine content of tea leaves varies from 2 to 5%. Commercially, caffeine is prepared from tea leaves and tea wastes.^{8, 17}

Amino Acids and Other Nitrogenous Compounds

The content of total nitrogen in green tea extract ranges from 4.5 to 6%, and about half of it comes from free amino acids.¹⁸ Theanine and glutamic acid are the major amino acids whereas aspartic acid and arginine are the minor amino acids present in tea.^{19, 20} Theanine is a unique amino acid, produced by the tea plant and certain other species of *Camellia*. The rate of metabolism of theanine in tea leaves is slow but its transport from root to leaf is rapid, leading to its accumulation in the leaves. It is also one of the major components that gives green tea its specific taste and is antagonistic in action to caffeine.²¹

Vitamins

Green tea contains about 0.3% of vitamin C, which is decomposed during manufacture of black and oolong tea. As a result, the vitamin C content of black and oolong tea is very low.²²

Inorganic Elements

Some specific inorganic elements present in tea are aluminium, fluorine and manganese.^{10, 22} The level of aluminium and fluorine in tea leaves is higher than in other plants. It is presumed that the tea plant has a biochemical mechanism to neutralize the toxicity of aluminium. It has been observed that aluminium in tea leaf exists mainly in a chelated form, indicating that catechins prevent aluminium toxicity. These findings are important in the physiological significance of tea polyphenols.²³

Carbohydrates and Lipids

The carbohydrate content of green tea is about 40% and one third of it is cellulose. Starch is also present and affects the quality of green tea. Tea harvested in the morning has less starch and is of better quality in comparison to afternoon collections. Tea leaves also contain about 4.0% oil.²⁴

3. Green Tea Polyphenols–Extraction and Estimation

Extraction Methods

Polyphenols are extracted with water at boiling or near boiling temperature. A mixture of acetonitrile and water (1 : 1) has been used to extract tea polyphenols at room temperature. Different workers have used different ratios of tea leaves to solvent (1 : 40 to 1 : 500) and varying periods of extraction (3 to 60 min). The extract is filtered preferably through 0.45 μ M membrane filter before being subjected to quantitative analysis in HPLC.²⁵⁻³²

Estimation of Tea Polyphenols

A considerable amount of work has been done and reviewed on the quantitative estimation of green tea polyphenols.^{33, 34} Tea polyphenols have been estimated by various methods, such as nuclear magnetic resonance,³⁵⁻³⁷ near-infrared reflectance spectroscopy,³⁸ spectrophotometry,³⁹ column liquid chromatography,³⁰ thin layer chromatography and high performance thin layer chromatography (HPTLC),⁴⁰ liquid chromatography coupled with mass spectroscopy (LC-MS),²⁶ high performance capillary electrophoresis (HPCE)³¹, and high performance liquid chromatography (HPLC).^{41, 42} The HPLC is the most frequently used technique for estimating tea polyphenols. Many detection methods have been employed including a post-column reaction with 4-methylaminocinnamaldehyde and detection at 640 nm,³⁹ electrochemical detector,^{43, 44} chemiluminiscent reaction⁴⁵ besides use of conventional UV-detectors.²⁹ In most cases reversed phase HPLC with UV-absorbance detection was the chosen method.³³ Separation is achieved using a C18 column. End capped, deactivated, monomeric C18 columns are preferred over non-deactivated or polymeric columns. Dalluge *et al.*²⁹ compared a variety of stationary phases and elution conditions. They observed that the stationary phases, with ultra pure silica and maximized coverage of the silica support, improved the separation. The presence of an acid in the mobile phase is essential for complete and efficient resolution of catechins, specifically for elimination of peak tailing and its detection at shorter wavelengths.^{26, 46}

Liquid chromatography coupled with mass spectrophotometry provides a reliable approach to the analysis of catechins in pico-molar quantities in the complex matrices.

The first report on the use of LC-MS for the identification of catechins in tea appeared in 1993.⁴⁷ This report demonstrated the separation of a mixture of (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and epicatechin (EC), employing thermo-spray ionization mass spectroscopy. Direct MS characterization of catechins in tea extracts without the use of LC has also been demonstrated. Electro-spray ionization, electron impact ionization, and fast atom bombardment mass spectroscopy have been employed to provide both molecular mass and structural information for catechins.^{26, 48}

High Performance Capillary Electrophoresis (HPCE) is another technique that offers the advantage of short analysis time as compared to HPLC. Capillary zone electrophoresis (CZE) and micellar electro kinetic capillary chromatography (MEKC) with UV absorbance detection are the preferred capillary electrophoresis methods for the determination of catechins. In most cases, uncoated fused-silica capillaries have been used to effect the separations. In general, the MEKC methods provide better separation, resolution and quantification than CZE methods.^{28, 31}

Gas chromatographic (GC) method utilizing glass columns or fused-silica capillary columns employing flame ionization detector (FID) has been developed for the estimation of catechins. As a prerequisite, a derivatization step is needed to convert catechins to trimethylsilyl (TMS) derivatives. Techniques of GC-MS and capillary column GC-FID were also used for the separation of catechins.^{49, 50}

Thin layer chromatography of tea has been used for qualitative and quantitative determination of individual catechins. Zhu and Xiao⁴⁰ used silica gel plates and chloroform : ethyl formate : n-butanol : formic acid as a mobile phase to separate catechins and caffeine in green tea samples. The separated components were visualized under UV and the quantitative measurements were made based on the areas under the curve of the catechins spots. Four catechins, EC, EGC, ECG and EGCG were separated on silica plate using a mixture of chloroform, methanol and water. Detection of the catechins was done by colour formation with vanillin-hydrochloric acid reagent.

Singh *et al.*³⁹ have developed a simple method for separating, identifying and quantifying individual catechins based on two-dimensional paper chromatography. The catechins are identified as bright yellow spots on the chromatographic paper by spraying the paper with diazotized sulfanilamide, a reagent specific and selective for catechins. The sensitivity of visual detection is less than 1 µg of catechins.

The spectrophotometric determinations of catechins are based on the formation of a green coloured complex with 4-dimethylaminocinnamaldehyde (DMACA), which is specific and selective for catechins.³⁹

Total catechins have also been measured using biosensor.⁵¹ Burdock tissue (*Arctium lappa* L., a biennial plant) contains polyphenol oxidase, which catalyses the oxidation of polyphenols. A catechin biosensor has been constructed that uses a slice of burdock tissue. As the catechins in a sample are oxidized, the oxygen electrode measures the amount of oxygen consumed. This biosensor was found to respond to various catechins namely, EC, EGC, ECG, EGCG and catechol. The biosensor has been applied for determination of total catechins in green tea infusions, but it is inadequate for accurate quantification because of the variability of response to the different catechins.

The quantitative determination of three catechins based on their chemiluminescent emission has also been reported. Catechins are reacted with hydrogen peroxide-acetaldehyde and horseradish peroxidase, which results in a distinct chemiluminescent emission at 630 nm.⁴⁵

4. Biological Significance of Green Tea

The health benefits of tea have long been recognized in China and Japan. Scientific reports in the last two decades have validated many beneficial claims for tea. Understanding the mechanisms of the biological effects has interested scientists worldwide. The majority of beneficial effects have been attributed to the polyphenolic constituents.

Phenolic compounds are widely distributed in food of plant origin and are regarded as effective antioxidants. Several studies suggest that these components may be of importance in reducing the incidence of degenerative diseases such as cancer and arteriosclerosis. The most relevant compounds in dietary regimens are cinnamic acid derivatives and flavonoids. As natural polyphenols remain unchanged in green tea, it can be said that green tea is more beneficial than black tea, where fermentation during manufacture leads to the oxidation of primary polyphenols. The strong antioxidant potential of tea polyphenols is thought to mediate most of the beneficial effects of tea.⁵²⁻⁵⁵ Health benefits in relation to cancer, arthritis, cardiovascular diseases, diabetes, obesity and dental caries have been focused on scientific investigations in the recent past.

Antioxidant Activity

The most potentially beneficial effects of tea catechins are attributed to their antioxidant properties that sequester metal ions, and scavenge oxygen species and free radicals.⁵⁴ The free radical scavenging property of catechins has been well studied particularly in the last decade. The early evidence of antioxidative property showed the EGCG-induced inhibition of soybean lipoxygenase ($IC_{50} = 10$ to $20 \mu M$).⁵⁵ Later it was reported that EGCG inhibited TPA-induced oxidative DNA base modification in HeLa cells,⁵⁶ inhibited Cu^{2+} -mediated oxidation of low-density lipoprotein (LDL),⁵⁷ reduced *tert*-butyl hydroperoxide-induced lipid peroxidation,⁵⁸ and blocked the production of reactive oxygen species derived from NADPH,⁵⁹ cytochrome P-450 mediated oxidation of the cooked meat carcinogen and 2-amino-3-methylimidazo[4,5-f]quinoline.^{60, 61} Low concentration of EGCG inhibited Jurkat T-cell DNA damage caused by hydrogen peroxide or 3-morpholinosydnonimine (a peroxynitrite generator) and at high concentration EGCG itself induced cellular DNA damage.⁶² EGCG, the most abundant component present in

tea extract, is also the most potent chemical of the epicatechin derivatives tested for biological activity. Along with other tea catechins and polyphenols, it is thought to prevent tumorigenesis by protecting cellular components from oxidative damage through free radical scavenging. Indeed, a number of studies have demonstrated the free radical scavenging activity of EGCG⁶³⁻⁶⁵ as well as its antimutagenic,^{66, 67} antiangiogenic,^{68, 69} antiproliferating and/or pro-apoptotic effects on mammalian cells both *in vitro* and *in vivo*.⁷⁰

Tea catechins have been found to be better antioxidants than vitamin C, E, tocopherols and β -carotene.⁷¹ The antioxidant property of the purified extract was less pronounced than the crude extract suggesting that many constituents contribute to the activity.⁷² The polyphenols block free radical damage to lipids (found in cell membranes and serum lipids), nucleic acids and proteins (like those found as cellular enzymes and structural proteins). Damage to these cell components can lead to tumour formation. The oxidative damage by oxygen free radicals of low-density lipoproteins (LDL) in serum leads to arteriosclerosis and coronary heart diseases. The oxidation of cell membranes and other cell components leads to ageing.⁷³

The highest level of total catechins (72.9 mg/g of tea leaves) has been reported in samples of Chinese green tea and consequently showed the maximal antioxidant potential.⁷⁴ Oolong and black tea show weak protective action. The antioxidant activity of tea polyphenols is not only due to their ability to scavenge superoxides but also due to their ability to block xanthine oxidase and related transducers.⁷⁵ The polyphenols increased the activity of antioxidant enzymes e.g. glutathione peroxidase, glutathione reductase, glutathione-S-transferase, catalase and quinone reductase in the small intestine, liver and lungs which are the detoxifying enzymes of the body.⁷⁶ The antioxidant activity of tea is diminished by the addition of milk to the infusion, due to binding of tea polyphenols to milk proteins.

Cardiovascular Activity

Several flavonoids and related phenolics have been reported to inhibit either enzymatic or non-enzymatic lipid peroxidation, an oxidative process implicated in several pathological conditions including atherosclerosis.⁷⁶ In particular, it has been suggested that tea polyphenols lower the oxidation of low-density lipoproteins (LDL) cholesterol, with a consequent decreased risk of heart diseases.⁷⁷ It has been observed that green tea polyphenols significantly reduce the levels of serum LDL, very low-density lipoproteins (VLDL) and triglycerides.⁷⁸ At the same time, they increase the levels of high-density lipoproteins (HDL).⁷⁹ A low ratio of triglycerides to HDL is an excellent marker for

cardiovascular health.⁸⁰⁻⁸² 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 due to coronary heart disease after 25 years of follow-up.^{83, 84} This observation has been strengthened by the finding that in hypercholesterolemic rats, green tea polyphenols lowered blood cholesterol levels and reduced blood pressure in spontaneously hypertensive animals.⁸⁵

The quantities of antioxidants in the diet are inversely related to the risk of death from heart disease and of non-fatal heart attacks.⁸⁶ Green tea inhibits vascular smooth muscle proliferation, which is another factor contributing to the formation of arteriosclerotic plaque.⁸⁷ Tea polyphenols also interfere with the absorption of dietary fat and cholesterol.⁸⁸

Green tea polyphenols have been found to play an important role in controlling essential hypertension by inhibiting angiotensin-I converting enzyme (ACE), which converts angiotensin-I to vasoconstrictive angiotensin-II.²

Anticancer Activity

Experimental evidence points to the potential of green tea to protect against cancer at several stages of carcinogenesis, including cancer prevention,⁸⁹ endogenous carcinogen activation,⁹⁰ DNA damage and destabilization,⁹¹ cell proliferation,⁹² neoplastic growth and metastasis.^{77, 89, 90, 93-97}

Tea, especially green tea, reduced the incidence of cancers of the stomach,^{98, 99} small intestine,⁹⁸ pancreas,^{100, 101} lung,¹⁰² breast,¹⁰³ skin,¹⁰⁴ urinary bladder,¹⁰³ prostate,¹⁰⁵ oesophagus⁹⁸ and mouth.^{106, 107} Also, it has been shown to reduce tumor size and growth in cancer-bearing animals.^{108, 109}

Green tea polyphenols directly inhibited the cytochrome P-450 enzyme systems (phase I enzyme) that play a pivotal role in carcinogen activation.⁶⁰ Concurrently, they boost the activity of phase II enzymes (e.g. glutathione transferase) that make xenobiotics hydrophilic for clearance from the body. The process is crucial for carcinogen detoxification.^{93, 110} Recently, it was proposed to associate the anticancer activity of EGCG with the inhibition of urokinase, one of the most frequently expressed enzymes in human cancers.¹¹¹ However, the practicability of this study was later challenged due to the required dose levels.¹¹⁰

Green tea polyphenols also inhibited the reactions that give rise to nitrosamines both *in vitro* and *in vivo*.¹¹²⁻¹¹⁴ Pretreatment with tea polyphenols resulted in substantial reduction of carcinogen binding to DNA.¹¹⁵ The increased activity of glutathione

peroxides and catalase in the intestine, liver and lungs of mice on pretreatment with tea polyphenols suggested that green tea polyphenols may suppress mutagenesis mediated by peroxides in the microenvironment of DNA.⁹⁵

Tea polyphenols have also been shown to promote apoptosis of cancer cell lines such as prostate, lymphoma, colon and lung.⁹⁴ Modulating apoptosis is useful in the management, therapy and prevention of cancer. Reduction and inexpression of tumour necrosis factor (TNF- α) may be the way polyphenols induce apoptosis. TNF- α is an endogenous tumour promoter and a central mediator in chronic inflammatory diseases like rheumatoid arthritis and multiple sclerosis.^{89, 108-110, 116, 117}

Green tea showed a protective effect against damage produced by UV radiation and reactive oxygen species to the dermis through apoptosis and inhibiting lipid peroxidation.⁹⁴ Topical application of EGCG decreased UV-induced erythema, edema and hypersensitivity.¹¹⁰ Tea polyphenols also inhibited tumour promoters like okadaic acid and teleocidin in the skin.¹¹⁷

DNA-reactive genotoxic carcinogens, which affect the DNA of a normal cell, are responsible for the majority of human cancers. Polyphenols have been shown to stimulate the repair process of nucleotide excision by removing DNA-adducts produced by carcinogens.⁹⁵ The structure of tea polyphenols possesses strong nucleophilic centres that react with electrophilic carcinogens to form an adduct, which results in the prevention of cancer.⁹⁶

Effects of polyphenols on nitric oxide (NO) induction have also been studied. EGCG inhibits the induction of NO synthase (NOS) via down regulation in the transcription nuclear factor, thus inhibiting induction of NOS. The NO is a bioactive molecule that plays an important role in inflammation and carcinogenesis.¹¹⁰

Green tea has been reported to be of great use in the modulation of cancer chemotherapy.^{89, 97, 118} It enhances the effect of antitumour drugs, which in many cases have resulted in serious complications as a result of effects on normal cells. Green tea has been reported to be beneficial as it increases the drug concentration (e.g. doxorubicin, sulindac) in the tumour cells with no increase in normal cells. As it is a beverage, it reduces the burden on the patient of taking too many medicines.^{97, 118, 119}

Bioavailability studies of polyphenols (especially EGCG) reported a wide distribution in multiple organs (digestive, liver, pancreas, mammary gland, and skin).¹²⁰ After intravenous administration of catechins in rats, it was seen that the half-life of EGCG, ECG and EC were 191, 362 and 45 min, respectively.¹²¹ When pure EGCG was

given, a shorter half-life was observed suggesting the effect of other components in the extract on the plasma concentration and the elimination of EGCG.¹²²⁻¹²⁴

Epidemiological studies in Japan and China suggest that the relatively high consumption of green tea (over 10 cups a day) is associated with substantial reduction in the risk of cancers of the skin, oesophagus, stomach, breast, lung and bladder.^{117, 122, 124-127} Theaflavins are also reported to inhibit cancer of the lung and oesophagus.^{89, 122} Thus therapeutic levels of polyphenols can exert important antimutagenic and anticarcinogenic effects in humans.

Most of the reports on cancer prevention came from Asians, who drink predominantly green tea, whereas studies involving black tea in Europeans observed infrequent protective effects.^{122, 128}

Antidiabetic Effect

The ability of polyphenols to lower blood glucose has been confirmed in diabetic rats. Both green tea and black tea were shown to possess antidiabetic activity and were effective in the prevention and treatment of diabetes.¹²⁹ Tea polyphenols lower the serum glucose by inhibiting the activity of the starch digesting enzyme, amylase. Tea inhibits both salivary and intestinal amylase. As a result, the starch is broken down more slowly and the sudden rise in serum glucose is minimized. In addition, tea also reduces the intestinal absorption of glucose.¹²⁹⁻¹³¹

Antiarthritic Activity

Polyphenols from green tea have demonstrated an exceptional protection against arthritis. The major polyphenols showing antiarthritic effect include epicatechin, epigallocatechin, epicatechin-3-gallate and epigallocatechin-3-gallate.

In a study conducted on mice, green tea polyphenols significantly reduced the incidence of arthritis (33 to 50%). Furthermore, the arthritic mice in the polyphenols fed group showed a less severe form of the disease. Histopathological examination of arthritic joints of mice from the control group revealed extensive cartilage and bone erosions with massive infiltration of mono-nuclear cells and fibroblasts, whereas the green tea polyphenols fed group showed a marked reduction in the number of infiltrating cells with no significant cartilage or bone erosions.¹³²

Expression of cyclooxygenase-2 enzyme (COX-2) is upregulated in arthritis. It has been found that green tea polyphenols inhibit the production of COX-2 in arthritic joints.^{132, 133} There was also a marked reduction in other inflammatory mediators such as

IFN- γ and TNF- α . The neutral endopeptidase activity is decreased on the administration of green tea polyphenols.¹³² It was observed that total IgG and type II collagen specific IgG levels were lower in the serum and arthritic joints of mice fed with green tea polyphenols.¹³² Catechins inhibited the release of lysosomal enzymes, the chemiluminescence response and the production of free radicals.^{134, 135}

The studies suggested that a polyphenolic fraction of green tea rich in antioxidants might be useful in the prevention and severity of arthritis.¹³² Since there is no cure for the rheumatoid and therapy aims at controlling the symptoms, a slight modification in lifestyle and the addition of green tea in the diet can reduce the risk of this disease. This has been demonstrated by a study conducted on mice, over a period of 85 days, which were administered an extract equivalent to a human drinking 4 cups of green tea per day.²

Antiplaque Activity

In the discovery of antibacterial activities of green tea polyphenols, it was found that these compounds inhibit the growth and adherence of oral bacteria.^{136, 137} Green tea extract acted in three ways. Firstly, it inhibited the growth of periodontal disease producing bacterium, *Porphyromonas gingivialis* and decay-causing bacteria such as *Streptococcus mutans*.^{138, 139} Therefore, green tea as a mouth rinse resulted in less plaque and periodontal diseases. Secondly, it inhibited the enzyme amylase present in the saliva, and the starch in the mouth did not get converted into glucose and maltose.^{130, 139} Less nutrition was thus available to decay-causing bacteria. Lastly, it increased the resistance of tooth enamel to acid-induced erosion.¹⁴⁰

Antiviral Activity

Tea extract has been shown to have virucidal activity against polio, influenza, vaccinia and herpes simplex virus.¹⁴¹

Anti-AIDS Activity

The long-term efficacy of new combination drug therapies for HIV infection is limited by the tendency of transfected HIV to mutate and become drug resistant. Green tea polyphenols are antimutagenic and act as effective adjuvants to drug therapy.⁹⁴ It has been discovered that polyphenols from green tea and their oxidation products could inhibit the reverse transcriptase or polymerase of several types of viruses, including HIV-1 and herpes simplex-1.^{130, 142, 143} However, research in this area is still in its initial stages.

Anorectic Effect

The catechin polyphenols inhibit catechol-*O*-methyl transferase¹⁴⁴ and caffeine inhibits transcellular phosphodiesterase, thus stimulating thermogenesis and assisting the management of obesity.^{145, 146} The release of glucose is slowed down by tea and thus harmful spiking of insulin is prevented. Since insulin is the most fattening hormone, fat burning overtakes fat storage.¹³⁰

Antimicrobial Activity

The crude catechins and theaflavins have been found to have an antibacterial activity. They are believed to damage bacterial cell membranes. Tea has been used in the treatment of diarrhoeal infections, cholera and typhus.¹⁴⁷ Polyphenols kill the spores of *Clostridium botulinum* and thus display antibacterial activity against food borne diseases and are also effective against heat-resistant bacilli like *Bacillus subtilis*, *B. cereus*, *Vibrio parahaemolyticus* and *Clostridium perfringens*.¹⁴⁸ Tea extracts also inhibited growth of *Staphylococcus aureus*, *S. epidemidis*, *Salmonella typhi*, *Shigella* spp., and *Streptococcus mutans*.¹⁴⁹ However, they show no activity against some common bacilli such as *Salmonella enteridis*, *Escherichia coli* or *Yersina enterocolitica*.^{2, 149} Green tea also has protozoacidal properties.^{2, 130}

Other Biological Effects

Recently, reports have been published showing that green tea polyphenols exhibit neuromuscular, antiangiogenic, antihepatotoxic, antiproliferative/apoptotic and immunomodulatory effects.¹⁵⁰

In summary, it can be concluded that a number of diseases can be prevented by the incorporation of green tea into the diet. It has been seen that at least 10 cups a day of 100 ml each (3 to 4 g of polyphenols) are needed to have a significant beneficial effect. However, theories on this aspect vary.¹⁰⁸

5. Experimental

5.1 Materials and Methods

Tea Samples

Samples of different cultivated varieties of green tea were collected from three major tea-growing areas in India including Palampur (North India), Assam and Darjeeling (Northeast India), Coonoor and Ooty (South India). Samples of three main cultivated varieties, i.e. *C. sinensis* var. *cambodiensis*, *C. sinensis* var. *assamica* and *C. sinensis* var. *sinensis* and their hybrids presently under cultivation in India were collected from these areas. The collection from Palampur was done at three different times of the plucking season, first in April (beginning), then in September (mid-season-growing burst after rainy season) and finally in October and November (end of season). Darjeeling samples were collected in June and samples from Coonoor and Ooty were collected in October. Details of the collections are provided in Table 3. The collected samples were treated as in the manufacture of green tea by subjecting them to steam and drying under controlled conditions. In addition, some samples were sun-dried without steaming. The leaves were powdered prior to extraction.

Solvents, Reagents and Chromatographic Plates

(-)-Epigallocatechin-3-gallate (EGCG) and (-)-epicatechin-3-gallate (ECG) were obtained from Sigma-Aldrich Chemicals. The solvents, chemicals and reagents were either obtained from E. Merck, or S. D. Fine Chemicals, India. Distilled water was used wherever water is mentioned. Laboratory-made TLC plates (silica gel G, 0.2 mm thickness) activated at 110°C for 30 min were used for qualitative work. For quantitative analysis, precoated silica gel G plates (20 x 20 cm, 0.2 mm thickness, plastic base, E. Merck) cut into desired size were used.

Preparation of Tea Extract

The tea extracts were prepared by hot maceration of tea leaves with the appropriate solvent. At the end of the extraction, the material was filtered under reduced

pressure and the marc was washed with a specified volume of fresh solvent while continuing filtration. The final volume of the filtrate was made to a specified volume before subjecting it to analysis. The quantity of tea leaves, its ratio to solvent, extraction period, temperature, volume of solvent used for washing the marc and the final volume of the extract were predetermined for each set of extraction. Initial experiments were conducted to optimize the extraction procedure with respect to the choice of solvent, temperature, period of extraction and particle size of tea leaves. The optimized extraction conditions were subsequently used to determine the amount of polyphenols in different varieties of tea.

Table 3: Collection details of tea samples

Area	Place of collection	Clone/variety	Month and year of collection
North	Palampur	TV-23	Sep. and Nov. 2001, Apr. 2002
		Kangra Asha	Nov. 2001, Apr. and Sep. 2002
		TV-1	Nov. 2001 and Apr. 2002
		Kangra Jawala	Apr. and Sep. 2002
Northeast	Darjeeling	P-126	Jun. 2002
		Takda-7, 8	Jun. 2002
		Tenali-17	Jun. 2002
		TV-1	Jun. 2002
		TV-9	Jun. 2002
		TV-18	Jun. 2002
		TV-20	Jun. 2002
		TV-23	Jun. 2002
		TV-26	Jun. 2002
		China Bush + Clones	Jun. 2002
South	Ooty	China Variety	Oct. 2002
		VP-Clones	Oct. 2002
		BSS-1	Oct. 2002
		C-1	Oct. 2002
		CR-6017	Oct. 2002
		UPASI-3	Oct. 2002
	Coonoor	TRI-2024	Oct. 2002
		TRI-2025	Oct. 2002
		TRI-2026	Oct. 2002
		UPASI-2	Oct. 2002
		UPASI-3	Oct. 2002
		UPASI-8	Oct. 2002
		UPASI-9	Oct. 2002
		UPASI-10	Oct. 2002

Quantitative Analysis

TLC Method

All quantitative estimations were made in triplicate, using a CAMAG TLC Scanner 3 and CATS software version 4.06. The known amount of tea extract was applied on a precoated TLC plate and the plate was developed in an appropriate solvent system. The plate was dried in a current of hot air and scanned in the TLC scanner at 280 nm. The area under the curve (AUC) for the peak corresponding to the spot of each polyphenol was noted and the concentration of different polyphenols in the extract was determined from their respective standard plots.

HPLC Method

The analytical determinations of EGCG and ECG were carried out using reverse phase-HPLC at isocratic mode. The Waters HPLC system equipped with automated gradient controller, 510 pumps, U6K injector, 481 detector and 746 data module was used for the analysis. The Waters μ -bondapak C18 (3.9 x 300 mm) column at ambient temperature 24 to 28°C, mobile phase water : methanol : acetic acid (70 : 30 : 0.5), flow rate 1.0 ml / min and UV detection at 280 nm were used in the HPLC analysis. All extracts were prepared and analyzed in triplicate. The extract was filtered through 0.45 μ m filter before injecting 5 μ L of the appropriately diluted sample.

Stability Studies

The degradation pattern of EGCG was studied in buffers of pH 1.2, 2.0, 4.0, 6.0, 7.0 and 8.0 (Appendix 1) over a period of 48 h. The change in concentration of EGCG in buffered solution was monitored through quantitative TLC using CAMAG TLC Scanner.

5.2 Development of TLC Fingerprint Profile of Green Tea

A methanol extract of green tea was prepared by refluxing 2 g tea leaves in 50 ml methanol for 1 h in a water bath at 80°C. A variety of solvent systems were tried in order to obtain the best resolution. The spots were visualized by spraying the plate with 1% vanillin in sulphuric acid, followed by heating for 10 min at 110°C. A solvent system comprised of chloroform : acetone : formic acid (5 : 4 : 1) produced the most resolved profile. The TLC fingerprint profiles of polyphenolic and non-polyphenolic constituents of tea are shown in Figure 5.

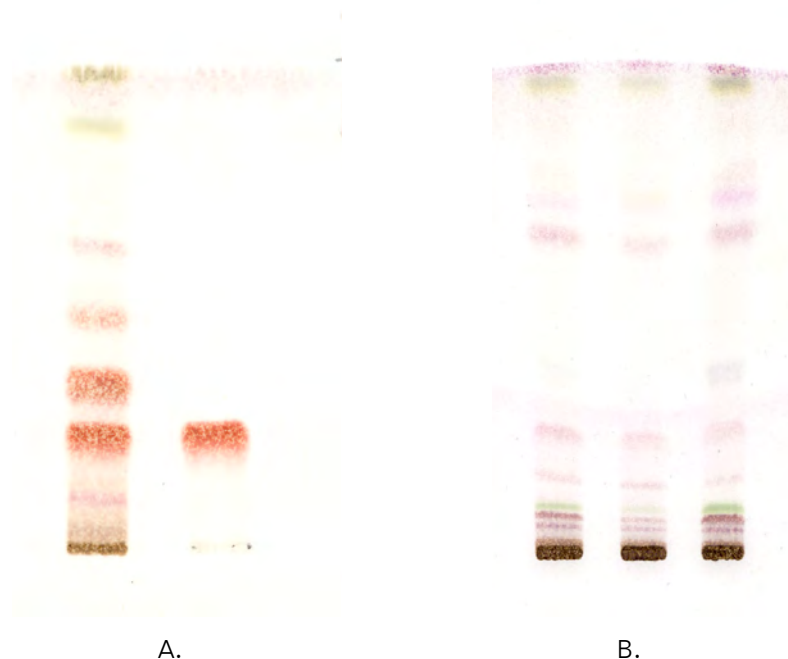


Figure 5: TLC fingerprint profile of polyphenolic (A) and non-polyphenolic (B) constituents of tea

5.3 Preparation of Standard Plots of EGCG and ECG

Preparation of Standard Plots of EGCG

A standard solution of EGCG was prepared by dissolving 2.41 mg of EGCG in 25 ml methanol. The varying volumes of standard solution 0.5 to 16.0 μL in geometric progression were applied in triplicate on a precoated TLC plate. The plate was developed in chloroform : acetone : formic acid (5 : 4 : 1), dried and then scanned at 280 nm in the TLC scanner. The AUC for each quantity was noted (Table 4 and Figure 6) to construct the standard plot.

Table 4: Mean peak areas of EGCG in TLC analysis

EGCG (μg)	^a Mean AUC \pm SD
0.0492	313 \pm 5.90
0.0984	631 \pm 26.71
0.1968	1219 \pm 49.08
0.3936	2471 \pm 23.61
0.7872	4808 \pm 190.59
1.5744	8853 ^b

^an= 3, ^bn= 1

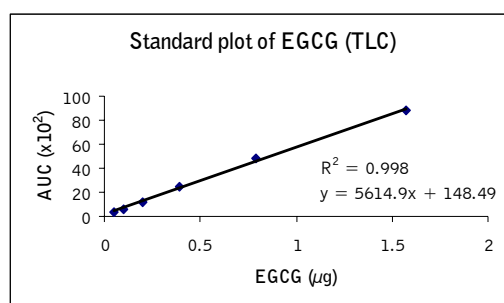


Figure 6: Standard plot of EGCG in TLC analysis

The standard plot for HPLC analysis was constructed by injecting in triplicate a constant volume of 5 μL of serially diluted concentrations and noting AUC corresponding to each concentration (Table 5 and Figure 7). Reverse phase C18 column and water : methanol : acetic acid (70 : 30 : 0.5) at 1 ml / min flow rate were used for the analysis.

Table 5: Mean peak areas of EGCG in HPLC analysis

EGCG (ng)	^a Mean AUC \pm SD
9.85	23471 \pm 19.0
19.70	46026 \pm 855
39.35	88815 \pm 36
78.50	175341 \pm 2502
157.50	355954 \pm 6708
283.20	635194 \pm 3077

^an = 3

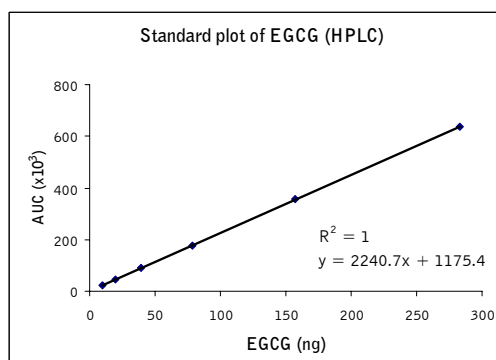


Figure 7: Standard plot of EGCG in HPLC analysis

Preparation of Standard Plots for ECG

The standard plots of ECG for TLC and HPLC analysis were constructed similarly as described for EGCG, using the same conditions of the analysis in TLC and HPLC. The standard solution of ECG was prepared by dissolving 3.02 mg of ECG in 10 ml methanol. In TLC 0.5 to 2.5 μL of standard solution in increments of 0.5 μL was applied and AUC was noted (Table 6) to construct the standard plot (Figure 8).

Table 6: Mean peak areas of ECG in TLC analysis

ECG (μg)	^a Mean AUC \pm SD
0.0302	211 \pm 12.28
0.0604	411 \pm 9.74
0.0906	571 \pm 26.0
0.1208	761 \pm 30.26
0.1510	953 \pm 31.47

^an = 3

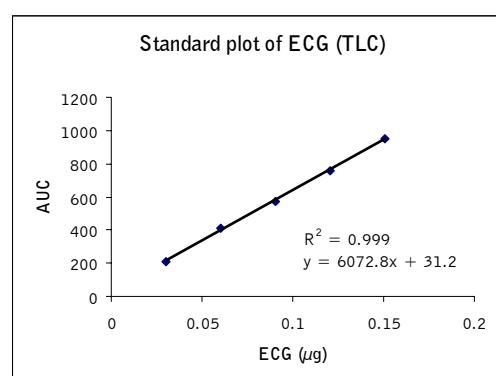


Figure 8: Standard plot of ECG in TLC analysis

In HPLC analysis, standard solution was diluted to inject a constant volume of 5 μL . The data and standard plot for HPLC are given in Table 7 and Figure 9, respectively.

Table 7: Mean peak areas of ECG in HPLC analysis

ECG (ng)	^a Mean AUC ± SD
09.40	26898 ± 736
18.85	48516 ± 1079
37.50	100503 ± 2205
75.50	188155 ± 2252
151.00	378395 ± 3563
302.00	761380 ± 5851

^an = 3

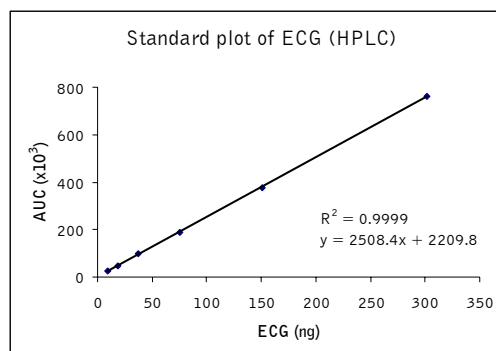


Figure 9: Standard plot of ECG in HPLC analysis

5.4 Optimization of Extraction Procedure for Tea Polyphenols

Effect of Solvent on EGCG Extraction

The tea sample (2 g) was refluxed separately in 50 ml each of methanol, water and ethyl acetate for 30 min at 60°C. The extract was filtered under vacuum while hot. The marc was washed with 20 ml of additional extraction solvent while continuing filtration. The volume of the filtrate was made up to 100 ml. 1 µL of this extract was applied on a precoated TLC plate, developed in a solvent system consisting of chloroform : acetone : formic acid (5 : 4 : 1) and scanned using TLC scanner. The AUC for peak corresponding to EGCG was noted and the concentration in the extract was calculated from the standard plot. The results are shown in Table 8 and Figure 10.

Table 8: Effect of solvent on extraction of EGCG

Solvent	EGCG (mg/g) ± SD ^a
Methanol	57.12 ± 0.68
Water	48.39 ± 0.20
Ethyl acetate	4.32 ± 0.30

^an = 3

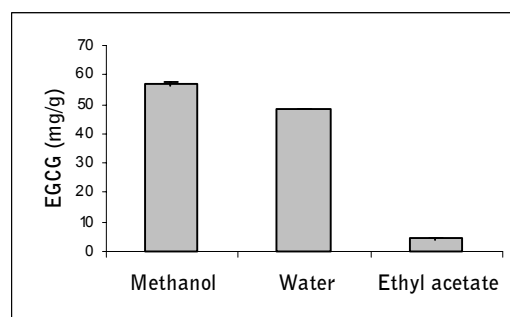


Figure 10: Effect of solvent on extraction of EGCG

Effect of Time and Temperature on Extraction of EGCG

Tea leaf powder (2 g) was refluxed separately with 50 ml each of methanol and water at different temperatures (40, 60, 80 and 98°C) on a water bath for different time intervals (15, 30, 45, 60, 75 and 90 min). After completion of each extraction, the final volume of the extract was made to 100 ml. 1 µL of this extract was applied on the TLC plate and the EGCG content was determined using the standard curve. Extractions were

carried out in triplicate and each extract was applied in triplicate. Table 9 shows the extracted amount of EGCG. In one study the marc was re-extracted with fresh solvent to determine if the extraction was complete.

Table 9: Effect of time and temperature on extraction of EGCG

Extraction time (min)	Solvent	EGCG (mg/g) \pm SD ^a			
		40°C	60°C	80°C	98°C
15	Methanol	15.25 \pm 0.37	54.12 \pm 0.06	42.73 \pm 1.08	
	Water	16.44 \pm 0.63	40.65 \pm 0.14	42.72 \pm 1.09	53.60 \pm 1.20
30	Methanol	20.55 \pm 0.20	57.12 \pm 0.68	47.55 \pm 0.36	
	Water	21.42 \pm 0.92	48.39 \pm 0.20	53.51 \pm 0.27	48.48 \pm 0.62
45	Methanol	23.00 \pm 1.16	58.24 \pm 0.03	48.22 \pm 0.59	
	Water	24.92 \pm 0.62	46.09 \pm 0.08	53.64 \pm 0.36	49.55 \pm 0.84
60	Methanol	29.98 \pm 0.22	59.06 \pm 0.26	48.68 \pm 0.35	
	Water	23.82 \pm 0.74	50.30 \pm 1.19	54.02 \pm 0.30	48.00 \pm 0.96
75	Methanol	24.14 \pm 0.31	58.74 \pm 0.93	40.66 \pm 0.65	
	Water	27.52 \pm 0.30	49.18 \pm 0.10	53.45 \pm 0.13	49.45 \pm 0.13
90	Methanol	26.52 \pm 0.51	58.70 \pm 0.52	39.96 \pm 0.31	
	Water	23.90 \pm 0.50	48.85 \pm 0.47	53.40 \pm 0.07	49.15 \pm 0.13

^an= 3

Effect of Time on Extraction of EGCG in Water

The tea leaf powder (2 g) was extracted with 50 ml of water at 98°C for varying period of extraction from 5 to 90 min. The amount of extracted EGCG was determined using the TLC method of analysis. The data are shown in Table 10 and Figure 11.

Table 10: Effect of time on extraction of EGCG in water

Extraction time (min)	EGCG (mg/g) \pm SD ^a
5	54.30 \pm 0.16
10	53.62 \pm 0.07
15	53.60 \pm 1.20
30	48.48 \pm 0.62
45	49.55 \pm 0.84
60	48.00 \pm 0.96
75	49.45 \pm 0.13
90	49.15 \pm 0.13

^an= 3

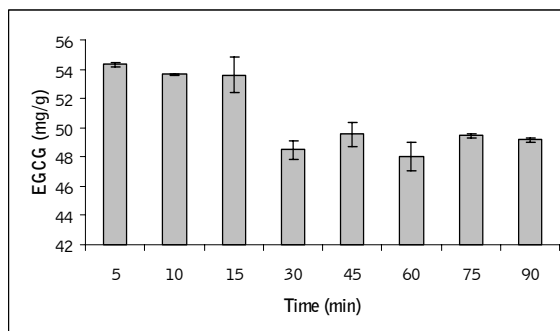


Figure 11: Effect of time on extraction of EGCG in water

Effect of Sample to Solvent Ratio on Extraction of EGCG

Different ratios of sample to water were used to extract EGCG and the other parameters were kept constant as optimized for the extraction with water. After completion of each extraction, the quantity of EGCG in the extract was determined by TLC. The effect of volume of water on extraction is given in Table 11 and Figure 12.

Table 11: Effect of sample: water ratio on extraction of EGCG

Sample : solvent (g : ml)	EGCG (mg/g) \pm SD ^a
1 : 10	40.21 \pm 2.40
1 : 25	53.27 \pm 0.58
1 : 50	54.58 \pm 0.18
1 : 100	55.62 \pm 0.67
1 : 150	61.01 \pm 2.66
1 : 200	57.64 \pm 0.60
1 : 400	24.98 \pm 0.45

^an= 3

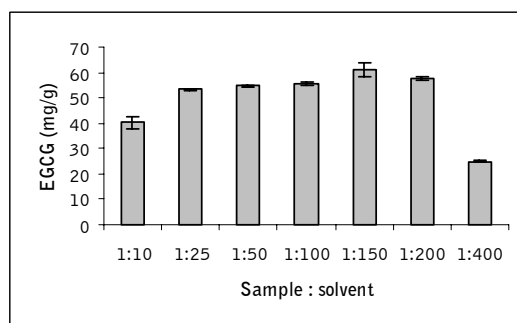


Figure 12: Effect of sample: water ratio on extraction of EGCG

Effect of Shaking on Extraction of EGCG

Tea leaves (4 g) were extracted with water at 98°C for 5 min. The flasks were shaken continuously in a shaker. The extraction yield was compared with the control yield. The results are given in Table 12 and Figure 13.

Table 12: Effect of shaking on extraction of EGCG

Treatment	EGCG (mg/g) \pm SD ^a
Control	14.80 \pm 0.06
Shaking	21.16 \pm 0.03

^an= 3

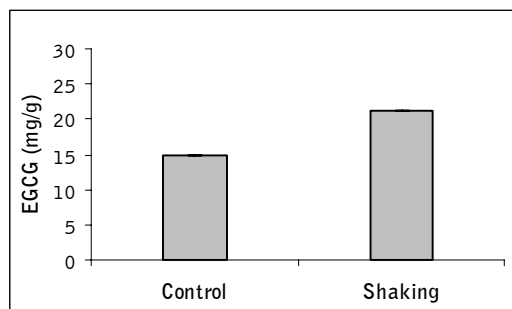


Figure 13: Effect of shaking on extraction of EGCG

Effect of Particle Size on Extraction of EGCG

The tea leaves (2 g each) powdered into coarse, moderately coarse, moderately fine and fine powder (Appendix 2) were extracted in methanol using optimized conditions of time and temperature. The results are shown in Table 13 and Figure 14.

Table 13: Effect of particle size on extraction of EGCG

Powder grade	EGCG (mg/g) \pm SD ^a
Coarse	55.88 \pm 0.73
Moderately coarse	56.54 \pm 0.80
Moderately fine	58.85 \pm 0.49
Fine	56.34 \pm 0.70

^an= 3

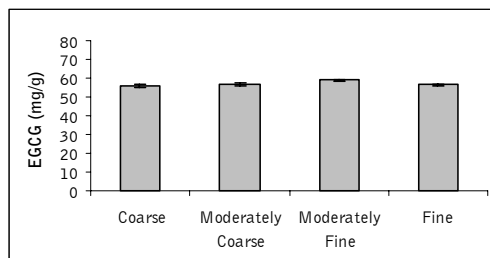


Figure 14: Effect of particle size on extraction of EGCG

5.5 Studies on Stability of Tea Polyphenols (EGCG)

Effect of pH on Degradation of EGCG

The tea extract was prepared using the optimized method. Different aliquots of 10 ml each of this extract were concentrated to dryness and dissolved separately in 10 ml buffer of pH 1.2, 2, 4, 6, 7 and 8. The buffered extracts were stored at room temperature (26 to 28°C) and the EGCG content was monitored through quantitative TLC at different time intervals (Table 14). The control extract was concentrated to dryness and dissolved in 10 ml of distilled water. The percentage of degradation of EGCG at different time intervals is given in Table 15 and Figure 15.

Table 14: Effect of pH on EGCG degradation

pH of solution	EGCG (mg/g) \pm SD ^a at different time intervals (h)						
	0	1	2	4	8	24	48
Control	40.86 \pm 0.85	36.07 \pm 0.43	32.63 \pm 0.44	30.80 \pm 0.10	29.98 \pm 0.78	25.35 \pm 0.18	23.18 \pm 0.56
1.2	50.88 \pm 0.88	49.55 \pm 0.78	49.20 \pm 0.53	49.18 \pm 1.05	49.12 \pm 0.44	49.14 \pm 1.11	47.22 \pm 0.84
2.0	44.84 \pm 0.81	43.60 \pm 0.52	43.44 \pm 0.85	43.42 \pm 0.96	43.44 \pm 0.80	43.40 \pm 1.10	41.96 \pm 0.53
4.0	61.75 \pm 0.53	60.01 \pm 1.02	59.93 \pm 0.95	59.96 \pm 0.66	59.88 \pm 0.89	59.86 \pm 0.59	46.12 \pm 0.82
6.0	50.65 \pm 0.75	48.98 \pm 0.96	48.98 \pm 0.75	48.78 \pm 1.05	48.75 \pm 1.06	40.70 \pm 1.02	39.54 \pm 0.64
7.0	39.95 \pm 0.37	35.62 \pm 0.26	35.64 \pm 0.77	35.58 \pm 0.34	32.40 \pm 0.54	25.19 \pm 0.95	25.00 \pm 0.46
8.0	28.15 \pm 0.53	22.14 \pm 0.84	19.95 \pm 0.75	17.60 \pm 0.46	16.65 \pm 0.85	12.25 \pm 0.52	09.64 \pm 0.46

^an=3

Table 15: Per cent degradation of EGCG at different pH

pH of solution	EGCG degradation (%) at time intervals (h)						
	0	1	2	4	8	24	48
Control	0	11.6	19.9	24.3	26.3	37.5	42.8
1.2	0	2.6	3.2	3.3	3.4	3.4	7.1
2.0	0	2.7	3.0	3.1	3.1	3.2	6.3
4.0	0	2.7	2.9	2.9	3.0	3.0	25.1
6.0	0	3.1	3.1	3.5	3.6	19.3	21.6
7.0	0	10.7	10.7	10.8	18.6	36.5	37.0
8.0	0	21.0	28.6	36.8	40.2	55.6	64.7

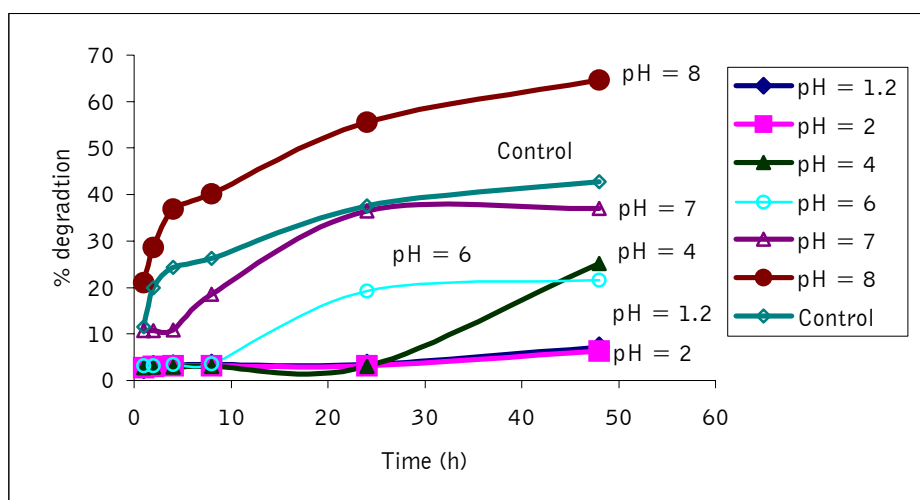


Figure 15: Effect of pH on stability of EGCG

Stability of EGCG in Ethyl Acetate

The stability of EGCG in the tea extracts stored in ethyl acetate was monitored until 48 h (Table 16 and Figure 16).

Table 16: Stability of EGCG in ethyl acetate

Time (h)	EGCG (mg/g) \pm SD ^a	Degradation %
0	14.66 \pm 0.50	0
1	14.50 \pm 0.56	1.1
2	14.48 \pm 0.28	1.2
4	14.48 \pm 0.17	1.2
8	14.48 \pm 0.53	1.2
24	14.48 \pm 0.38	1.2
48	14.43 \pm 0.54	1.5

^an = 3

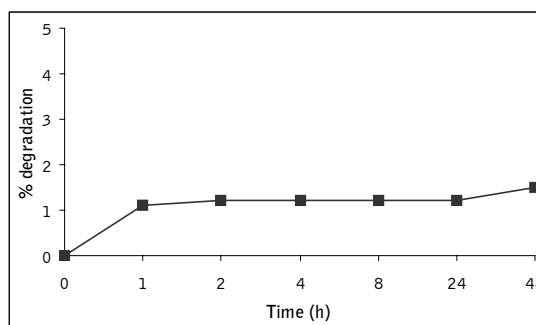


Figure 16: Stability of EGCG in ethyl acetate extract

5.6 Polyphenol Content in Cultivated Varieties of Indian Tea

The EGCG and ECG content was estimated by HPLC in the different samples of cultivated Indian varieties of tea using the optimized procedure of extraction and analysis developed in the laboratory. Accurately weighed (2 g) moderately fine powder of tea sample was put in a vacuum flask and 100 ml of boiling water was added to it. The flask was stoppered and shaken for 5 min. The extract was filtered while hot and the marc was washed with 10 ml of boiling water. The volume of extract was adjusted to 100 ml with refrigerated water. The extract was diluted (1 to 25) with a mobile phase of HPLC analysis (water : methanol : acetic acid 70 : 30 : 0.5). The diluted extract was filtered through a 0.45 μm filter and used for the HPLC analysis (Figure 17). The amount of EGCG and ECG in the extract was calculated from the area under the curve corresponding to the peaks of EGCG and ECG and using the standard plots (Table 17).

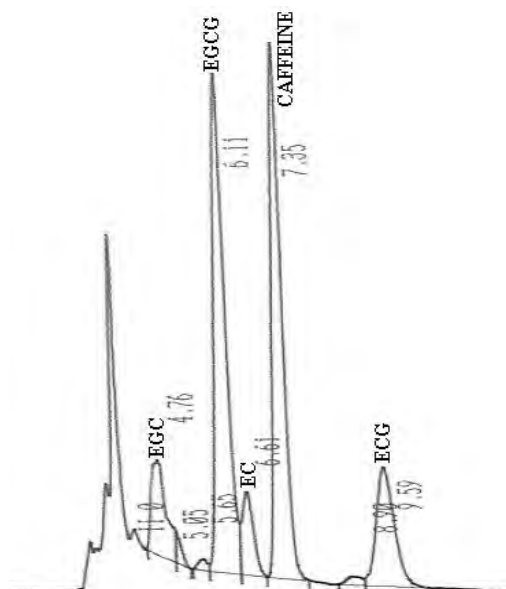


Figure 17: A typical HPLC chromatogram of green tea extract

Table 17: Polyphenol content of tea samples of different varieties cultivated in India

Area	Clone/variety	Period of collection	Processing method	EGCG (mg/g)	ECG (mg/g)
North	TV-23	Sep. 2001	Sun drying	2.70 \pm 0.50	Not detected
		Sep. 2001	Steam drying	24.45 \pm 2.48	6.36 \pm 0.50
		Nov. 2001	Sun drying	19.82 \pm 0.20	9.58 \pm 0.11
		Nov. 2001	Steam drying	43.60 \pm 0.25	16.40 \pm 0.30
		Apr. 2002	Sun drying	31.87 \pm 1.63	12.18 \pm 0.22
		Apr. 2002	Steam drying	58.52 \pm 1.34	18.95 \pm 0.33

Continued

Table 17 continued

	Kangra Asha	Nov. 2001	Sun drying	34.94 ± 0.23	8.13 ± 0.16
		Nov. 2001	Steam drying	32.00 ± 2.93	7.04 ± 0.51
		Apr. 2002	Sun drying	52.35 ± 1.41	10.62 ± 0.56
		Apr. 2002	Steam drying	45.61 ± 1.67	9.56 ± 0.32
		Sep. 2002	Sun drying	23.50 ± 0.85	11.39 ± 0.85
		Sep. 2002	Steam drying	64.38 ± 1.52	14.04 ± 0.90
	Kangra Jawala	Apr. 2002	Sun drying	26.64 ± 2.52	6.37 ± 0.58
		Apr. 2002	Steam drying	68.89 ± 2.77	19.04 ± 0.35
		Sep. 2002	Sun drying	27.14 ± 2.07	10.88 ± 0.38
		Sep. 2002	Steam drying	62.01 ± 4.52	14.30 ± 1.15
	TV-1	Nov. 2001	Sun drying	29.61 ± 2.74	20.63 ± 2.63
		Nov. 2001	Steam drying	42.54 ± 0.43	25.36 ± 0.07
		Apr. 2002	Sun drying	22.16 ± 0.52	15.56 ± 0.67
		Apr. 2002	Steam drying	43.22 ± 2.80	27.39 ± 2.20
Northeast	TV-1	Jun. 2002	Steam drying	27.48 ± 2.32	19.64 ± 1.71
	TV-9	Jun. 2002	Steam drying	33.91 ± 3.67	10.72 ± 1.18
	TV-18	Jun. 2002	Steam drying	50.18 ± 1.61	16.22 ± 1.52
	TV-20	Jun. 2002	Steam drying	19.18 ± 0.82	6.60 ± 0.86
	TV-23	Jun. 2002	Steam drying	23.52 ± 0.61	13.71 ± 1.49
	TV-26	Jun. 2002	Steam drying	18.46 ± 1.40	7.33 ± 0.53
	P-126	Jun. 2002	Steam drying	12.89 ± 1.24	7.16 ± 0.71
	Takda-7,8	Jun. 2002	Steam drying	19.48 ± 0.59	6.45 ± 0.30
	Tenali-17	Jun. 2002	Steam drying	17.62 ± 1.48	5.63 ± 0.69
	China Bush + Clones	Jun. 2002	Steam drying	30.36 ± 3.49	8.50 ± 0.85
South	China Variety	Oct. 2002	Sun drying	1.65 ± 0.07	1.37 ± 0.04
	UPASI-3	Oct. 2002	Sun drying	1.36 ± 0.22	0.32 ± 0.00
	VP-Clones	Oct. 2002	Sun drying	2.14 ± 0.50	0.71 ± 0.16
	BSS-1	Oct. 2002	Steam drying	24.32 ± 0.86	8.04 ± 0.56
	C-1	Oct. 2002	Steam drying	30.87 ± 0.89	10.54 ± 0.55
	China Variety	Oct. 2002	Steam drying	38.12 ± 2.35	14.24 ± 1.08
	CR-6017	Oct. 2002	Steam drying	44.18 ± 3.58	11.60 ± 1.20
	TRI-2024	Oct. 2002	Steam drying	33.43 ± 3.60	12.76 ± 0.89
	TRI-2025	Oct. 2002	Steam drying	42.31 ± 5.09	10.29 ± 0.82
	TRI-2026	Oct. 2002	Steam drying	68.35 ± 5.21	22.20 ± 1.91
	UPASI-2	Oct. 2002	Steam drying	51.47 ± 0.74	12.15 ± 0.33
	UPASI-3	Oct. 2002	Steam drying	19.75 ± 6.99	5.53 ± 2.02
	UPASI-8	Oct. 2002	Steam drying	45.44 ± 3.09	14.68 ± 1.26
	UPASI-9	Oct. 2002	Steam drying	57.82 ± 1.62	14.24 ± 0.98
	UPASI-10	Oct. 2002	Steam drying	21.64 ± 2.24	8.33 ± 0.76

6. Survey Data

The tea producing areas in North, Northeast and South India were surveyed to collect information on the cultivated varieties of tea, the area under cultivation, the production, processing and marketing of green tea, and the problems of the green tea manufacturers. The information was procured from the Tea Board of India, the tea processing companies and other governmental and non-governmental organizations dealing with tea. The production data, including the total tea produced, the areas under tea cultivation, the tea yields, the monthly and annual tea sales at different auctions and the average prices of tea (Appendix 3) were provided by the Tea Board of India and J. Thomas and Private Company Limited, Kolkata. Various tea companies (Makaibari Tea Estates, Sannyasithan Tea Co. Pvt. Ltd., Maud Tea & Seed Co. Ltd., Tea Promoters-India Pvt. Ltd., Ambari Tea Co. Ltd., Sublime Agro Ltd., Sepoydhoorah Tea Co. Pvt. Ltd., Goodricke Group Ltd., Duncans Industries Ltd., United Planter's Association of Southern India, Indcoserve, The Peria Karamalai Tea & Produce Co. Ltd., and Mahalinga Indco Tea) and tea gardens were visited and surveyed using a questionnaire (Appendix 4) and to obtain processed green tea samples of different varieties for analysis. Information was gathered on marketing channels and problems in green tea processing and marketing (Appendix 5).

India is the world's largest producer and consumer of tea and is known to produce the finest qualities of tea.¹⁵⁰ It also produces the largest number of tea varieties. Indian tea is best known for its flavour, taste and colour. The three world famous types of Indian tea are Assam, Darjeeling and Nilgiri. According to an estimate, 673 thousand tonnes of tea were consumed in India in 2001, ranking it at the top of the tea consuming nations of the world. In India, the tea producing areas are located in the north, northeast and south (Figures 18-20). The largest producer is the northeastern area of India. The tea producing states and regions under each area are presented in Table 18.

6.1 Cultivated Varieties

In the north, Palampur in the Kangra Valley in the state of Himachal Pradesh, and Nainital in the state of Uttaranchal are engaged in tea production. The region largely

cultivates the Chinese variety of tea (*Camellia sinensis* (L.) O. Kuntze var. *sinensis*), which is suited to the prevailing agro-climatic conditions of the area.



Figure 18: A tea garden in Palampur (North India)



Figure 19: A tea garden in Darjeeling (Northeast India)

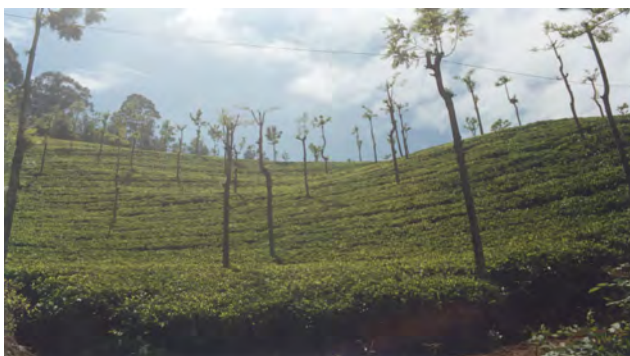


Figure 20: A tea garden in Ooty (South India)

Table 18: Tea producing areas of India

Area	State	District
North	Himachal Pradesh	Kangra
	Uttaranchal	Nainital
Northeast	Arunachal Pradesh Assam	-
		Cachar
		Darrang*
		Dibrugarh*
		Goalpara*
		Kamrup*
		Karbi Anlong
		Lakhimpur*
		North Cachar
		Nowgong*
	Sibsagar * ^a	
	Bihar	Kisanganj
	Manipur	-
	Meghalaya	-
	Mizoram	-
Nagaland	-	
Orissa	-	
Sikkim	-	
Tripura	-	
	West Bengal	Darjeeling
		Dooars ^b
Teraï ^c		
South	Tamilnadu	Coimbatore
		Kanyakumari
		Madurai
		Nilgiris
		Tirunelveli
	Kerala	Cannanore
		Ernakulam
		Idukki
		Kottayam
		Kozhikode
		Malapuram
		Palghat
		Quilon
		Trichur
		Trivandrum
		Wynaad
		Karnataka
Coorg		
Hassan		

* Collectively known as Assam Valley

^a Including Mikhir Hills and North Cachar

^b Including Cooch Behar

^c Including West Dinajpur

Assam and West Bengal are the main tea producing states in the northeast area (Table 18). In Assam, the Assam variety (*Camellia sinensis* (L.) O. Kuntze var. *assamica*) and its hybrids are cultivated, whereas in Darjeeling and the surrounding areas in West Bengal, the Chinese variety is cultivated. A number of clones and cultivars have been developed for cultivation in India (Table 19). The Department of Tea Husbandary and Technology of Himachal Pradesh Krishi Vishwa Vidhalaya in Palampur, the Tocklai Experimental Station in Jorhat, and the United Planters Association of South India (UPASI), and the Tea Research Institute in Coonoor have developed these clones.

Table 19: Important clones/cultivars of tea in different tea producing areas of India

Area	Clone/cultivar	Area	Clone/cultivar
North	Kangra Asha	South	C-1
	Kangra Jwala		CR-6017
	TV-1		TRI 2024
TV-23	TRI 2025		
Northeast	TV-1		TRI 2026
	TV-9		UPASI-1
	TV-18		UPASI-2
	TV-19		UPASI-3
	TV-20		UPASI-4
	TV-23		UPASI-5
	TV-26	UPASI-6	
	TV-27	UPASI-7	
	TV-29	UPASI-8	
	TV-30	UPASI-9	
	Takda-7-8	UPASI-10	
	Tenali-17	UPASI-15	
South	BSS-1		UPASI-20

6.2 Tea Production and Area Under Tea Cultivation in India

North and Northeast India

The zone under tea production in the north and northeast areas from 1998 to 2000 is presented in Table 20.¹⁵¹ No change in the area under tea cultivation was observed in the northern zone during this period. However, tea production decreased by 529 tonnes (25.6%) in 1999 while in 2000 a decrease of 20 tonnes (1.30%) was observed.

Table 20: Production and area under tea cultivation in North and Northeast India

Area	State District	1998		1999		2000	
		Area (ha)	Quantity (tonnes)	Area (ha)	Quantity (tonnes)	Area (ha)	Quantity (tonnes)
North	Himachal Pradesh						
	Kangra	2,325	1,711	2,325	1,222	2,325	1,247
Northeast	Uttaranchal						
	Nainital	1,068	349	1,068	309	1,068	264
	Total North	3,393	2,060	3,393	1,531	3,393	1,511
	Arunachal Pradesh	1,953	965	2,179	1,063	2,176	993
	Assam						
	Cachar	30,565	51,850	30,938	49,119	31,116	53,722
	Darrang	40,950	86,942	41,393	78,501	41,968	80,227
	Dibrugarh	83,380	164,463	88,291	150,469	93,138	155,932
	Goalpara	3,338	6,099	3,357	5,436	3,522	5,855
	Kamrup	3,307	5,146	3,289	4,941	3,300	5,003
	Karbi Anlong	1,652	1,375	1,781	1,301	2,005	1,878
	Lakhimpur	4,809	9,995	5,046	9,413	5,230	9,701
	North Cachar	4,516	6,743	4,523	6,367	4,524	6,521
	Nowgong	7,746	14,684	7,850	13,559	8,014	14,073
	Sibsagar	71,362	119,749	71,267	113,819	74,575	118,324
	Total Assam	251,625	467,046	257,735	432,925	267,392	451,236
	Bihar	762	138	1,348	473	1,350	538
	Manipur	536	76	746	97	907	96
	Meghalaya	145	127	215	135	351	140
	Mizoram	350	23	360	35	391	39
	Nagaland	472	29	1,012	39	1,214	43
	Orissa	214	94	214	100	214	105
	Sikkim	202	112	296	102	296	105
	Tripura	6,355	6,099	6,482	6,385	6,623	6,431
	West Bengal						
	Darjeeling	17,830	10,253	17,968	9,294	18,109	9,814
	Dooars	70,479	147,133	70,996	133,803	71,225	135,963
	Terai	17,315	36,403	19,790	37,115	20,356	34,947
	Total West Bengal	105,624	193,789	108,754	180,212	109,690	180,724
	Total Northeast	368,238	668,598	379,341	631,566	390,604	640,450
North and Northeast		371,631	670,658	382,734	623,097	393,997	641,961

In the northeast, the area under tea cultivation in 1998 ranged up to 368,238 hectares, it increased by 11,103 hectares (3.0%) in 1999, and 11,263 hectares (2.96%) in 2000. In Assam, the area under tea cultivation increased by 6,110 hectares (2.42%) in 1999, and 9,657 hectares in 2000, whereas in West Bengal an increase of 936 hectares (0.8%) was observed in 2000 as compared to 3,130 hectares (2.96%) in 1999. Except for the state of Orrisa (for which no change was registered), all the other states in the northeast observed an increase in the areas under cultivation.

All the tea producing districts in West Bengal with the exception of Terai, the states of Manipur and Arunachal Pradesh recorded a higher crop production. In 2000, the maximum increase was recorded in Assam (4.22%) and the minimum in West Bengal (0.284%) (Table 20).¹⁵¹ Production in the northeast declined from 668,598 tonnes in 1998 to 621,566 tonnes (7.0%) in 1999; whereas an increase of 18,884 tonnes (3.04%) in production was witnessed in 2000 over the level of the previous year.

A decrease of 9.8% in yield was observed in North India (north and northeast) in 1998, while a negligible change (increase of 0.06%) was observed in 2000 (Table 21).¹⁵¹

South India

The state of Tamilnadu witnessed the maximum increase in the area under tea cultivation, 5,612 hectares in 1999 and 5,176 hectares in 2000 (Table 22).¹⁵² In Kerala, a marginal increase of 0.04% was observed from 1998 to 2000 and no change was observed in Karnataka. Tea production in South India increased by 1.59% in 1999–2000 and stood at 204,522 tonnes in 2000. The higher increase was reported in Kerala with marginal contribution from other tea producing states of South India. The average tea production and yield of South India are given in Table 22 and 23, respectively.¹⁵¹

Total Tea Production in India

The total area under tea cultivation increased from 490,747 hectares in 1999 to 507,196 hectares in 2000. Area-wise production of tea in India is given in Figure 21. Tea production increased by 2.67% in 2000 over the previous year and reached 846,483 tonnes in 2000, although the yield of tea recorded a negligible growth during the same period. Only the state of Kerala recorded an increase (5.18%) in yield in 2000.

Table 21: Average tea yield in North and Northeast India

Area	State District	Average yield (kg/ha)		
		1998	1999	2000
North	Himachal Pradesh			
	Kangra	736	526	536
	Uttaranchal			
	Nainital	327	289	247
Northeast	Arunachal Pradesh	494	488	456
	Assam			
	Cachar	1,696	1,588	1,727
	Darrang	2,123	1,896	1,912
	Dibrugarh	1,972	1,704	1,674
	Goalpara	1,827	1,619	1,662
	Kamrup	1,556	1,502	1,516
	Karbi Anlong	832	730	937
	Lakhimpur	2,078	1,865	1,855
	North Cachar	1,493	1,408	1,441
	Nowgong	1,896	1,727	1,756
	Sibsagar	1,678	1,597	1,587
	Average Assam	1,856	1,680	1,688
	Bihar	181	351	399
	Manipur	142	130	106
	Meghalaya	876	628	399
	Mizoram	66	97	100
	Nagaland	61	39	35
	Orissa	439	467	491
	Sikkim	554	345	355
	Tripura	975	985	971
	West Bengal			
	Darjeeling	575	517	542
	Dooars	2,088	1,885	1,909
	Terai	2,102	1,875	1,717
Average W. Bengal	1,835	1,657	1,648	
North and Northeast		1,805	1,628	1,629

Table 22: Tea production in South India

State District	1998		1999		2000	
	Area (ha)	Quantity (tonnes)	Area (ha)	Quantity (tonnes)	Area (ha)	Quantity (tonnes)
Tamilnadu						
Coimbatore	11,609	32,997	11,650	32,000	11,650	32,100
Kanyakumari	434	119	434	101	434	120
Madurai	941	3,075	941	3,600	941	3,650
Nilgiris	49,759	93,972	55,330	90,375	60,506	90,729
Tirunelveli	800	1,883	800	2,012	800	2,100
Total Tamilnadu	63,543	132,046	69,155	128,088	74,331	129,699
Kerala						
Cannanore	-	-	-	-	-	-
Ernakulam	2	-	2	-	2	-
Idukki	26,608	48,254	26,610	51,200	26,615	52,000
Kottayam	840	244	840	300	840	320
Kozhikode	-	-	-	-	-	1,420
Malapuram	174	-	174	-	174	5,468
Malapulam	174	-	174	-	174	-
Palghat	841	2,398	841	1,800	841	1,950
Quilon	1,348	366	1,348	360	1,348	375
Trichur	523	1,951	523	1,500	523	1,650
Trivendrum	965	582	965	440	965	475
Wynaad	5,447	12,148	5,449	12,196	5,454	12,585
Total Kerala	36,748	65,943	36,752	67,796	36,762	69,355
Karnataka						
Chikmagalur	1,420	3,484	1,421	3,269	1,421	3,288
Coorg	290	768	290	750	290	760
Hassan	395	1,209	395	1,408	395	1,420
Total Karnataka	2,105	5,461	2,106	5,427	2,106	5,468
Total South India	102,396	203,450	108,013	201,311	113,199	204,522

Table 23: Average tea yield in South India

State District	Average yield (kg/ha)		
	1998	1999	2000
Tamilnadu			
Coimbatore	2,842	2,747	2,755
Kanyakumari	274	233	276
Madurai	3,268	3,826	3,879
Nilgiris	1,889	1,633	1,516
Tirunelveli	2,354	2,515	2,625
Average Tamilnadu	2,078	1,852	1,745
Kerala			
Cannanore	-	-	-
Ernakulam	-	-	-
Idukki	1,814	1,924	1,954
Kottayam	290	357	381
Kozhikode	-	-	-
Malapuram	-	-	-
Palghat	2,851	2,140	2,319
Quilon	272	267	278
Trichur	3,730	2,868	3,155
Trivendrum	603	456	492
Wynaad	2,230	2,238	2,307
Average Kerala	1,794	1,845	1,887
Karnataka			
Chikmagalur	2,454	2,300	2,314
Coorg	2,648	2,586	2,621
Hassan	3,061	3,565	3,595
Average Karnataka	2,594	2,577	2,596
South India	1,987	1,864	1,807

6.3 Production Trends of Different Types of Indian Tea

Production of cut tear curl (CTC) black tea in North India decreased by 35,514 tonnes (5.99%) in 1999 and increased by 34,024 tonnes (5.75%) in 2000 (Table 24).^{151, 152} In South India however, a reverse trend was observed as CTC tea production increased by 3,815 (2.30%) in 1999 and decreased by 1,871 tonnes (1.10%) in 2000. In India as a whole, production of CTC tea decreased by 31,699 tonnes (4.18%) in 1999 and increased by 32,153 tonnes (4.4%) in 2000. Orthodox tea manufacture of black tea in North India decreased by 28,788 tonnes (41.21%) in 1999 and increased by 39,370 tonnes (9.6%) in 2000. Similar trends were observed in the south and for the country as a whole.

A continuous decrease in green tea production since 1998 was observed in North India, (6.3%) in 1999 and (5.27%) in 2000. In South India, the production of green tea stayed nearly static between 1998 and 2000 and stood at 1,819 tonnes in 2000.

Table 24: Production of different types of tea in India

Area Tea type	1998		1999		2000	
	Tonnes	% share	Tonnes	% share	Tonnes	% share
North						
CTC black	592,421	88.5	556,907	92.2	590,931	92.1
Orthodox black	69,845	10.5	41,057	6.8	45,027	7.0
Green tea	6,764	1.0	6,337	1.0	6,003	0.9
Total	669,030	100.0	604,301	100.0	641,961	100.0
South						
CTC black	165,737	82.3	169,552	84.2	167,681	82.0
Orthodox black	33,786	16.8	29,905	14.9	35,022	17.1
Green tea	1,852	0.9	1,854	0.9	1,819	0.9
Total	201,375	100.0	201,311	100.0	204,522	100.0
All India						
CTC black	758,158	87.1	726,459	90.2	758,612	89.6
Orthodox black	103,631	11.9	70,962	8.8	80,049	9.5
Green tea	8,616	1.0	8,191	1.0	7,822	0.9
Total	870,405	100.0	805,612	100.0	846,483	100.0

Amritsar in Panjab, Kolkata and Siliguri in West Bengal, Guwahati in Assam, Cochin in Kerala and Coonoor and Coimbatore in Tamil Nadu are the Indian trading centres of tea, where tea is sold at open auctions. The sale of tea has decreased in Amritsar auctions since 1998. The tea sale price has also followed a similar trend. The different kinds of tea at other markets followed a variable trend both for sale and average price. Orthodox and all leaf tea generally fetched a higher price compared to other grades (Table 25 and Figure 22).^{151, 152} Sale and average price trends were variable at south Indian tea auctions (Figure 23).^{151, 152} At all the auctions, orthodox tea's average sale price was the highest.

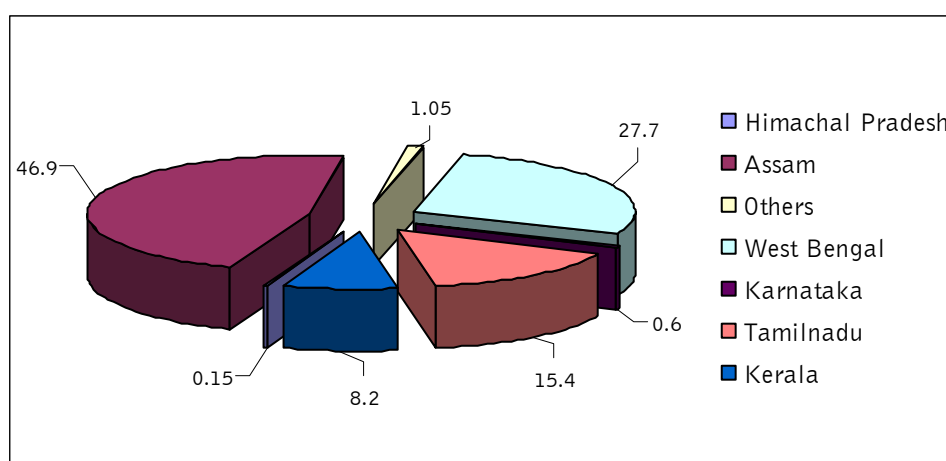


Figure 21: State-wise production share of tea in India (2000)

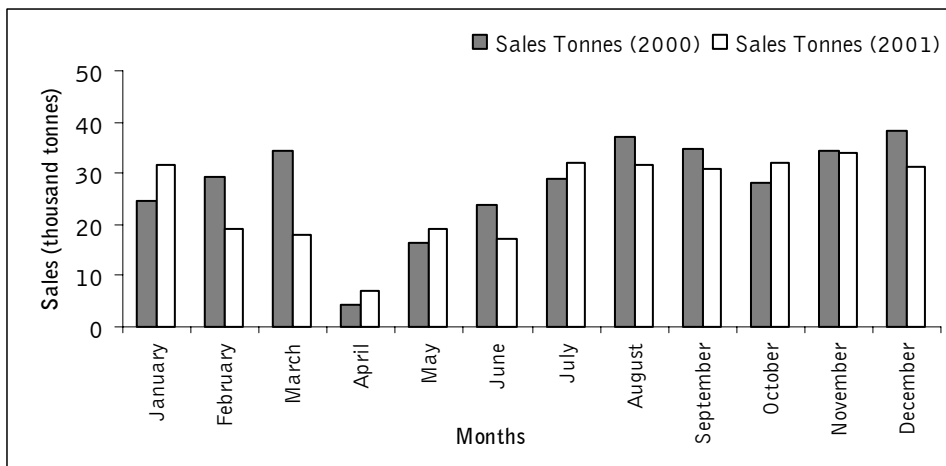


Figure 22: Month-wise sales of tea at North Indian auctions

6.4 Green Tea Production in India

In South India, green tea is produced only in the state of Tamilnadu. In India as a whole, a 19.39% decrease in green tea production was observed during the period 1997 to 1999 (Table 26).¹⁵¹

The state of Himachal Pradesh and Uttaranchal in the north and the state of Assam, West Bengal and Tripura in the northeastern zone are engaged in production of green tea. West Bengal is the largest producer of green tea. The production of green tea has declined in North India since 1997.

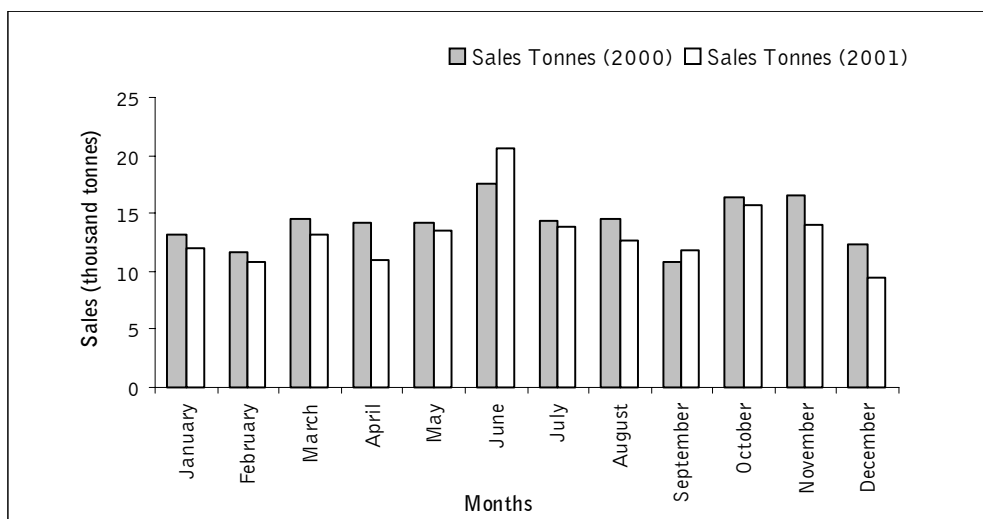


Figure 23: Month-wise sales of tea at South Indian auctions

Table 25: Sales of different kinds of black tea through auctions in India

Market	Type of tea	1998			1999			2000			2001		
		Quantity (tonnes)	Price (INR/kg)	Quantity (tonnes)	Price (INR/kg)	Quantity (tonnes)	Price (INR/kg)	Quantity (tonnes)	Price (INR/kg)	Quantity (tonnes)	Price (INR/kg)	Quantity (tonnes)	Price (INR/kg)
Amritsar	All types of tea	718	50.83	472	49.31	405	48.56	319	43.05				
Kolkata	Orthodox	24,469	85.48	11,669	94.57	18,330	95.77	19,277	79.80				
	CTC	34,792	84.81	49,881	83.98	52,147	71.74	47,635	73.69				
	All leaf	67,560	91.56	67,146	93.51	75,674	84.93	71,563	81.56				
	All dust	18,058	79.61	22,026	79.86	22,439	70.08	23,213	66.48				
Guwahati	CTC	92,377	79.98	98,618	82.35	101,196	69.99	94,368	71.47				
	All leaf	93,222	79.94	99,044	82.24	111,797	70.08	94,995	71.47				
Silliguri	All leaf	58,192	77.29	71,630	74.82	64,977	63.37	58,449	66.71				
	All dust	14,164	69.56	15,163	65.09	13,859	53.93	13,342	57.55				
Cochin	Orthodox	14,100	80.42	14,855	68.03	17,664	58.10	13,935	56.67				
	CTC	9,607	64.94	7,791	52.81	5,966	38.09	4,845	40.98				
	All leaf	23,707	74.22	22,646	62.80	23,633	52.87	18,780	52.59				
Coonoor	Orthodox	2,110	69.08	1,720	59.75	1,882	49.89	1,663	47.62				
	CTC	56,384	64.78	68,366	53.98	57,187	39.12	57,351	42.25				
Coimbatore	Orthodox	4,038	75.87	4,555	64.54	6,678	56.32	4,023	54.75				
	CTC	7,449	65.72	9,566	53.71	15,834	38.16	11,978	40.79				
	All dust	5,759	67.76	6,141	56.84	10,763	43.64	9,117	47.39				

6.5 Problems and Prospects of Green Tea Production

Indian green tea had a good market in Afghanistan and Morocco and 50% of green tea production in India was exported to these two countries. Exports to Afghanistan suffered a major setback following political disturbances in that country. Some other potential markets for Indian green tea are Germany, France, USA, Tunisia, Algeria and Middle East countries. Initiatives need to be taken to promote the production and export of green tea. In India, green tea is mainly produced by small tea companies, which are financially weak. As a result, it is not possible for them to undertake R&D to improve the quality or technique of green tea manufacture. Old methods of manufacture are used. Green tea is manufactured by steaming, followed by rolling. A substantial amount of components are lost from the leaf during the process leading to lower quality, which is the major drawback of this process. Other methods such as frying or panning and devising sophisticated sorting machines to remove both smaller and bigger stalks can produce better grades of tea.

Table 26: Green tea production in India

Area	State District	Production (Tonnes)		
		1997	1998	1999
North	Himachal Pradesh			
	Kangra	755	648	600
	Uttaranchal	260	210	175
Northeast	Assam			
	Cachar	212	178	160
	Darrang	289	243	233
	Dibrugarh	407	342	325
	Goalpara	218	185	175
	Kamrup	12	10	7
	Sibsagar	506	437	415
	Total Assam	1,644	1,395	1,315
	West Bengal			
	Dooars	1,926	2,755	2,221
Terai	3,282	1,658	1,600	
Total W. Bengal	5,208	4,413	3,821	
South	Tamilnadu			
	Madurai	45	43	47
	Nilgiris	455	482	453
	Total Tamilnadu	500	525	500
Total		500	525	500

The lack of proper auction centres for green tea, the low consumption of green tea on the domestic market, the lack of knowledge regarding the technical aspects of green tea manufacture, and the lack of R&D facilities for the upgrading of manufacturing

processes are some of the main problems faced by the green tea industry in India. In order to overcome these problems and to increase green tea production, there is a need to identify potential areas for green tea production in each region and to cultivate the best variety of tea. At present, Kashmir Valley has an annual domestic market of about 500 tonnes. Other markets in the country should be developed through promotional campaigns to increase domestic consumption of green tea and make people aware of its health benefits. R&D activities are urgently required to develop cultivars suited to green tea production and to improve the existing manufacturing process of green tea.

7. Conclusions

Samples of different cultivated varieties of tea were collected from all tea growing areas of the country. In one case, collection was repeated at the beginning, in the middle and at the end of the plucking season. The hand-picked tea leaves were processed for sample preparation following the usual manufacturing procedure of green tea. The leaves were steamed for 5 min and then dried in an oven at a temperature not exceeding 65°C. Samples were also prepared by directly drying the leaves in the sun immediately after plucking to compare their polyphenol profile.

Quantitative TLC was used for standardizing the extraction procedure and the HPLC method was used for estimating the polyphenol content of tea samples. The TLC solvent system for the best resolution of polyphenols in tea was developed in the laboratory. The best results were obtained using a mixture of chloroform : acetone : formic acid (5 : 4 : 1), which gave R_f values of 0.22 for EGCG, 0.32 for ECG, 0.27 for EGC and 0.46 for caffeine. In HPLC analysis, the mixture of water : methanol : acetic acid (70 : 30 : 0.5) as mobile phase gave a good separation of tea polyphenols in a 15 min run. It was observed that presence of acid in the solvent system of TLC and in the mobile phase of HPLC was essential for obtaining good chromatograms.

All analyses were performed in triplicate. The standard plots were constructed using reference samples of EGCG and ECG obtained from Sigma-Aldrich Chemicals. The estimations of EGCG and ECG in standard solutions showed an excellent coefficient of correlation both in TLC ($R^2 = 0.9998$ and 0.999 for EGCG and ECG respectively) and HPLC ($R^2 = 1.0$ and 0.9999 for EGCG and ECG respectively) over a wide range of concentration. It was therefore satisfactory to use the standard curve to determine the amount of EGCG and ECG in tea extracts. The concentration of extract was adjusted to fall in the middle range of the standard plot for carrying out the analysis.

In the initial experiments to optimize the extraction procedure, the EGCG extraction from tea samples was tried using methanol, water and ethyl acetate. It was observed (Table 8 and Figure 10) that methanol was the most suitable for extraction of tea polyphenols followed by water, whereas ethyl acetate proved to be a very poor solvent.

Therefore, water and methanol were further compared and investigated to optimize the period and temperature of extraction.

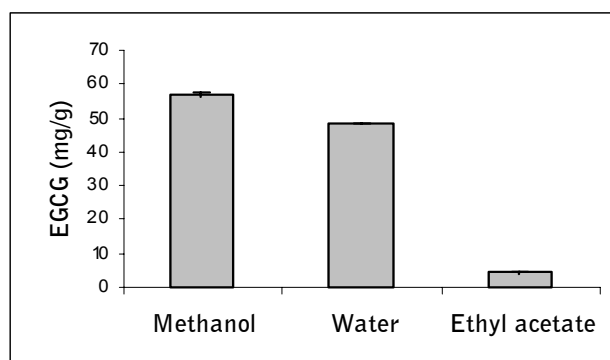


Figure 10: Effect of solvent on extraction of EGCG

The extractions in these two solvents were done at different temperatures (40, 60, 80 and 98°C) and time periods (15, 30, 45, 60, 75 and 90 min). In the case of methanol, there was a sharp rise in the EGCG extraction as the temperature was increased from 40 to 60°C and the maximum extraction was achieved in 60 min before it started declining. In the case of water, a similar trend was noticed as the temperature was increased from 40 to 98°C. The maximum extraction was achieved at 98°C in 15 min and thereafter it remained more or less static. However, in the case of methanol it started declining after attaining the maxima. These observations indicate different rates of EGCG degradation in water and methanol. The maximum EGCG extracted in methanol was slightly higher than the one obtained in water but it required 60 min extraction as compared to water which produced the maximum extraction in a short period of 5 min. Safety, acceptability and economical parameters of water off set the small advantage of a slightly higher extraction in methanol. Therefore, water became the logical choice as an extraction solvent.

The extraction of EGCG in water for time periods of 5, 10 and 15 min showed that the amount of EGCG obtained in 5 min did not improve further as the extraction time was increased to 15 min. Therefore, extraction in boiling water for 5 min was selected to extract and compare the polyphenols content of different tea samples.

The sample to water ratio of 1 : 50 was found the most appropriate to extract EGCG. No significant increase was observed in increasing the water proportion, although at a lower proportion it resulted in incomplete extraction (Table 11 and Figure 12).

Shaking during extraction resulted in a highly significant increase of 43% in EGCG extraction in comparison to still extraction (Table 12 and Figure 13).

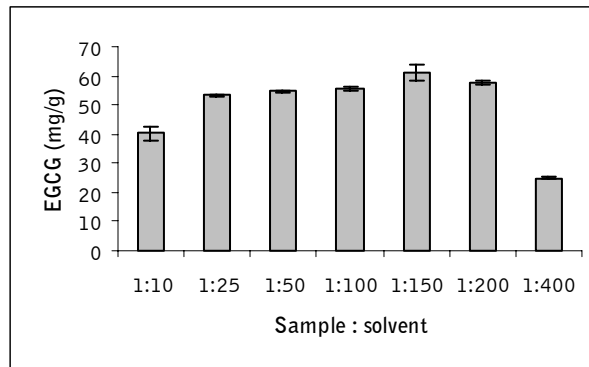


Figure 12: Effect of sample: solvent ratio on extraction of EGCG

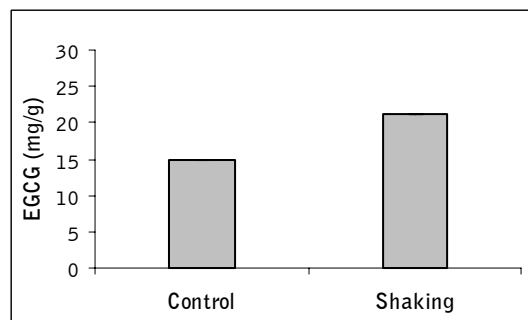


Figure 13: Effect of shaking on extraction of EGCG

The extraction of EGCG was also affected by the particle size of the powder (Table 13 and Figure 14). Fine powder gave a better extraction and moderately fine powder gave the maximum yield. Further reduction in the particle size of the powder adversely affected the extraction. These observations indicate that the solvent, the temperature, the duration of extraction, the particle size and shaking during extraction are critical for extraction of EGCG from tea leaves. The preliminary experiments established that boiling water, 1 : 50 sample to water ratio, 5 min extraction time, and moderately fine powder were the most appropriate for extracting EGCG from tea leaves.

Stability of EGCG as mixture of polyphenols in tea extract was monitored using buffers of different pH. The results indicated that there was marginal (3%) degradation of EGCG at pH 4 or below during the first 24 h, which increased to around 7% in the next 24 h at pH 1.2 and 2.0 but increased to 25% at pH 4 (Table 15 and Figure 15). The degradation was faster in alkaline pH and 65% of EGCG degraded during the first 48 h at pH 8. In water extract, pH 5.5, 43% degradation was observed after 48 h.

The EGCG in ethyl acetate extract of tea was stable and it was found that only 1.5% of it degraded during the first 48 h (Table 16 and Figure 16). Therefore, although

ethyl acetate is not a good solvent for extraction, it can be used as a solvent for short storage of the EGCG solution.

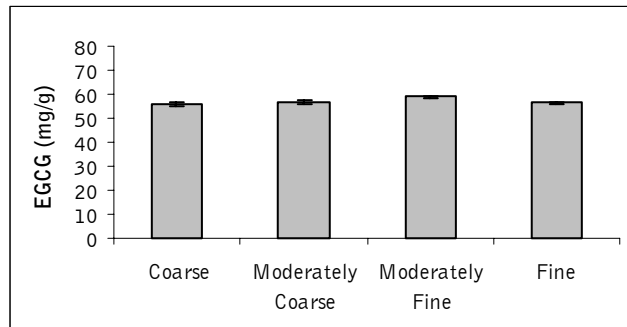


Figure 14: Effect of particle size on extraction of EGCG

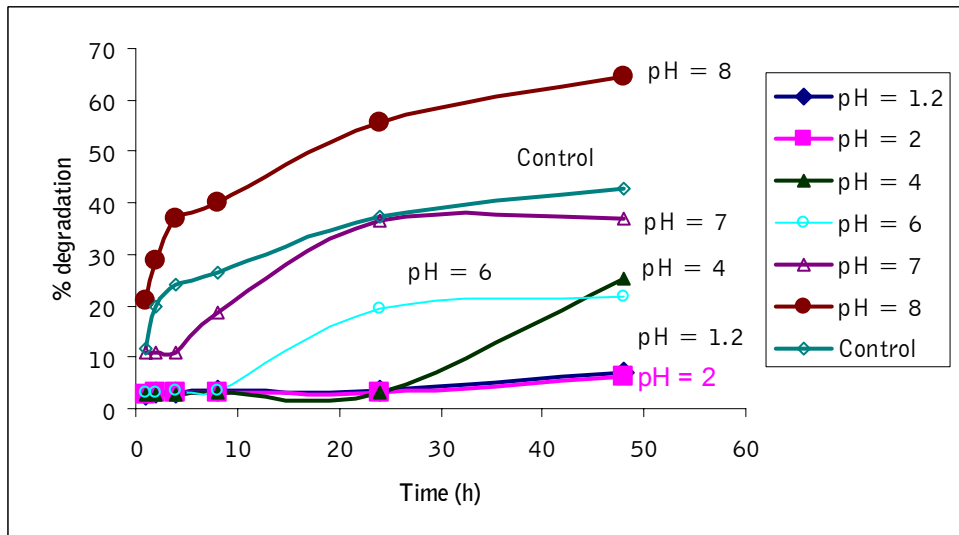


Figure 15: Effect of pH on stability of EGCG

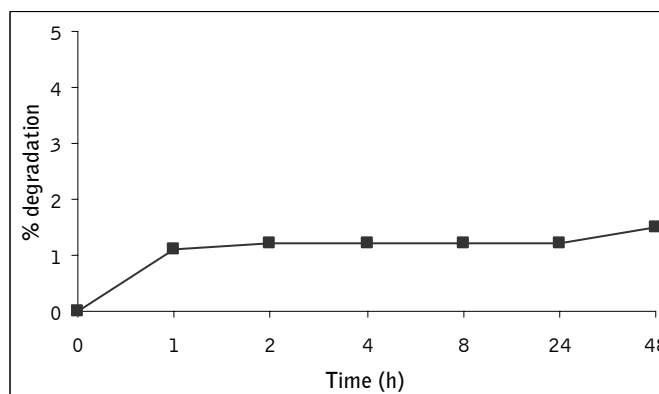


Figure 16: Stability of EGCG in ethyl acetate extract

The EGCG and ECG content of different cultivars were determined using optimized conditions of extraction and analysis developed in the laboratory. The content of EGCG and ECG in analyzed samples varied over a wide range (0.14 to 6.88% for EGCG and 0.03 to 2.74% for ECG). In general, samples prepared by the method of steaming and drying under controlled temperature showed a higher content of these two polyphenols. In samples collected from North India, the content of EGCG and ECG varied from 0.27 to 6.88% and 0.64 to 2.73% respectively. The comparison of EGCG and ECG content in tea samples collected at the beginning and at the end of the plucking season showed that the polyphenol content was higher in samples collected at the beginning of the plucking season. The EGCG and ECG content of samples from the northeast ranged from 1.28 to 5.01% and 0.56 to 1.96% respectively, whereas it ranged from 0.14 to 6.83% and 0.03 to 2.22% in samples from South India.

Among different cultivars, steamed samples of Kangra Jawala, a cultivar developed at the Department of Tea Husbandry and Technology, Himachal Pradesh Krishi Vishvavidalya, Palampur, North India, and TRI-2026 developed at the United Planters Association of South India, showed the highest content of EGCG (6.88 and 6.83% respectively). The TV-1 cultivar from Palampur showed the highest content of ECG (2.74%) among all the samples analyzed. The EGCG and ECG content of TV-1 and TV-23 in samples from Palampur was marginally higher than the content of samples from the northeast.

8. Summary

There are three areas of cultivation and production of tea in India. In the year 2000, black tea constituted 99.07% of the total production of tea. The bulk of green tea is produced in the northeast (West Bengal and Assam), followed by the northern area (Himachal Pradesh), and very small quantities are produced in the southern area (Tamilnadu). The production figures from 1998 to 2000 are compiled in this report along with the auction prices. In this period, the production of green tea decreased from 8,616 to 7,822 tonnes. Various factors responsible for low production of green tea in India have been highlighted and the comments of some of the companies visited are available in Appendix 5.

The extraction procedure of EGCG from green tea leaves was optimized with respect to solvent, time, temperature, particle size and sample to solvent ratio. EGCG can be extracted with boiling methanol or water. The extraction is quicker in water. The maximum amount of EGCG is extracted in 50 times by weight of water in 5 min using moderately fine powder of tea leaves. The ethyl acetate has proved to be a poor solvent for extraction of polyphenols. These constituents were found to degrade rapidly in weakly acidic to alkaline solution.

A large number of cultivars of tea have been developed in India. In the present study, 26 prominent cultivars were analyzed. Region-wise, Kangra Jawala grown in Palampur (North India) showed the highest content of EGCG (6.8%), followed by TRI-2026 (6.83%) grown in Coonor (South India), and Kangra Asha (6.4%) grown in Palampur. The lowest content of EGCG was recorded in P-126 (1.29%), Tenali-17 (1.76%) and TV-20 (1.92%) grown in Darjeeling. The samples of two cultivars, TV-1 and TV-23 were collected from two regions, north (Palampur) and northeast (Darjeeling). The content of EGCG was lower in northeast samples, suggesting the possible role of agro-climatic conditions. The deactivation of enzymes by steaming immediately after plucking is essential as observed from the very low content of EGCG in sun dried samples.

REFERENCES

- 1 Anonymous, 2003, History of tea. Available at: <http://www.inpursuitof tea.com/history.htm> (12 Feb. 2003)
- 2 Anonymous, 2001, Tea and health. Available at: <http://www.tea-directory.com/sciencetea/15teaandhealthleafcontents.htm> (2 Aug. 2001)
- 3 Mahindroo, N., 2000, Tea - *Camellia sinensis* - report, ICS-UNIDO, Trieste, Italy
- 4 Anonymous, 2001, A flavourful guide to green teas. Available at: www.holymntn.com/guide2green tea.htm (18 Jul. 2001)
- 5 Eden, T., 1976, Pruning and plucking, in, Tea, 3rd edn., Longman Group Ltd., London, UK, pp. 54-68
- 6 Anonymous, 2003, Tea info-tea grades. Available at: www.tea-experience.com/tea-grades.html (13 Feb. 2003)
- 7 Haslam, E., 1989, Polyphenols - vegetable tannins, in, Plant Polyphenols, Cambridge University Press, Cambridge, UK, p. 9
- 8 Chu, D. C. and Juneja, L. R., 1997, General composition of green tea and its infusion, in, chemistry and applications of green tea, in, Yamamoto, T., Juneja, L. R., Chu, D. C. and Kim, M. (eds.), Chemistry and Applications of Green Tea, CRC Press, New York, USA, pp. 13-22
- 9 Roberts, E. A. H. and Wood, D. J., 1951, Study of polyphenols in tea leaf by paper chromatography, *Biochem. J.*, 49: 414-22
- 10 Nakabayashi, T., 1991, Chemical components in tea leaves, in, Nakabayashi, T., Ina, K. and Sakata, K. (eds.), Chemistry and Function of Green Tea, Black Tea and Oolong Tea (in Japanese), Kogaku Shuppan, Kawasaki, Japan, p. 20
- 11 Oshima, Y., 1936, Chemical studies on the tannin substances of Formosan tea leaves, *Bull. Agr. Chem. Soc. Japan*, 12: 103
- 12 Bradfield, A. E., Penny, M. and Wright, W. B., 1947, The catechins of green tea Part I, *J. Chem. Soc.*: 32
- 13 Bradfield, A. E., Penny, M. and Wright, W. B., 1948, The catechins of green tea Part II, *J. Chem. Soc.*: 2249
- 14 Balentine, D. A., Wiseman, S. A. and Bouwens, L. C., 1997, The chemistry of tea flavonoids, *Crit. Rev. Food Sci. Nutr.*, 37: 693-704
- 15 Eden, T., 1976, The chemistry of the tea leaf and of its manufacture, in, Tea, 3rd edn., Longman Group Ltd., London, UK, pp. 153-65
- 16 Harler, C. R., 1964, The chemistry of tea, in, The Culture and Marketing of Tea, Oxford University Press, UK, pp. 67-71
- 17 Schery, W. R., 1954, Beverage plants, in, Plants for Man, George Allen and Unwin Ltd., London, UK, pp. 510-5
- 18 Nagata, T. and Sakai, S., 1985, Caffeine, flavonol and amino acid contents in leaves of hybrids and species of selection dubial in the genus *Camellia*, *Jpn. J. Breed.*, 35: 1
- 19 Mukai, T., Horie, H. and Goto, T., 1992, Differences in free amino acids and total nitrogen contents among various prices of green tea, *Tea Res. J.*, 76: 45
- 20 Ikegaya, K., Takayanagi, H., Anan, T., Iwamoto, M., Uozumi, J., Niahinari, K. and Cho, R., 1988, Determination of the content of total nitrogen, caffeine, total free amino acids, theanine and tannin of sencha and matcha by near infrared spectroscopy, *Bulletin of the National Research Institute of Vegetables, Ornamental Plants and Tea*, p. 47
- 21 Kimura, R. and Murata, T., 1986, Effect of theanine on norepinephrine and serotonin levels in rat brain, *Chem. Pharma. Bull.*, 34: 3053
- 22 Anonymous, 1991, Standard tables of food composition (in Japanese), Resources Council, Science and Technology Agency, Tokyo, Japan

- 23 Nagata, T., 1990, New analytical methods for studying tea quality components, *Tea Res. J.*, 72: 53
- 24 Mizuno, T., Katayama, Y. and Funaki, T., 1965, Studies on the carbohydrates of tea. Part XI. The contents of starch in green tea, *Nisshokukoshi* (in Japanese), 12: 373
- 25 Goto, T., Yoshida, Y., Kiso, M. and Nagashima, H., 1996, Simultaneous analysis of individual catechins and caffeine in green tea, *J. Chrom. A*, 749: 295-9
- 26 Poon, G. K., 1998, Analysis of catechins in tea extracts by liquid chromatography-electrospray ionization mass spectrometry, *J. Chrom. A*, 794: 63-74
- 27 Bronner, W. E. and Beecher, G. R., 1998, Method for determining the content of catechins in tea infusions by high-performance liquid chromatography, *J. Chromatogr. A*, 805: 137-42
- 28 Larger, P. J., Jones, A. D. and Dacombe, C., 1998, Separation of tea polyphenols using micellar electrokinetic chromatography with diode array detection, *J. Chrom. A*, 799: 309-20
- 29 Dalluge, J. J., Nelson, B. C., Thomas, J. B. and Sander, L. C., 1998, Selection of column and gradient elution system for the separation of catechins in green tea using high-performance liquid chromatography, *J. Chrom. A*, 793: 265-74
- 30 Ding, M., Yang, H. and Xiao, S., 1999, Rapid, direct determination of polyphenols in tea by reversed-phase column liquid chromatography, *J. Chrom. A*, 849: 637-40
- 31 Lee, B. L. and Ong, C. N., 2000, Comparative analysis of tea catechins and theaflavins by high-performance liquid chromatography and capillary electrophoresis, *J. Chrom. A*, 881: 439-47
- 32 Kim, S. H., Jong-Dae, P., Lee, L. S. and Daeseok, H., 2000, Effect of heat processing on the chemical composition of green tea extract, *Food Sci. Biotechnol.*, 9: 214-7
- 33 Dalluge, J. J. and Nelson, B. C., 2000, Determination of tea catechins, *J. Chrom. A*, 881: 411-24
- 34 Horie, H. and Kohata, K., 2000, Analysis of tea components by high-performance liquid chromatography and high-performance capillary electrophoresis, *J. Chrom. A*, 881: 425-38
- 35 Sakata, K., Yamauchi, H., Yagi, A., Ina, K., Parkanyi, L. and Clardy, 1989, 2-O-(β -L-arabinopyronosyl)-myo-inositol as a main constituent of tea (*Camellia sinensis*), *J. Agric. Biol. Chem.*, 53: 2975
- 36 Gong, Z., Watanabe, N., Yagi, A., Etoh, H., Sakata, K., Iria, K. and Liu, Q., 1993, Compositional change of Pu-erh tea during processing, *Biosci. Biotech. Biochem.*, 57: 1745
- 37 Davis, A. L., Cai, Y., Davies, A. P. and Lewis, J. R., 1996, ^1H and ^{13}C NMR assignment of some green tea polyphenols, *Mag. Res. Chem.*, 34: 887
- 38 Schulz, H., Engelhardt, U. H., Wegent, A., Drews, H. and Lapczynski, S., 1999, Application of near-infrared reflectance spectroscopy to the simultaneous prediction of alkaloids and phenolic substances in green tea leaves, *J. Agric. Food Chem.*, 47: 5064-7
- 39 Singh, H. P., Ravindranath, S. D. and Singh, C., 1999, Analysis of tea shoot catechins: Spectrophotometric quantitation and selective visualization on two-dimensional paper chromatograms using diazotized sulfanilamide, *J. Agric. Food Chem.*, 47: 1041-5
- 40 Zhu, M. and Xiao, P. G., 1991, Quantitative analysis of active constituents of green tea, *Phytother. Res.*, 5: 239
- 41 Khokhar, S. and Magnusdottir, S. G., 2002, Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom, *J. Agric. Food Chem.*, 50: 565-70
- 42 Lakenbrink, C., Llapczynski, S., Maiwald, B. and Englehardt, U. H., 2000, Flavonoid and other polyphenols in consumer brews of tea and other caffeinated beverages, *J. Agric. Food Chem.*, 48: 2848-52
- 43 Maiani, G., Serafini, M., Salucci, M., Azzini, E. and Ferro-Luzzi, A., 1997, Application of a new high-performance liquid chromatographic method for measuring selected polyphenols in human plasma, *J. Chrom. B*, pp. 311-7
- 44 Kumamoto, M., Sonda, T., Takedomi, K. and Tabata, M., 2000, Enhanced separation and elution of catechins in HPLC using mixed solvents of water, acetonitrile and ethyl acetate as the mobile phase, *Analytical Sciences*, 16: 139-44

- 45 Miyazawa, T. and Nakagawa, K., 1998, Structure-related emission spectrometric analysis of the chemiluminescence of catechins, theaflavins and anthocyanins, *Biosci. Biotechnol. Biochem.*, 62: 829-32
- 46 Beecher, G. R., Warden, B. A. and Merken, H., 1999, Analysis of tea polyphenols, *Proc. Soc. Exp. Biol. Med.*, 220: 267-70
- 47 Lin, Y. Y., Ng, K. J. and Yang, S., 1993, Characterization of flavonoids by LC-tandem mass spectrometry, *J. Chrom.*, 629: 389
- 48 Miketova, P., Schram, K. H., Whitney, J. L., Kerns, E. H., Valcic, S., Timmermann, B. N. and Volk, K. J., 1998, Mass spectrometry of selected components of biological interest in green tea extracts, *J. Nat. Prod.*, 6: 461-7
- 49 Lee, M. J., Wang, Z. Y., Li, H., Chen, L., Sun, Y., Gabbo, S., Balentine, D. A. and Yang, C. S., 1995, Analysis of plasma and urinary tea polyphenols in human subjects, *Cancer Epidemiology, Biomarkers and Preventors*, 4: 393-9
- 50 Luthria, D. L., Jones, A. D., Donovan, J. L. and Waterhouse, A. L., 1997, Determination of catechin and epicatechin levels in human plasma, *J. High Resolut. Chromato.*, 20: 621
- 51 Horie, H., Mukai, T., Goto, T., Mkawanaka and Shimohara, T., 1994, Measurement of tea catechins using biosensors, *Nippon Shokuhin Kogyo Gakkaishi*, 41: 433
- 52 Serafini, M., Ghiselli, A. and Ferro-Luzzi, A., 1996, In vivo antioxidant effect of green and black tea in man, *Eur. J. Clin. Nutr.*, 50: 28-32
- 53 Weisburger, J. H., 1999, Mechanisms of action of antioxidants as exemplified in vegetables, tomatoes and tea, *Food Chem. Toxicol.*, 37: 943-8
- 54 Wiseman, S. A., Balentine, D. A. and Frei, B., 1997, Antioxidants in tea, *Crit. Rev. Food Sci. Nutr.*, 37: 705-18
- 55 Ho, C. T., Chen, Q., Shi, H., Zhang, K. Q. and Rosen, R. T., 1992, Antioxidative effect of polyphenol extract prepared from various Chinese teas, *Prev. Med.*, 21: 520-5
- 56 Bhimani, R. S., Troll, W., Grunberger, D. and Frenkel, K., 1993, Inhibition of oxidative stress in HeLa cells by chemopreventive agents, *Cancer Res.*, 53: 4528-33
- 57 Miura, S., Watanabe, J., Sano, M., Tomita, T., Osawa, T., Hara, Y. and Tomita, I., 1995, Effects of various natural antioxidants on the Cu(2+)-mediated oxidative modification of low density lipoprotein, *Biol. Pharm. Bull.*, 18: 1-4
- 58 Yoshino, K., Hara, Y., Sano, M. and Tomita, I., 1994, Antioxidative effects of black tea theaflavins and thearubigin on lipid peroxidation of rat liver homogenates induced by tert-butyl hydroperoxide, *Biol. Pharm. Bull.*, 17: 146-9
- 59 Blazovics, A., Lugasi, A., Kemeny, T., Hagymasi, K. and Kery, A., 2000, Membrane stabilising effects of natural polyphenols and flavonoids from *sempervivum tectorum* on hepatic microsomal mixed-function oxidase system in hyperlipidemic rats, *J. Ethnopharmacol.*, 73: 479-85
- 60 Hasaniya, N., Youn, K., Xu, M., Hernaez, J. and Dashwood, R., 1997, Inhibitory activity of green and black tea in a free radical-generating system using 2-amino-3-methylimidazo[4,5-f]quinoline as substrate, *Jpn. J. Cancer Res.*, 88: 553-8
- 61 Surh, Y., 1999, Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances, *Mutat. Res.*, 428: 305-27
- 62 Johnson, M. K. and Loo, G., 2000, Effects of epigallocatechin gallate and quercetin on oxidative damage to cellular DNA, *Mutat. Res.*, 459: 211-8
- 63 Choi, H. Y., Jhun, E. J., Lim, B. O., Chung, I. M., Kyung, S. H. and Park, D. K., 2000, Application of flow injection - Chemiluminescence to the study of radical scavenging activity in plants, *Phytother. Res.*, 14: 250-3
- 64 Yang, X. Q., Shen, S. R., Hou, J. W., Zhao, B. L. and Xin, W. J., 1994, [Mechanism of scavenging effects of (-)-epigallocatechin gallate on active oxygen free radicals], *Zhongguo Yao Li Xue Bao*, 15: 350-3
- 65 Zhao, B., Guo, Q. and Xin, W., 2001, Free radical scavenging by green tea polyphenols, *Methods Enzymol.*, 335: 217-31

- 66 Krul, C., Luiten-Schuite, A., Tenfelde, A., van Ommen, B., Verhagen, H. and Havenaar, R., 2001, Antimutagenic activity of green tea and black tea extracts studied in a dynamic in vitro gastrointestinal model, *Mutat. Res.*, 474: 71-85
- 67 Santana-Rios, G., Orner, G. A., Amantana, A., Provost, C., Wu, S. Y. and Dashwood, R. H., 2001, Potent antimutagenic activity of white tea in comparison with green tea in the salmonella assay, *Mutat. Res.*, 495: 61-74
- 68 Sachinidis, A. and Hescheler, J., 2002, Are catechins natural tyrosine kinase inhibitors?, *Drug News Perspect*, 15: 432-8
- 69 Garbisa, S., Sartor, L., Biggin, S., Salvato, B., Benelli, R. and Albini, A., 2001, Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate, *Cancer*, 91: 822-32
- 70 L'Allemain, G., 1999, [Multiple actions of EGCG, the main component of green tea], *Bull. Cancer*, 86: 721-4
- 71 Tang, F. Y. and Meydani, M., 2001, Green tea catechins and vitamin E inhibit angiogenesis of human microvascular endothelial cells through suppression of il-8 production, *Nutr. Cancer*, 41: 119-25
- 72 Vinson, J. and Dabbagh, Y. A., 1998, Tea phenols: Antioxidant effectiveness of teas, tea components, tea fractions and their binding with lipoproteins, *Nutr. Res.*, 18: 1067-75
- 73 Itaro, O., Nobutaka, S. and Kazuhiko, K., 1999, Prevention of ageing by tea catechins, *Roka Yobo Shokuhin no Kaihatsu*, pp. 139-55
- 74 Lin, J. K., Chen, P. C., Ho, C. T. and Lin-Shiau, S. Y., 2000, Inhibition of xanthine oxidase and suppression of intracellular reactive oxygen species in hl-60 cells by theaflavin-3,3'-digallate, (-)-epigallocatechin-3-gallate, and propyl gallate, *J. Agric. Food Chem.*, 48: 2736-43
- 75 Langley-Evans, S. C., 2000, Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay, *Int. J. Food Sci. Nutr.*, 51: 181-8
- 76 Pietta, P., Simonetti, P., Roggi, C., Brusamolino, A., Pellegrini, N., Maccarini, L. and Testolin, G., 1996, Dietary flavonoids and oxidative stress, in, Kumpulainen, J. T. and Salonen, J. T., (eds.), Proc. on the Natural Antioxidants and Food Quality in Atherosclerosis and Cancer Prevention, Cambridge, UK
- 77 Weisburger, J. H., 1999, Tea and health: The underlying mechanisms, *Proc. Soc. Exp. Biol. Med.*, 220: 271-5
- 78 Muramatsu, K., Fukuyo, M. and Hara, Y., 1986, Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats, *J. Nutr. Sci. Vitaminol.*, 32: 613-22
- 79 Yokozawa, T., Nakagawa, T. and Kitani, K., 2002, Antioxidative activity of green tea polyphenol in cholesterol-fed rats, *J. Agric. Food. Chem.*, 50: 3549-52
- 80 Shen, X., Lu, R. and Tang, J., 1993, Hypolipidemic and anti-coagulant effects of tea polyphenol in rats, *Yingyang Xuebao*, 15: 147-51
- 81 Hodgson, J. M., Prondfoot, J. M., Croft, K. D., Puddey, B. I., Mori, A. and Beilin, J., 1999, Comparison of effects of black and green tea on in vitro lipoprotein oxidation in human serum, *J. Sci. Food Agri.*, 79: 561-6
- 82 Anderson, J. W., 1998, Selective effects of different anti-oxidants on oxidation of lipoproteins from rats, *Proc. Soc. Exp. Biol. Med.*, 218: 376-81
- 83 Hertog, M. G., Kromhout, D., C. A., Blackburn, H., Buzina, R., Fidanza, F., Giampaoli, S., Jansen, A., Menotti, A., Nedeljkovic, S., Pekkarinen, M., Simic, B. S., Toshima, H., Feskens, E. J. M., Hollman, P. C. H. and Katan, M. B., 1995, Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study, *Arch. Intern. Med.*, 155: 381-6
- 84 Hollman, P. C., Feskens, E. J. and Katan, M. B., 1999, Tea flavonols in cardiovascular disease and cancer epidemiology, *Proc. Soc. Exp. Biol. Med.*, 220: 198-202
- 85 Dreosti, I. E., 1996, Bioactive ingredients: Antioxidants and polyphenols in tea, *Nutr. Rev.*, 54: S51-8
- 86 Turner, L., 2001, Spotlight: Green tea extract. Available at: www.Body21a.com/herbs/supplement/greentea.htm (18 Jul. 2001)

- 87 Hofmann, C. S. and Sonenshein, G. E., 2003, Green tea polyphenol epigallocatechin-3-gallate induces apoptosis of proliferating vascular smooth muscle cells via activation of p53, *Faseb. J.*, 17: 702-4
- 88 Chen, Z. Y., Law, W. I., Yao, X. Q., Lau, C. W., Ho, W. K. and Huang, Y., 2000, Inhibitory effects of purified green tea epicatechins on contraction and proliferation of arterial smooth muscle cells, *Acta Pharmacol. Sin.*, 21: 835-40
- 89 Suganuma, M., Sueoka, E., Sueoka, N., Okabe, S. and Fujiki, H., 2000, Mechanisms of cancer prevention by tea polyphenols based on inhibition of TNF-alpha expression, *Biofactors*, 13: 67-72
- 90 Dreosti, I. E., 1997, Cancer biomarkers in the field of tea, *Cancer Lett.*, 114: 319-21
- 91 Parshad, R., Sanford, K. K., Price, F. M., Steele, V. E., Tarone, R. E., Kelloff, G. J. and Boone, C. W., 1998, Protective action of plant polyphenols on radiation-induced chromatid breaks in cultured human cells, *Anticancer Res.*, 18: 3263-6
- 92 Kuo, P. L. and Lin, C. C., 2003, Green tea constituent (-)-epigallocatechin-3-gallate inhibits hep g2 cell proliferation and induces apoptosis through p53-dependent and fas-mediated pathways, *J. Biomed. Sci.*, 10: 219-27
- 93 McCarty, M. F., 1997, Natural antimutagenic agents may prolong efficacy of human immunodeficiency virus drug therapy, *Med. Hypotheses*, 48: 215-20
- 94 Taraphdar, A. K., Madhumita, R. and Bhattacharaya, R. K., 2001, Natural products as inducers of apoptosis: Implication for cancer therapy and prevention, *Curr. Sci.*, 80: 1391-6
- 95 Miyajima, M. and Hara, T., 1999, Excision repair activity of green tea extract in 4 nqo-induced mutations of cultured Chinese hamster v79 cells, *Kankyo Heig'n Kenkyu*, 21: 95-102
- 96 Kim, M. and Masuda, M., 1997, Cancer chemoprevention by green tea polyphenols, in, *Chemistry and Applications of Green Tea*, CRC Press, pp. 61-73
- 97 Sadzuka, Y., Sugiyama, T. and Hirota, S., 1998, Modulation of cancer chemotherapy by green tea, *Clin. Cancer Res.*, 4: 153-6
- 98 Sun, C. L., Yuan, J. M., Lee, M. J., Yang, C. S., Gao, Y. T., Ross, R. K. and Yu, M. C., 2002, Urinary tea polyphenols in relation to gastric and esophageal cancers: A prospective study of men in Shanghai, China, *Carcinogenesis*, 23: 1497-503
- 99 Kinjo, J., Nagao, T., Tanaka, T., Nonaka, G., Okawa, M., Nohara, T. and Okabe, H., 2002, Activity-guided fractionation of green tea extract with antiproliferative activity against human stomach cancer cells, *Biol. Pharm. Bull.*, 25: 1238-40
- 100 Hara, Y., 1997, Influence of tea catechins on the digestive tract, *J. Cell Biochem. Suppl.*, 27: 52-8
- 101 Takada, M., Nakamura, Y., Koizumi, T., Toyama, H., Kamigaki, T., Suzuki, Y., Takeyama, Y. and Kuroda, Y., 2002, Suppression of human pancreatic carcinoma cell growth and invasion by epigallocatechin-3-gallate, *Pancreas*, 25: 45-8
- 102 Chung, F. L., 1999, The prevention of lung cancer induced by a tobacco-specific carcinogen in rodents by green and black tea, *Proc. Soc. Exp. Biol. Med.*, 220: 244-8
- 103 Yang, C. S., Maliakal, P. and Meng, X., 2002, Inhibition of carcinogenesis by tea, *Annu. Rev. Pharmacol. Toxicol.*, 42: 25-54
- 104 Fujiki, H., Yoshizawa, S., Horiuchi, T., Suganuma, M., Yatsunami, J., Nishiwaki, S., Okabe, S., Nishiwaki-Matsushima, R., Okuda, T. and Sugimura, T., 1992, Anticarcinogenic effects of (-)-epigallocatechin gallate, *Prev. Med.*, 21: 503-9
- 105 Gupta, S., Ahmad, N., Mohan, R. R., Husain, M. M. and Mukhtar, H., 1999, Prostate cancer chemoprevention by green tea: In vitro and in vivo inhibition of testosterone-mediated induction of ornithine decarboxylase, *Cancer Res.*, 59: 2115-20
- 106 Bushman, J. L., 1998, Green tea and cancer in humans: A review of the literature, *Nutr. Cancer*, 31: 151-9
- 107 Komori, A., Yatsunami, J., Okabe, S., Abe, S., Hara, K., Suganuma, M., Kim, S. J. and Fujiki, H., 1993, Anticarcinogenic activity of green tea polyphenols, *Jpn. J. Clin. Oncol.*, 23: 186-90

- 108 Sueoka, N., Suganuma, M., Sueoka, E., Okabe, S., Matsuyama, S., Imai, K., Nakachi, K. and Fujiki, H., 2001, A new function of green tea: Prevention of lifestyle-related diseases, *Ann. N. Y. Acad. Sci.*, 928: 274-80
- 109 Siro, I. T. and Young, I. K., 2000, Tea and health, *Nutr. Rev.*, 58: 1-10
- 110 Mukhtar, H. and Ahmad, N., 1999, Green tea in chemoprevention of cancer, *Toxicol. Sci.*, 52: 111-7
- 111 Nakachi, K., Suemasu, K., Suga, K., Takeo, T., Imai, K. and Higashi, Y., 1998, Influence of drinking green tea on breast cancer malignancy among Japanese patients, *Jpn. J. Cancer Res.*, 89: 254-61
- 112 Tanaka, K., Hayatsu, T., Negishi, T. and Hayatsu, H., 1998, Inhibition of n-nitrosation of secondary amines in vitro by tea extracts and catechins, *Mutat. Res.*, 412: 91-8
- 113 Wang, H. and Wu, Y., 1991, Inhibitory effect of Chinese tea on n-nitrosation in vitro and in vivo, *IARC Sci. Publ.*: 546-9
- 114 Wu, Y. N., Wang, H. Z., Li, J. S. and Han, C., 1993, The inhibitory effect of Chinese tea and its polyphenols on in vitro and in vivo n-nitrosation, *Biomed. Environ. Sci.*, 6: 237-58
- 115 Katiyar, S. K., Agarwal, R., Wang, Z. Y., Bhatia, A. K. and Mukhtar, H., 1992, (-)-epigallocatechin-3-gallate in *Camellia sinensis* leaves from Himalayan region of Sikkim: Inhibitory effects against biochemical events and tumor initiation in senear mouse skin, *Nutr. Cancer*, 18: 73-83
- 116 Fujiki, H., Suganuma, M., Okabe, S., Sueoka, N., Komori, A., Sueoka, E., Kozu, T., Tada, Y., Suga, K., Imai, K. and Nakachi, K., 1998, Cancer inhibition by green tea, *Mutat. Res.*, 402: 307-10
- 117 Fujiki, H., Suganuma, M., Okabe, S., Sueoka, E., Suga, K., Imai, K. and Nakachi, K., 2000, A new concept of tumor promotion by tumor necrosis factor-alpha, and cancer preventive agents (-)-epigallocatechin gallate and green tea-a review, *Cancer Detect Prev.*, 24: 91-9
- 118 Suganuma, M., Ohkura, Y., Okabe, S. and Fujiki, H., 2001, Combination cancer chemoprevention with green tea extract and sulindac shown in intestinal tumor formation in min mice, *J. Cancer Res. Clin. Oncol.*, 127: 69-72
- 119 Suganuma, M., Okabe, S., Kai, Y., Sueoka, N., Sueoka, E. and Fujiki, H., 1999, Synergistic effects of (-)-epigallocatechin gallate with (-)-epicatechin, sulindac, or tamoxifen on cancer-preventive activity in the human lung cancer cell line PC-9, *Cancer Res.*, 59: 44-7
- 120 Suganuma, M., Okabe, S., Oniyama, M., Tada, Y., Ito, H. and Fujiki, H., 1998, Wide distribution of [3H](-)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue, *Carcinogenesis*, 19: 1771-6
- 121 Zhu, M., Chen, Y. and Li, R. C., 2000, Oral absorption and bioavailability of tea catechins, *Planta Medica*, 66: 444-7
- 122 Yang, C. S., Chung, J. Y., Yang, G., Chhabra, S. K. and Lee, M. J., 2000, Tea and tea polyphenols in cancer prevention, *J. Nutr.*, 130: 472S-8S
- 123 Suganuma, M., Sueoka, N., Sueoka, E., Matsuyama, S., Imai, K. and Fuji, H. S., 1999, Green tea and cancer chemo prevention, *Mutat. Res.*, 428: 334-9
- 124 Hirota, F., Suganuma, M., Okabe, S., Sueska, E., Sueoka, N., Matsuyama, S., Imai, K. and Nakachi, K., 1999, Green tea as cancer preventive, *Chem. Biol. Asp.*: 12-7
- 125 Yamane, T., 2000, Cancer prevention by green tea polyphenols, *Gan Kagaku Yobu no Saizensen*, pp. 18-28
- 126 Suga, K., Imai, K., Sueoka, N. and Nakachi, K., 1998, Phase I clinical trial with green tea tablets in Japanese healthy population, *Cancer Prev. Intern.*, 3: 79-88
- 127 Hirota, F., Sugunama, M., Komori, A., Okabe, S., Sueoka, E., Sueoka, N., Kozu, T. and Tada, Y., 1998, Natural inhibitors of carcinogenesis, in, *Clinical and Biological Basis of Lung Cancer Prevention*, pp. 285-90
- 128 Blot, W. J., McLaughlin, J. K. and Chow, W. H., 1997, Cancer rates among drinkers of black tea, *Crit. Rev. Food Sci. Nutr.*, 37: 739-60
- 129 Gomes, A., Vedasiromoni, J. R., Das, M., Sharma, R. M. and Ganguly, D. K., 1995, Anti-hyperglycemic effect of black tea (*Camellia sinensis*) in rat, *J. Ethnopharmacol.*, 45: 223-6

- 130 Anonymous, 2002, Green tea. Available at: www.lef.org/magazine/mag99/june99-report3.html (21 Jun. 2002)
- 131 Deng, Z. Y. and Tao, B. Y., 1998, Effect of green tea and black tea on blood glucose, triglycerides and antioxidants in aged rats, *J. Agri. Food Chem*, 46: 3875-8
- 132 Haqqi, T. M., Anthony, D. D., Gupta, S., Ahmad, N., Lee, M. S., Kumar, G. K. and Mukhtar, H., 1999, Prevention of collagen-induced arthritis in mice by a polyphenolic fraction from green tea, *Proc. Natl. Acad. Sci., USA*, 96: 4524-9
- 133 Crafford, L. J., 1997, COX-1 and COX-2 tissue statement: Implications and predictions, *J. Rheum.*, 49: 15-9
- 134 Tijburg, L. B., Mattern, T., Folts, J. D., Weisgerber, U. M. and Katan, M. B., 1997, Tea flavonoids and cardiovascular disease: A review, *Crit Rev Food Sci Nutr*, 37: 771-85
- 135 Tapiero, H., Tew, K. D., Ba, G. N. and Mathe, G., 2002, Polyphenols: Do they play a role in the prevention of human pathologies?, *Biomed. Pharmacother*, 56: 200-7
- 136 Sakanaka, S., Aizawa, M., Kim, M. and Yamamoto, T., 1996, Inhibitory effects of green tea polyphenols on growth and cellular adherence of an oral bacterium, *Porphyromonas gingivalis*, *Biosci. Biotechnol. Biochem.*, 60: 745-9
- 137 Xiao, Y., Liu, T. and Zhan, L., 2000, [The effects of tea polyphenols on the adherence of cariogenic bacterium to the collagen in vitro], *Hua Xi Kou Qiang Yi Xue Za Zhi*, 18: 340-2
- 138 Hara, Y., 1997, Actions of tea polyphenols in oral hygiene, antioxidant- Food suppl., *Hum. Health*, pp. 429-43
- 139 Sakanaka, S., 1997, Green tea polyphenols for prevention of dental caries, in, *Chemistry and Application of Green Tea*, CRC Press, pp. 87-99
- 140 Yu, H., Oho, T. and Xu, L. X., 1995, Effects of several tea components on acid resistance of human tooth enamel, *J. Dent.*, 23: 101-5
- 141 Okubo, T. and Juneja, L. R., 1997, Effects of green tea polyphenols on human intestinal microflora, in, *Chemistry and Application of Green Tea*, CRC Press, pp. 109-21
- 142 Hashimoto, F., Kashiwada, Y., Nonaka, G. I., Nishioka, I., Nohara, T., Cosentino, L. M. and Lee, K. H., 1996, Evaluation of tea polyphenols as anti-HIV agents, *Bioorg. Med. Chem. Lett.*, 6960: 695-700
- 143 Chang, C. W., Hsu, F. L. and Lin, J. Y., 1994, Inhibitory effects of polyphenolic catechins from Chinese green tea on HIV reverse transcriptase activity, *J. Biomed. Sci.*, 1: 163-6
- 144 Baldessarini, R. J. and Greiner, E., 1973, Inhibition of catechol-o-methyl transferase by catechols and polyphenols, *Biochem. Pharmacol.*, 22: 247-56
- 145 Dulloo, A. G., Seydoux, J., Girardier, L., Chantre, P. and Vandermander, J., 2000, Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity, *Int. J. Obes. Relat. Metab. Disord.*, 24: 252-8
- 146 Kwanashie, H. O., Usman, H. and Nkim, S. A., 1989, Screening of 'Kargasok tea': anorexia and obesity, *Biochem. Soc. Trans.*, 17: 1132-3
- 147 Shetty, M., Subbannayya, K. and Shivananda, P. G., 1994, Antibacterial activity of tea (*Camellia sinensis*) and coffee (*Coffea arabica*) with special reference to *Salmonella typhimurium*, *J. Commun. Dis.*, 26: 147-50
- 148 Chou, C. C., Lin, L. L. and Chung, K. T., 1999, Antimicrobial activity of tea as affected by the degree of fermentation and manufacturing season, *Int. J. Food Microbiol.*, 48: 125-30
- 149 Hamilton-Miller, J. M., 1995, Antimicrobial properties of tea (*Camellia sinensis* L.), *Antimicrob Agents Chemother.*, 39: 2375-7
- 150 Anonymous, 2002, Indians drink tea the most, *The Tribune*
- 151 Anonymous, 2000, Tea Digest 2000, Tea Board of India, Kolkata, India
- 152 Suchanti, R., 2001, Tea Market Annual Report and Statistics 2001, J. Thomas & Company Private Limited, Kolkata, India

APPENDIX 1

BUFFERS USED IN STABILITY STUDIES OF EGCG

Hydrochloric acid buffer pH 1.2	Add 85 ml of 0.2 M hydrochloric acid to 50 ml of 0.2 M potassium chloride solution. Make the volume up to 200 ml with water.
Hydrochloric acid buffer pH 2.0	Add 13 ml of 0.2 M hydrochloric acid to 50 ml of 0.2 M potassium chloride solution. Make the volume up to 200 ml with water.
Phosphate buffer pH 4.0	Dissolve 6.8 g of potassium dihydrogen orthophosphate in 700 ml of water. Adjust the pH with 10% v/v orthophosphoric acid. Add water to make 1,000 ml.
Phosphate buffer pH 6.0	Dissolve 5.6 ml of 0.2 M NaOH solution to 50 ml monobasic potassium phosphate. Add water to make 200 ml.
Phosphate buffer pH 7.0	Add 29.1 ml of 0.2 M NaOH solution to 50 ml monobasic potassium phosphate. Add water to make 200 ml.
Phosphate buffer pH 8.0	Add 46.1 ml of 0.2 M NaOH solution to 50 ml monobasic potassium phosphate. Add water to make 200 ml.

APPENDIX 2

PARTICLE SIZE DESCRIPTION OF POWDER USED IN EXTRACTION

Coarse powder	All passes through 2,000 μ sieve and not more than 40% passes through 350 μ sieve.
Moderately coarse powder	All passes through 710 μ sieve and not more than 40% passes through 250 μ sieve.
Moderately fine powder	All passes through 355 μ sieve and not more than 40% passes through 180 μ sieve.
Fine powder	All passes through 180 μ sieve.

APPENDIX 3

MONTHLY SALES OF ORTHODOX, CTC, ALL LEAF AND ALL DUST TEA AT AUCTION MARKETS IN THE NORTHEAST (KOLKATA, GUWAHATI AND SILIGURI)¹⁵²

Monthly sales of orthodox tea at Kolkata auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	3,232	91.48	1,672	67.63	1,314	84.50	2,328	66.05
February	2,296	89.02	1,571	65.02	765	78.30	2,379	58.23
March	822	76.94	789	64.74	358	83.10	851	57.33
April	592	93.39	372	62.28	178	101.32	244	75.64
May	1,526	80.23	486	96.84	1,246	99.65	719	83.46
June	2,468	85.16	373	106.74	1,192	99.41	874	108.59
July	2,779	95.12	602	133.74	2,227	121.77	1,845	104.18
August	3,110	96.37	1,086	116.79	2,110	111.98	1,905	95.79
September	1,692	84.81	764	115.89	2,423	104.55	2,224	91.02
October	1,315	80.08	871	112.48	2,065	100.37	2,053	77.14
November	2,484	80.24	1,644	102.90	2,148	87.11	1,719	70.88
December	2,153	72.98	1,439	89.88	2,304	77.24	2,136	68.12

Monthly sales of CTC tea at Kolkata auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	2,970	101.41	5,116	74.46	5,588	77.74	5,684	88.80
February	1,668	95.29	5,134	72.01	5,666	64.27	4,588	82.13
March	1,511	83.95	3,401	63.63	3,480	57.79	3,998	63.51
April	1,502	90.06	832	80.58	703	60.61	1,070	72.69
May	2,585	83.47	1,857	99.74	2,544	78.26	2,846	79.54
June	3,185	87.47	3,834	91.20	2,966	77.23	2,350	84.75
July	3,025	80.92	4,413	88.73	4,978	82.13	4,228	80.33
August	4,140	80.41	6,311	83.90	4,503	75.33	3,887	70.90
September	2,722	81.51	3,798	88.08	4,544	74.83	4,154	66.51
October	2,007	81.20	4,134	93.34	5,064	74.58	4,861	59.85
November	4,915	79.13	6,095	91.01	5,874	67.40	4,529	64.56
December	4,562	73.97	5,356	81.09	6,237	70.82	5,440	70.73

Monthly sales of all leaf tea at Kolkata auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	6,551	98.54	7,261	73.73	7,313	80.93	8,400	84.26
February	4,136	92.86	7,011	70.75	6,648	66.54	7,143	74.60
March	2,400	83.53	4,358	65.06	3,990	60.72	4,957	64.11
April	2,284	111.62	1,310	98.39	957	95.21	1,497	96.85
May	4,619	95.57	2,567	117.39	4,169	99.16	3,904	91.28
June	6,201	93.67	4,613	106.41	4,590	92.85	3,532	98.29
July	6,415	99.34	5,589	110.30	7,933	106.77	6,818	99.27
August	7,939	92.10	8,095	95.49	7,187	92.66	6,338	85.14
September	4,798	87.46	5,048	98.39	7,614	88.63	6,833	77.57
October	3,714	85.88	5,535	101.69	7,678	84.28	7,331	67.09
November	8,012	82.45	8,358	96.96	8,555	75.85	6,635	68.12
December	7,254	75.76	7,401	87.63	9,040	75.61	8,175	72.24

Monthly sales of all dust tea at Kolkata auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	1,561	97.19	2,314	65.35	2,522	74.09	3,081	86.47
February	1,457	90.67	2,091	58.20	2,347	59.63	2,368	74.67
March	775	75.00	1,753	51.26	2,056	50.28	2,346	50.21
April	322	86.06	231	63.47	324	50.27	486	47.26
May	938	78.62	536	100.61	658	83.93	855	77.89
June	1,270	82.97	1,319	93.51	1,039	76.80	1,049	86.16
July	1,524	76.86	1,817	91.93	1,985	82.56	2,183	77.45
August	2,441	73.52	2,974	83.99	2,024	72.22	1,764	66.77
September	1,728	76.40	1,969	89.99	2,550	75.63	1,839	62.40
October	767	77.67	2,006	92.58	1,751	73.98	2,116	56.84
November	3,157	72.94	2,767	88.45	2,300	70.86	2,147	55.50
December	2,118	67.73	2,249	79.06	2,883	70.80	2,979	56.22

Monthly sales of CTC leaf tea at Guwahati auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	8,509	98.33	8,292	70.90	8,279	75.73	9,322	87.27
February	6,477	93.87	8,014	68.67	9,882	66.05	4,323	76.60
March	4,114	75.64	7,076	63.41	13,036	55.12	3,960	56.79
April	4,035	82.97	1,070	85.32	1,246	68.07	2,081	79.02
May	7,758	77.50	2,964	99.12	6,767	76.95	6,704	78.16
June	9,240	81.71	8,960	89.59	7,598	76.13	6,262	83.18
July	8,457	76.11	9,711	82.31	9,118	80.39	11,403	75.73
August	8,885	76.06	14,802	80.42	12,787	71.40	9,343	68.40
September	9,131	74.44	9,496	89.17	10,960	69.31	9,947	64.27
October	8,529	76.69	7,499	92.42	9,738	68.07	11,132	59.36
November	8,513	75.63	10,770	88.20	10,674	63.71	10,493	62.34
December	8,729	70.82	9,964	78.68	10,981	69.00	9,398	66.53

Monthly sales of all leaf tea at Guwahati auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	8,597	98.30	8,333	70.94	8,313	75.69	9,405	86.99
February	6,495	93.67	8,057	68.50	9,905	66.04	4,391	75.97
March	4,114	75.74	7,093	63.43	13,063	55.14	3,982	56.70
April	4,092	82.99	1,095	84.14	1,257	67.98	2,091	78.90
May	7,809	77.44	2,973	99.04	6,817	76.96	6,718	78.17
June	9,377	81.55	8,999	89.50	7,632	76.13	6,292	83.13
July	8,545	76.13	9,727	82.32	9,187	80.39	11,478	75.66
August	8,937	76.12	14,859	80.43	12,859	71.48	9,416	68.37
September	9,172	74.46	9,555	89.16	11,035	69.34	10,015	64.27
October	8,679	76.63	7,526	92.48	9,857	69.17	11,179	59.37
November	8,568	75.61	10,825	88.22	10,803	63.72	10,560	62.36
December	8,837	70.64	10,002	78.72	11,069	68.96	9,468	66.47

Monthly sales of all leaf tea at Siliguri auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	5,760	91.86	5,740	70.72	4,926	69.76	5,092	78.01
February	3,142	83.87	5,213	65.73	5,724	59.52	2,246	75.04
March	1,532	72.01	4,893	57.88	6,752	50.57	3,016	59.74
April	2,009	86.79	2,286	79.76	891	71.08	1,866	71.78
May	4,952	77.94	2,670	86.96	2,433	70.36	4,705	72.96
June	3,593	82.07	5,182	82.19	6,061	70.64	3,218	77.73
July	6,300	74.54	7,968	75.95	4,650	68.58	5,549	69.43
August	5,741	72.78	7,094	71.29	7,567	62.83	7,354	63.73
September	6,007	72.23	10,228	75.77	6,937	60.22	6,234	56.94
October	6,179	72.89	5,928	82.21	3,554	61.41	5,909	54.63
November	6,601	70.77	6,634	78.15	6,647	55.90	7,983	59.93
December	6,376	69.75	7,794	71.26	8,835	59.57	5,277	60.86

Monthly sales of all dust tea at Siliguri auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	1,500	88.02	1,110	56.99	846	66.91	1,260	66.11
February	827	78.31	1,273	48.22	955	55.24	625	64.69
March	421	67.78	1,413	43.3	1,612	39.82	718	49.53
April	238	82.50	376	60.87	150	49.42	350	57.94
May	1,000	70.80	401	80.94	375	64.82	812	66.38
June	888	70.22	894	76.07	1,215	64.08	723	71.91
July	2,025	61.23	1,815	70.47	1,168	60.85	1,232	62.33
August	1,746	60.95	1,281	64.59	1,873	52.23	2,017	53.70
September	1,570	63.95	2,446	66.61	1,626	47.62	1,514	49.94
October	1,365	65.75	1,475	72.98	833	49.30	1,198	45.65
November	1,393	63.38	1,304	72.52	1,576	45.49	1,835	49.19
December	1,191	61.83	1,375	67.53	1,630	51.39	1,058	53.30

MONTHLY SALES OF ALL TEA (BLACK AND GREEN) AT AUCTION MARKET IN THE NORTH (AMRITSAR)¹⁵²

Monthly sales of all tea at Amritsar auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	45	32.36	21	36.18	38	40.26	14	24.53
February	54	29.54	10	32.44	11	34.68	14	26.49
March	46	17.52	30	23.41	8	23.64	7	26.56
April	30	89.25	4	111.29	9	111.05	3	82.34
May	89	65.09	22	69.74	79	71.39	38	64.62
June	101	60.41	34	57.43	87	50.38	31	59.39
July	66	60.10	51	66.64	47	58.94	33	53.28
August	52	56.81	31	64.85	27	52.69	45	44.89
September	77	58.19	90	47.84	28	45.41	32	50.25
October	64	54.11	65	49.34	27	44.69	36	39.18
November	36	46.33	78	44.84	21	25.66	38	25.38
December	58	40.26	36	37.05	23	24.02	28	19.76

Total sales of different types of tea at Amritsar auctions

Year	Quantity (tonnes)			Average price (INR/kg)		
	Black tea	Green tea	Total	Black tea	Green tea	Total
1997	23	721	744	18.24	42.65	60.89
1998	55	662	717	18.42	54.74	73.16
1999	10	546	556	47.31	12.48	59.79

Monthly sales of different types of tea at Amritsar auctions

BLACK TEA						
Months	Quantity (tonnes)			Average price (INR/kg)		
	1997	1998	1999	1997	1998	1999
January	9	18	2.70	23.91	24.88	36.64
February	8	-	-	12.60	-	31.06
March	5	32	5.70	12.01	13.76	25.64
April	-	-	-	-	-	111.29
May	-	-	-	-	-	69.74
June	-	-	-	-	-	57.42
July	1	-	-	-	-	66.64
August	-	3	-	28.02	27.95	64.89
September	0	-	-	37.04	-	38.80
October	-	1	-	-	23.36	49.20
November	-	-	2.00	-	-	44.78
December	-	1	-	-	17.62	42.98
GREEN TEA						
January	44	27	36.20	22.10	37.36	12.50
February	17	54	15.10	29.65	29.58	-
March	24	14	24.90	25.25	26.32	12.35
April	11	30	3.40	73.87	89.24	-
May	62	89	22.10	59.01	65.09	-
June	79	102	34.00	45.98	60.42	-
July	80	65	51.50	39.21	60.10	-
August	49	49	31.10	41.31	58.64	-
September	60	77	143.20	41.32	58.18	-
October	137	63	65.40	42.43	54.35	-
November	83	36	100.50	40.88	46.41	12.48
December	75	56	18.50	49.62	40.64	-

MONTHLY SALES OF ORTHODOX, CTC, ALL LEAF AND ALL DUST TEA AT AUCTION MARKETS IN THE SOUTH (COCHIN, COONNOOR AND COIMBTORE)¹⁵²

Monthly sales of CTC leaf tea at Cochin auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	1,376	82.42	651	58.64	542	43.99	595	45.25
February	927	83.26	837	59.17	642	41.48	473	49.11
March	837	82.46	716	55.03	402	40.9	398	49.21
April	864	69.1	664	51.58	329	38.11	365	44.16
May	602	56.89	539	51.46	653	36.36	676	38.92
June	660	55.76	550	47.59	462	37.11	537	37.37
July	946	55.03	588	49.14	417	37.48	350	38.98
August	667	64.2	607	53.8	640	38.3	301	40.16
September	884	52.62	562	56.88	341	38.62	315	37.66
October	647	53.55	549	50.28	582	36.98	362	35.2
November	571	63.1	857	54.46	500	33.56	264	36.65
December	626	60.94	671	45.79	458	34.3	209	38.2

Monthly sales of orthodox leaf tea at Cochin auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	1,535	87.89	1,062	69.57	1,285	61.54	1,922	53.49
February	1,135	92.2	1,094	71.38	1,110	69.37	1,229	56.87
March	1,062	93.62	1,043	67.79	1,411	63.55	1,101	58.79
April	1,082	87.49	1,002	67.81	1,909	54.6	1,055	57.45
May	746	80.54	911	63.63	1,997	54.44	1,303	55.35
June	1,197	80.17	1,364	57.08	1,490	54.23	1,835	49.79
July	1,640	81.23	1,058	65.99	1,407	56.74	856	50.52
August	1,093	84.35	1,083	76.3	1,533	58.22	530	59.65
September	1,271	71.15	1,278	78.33	813	61.07	975	62.98
October	1,342	62.05	1,546	72.47	2,040	60.37	1,068	59.66
November	825	71.51	1,798	67.81	1,411	51.81	1,194	55.49
December	1,172	72.51	1,616	58.3	1,258	51.30	867	60.03

Monthly sales of all leaf tea at Cochin auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	2,912	85.3	1,712	65.42	1,827	56.34	2,517	51.54
February	2,061	88.2	1,932	66.09	1,752	59.15	1,702	54.71
March	1,899	88.71	1,760	62.6	1,814	58.52	1,499	56.25
April	1,946	79.32	1,665	61.34	2,238	52.18	1,420	54.04
May	1,348	69.93	1,450	59.06	2,649	49.99	1,979	49.74
June	2,455	71.72	1,914	54.39	1,952	50.17	2,372	46.98
July	1,989	71.46	1,646	59.97	1,824	52.34	1,206	47.17
August	1,759	76.7	1,690	68.23	2,173	52.35	831	52.58
September	2,155	63.54	1,840	71.74	1,154	54.44	1,290	56.79
October	1,989	59.23	2,095	66.66	2,621	55.18	1,430	53.47
November	1,396	68.12	2,655	63.53	1,912	47.03	1,458	52.08
December	1,798	68.46	2,287	54.63	1,717	46.76	1,076	55.8

Monthly sales of CTC leaf tea at Coonoor auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	6,445	82.37	5,381	61.89	4,705	45.47	2,945	46.21
February	4,276	84.35	4,015	62.45	2,785	44.07	3,284	51.73
March	3,919	82.82	5,321	56.24	4,644	42.18	4,740	52.08
April	4,597	70.14	4,969	52.75	5,021	36.68	3,996	47.63
May	3,140	57.32	4,909	51.96	3,835	38.44	4,611	42.23
June	4,063	59.49	5,759	47.97	6,749	37.55	8,571	39.41
July	5,514	58.84	8,108	51.44	5,132	37.66	5,772	40.21
August	4,664	61.69	5,957	55.1	4,377	40.48	5,244	40.64
September	4,983	53.64	6,763	57.77	3,969	38.91	4,321	38.91
October	5,784	53.92	6,613	53.64	5,443	38.83	5,731	34.25
November	4,575	50.46	3,667	52.29	6,165	33.26	4,974	36.49
December	4,424	62.39	6,904	44.35	4,362	35.95	3,162	37.3

Monthly sales of orthodox leaf tea at Coonoor auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	200	81.07	130	60.78	134	56.35	114	46.39
February	109	84.77	62	64.12	79	63.44	82	45.69
March	188	79.18	140	60.15	169	55.11	146	49.84
April	276	69.97	164	56.97	225	45.89	102	46.00
May	128	65.83	158	55.58	102	50.69	122	47.31
June	167	67.28	137	49.63	180	48.65	263	44.22
July	192	69.22	182	57.65	135	48.08	128	44.01
August	118	76.77	118	66.16	148	46.81	104	46.83
September	173	61.81	147	70.95	134	52.47	98	52.13
October	291	55.40	156	63.85	162	47.28	125	50.49
November	163	58.55	109	59.23	240	43.79	220	47.95
December	105	59.19	217	51.97	174	40.12	159	50.58

Monthly sales of CTC leaf tea at Coimbatore auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	1,193	83.06	899	61.37	1,026	45.49	785	44.86
February	318	85.67	703	61.71	1,146	42.87	692	50.35
March	634	82.66	628	56.72	1,498	41.17	1,155	50.23
April	530	70.81	722	53.14	1,055	35.84	845	46.43
May	423	57.05	416	50.27	1,143	37.02	1,069	39.60
June	261	56.62	373	47.12	1,822	36.06	1,449	37.30
July	634	58.15	638	51.19	1,514	36.89	1,173	39.33
August	493	61.67	549	54.57	1,479	38.66	1,262	38.40
September	645	53.90	816	57.36	1,078	38.10	930	37.67
October	895	52.79	1,336	52.72	1,262	38.68	1,110	33.53
November	588	64.65	1,558	52.33	1,494	32.94	897	35.80
December	835	61.64	928	46.13	1,317	34.29	611	36.00

Monthly sales of orthodox leaf tea at Coimbatore auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	683	85.68	384	64.20	389	61.73	212	50.42
February	228	89.64	173	64.91	494	68.69	352	52.47
March	441	87.56	353	60.42	736	61.52	480	57.13
April	250	81.58	325	59.87	526	51.89	304	54.47
May	222	76.51	305	59.25	587	54.14	283	55.29
June	325	75.16	215	55.23	704	52.82	771	49.85
July	391	77.95	197	64.52	612	55.88	407	50.74
August	214	81.08	165	74.98	430	58.04	238	57.75
September	340	64.48	506	76.99	519	58.34	199	63.62
October	400	56.76	472	72.72	620	57.28	235	58.70
November	238	66.98	854	65.91	614	49.34	304	52.86
December	306	67.08	606	58.49	447	46.18	238	53.72

Monthly sales of all dust tea at Coimbatore auctions

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	595	81.38	597	61.76	799	50.93	622	52.09
February	276	85.32	377	58.22	697	50.18	605	54.96
March	416	78.58	350	58.88	1,039	44.72	902	48.16
April	412	74.52	465	57.88	615	40.75	529	46.00
May	439	68.71	459	55.13	662	43.74	502	47.00
June	497	57.54	514	51.22	1,131	41.23	971	42.25
July	676	55.06	590	53.21	1,075	39.47	706	46.11
August	349	65.36	417	53.96	1,030	42.58	763	49.47
September	487	61.01	386	62.35	918	42.81	764	49.27
October	676	59.39	659	56.20	877	41.71	932	44.21
November	432	63.17	666	59.17	1,086	39.87	1,231	44.60
December	504	63.19	661	54.21	833	45.71	590	44.59

Monthly sales of North Indian tea at auctions (all tea)

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	28,084	96.58	28,494	69.34	27,429	75.39	31,820	83.33
February	19,432	90.09	27,891	64.68	29,382	63.49	19,034	73.85
March	11,496	76.14	23,543	58.91	34,201	52.25	17,908	56.57
April	10,023	90.29	5,961	80.78	4,219	70.45	7,176	75.61
May	22,915	80.05	10,149	99.53	16,559	81.47	19,221	78.49
June	25,908	83.29	24,468	91.11	23,636	77.53	17,325	85.19
July	29,776	78.95	31,140	86.38	28,948	84.87	32,121	78.77
August	32,203	77.49	41,462	81.65	37,032	72.54	31,617	68.97
September	28,771	75.54	33,923	84.91	34,621	71.10	30,982	64.45
October	25,406	76.53	26,273	91.06	28,125	72.61	32,225	58.73
November	32,189	75.52	34,812	87.90	34,299	65.48	34,037	60.54
December	29,478	70.85	33,393	78.08	38,331	67.66	31,214	64.36

Monthly sales of South Indian tea at auctions (all tea)

Months	1998		1999		2000		2001	
	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)	Tonnes	Price (INR)
January	16,397	83.71	12,321	63.26	13,147	51.26	12,040	51.31
February	10,883	85.55	10,559	63.13	11,714	52.07	10,879	53.69
March	10,952	83.06	12,235	58.61	14,527	47.74	13,193	52.54
April	11,671	75.07	11,753	57.00	14,238	42.70	11,003	49.62
May	9,109	63.81	11,508	54.92	14,199	44.58	13,528	46.13
June	12,952	61.66	13,841	51.39	17,602	41.53	20,536	41.92
July	13,681	60.54	15,581	53.77	14,299	42.25	13,775	43.61
August	10,701	66.18	12,472	57.99	14,602	45.43	12,753	45.39
September	12,375	59.29	13,829	61.77	10,733	45.40	11,789	46.35
October	14,518	57.41	15,363	58.09	16,443	42.00	15,653	41.27
November	11,344	64.99	13,243	58.78	16,599	40.90	14,007	41.81
December	12,097	64.45	16,474	50.15	12,270	43.35	9,512	43.87

APPENDIX 4

SURVEY QUESTIONNAIRE

- 1) **Name of the company/firm**
- 2) **Tea brands marketed:**
 - Green Tea
 - Black Tea
- 3) **Total tea production:**
 - Type of Tea (green/black)
 - Production (tonnes)
 - Year
- 4) **Tea varieties cultivated:**
 - Variety/clone
 - Area under cultivation (ha)
- 5) **Total area (ha) under tea cultivation**
- 6) **Tea consumption in the market:**
 - National
 - International
 - Countries to which tea is exported
- 7) **Production processes for green tea**
- 8) **Problems related to green tea**
- 9) **Additional information, if any**
- 10) **Contact address**

APPENDIX 5

- 1. Name of the firm:** Makaibari Tea Estates.

Tea brands marketed: Makaibari tea (for both green and black tea).

Total tea production during (2001): 110 tonnes of which 40% is green tea.

Tea varieties cultivated: Chinese type, Assam type and their clones.

Area under cultivation (ha): 570.12 ha

Tea consumption in the market (national and international): 80% of the total green tea production is exported to Japan, USA, UK and Germany; only 20% is consumed on the domestic market. Steaming is the basic step in green tea manufacturing.

Production processes for green tea: Green tea is produced against order; the main importer is Japan.

Problems related to green tea production, plucking, packing and marketing: Major green tea grades produced by the company are: Tips Golden Flowery Orange Pekoe (TGFP), Golden Flowery Orange Pekoe (GFOP), Broken Orange Pekoe (BOP), Orange Fanning (OF), and dust; TGFP is the most expensive grade while dust is the cheapest.

Additional information: Mr. P. N. Banerjee

Contact address: Director
Makaibari Tea Estates
Flat No. 7, 184, Lenin Sarani
Kolkata 700013
- 2. Name of the firm:** Sannyasithan Tea Co. Pvt. Ltd.

Tea brands marketed: Hind tea.

Total tea production during (2001): 300 tonnes per year of green tea.

Tea varieties cultivated: Chinese seed, clones developed by Tocklai Research Association.

Area under cultivation (ha): 125 ha

Tea consumption in the market (national and international):

Production processes for green tea:

Problems related to green tea production, plucking, packing and marketing:

Additional information:

Contact address:

Almost all green tea produced by the company is auctioned at Amritsar market; Kashmir is the main domestic market for the product; small quantities are also exported to Europe.
Steaming is the basic step in green tea manufacturing.

Lack of awareness of the health benefits among the consumers, organized marketing, auction centres and market statistics for green tea; manufacturing is costly; sale price is lower than manufacturing price.

Mr. P. Bhartia

C. F. A.

Sannyasithan Tea Co. Pvt. Ltd.

B- $\frac{1}{2}$, Gillanders House, 1st Floor

8, Netaji Subhas Road

Kolkata 700001

3. Name of the firm:

Tea brands marketed:

Total tea production during (2001):

Tea varieties cultivated:

Area under cultivation (ha):

Tea consumption in the market (national and international):

Production processes for green tea:

Problems related to green tea production, plucking, packing and marketing:

Maud Tea & Seed Co. Ltd. (also known as Biotea Estates Ltd.).

Directly sell tea in bulk from the gardens.

2,500 tonnes, including 30 tonnes of green tea.

China variety in Darjeeling gardens and Assam variety in Assam gardens.
1,700 ha

80% of the production is exported: Germany (50%), USA (20-30%), UK, Japan and Australia.

Steaming is the basic step in green tea manufacturing.

Green tea is produced against order; production is costly; crop losses are high; green tea is not advertised properly; people are not used to the taste of green tea.

Additional information:
Contact address:

Green tea is made from leaves plucked just before the "Banji period".
Mr. S. Lohia and Y. Lohia
Maud Tea & Seed Co. Ltd.
1 & 2, Old Court House Corner
Kolkata 700001

4. Name of the firm:
Tea brands marketed:
Total tea production during (2001):
Tea varieties cultivated:

Tea Promoters (India) Pvt. Ltd.
Bulk green tea.
100 tonnes, which includes 55 tonnes of green tea.
China type in Darjeeling gardens and Assam type and its hybrids in Assam gardens.
900 ha

Area under cultivation (ha):
Tea consumption in the market (national and international):

All tea production is exported to Europe, mainly Germany, Italy, Switzerland and UK.

Production processes for green tea:
Problems related to green tea production, plucking, packing and marketing:
Additional information:

Steaming or roasting are the basic steps used in green tea manufacturing.

No market for green tea.

Started green tea production in 1988; since then production has increased significantly; almost all (99.9%) green tea production is exported to Europe and the rest is auctioned at Amritsar market; selling price is INR 300 per kg for leaf grade and INR 150 per kg for Fannings; green tea is mainly produced in July; the company also produces organic tea made from plants that are cultivated without fertilizers.

Contact address:

Mr. Binod. K. Mohan
Director
Tea Promoters (India) Pvt. Ltd.
17, Chowringhee Mansions
30, Jawaharlal Nehru Road
Kolkata 700016

- 5.** Name of the firm:
 Tea brands marketed:
 Total tea production during (2001):
 Tea varieties cultivated:
 Area under cultivation (ha):
 Tea consumption in the market (national and international):
 Production processes for green tea:
 Problems related to green tea production, plucking, packing and marketing:
 Additional information:
 Contact address:
- 6.** Name of the firm:
 Tea brands marketed:
 Total tea production during (2001):
 Tea varieties cultivated:
 Area under cultivation (ha):

Ambari Tea Co. Ltd.
 Cooch Behar green tea, Long view/Snow view green tea.
 380 tonnes (300 tonnes green tea and 80 tonnes black tea) from Cooch Behar Tea Estates, 710 tonnes (300 tonnes green tea and 410 tonnes black tea) from Longview Tea Estates.
 Assam type and its hybrids developed by Tocklai Research Association. 700 ha (both the gardens)

90% of total production (green and black tea) is consumed locally and the rest is exported to Germany and Japan.
 Steaming is the basic step used in green tea manufacturing.

Costly production processes, sorting of green tea is slow and expensive; high stocks of green tea are held due to lack of market; from a given quantity of tea leaves 45% of black tea can be made; however, only 21% of green tea is produced resulting in considerable crop losses due to loss of water; steaming and firing are also costly.
 Green tea is sold at INR 80 per kg.
 Mr. S. Agarwal
 Director
 Ambari Tea Co. Ltd.
 5/2, Garstian Place
 Kolkata 700001

Sublime Agro. Ltd.
 Bulk producer of tea.
 250 tonnes green tea and 100 tonnes black tea.
 Assam type, China type, and clones (TV1-30, Tenali-17).
 300 ha

Tea consumption in the market (national and international):
 Production processes for green tea:
 Problems related to green tea production, plucking, packing and marketing:
 Additional information:
 Contact address:

Exported directly to Germany (about 3 tonnes of green tea), USA, Japan.
 Steaming.

-
 -
 Mr. Raja Ram Upadhyay
 Supervisor
 Dagapur Tea Estate
 Siliguri

7. Name of the firm:
 Tea brands marketed:
 Total tea production during (2001):
 Tea varieties cultivated:
 Area under cultivation (ha):
 Tea consumption in the market (national and international):
 Production processes for green tea:
 Problems related to green tea production, plucking, packing and marketing:
 Additional information:

Sepoydhoorah Tea Co. Pvt. Ltd.
 Chamling for both green and black tea.
 100 tonnes and small quantity of green tea.
 Assam type (14%), China type (32.3%), hybrids (53%) and clones (TV-9, TV-29).

-
 Auctioned on Amritsar market.
 Steaming.

Low market demand.
 Started green tea production in 1998 but it has been abandoned in the last three years.

8. Name of the firm:
 Tea brands marketed:
 Total tea production during (2001):
 Tea varieties cultivated:

Goodricke Group Ltd.
 Goodricke tea.
 27,830 tonnes.

Area under cultivation (ha):
 Tea consumption in the market (national and international):
 Production processes for green tea:
 Problems related to green tea production, plucking, packing and marketing:
 Additional information:
 Contact address:

-
 Green tea is produced against order.
 Steaming, panning, roasting.
 Limited market for green tea.

-
 Mr. A. N. Singh
 14, Gurusadaya Road
 Kolkata 700001

9. Name of the firm:
 Tea brands marketed:
 Total tea production during (2001):
 Tea varieties cultivated:
 Area under cultivation (ha):
 Tea consumption in the market (national and international):
 Production processes for green tea:
 Problems related to green tea production, plucking, packing and marketing:
 Additional information:
 Contact address:

Duncans Industries Ltd.
 Duncans tea, Sargam, Shakti, etc.
 17,000 tonnes.
 TV-1, TV-20, TV-27, TV-19.
 7,000 ha

Domestic market.

-
 Do not produce green tea.

-
 Mr. M. Gurbaxani
 31, Netaji Subash Road
 Kolkata 700001

10. **Name of the firm:** The Peria Karamalai Tea & Produce Co. Ltd.
Tea brands marketed: CTC.
Total tea production during (2001): 4,500 tonnes per year.
Tea varieties cultivated: Assam light leaf, Vetjan- khorjian, UPASI-clones, Akamalai clones.
Area under cultivation (ha): Approx. 1600 ha in Anmalai hills.
Tea consumption in the market (national and international): Local consumption and export to Russia.
Production processes for green tea: Do not produce green tea.
Problems related to green tea production, plucking, packing and marketing: Crop loss and marketing problems due to fine plucking.
Additional information: -
Contact address: Mr. Prakash Prashar
Vice President
Cowcoody Chambers
234-A, Race Course Road
Coimbatore 641018
11. **Name of the firm:** United Planter's Association of Southern India (UPASI).
Tea brands marketed: -
Total tea production during (2001): -
Tea varieties cultivated: 32 cultivars have been recommended for cultivation in South India. UPASI-02, 03, 08, 09, 10; TRI- 2024, 2025, 2026; CR-6017 and Sri Lankan clones.
Area under cultivation (ha): -
Tea consumption in the market (national and international): -
Production processes for green tea: -
Problems related to green tea production, plucking, packing and marketing: Lack of market and technical know-how for green tea production.

Additional information:
Contact address:

Mr. J. D. Hudson
Assistant Director
UPASI Tea Research Foundation
Regional Centre
Glenview
Coonoor 643101

12. Name of the firm:
Tea brands marketed:
Total tea production during (2001):
Tea varieties cultivated:
Area under cultivation (ha):
Tea consumption in the market (national and international):
Production processes for green tea:
Problems related to green tea production, plucking, packing and marketing:
Additional information:
Contact address:

The IndcoServe.
CTC and Orthodox.
Data provided and included in Table 22.
UPASI varieties.

Most of green tea production is exported to Russia.
Roasting and steaming; Roasting is preferred over steaming.
Lack of basic knowledge of green tea production technology.
Different grades of tea have been provided for analysis.
Mr. K. Swaminaathan
General Manager
The Indcoserve
Church Road
Coonoor 643101

13. Name of the firm:
Tea brands marketed:
Total tea production during (2001):
Tea varieties cultivated:

Mahalinga Indco Tea.
CTC and Orthodox.
700 tonnes.
China bush and VP-clones.

Area under cultivation (ha):
Tea consumption in the market (national and international):
Production processes for green tea:
Problems related to green tea production, plucking, packing and marketing:
Additional information:
Contact address:

1.22 ha
Produces green tea by order.
Steaming.
Lack of market.
-
Mr. Pitchai Pillai
Special Officer
Mahalinga Ind. Co. Tea
Coonoor 643101