Chapter -I

INTRODUCTION

1. INTRODUCTION

Tea is the most popular nonalcoholic beverages in the world. Near about two-third of the world population uses tea as a beverage. Tea *'Camellia sinensis,'* is believed to have originated from South East Asia. However, the center of origin is not clearly known. It may be the Tibetan Plateau including Sze-Chuan, Yu-nan, Sain, North East India or China where tea could be originated. Chinese people were the pioneers in using tea for medicinal purposes. By the end of the sixth century, the Chinese began to regard tea as a beverage. Thea or tea [*Camellia sinensis* (L.) O. Kuntze] family: Theaceae), is an acid loving long shrub or small tree with alternate evergreen leaves, indigenously grown in Eastern Asia. But, in 1823 Major R. Bruce discovered tea plants from North East India. Tea has become one of the powerful commodities of commercial value during the colonial period. At that time, only luxurious and rich people could afford tea as a beverage. Tea has now become the world's cheapest and most widely used drink other than water. It has truly become a beverage of international fellowship, a bond that brings people together. Economically speaking too, tea is an extremely valuable source of much needed foreign exchange. Tea plantation industry is a combination of industry and agriculture (plantation is a large estate on which crops such as tea. coffee, rubber etc. are grown). Production of the leaf is an agricultural activity while its processing is an industrial activity. Most of the large estates process raw leaf in their own factories. The tea industry is of considerable importance in the national economy of India in terms of income generation, earning foreign exchange, employment generation-end contribution to the national exchequer. Now it is extensively cultivated in the sub-tropical belt slops of hills, plateaus at an altitude varying from 10 meters to 2400 meter above sea level. In India, there are three distinctly different tea growing regions. These regions are geographically separated, thereby producing three entirely different teas both in style and in taste/flavour. The three regions of tea production areas are Darjeeling (North-Eastern India), Assam (far North-East India) and Nilgiri (South India). The cool and moist climate, the soil, the rainfall and the sloping terrain all combine to give Darjeeling its unique "Muscatel" flavour and exquisite bouquet. The combination of natural factors that gives Darjeeling tea its unique destination and is not found anywhere else in the world, hence this finest and most delicately flavored of all teas has over the years acquired such reputation as "Champagne enjoys amongst wine".

Assam rich in nature's bounty and a rainfall ranging from 100 to 150 inches per year ensuring a very special place for the teas grown here. These teas are referred to simply as "Assam" and offer rich, full-bodied, bright tea liquor. For those who favour a bright, strong cup of tea, Assam is "your cup of tea". Assam is the single largest contiguous tea growing area in the world. The Blue Mountains or the Nilgiris are situated in South India. They are a picturesque range of undulating hilly landscapes where tea is grown at elevations ranging from 1,000 meters to 2,500 meters. Rainfall varies from 60 inches to 90 inches annually. These conditions favour the fine, elegant flavour and brisk liquor of Nilgiri teas. The combination of fragrance and briskness makes Nilgiri a truly unique tea, the like of which can be found nowhere else in the world.

Kangra tea is grown in the foothills of Himalayas in Himachal Pradesh, cultivated first in the Kangra valley of the region. Kangra Tea has floral aroma and great taste. These Kangra teas are famous for their wide varieties in taste, color and flavour. The generic name of tea '*Thea*' is from the Greek meaning goddess; '*Sinensis*' refers to its Chinese origin, although the origin of tea is still cloaked in mystery. The main medicinal property ascribed to this plant is antioxidative, reported in the many scientific literatures of the world as typical remedies by a decoction of its prepared leaves and bud (Mabe *et al.,* 1999; Hitchen *et al., 2004;* Pajohk *et al.,* 2006). In therapeutics, various kinds of tea matrices obtained from different grades/variety of tea - are most often employed in prophylactic purposes (Chakraborty, 1995) and in the formulation of plant derived elixir to protect human diseases (Misra *et al.,* 2003; Carol *et al.,* 2004). Scientists and medical practitioners generated maximum data for claiming the health benefits of tea consumption and many scientific experimental evidences support their claim.

Antioxidants are substances that reduce damage due to oxygen, such as that caused by free radicals. Tea is well-known antioxidants which are capable of counteracting the damaging effects of oxidation. Mandal *et al*. (2010) revealed that there has been increasing interest in finding plants with high antioxidant capacities since they can be responsible for the progression of many chronic diseases. Serafini *et al*. (1996) analyzed young tea shoots containing more than 35% of their dry weight in polyphenols. Non-fermented green tea contains predominantly flavonols, flavan-diols and phenolic acids like gallic acid, coumaric acid or caffeic acid, with those in green

tea is higher than those in black tea. Misra *et al*. (2008) reported that phenolic compounds that are present in young tea shoots are known to be one of the main factors in determining the quality of the resulting tea drink. The bioactive food component refers to nonessential bio-molecules which are present in tea, exhibit the capacity to modulate several metabolic processes that result in the promotion of better health. In the present day, it is very essential to assess the antioxidant activity of different grades and types of tea and also during cultivation to processed; because an important factor controlling bioactive content in plant-based foods is post-harvest processing, particularly drying. It is also required to judge the optimal concentration, persistence and thermo-stability of antioxidants, nutraceutical fate and bio-availability antioxidants present in tea. There are several diseases in which tea is used generally for therapeutic purposes by the ethnic people in the Tea Garden. Therefore, the people depend on their traditional knowledge for the treatment of their ailments. The ethnic group of inhabitants has gathered this knowledge through trial and error, during their survival in such inhospitable environments for hundreds of years. Unfortunately, this traditional knowledge is masked under the light of modern medication and therapy. Although, recently the importance of plant based traditional medicine is being realized by the masses; people of different parts of the world have started recording and evaluating such herbal knowledge through assessing antioxidants as well as different pharmacological properties (Saha *et al*., 2011). Those who are unable to collect their staple foods or not getting the chance to take lunch every day; at least they consumed a cup of tea per day. Therefore, tea is most popular drink and that is why necessary to evaluate antioxidant activity of tea under various agro-climatic conditions of North Bengal. But unfortunately, through literature survey, it has to be known that the tea of Terai, Dooars and Hills are almost untouched.

In this context, the study was designed for in-depth analysis of antioxidant activities along with the quantity of bioactive phytochemicals of under-explored tea under various agro-climatic conditions of North Bengal especially a few selected TE in Terai, Dooars and Hills of Darjeeling and also the dynamic alteration of bioactive substances during processing.

1.1 ORIGIN AND HISTORY OF TEA

“Tea” is derived from “T”e – Amoy Language and CHA from Cantanese Language. It is native to South East Asia - China as early as 2737 BC, later on China People used

as medicine and became common beverage from fourth and seventh century. Dutch traders were introduced Tea to Europe in 1610 and became a popular drink in England in 1664. Robert Kyd experimented with Tea in Calcutta in 1780.

1.2 DISCOVERY AND GROWTH OF TEA IN INDIA

The event which marked the birth of the Indian tea industry was the discovery by Major Bruce in 1823, of the indigenous tea plants in Assam. Major Robert Bruce made friendship with a Singpho (a tribal community) Chief known as Bessagaum and obtained indigenous plants and seeds in 1823. The exact location was at a place near Sadiya in north east Assam, adjacent to Burma. In the following year, Robert Bruce showed the wild tea plants to his brother Charles Alexander Bruce. Some of these plants were sent down to the Botanical Gardens, Calcutta, where upon close examination they were pronounced to be of the same family, but not the same species from which the Chinese manufactured tea. But this discovery went unrecognized and no official action was taken at that time (Sarkar, 1984). Then in 1832, Lieutenant Charlton of the Assam Light Infantry at Sadiya, found similar tea plants growing in the jungle close to his garrison. He also sent some seeds and leaf samples of this *Camellia* to the Botanical Gardens, Calcutta. East India Company lost its monopoly of tea trade by the year 1833. When Parliament abolished the company's monopoly with China in 1833 they were ready to take some positive actions with regard to replacing that trade in some way. In January of 1834, Lord William Bentinck proposed to the Council of the East India Company, the setting up of a Tea Committee to investigate and make recommendations to the most suitable areas in which to grow tea. The Tea Committee decided to send their secretary G.J. Gordon to China in order to acquire tea seeds as well as tea makers and those familiar with the cultivation of the tea plant. Gordon left Calcutta in June 1834 on the sailing ship 'Water Witch' (Wilson *et al*., 1992). The Tea Committee also decided to prepare suitable sites at chosen places in India where the imported China plant would flourish. This is the idea that, if successful, these experimental 'tea land' could later be handed over to private enterprise for future development. To this effect, the Government secured the services of Charles Bruce, and he was appointed as 'superintendent of tea culture' in 1835. The cultivation of tea in China was centuries old and it was also a large and flourishing industry; its secret had been jealously guarded by the Chinese people. For the Government, it was a bold undertaking to enter into competitions with that country

(Wilson *et al*., 1992). The committee was able to inform the Government, that the so called tea plant found near Sadiya was indigenous and was the true tea, *Camellia* of commerce, only after Gordon left to China for procuring tea seeds. The seeds brought by Gordon in 1835, were sent to the Botanical Gardens, Calcutta for germination. From this original consignment of 80,000 China seeds, the resultant 42,000 young plants were allocated to three main areas: 20,000 seedlings to the hill districts in Kumaon in North India; 2,000 to the hill districts of South India and the remaining 20,000 to Upper Assam on the North East Frontier.

Among the seedlings of China tea planted in different hill districts of North India, only those planted at Ghurwal and Sirmoor were met with any degree of success. Places like Ranchi, Dehra Dun and Kangra Valley never developed the tea plants on a large scale equivalent to the hills around Darjeeling. In South India, nearly all the plants sent to Nilgiri Hills died but those put out at an experimental farm near Ootacamund fared best. Seedlings planted in Wayanad were also successfully established, but tea was commercially planted in this region only after 1853.1t was planted alongside coffee and was only in the late 1890's large acreages of tea were opened in Wayanad (Wilson *et al*., 1992).

Indigenous plants of Assam and the China plants were planted in North - East India on a trial basis. Dr. Wallich, assistant surgeon and botanist, Dr. McClelland, a geologist and Dr. William Griffith, a botanist were deputed by the tea committee for investigating the wild tea plants grew in the Upper Assam in the year 1835. They decided to plant China tea plants next to a plot of Assam indigenous tea plants, which were collected from the surrounding jungle by Charles Bruce, the superintended of tea culture. It was found that the Assam indigenous plants are thrived well than the sick China type. But the China plants were very prolific seed - bears and it caused rapid spreading of the plants (Wilson *et al*., 1992).

1.3 TAXONOMY AND SYSTEMATIC POSITION:

The genus *Camellia* with its 82 species belongs to the family *Theaceae.* All the tea clones in cultivation are botanically called *C. sinensis* (L) O. Kuntze, irrespective of species-specific differences. Tea is a heterogeneous plant with many overlapping morphological, biochemical and physiological attributes. Based on leaf pose and growth habitat, Kitamura and later Sealy identified two intra-specific forms of *C.*

sinensis (L.): the China variety, *Camellia sinensis* var. *sinensis* (L.) and the Assam variety, *Camellia sinensis* var. *assamica* (Masters) Kitamura. The grouping of tea into an erect small-leafed China variety and a horizontal broad-leafed Assam variety was rather subjective. Plants with intermediate leaf characteristics could not always be assigned to either of these two varieties. Relying mostly on characteristics of styles, (Wight, 1962) divided tea plants into *C. sinensis* (L.), *C. assamica* (Masters) and a third southern form of tea or Cambod race, the sub-species *C. assamica* sub species *lasiocalyx* (Singh, 2005).

1.4 MORPHOLOGY OF TEA

Evergreen shrubs or trees, C3 plants, leaves simple, alternate, serrate, flowers bisexual, regular; sepals and petals usually five, stamens many, anthers two celled, ovary superior, 2-4 locular, rarely solitary axile; fruit capsule, seeds recalcitrant (loss viabilities on dry). Chromosomes 2n=30, diploid. Cultivated tea plants are cross pollinated, self incompatible, extent of selfing was varying between 6 – 40%; all these attributes are lowest in Assam type and highest in China type. Breeding depression is very high, leads to loss of vigour and quality. Hence self seeds for raising plants are to be avoided.

1.5 MAINTENANCE OF FOLIAGE, PHOTOSYNTHESIS, BUD DORMANCY AND PRODUCTIVITY

Permanent leaves kept retain on the bush below the plucking table constitute the maintenance foliage. A leaf does not attain full photosynthetic efficiency until it expands to more than half its maximum size. Young shoots grow at the expanses for the photosynthesis by the maintenance foliage. Adequate maintenance foliage is a must for high and sustainable productivity. The maximum life span of a tea leaf on a tipped primary is about ten months which remains on its peak of photosynthesis up to six months after full expansion beyond which aging starts and efficiency declined gradually. Top ten cm layer of the maintenance foliage on 20 cm tipped primary after pruning, giving about 70% of the crops. The optimum temperature for maximum photosynthesis is 25 – 30° C, which was declined fast above 37° C and no net photosynthesis at or above 42° C, is observed. The lowest temperature for photosynthesis is about 10–12° C, temperature above the level requires appropriate shade in North East India for higher productivity. Growing shoots are the strong sinks

and the maintenance foliage is the source. So, ideally, 2 leaves + Bud, and 3 leaves + bud are the best stages of plucking. The first start of bidirectional flow of photosynthate in North East India is mid-October and next bidirectional flow is May. The average flush period is 31 days and growth cycle is 57 days, four numbers of flushes can be obtained per year.

1.6 CONCEPTS OF FREE RADICALS

A free radical is defined as a molecular species which is capable of independent existence and posses an unpaired electron in its outermost atomic orbital. This unpaired electron results in presence of certain common properties that are shared by most of the radicals. These free radicals are highly unstable as well as highly reactive. They have the capability to either donate an electron or accept an electron from other molecules, therefore altering their native properties. The free radicals generated from oxygen are called reactive oxygen species (ROS) and those from nitrogen are termed as reactive nitrogen species (RNS). ROS includes various forms of activated oxygen molecules, such as superoxide ($O^{2\bullet -}$), hydroxyl ($^{\bullet}OH$) and peroxyl ($ROO^{\bullet}$), as well as non-free radicals hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). Likewise, RNS includes nitric oxide ($NO^{\bullet}$) and nitrogen dioxide ($NO_2^{\bullet}$) and free radicals such as nitrous acid (HNO_2) as peroxynitrite ($ONOO^-$) (Halliwell, 1994). These free radicals are generated under normal physiological conditions but become harmful when not being eliminated from the cellular systems. In fact, such imbalance between the production and elimination of reactive oxygen species in the cell system leads to a condition known as oxidative stress. After excessive accumulation, they attack vital biomolecules leading to cell damage and homeostatic disruption. The major targets of these free radicals are lipids, nucleic acids, proteins and carbohydrates (Aruoma, 1994). The formation of free radical is a consequence of both enzymatic and non-enzymatic reactions which occurs continuously in the cell system. Enzymatic reactions include those phenomena involved in the phagocytosis, respiratory chain, synthesis of prostaglandin also in the cytochrome P450 system (Lui *et al*., 2010) Free radicals can also be produced in non-enzymatic reactions between oxygen and organic compounds as well as those initiated by ionizing reactions. In the cellular system, free radicals can be derived from two sources either endogenous sources such as nutrient metabolism, ageing process etc or exogenous sources which include tobacco smoking, radiation ionization, pollution, organic solvents, etc.

1.7 CONCEPTS OF PHYTOCONSTITUENTS PRODUCTION IN TEA

Biosynthesis of polyphenols in tea plants is a combination of Shikimate and Acetate pathways depicted in Figure 1.1 and the classification of tea polyphenols was schematically represented in Figure 1.2**.** Flavonoids are derived from variety of simple poly-phenolic units. The hydroxylation pattern of β-ring of flavonoids and their different substitution are principally important for executing their bioactivity. They have a potential role in plant defense mechanisms, reproduction and antioxidants activity.

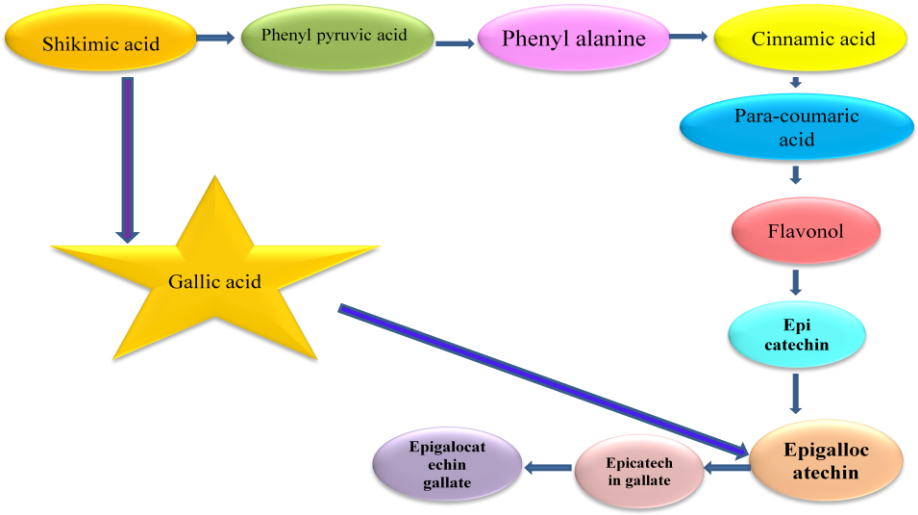

Figure 1.1 Shikimate - acetate Pathway

More often polyphenols are found in vacuoles and tea leaf cell, epidermal layers are the sites of their oxidation which are performed by the enzyme Poly Phenol Oxidases. Flavonoids are probably turned over more slowly than floral pigments but there are good evidence that metabolism occurs. A pulse-labeling measurement on Kaempferol and quercetin glycosides in *Cicer arietinum* leaves clearly indicate the half life of 7 to 12 days (Berlin *et al*., 1971). The early stage of the catabolism of flavonoids involves the addition of oxygen to the 2,3 – double bond to give a 2-hydroxy derivative. This 2, 3-dyhydroxy flavonone is then undergoing ring-cleavage to give a substituted benzoic acid and a phloroglucinol derivative, which may not be detectable because of its immediate oxidation. Undoubtedly the end products are eventually returned to the atmosphere as respired CO_2 (Figure 1.3). The catabolism of other leaf flavonoids has been less studied but it appears that flavonones may be oxidized by ring cleavage at the 2-position to 5, 7-dihydroxy chromones and hydroxy benzenes, which are then

further broken down, in the same way, to give CO_2 as their final fate. Alternatively, flavonones may undergo isomerisation to chalcones, which are then metabolized by the pathway: hydroxy cinnamic acid to hydroxy benzoic acid and converted to CO_2.

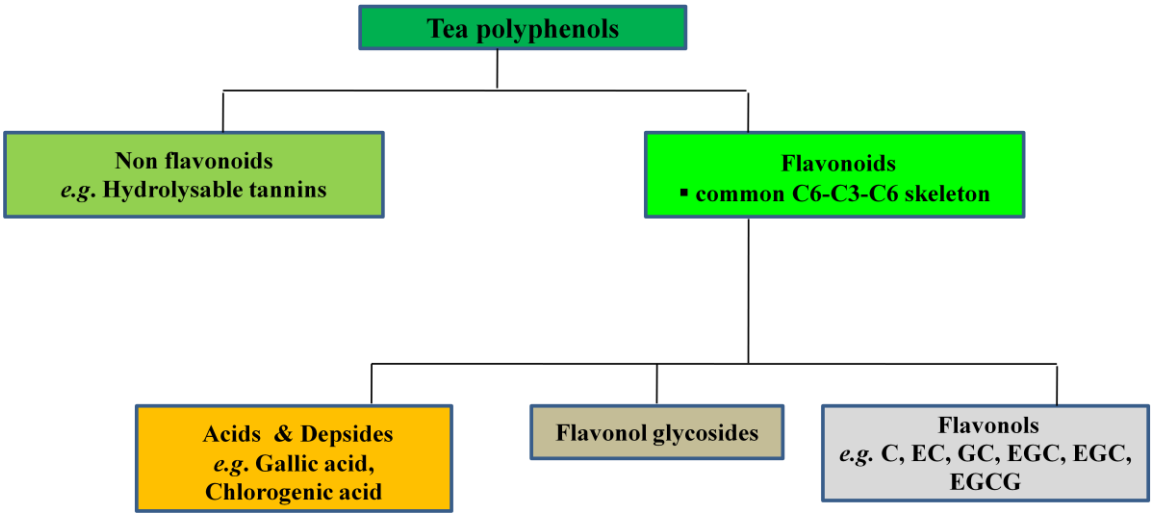

Figure 1.2 Classification of tea poly phenols

1.8 TEA IS A HEALTH ELIXIR/PANACEA

Presently, scientists are very concerned about escalating occurrence of age related

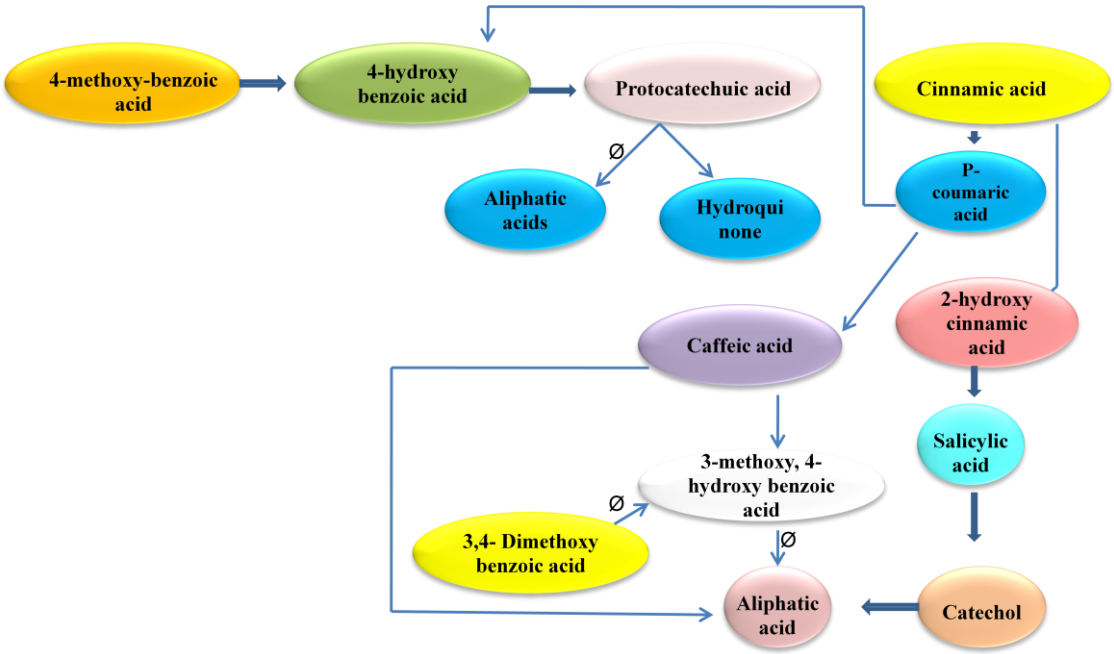

Figure1.3 Storage and degradation of polyphenol in plants, (Ø) Ring cleavage

diseases like hyperlipidemia, hypertension, hyperglycemia, cardiovascular diseases, and oxidation in rheumatoid arthritis (Carol *et al.*, 2004). Tea extracts directly control pro-inflammatory signaling (Frank *et al.*, 2006), green tea poly-phenols prevent toxin-induce hepatotoxicity and black tea prevents cigarette smoke induced oxidative damage of proteins. It is reported that polyphenols decrease capillary fragility and likely to accumulate the flavonoids like vitamin-P during apoptosis, anti inflammatory and antiviral capacity anti-carcinogenic activity, alter enzyme activity affecting cell division, proliferation, platelet aggregation and immune response.

The prophylactic function of tea poly-phenols has been established like:

(i) Anti-oxidative action

(ii) Radio-protective action

(iii) Anti-mutagenic action

(iv) Anti-tumor action

(v) Enzyme inhibitory action

(vi) Anti-hyperglycemic action

(vii) Anti-hypercholesterolemia action

(viii) Fat-reducing action

(ix) Anti-hypertensive action

(x) Anti-ulcer action

(xi) Anti-bacterial action

(xii) Anti-viral action (influenza)

(xiii) Deodorant

(xiv) Molueskcidal action

Poly-phenols are a group of chemicals substances found in plants, characterized by the present more than one phenol group per molecule. Recent scientific studies of food research laboratory, Japan revealed that intake of at least three cups of tea has a variety of physiologically beneficial action which works to prevent the infection of pathogenic bacteria like *H. pylori* (Katsuhiro et al., 1999). Medicinal properties of tea have been widely established in past few years, tea as a popular health drink (Table

1.1) get special attention in the world, so 100% attention must be taken to make tea free from hazardous chemicals and residues that must be safe to human health and also for wild life.

Table 1.1 Annual per capita tea consumption (*Source*: Tea Board of India)

Country	Per capita consumption/annum (kg)
Ireland	2.69
Turkey	2.56
U.K.	2.33
Morocco	1.40
Sri Lanka	1.28
Tunisia	1.25
Japan	1.08
New Zealand	1.03
Pakistan	0.82
India	0.64

1.9 TEA CULTIVATION AT A GLANCE IN THE WORLD

The Tea tree is indigenous to Eastern Asia and is now extensively cultivated in China, Japan, South Africa, Srilanka, Nepal, Indonesia and India. Areas under tea and per hectare production are listed in Table 1.2 and 1.3. Tea (*Camellia sinensis*) is one for the most important Agro industrial crops in India (Table 1.4).

Table 1.2 Area under Tea: World & India (1000 hectares) (*Source*: Tea Board of India)

Country	1998	2003
China	382	518
Srilanka	245	188
Indonesia	111	155
Kenya	077	131
Japan	061	050
Bangladesh	044	050
Argentina	041	036
Iran	032	035
India	382	518
Total	**2264**	**2720**

The tea industry is a labour intensive job and as such, it generates employment for a large number of people. Also, tea is one of the top foreign exchange earning commodities of the country. Competition from Sri Lanka, Kenya and other countries notwithstanding, Indian tea is still sought after by the tea drinking community through the world. Being brought up in North Bengal which occupies such an important position as far as Indian tea is concerned.

Table 1.3 Average Yield/Hectare. (MTKHY) (*Source*: Tea Board of India)

Country	**1998**	**2003**
Srilanka	782	1611
Indonesia	1118	1084
Kenya	1049	2235
Bangladesh	911	1137

Table 1.4 Number of Tea garden in India (2003) (*Source:* Tea Board of India)

State	**Big**	**Small**	**Total**
Assam	766	38371	39137
Darjeeling	85	-	85
Terai	63	845	908
Dooars	158	387	545
West Bengal	306	1232	1538
North India	1194	43992	45186
South India	404	56556	56960
Total India	**1598**	**100548**	**102146**

1.10 EXISTING KNOWLEDGE GAP

However, the variation of chemical content, as well as antioxidant potentiality present in tea shoots in various agro climatic conditions of North Bengal, has not been studied so far. In this context, the study was designed for in-depth analysis of antioxidants and region wise seasonal variation along with the quantity of bioactive phytochemicals of under-explored tea of Darjeeling Himalaya, Dooars and Terai and also the dynamic alteration of bioactive substances during industrial processing particularly processed intermediate and grades of tea.