Chapter 2
Review of Literature

"The universe is not only queerer than we suppose, but queerer than we can suppose."

-J. B. S. Haldane

2.1. History of tea

Historical evidence from Chinese mythology states that tea was first discovered in the year 2737 B.C., by Emperor Shen Nung (Han dynasty) as mentioned in the Pen T'sao (a Chinese medical book written during the Han Dynasty, circa 25 to 221 A.D). Tea is believed to be first mentioned in the ErhYa, (a Chinese dictionary circa 400 B.C). However, the term ch'a used for tea was not so popular before until the writing of Lu Yu (*The Classic of Tea, Ch'a Ching)* which was published during 780A.D. As cited in *The Story of Tea* (Heiss and Heiss, 2007), the consumption of tea as a medicinal drink began in Yunnan province during the Shang Dynasty (1500 BC–1046 BC). After the Tang dynasty (618-906 A.D.), tea became popular as a refreshing beverage (Benn, 2015). Tea's origin is likewise a point of contention that has to be clarified. Although the Indo-Burma region around the Irrawaddy River is thought to be the genesis point (Mondal, 2009), it is unclear if Assam and China type of tea have the same or separate domestication origins (Rawal *et al.,* 2021).

The famous tea transferred from one culture to another so the brewing, consuming method and chemical aspects also varied among different cultures. However, the modern way of tea brewing technique by infusing tea leaves into hot water arose during the

Ming dynasty (1368 to 1644) and subsequently got popular throughout the world.

During the Tang dynasty tea was believed to be consumed as a vegetable with which they prepared soups mixing with other ingredients. Later Brick tea (Mair and Hoh, 2009) made by steaming and compressing the tea leaves into bricks, got popularized. The powdered form of tea with bright tea green color and low astringency got popularized during the Song Dynasty (960 to 1279 A.D.). In the mid 13th century, the Chinese discovered a new way to process tea. Rather than being steamed, tea leaves were roasted and crushed. Unfermented tea leaves were pan-fried, then rolled and dried throughout the Yuan and Ming dynasties. This inhibits the oxidation process that darkens the leaves, allowing the tea to remain green. Oolong tea was invented in the 15th century when the tea leaves were allowed to partially ferment before being pan-fried (Benn, 2015). Yellow tea was discovered by accident during the Ming dynasty's manufacture of green tea, when seemingly poor techniques caused the leaves to turn yellow, thus yielding a distinctive flavor (Mair and Hoh,2009).

Tea drinking and tea culture transferred to Japan, Korea, Portugal, Italy, Iran, Taiwan, United Kingdom, America, Australia, Africa, Srilanka, India, and so on to many other parts of the world.

While attempting to introduce tea to India, British colonists discovered that tea plants with thicker leaves flourished in Assam as well and that these, when planted in India, thrived. The Singphos tribe of Assam had long grown the same plants, using tea chests provided by tribal chief Ningroola (Acharya, 1998).

2.2. Tea taxonomy

Tea, used as a popular beverage worldwide is distinguished as three taxa, namely *C. sinensis* (L.) O, Kuntze ("China type"), *C. assamica* (Masters) Wight ("Assam type"), and *C. assamica* ssp. *lasiocalyx* ("Southern form" or "Cambod type"). A careful observation, however, indicates an entirely different situation in the field, where one comes across a wide spectrum of tea populations, particularly in leaf form and other morphological characters of taxonomic significance. We have in our collection tea selections varying widely from the small-leaved "China type" to the large-leaved "Assam type", with innumerable intermediates between these two extreme forms. Apart from variations in leaf size and form, one often recognizes certain other features in cultivated tea populations namely, pigmented young shoots and leaves, punctuations on the leaf either present or absent, varying number of styles, nature of the stylar arms, glabrous ovary, or pubescent to different degrees, which are not readily visible in the typical forms of the three taxa mentioned above (Sharma and Venkataramani, 1974). The characters of *C. sinensis*, *C. assamica* and *C. assamica* ssp. *lasiocalyx* often overlap and also a few other characters unknown to these taxa appear in some populations of the cultivated tea. One is thus left perplexed with the taxonomy of the cultivated tea plant. Tea reproduces freely among themselves because of their high out-crossing tendencies, resulting in plant types that are midway between the two extreme forms, i.e., Assam type huge leaf and China type small leaf. As a result, tea taxonomic categorization based on morphology remains a muddle, and the species name is also a misnomer. Even though China and Assam teas are classified as distinct species, there is no limit on gene transfer between them (Rawal *et al.,* 2021). Desirable characteristics of Darjeeling tea, such as anthocyanin coloration or particular quality, may have been introduced from two wild species, *C. irrawadiensis* and *C. taliensis* (Wood and Barua, 1958).

2.3. Tea morphology

Camellia sinensis (Figure 2.1) is small and nearly 1-2 m tall with numerous stems and 2-inch leaves resistant to cold weather. The leaves are leathery, hard, and thick with a matt surface. Due to its thickness, it is very difficult to distinguish the veins present in the lamina. The leaf blade is elliptical with an obtuse end and square base with teeth present in the edges. The young leaves are smooth with hairs in the bottom, whereas the older leaves have few hairs or eventually it disappears. The Assam tea plant widely known as *Camellia assamica* (Figure 2.2) belongs to North East India and is considered small even though it has a height ranging between 10-15m. Warm weather environments are best suited for this plant. The branching system is strong with thick-thin, shiny, dependent, and pointed leaves, which are hairless or hairy on the vein below. The marginal veins are distinguishable. The leaf blade is oval with leaves 8-20 cm in length and 3.5-7.5 cm wide. *Camellia assamica* sub sp. *Lasiocalyx* (Figure 2.3) or southern form of tea is a small tree with upright branches between 6–10 m tall (Hilal, 2017).

Fig.2.1. *Camellia sinensis* var *sinensis* with small leaves

Fig.2.2. *Camellia sinensis* var *assamica* with large leaves

2.4. Importance of germplasm collection and characterization

Germplasm characterization is very much essential for plant breeders to effectively utilize and manage the gene banks to enhance the improvement of cultivars for higher crop productivity (Piyasundara *et al.,* 2006). The information gathered from the characterization of accessions will also be beneficial to understand genetic variation and select genetically distant relatives for successful hybridization (Anandappa, 1993). Morphological descriptors are the important preliminary characterization of the genetic resources and it is a cost-effective method when compared to the biochemical and molecular markers (Martinez *et al.,* 2003). It surely has many disadvantages under influence of environmental factors or parameters which results in continuous variations and increased plasticity. Despite such shortcomings or disadvantages, morphological traits are utilized in tea for its characterization and identification as

Fig.2.3.*Camellia assamica* subsp. *Lasiocalyx* with intermediate leaves

well as for the study of genetic diversity, and phylogeny (Ranatunga *et al.,* 2017). Piyasundara *et al.* (2006) characterized 20 accessions of *Camellia sinensis* using 13 standard morphological descriptors (Table 2.1). Out of these 13 morphological descriptors, a total of 11 descriptors contributed to the phenotypic variation where width, leaf shape, leaf, and petiole pigmentation mainly contributed to the variation. Likewise, 89 accessions from tea germplasm have been characterized employing 16 floral traits (Table 2.1) comprising of both qualitative and quantitative traits and the result identified considerable variations among the accessions with the pistil trait being highly variable (Ranatunga *et al.,* 2017).

The role of tea germplasm in crop improvement is well recognized but it still lacks adequate information so it cannot be utilized to its full potential. Sri Lanka has reported a total of 600 accessions but the narrow genetic choices for the breeding program have led to the utilization of only 4% of accessions (Ranatunga *et al.,* 2017). These accessions also lack adequate evaluation in the biochemical, agronomic, and molecular aspects (Gunasekare, 2007; Ranatunga and Gunesekera, 2008). Tea being an allogamous plant shows many overlapping, phenotypic and biochemical characteristics and the excessive hybridization may cease the existence of pure archetypes of tea (Banerjee, 1992). Study regarding the characterization of tea germplasm based on morphological descriptors not only helped in the study of variation but also put some light on the collection gap where the majority of the accessions collected were Cambod followed by Assam, showing more preference and bias towards these two types showing void or less preference in China type collection (Ranatunga *et al.,* 2017). Therefore, morphological characterization can generate valuable information regarding the genotypes that predominate in a particular region and thus help to fill the gap in the core collection of germplasm.

Table 2.1. Morphological descriptors used for characterizing tea accessions

(Piyasundara *et al.,* 2006)	(Ranatunga *et al.,* 2007)
Leaf length	**Qualitative traits:**
Leaf width	Ovary pubescence
Ratio of leaf length to leaf width	Stigma position
Leaf shape	Split pattern of style
Leaf pose	**Quantitative traits:**
Immature leaf color	Bud length
Mature leaf color	Bud width
Length of the mature leaf petiole	Petal length
Leaf pigmentation	Petal width
Petiole pigmentation	Total number of sepals
Appearance of the leaf upper surface	Total number of petals
Young shoot pubescent	Number of whorls of sepals
Leaf apex habit	Number of whorls of petals
	Number of style arms
	Style length
	Style column length
	Style arm lengths
	Corolla diameter

2.5. Genetic diversity of tea using molecular markers

The tea germplasm can be characterized using morphological, phytochemical, and molecular descriptors but among the former descriptors, namely the morphological and phytochemical is affected or influenced by environmental factors (Hyun *et al.,* 2020).

The morphological and geographical characters are also considered to be less beneficial when studying the relationships among the cultivars (Katoh *et al.,* 2003). The genetic diversity of tea also has been studied using amplified fragment length polymorph (AFLP) (Paul *et al.,* 1997) but the requirement of radioactive labeling and comparatively better need of quantitative and qualitative DNA limits the utilization of AFLP in every research laboratory. The RAPD technique has been successfully employed to determine the relationships between the species of *Camellia* (Wachira *et al.,* 1997) along with its successful utilization to differentiate between varieties and clones of *C. sinensis* (Wachira *et al.,* 1995). Additionally, we can develop locus-specific probes and other SSR sequences of interest by isolating and reamplifying individual bands (Wu *et al.,* 1994; Zietkiewicz *et al.,* 1994). Previous work on Darjeeling tea reports the study of genetic diversity using RAPD (Roy and Chakraborty, 2009; Baruah *et al.,*2010), ISSR (Roy and Chakraborty, 2009), and AFLP (Mishra and Sen-Mandi, 2004) markers.

Genetic diversity study has its importance. Genetic diversity and taxonomic relationships were estimated in 38 clones (Wachira *et al.,* 1995) belonging to three tea varieties through RAPD analysis and reported genetic variability between species partitioned between and within-population with 70 percent detected in the latter. Band sharing analysis separated three populations according to present taxonomy and known pedigree of some clones and RAPD analyses also discriminated against 38 commercial clones including the clone which was morphologically and phenotypically indistinguishable (Wachira *et al.,* 1995). Genetic diversity study of 27 superior tea (*Camellia sinensis* var. *sinensis*) accessions from Korea, Japan, and Taiwan using RAPD markers led to the suggestion that the Taiwan tea studied may have a different origin from that of Korea and Japan and also reported 71 percent of variability residing within groups and rest between groups with the greatest variation within the Korean group followed by Taiwan and Japan (Kaundun *et al.,* 2000). Random amplified polymorphic DNA (RAPD) techniques were applied to assess genetic instability among micro propagated tea [*Camellia sinensis* (L.) O. Kuntze] cultivar 'T-78' and suggested RAPD be used successfully to determine the genetic instability among micropropagated plants which otherwise were morphologically indistinguishable (Nondal and Chand, 2002). RAPD markers provided a practical method not only to estimate the genetic diversity and relationship but also to identify tea genetic resources of the 15 well-known, widely planted traditional Chinese elite tea genetic resources [*Camellia sinensis* (L.) O. Kuntze] (Chen *et al.,* 2005). Inter simple sequence repeats (ISSR) technique was adopted to analyze the genetic variability of somatic embryo-derived tea plants and the study confirmed the existence of wide genetic variation among the somaclones (Thomas *et al.,* 2006). Genetic diversity and differentiation were examined in 10 ancient tea populations (*Camellia sinensis* var. *assamica*) by using ISSR markers and found a moderate level of genetic differentiation among the population (Ji *et al.,* 2011). The genetic fidelity of the micropropagated plants of three tea clones was assessed by analyzing their nuclear, mitochondrial (mt), and chloroplast (cp) genomes using multiple molecular DNA markers like RFLP, RAPD, SSR, and ISSR and revealed that genomic changes in tea clones are genotype-dependent rather than culture condition-dependent (Devarumath *et al.,* 2002). RFLP analysis with phenylalanine ammonia-lyase *(PAL)*cDNA as a probe was used to evaluate the genetic diversity of the Korean tea plant *(Camellia sinensis* var. *sinensis*) and reported the genetic background of Korean teas, to differ from those of Japanese teas along with different morphological characters (Matsumoto *et al.,* 2004). Genomic fingerprinting in 21 tea genotypes was carried out using 7 ISSR and 12 RAPD primers with China variety showing the largest within-group diversity, followed by Assam tea and Cambod tea (Roy and Chakraborty, 2009). Recovery of standard DNA barcodes was tested for land plants from a large array of commercial tea products their performance was analyzed in identifying tea constituents using existing databases and thus indicated that unlisted ingredients are common in herbal teas (Stoeckle *et al.,* 2011)**.** Use of ITS2 barcode accurately and effectively to distinguish herbal tea ingredient *Plumeria rubra* from its adulterants was reported, which provided a new molecular method to identify *P. rubra* and ensure its safety in use (Shi *et al.,* 2014). Four candidate regions (*rbc*L, *mat*K, ITS2, *psb*A-*trn*H) were amplified by a polymerase chain reaction and DNA barcodes were used for the first time to discriminate the commercial non-*Camellia* tea and their adulterants, and to evaluate their safety and suggested non-limitation of the sequence of original plants in GenBank and thus requires submission of more original plant sequences to the GenBank for evaluating the safety of non-*Camellia* teas (Long *et al.,* 2014). A total of 113 tea plants [*Camellia sinensis* (L.)O. Kuntze] housed at the Tea Research and Extension Substation (Taiwan), was used in the experiment from which 113 internal transcribed spacer 2 (ITS2) fragments, 104 *trnL* intron, and 98 *trnL-trnF* intergenic sequence regions were successfully sequenced and found ITS2 nucleotide sequence variation larger compared to trnL intron and trnL intergenic sequence fragments of chloroplast cpDNA, thus suggesting it more suitable for establishing a DNA barcode database to identify tea plant varieties (Lee *et al.,* 2017).

2.6. Different types of tea

Sharangi (2009), elaborated on different types of tea consumed worldwide which are discussed briefly below. The different forms of tea are green tea, black tea, white tea, oolong tea, pu'erh tea, etc. The process of production of respective teas is provided in Table 2.2.

The consumption of tea imparts many

potential health benefits. The health benefits of a different form of tea are enlisted in Table 2.3. Green tea is loaded with beneficial chemicals with maximum health benefits (Sinija and Mishra, 2008). Due to no fermentation, it has more polyphenols and less caffeine which imparts various positive health effects. White tea on the other hand retains high levels of antioxidants than other types of tea due to its unique process of selection and production. Black tea accounts for the largest production worldwide i.e., 72% of total tea production (Sharangi, 2009). Black tea requires longer fermentation so most of the antioxidants mostly the EGCG antioxidants are oxidized although it retains a higher number of the antioxidant compounds especially the flavonoids which helps in the elimination of various harmful toxins from the body. The partially fermented tea i.e., the oolong tea has the flavor and health attributes of both black tea and green tea. It is rich in antioxidants beneficial for skin cells and slowing down the aging process. Another form of tea i.e., the Pu'erh tea is unique in its way due to its aged consumption since the large tea leaves once plucked are piled and stored for 50-100 years. The consumption of green tea and oolong tea is mainly prominent in Asian countries whereas black tea is popular in Western countries (Sharangi, 2009). The polyphenol content decreases and the caffeine level increases with the increase in fermentation process, the lower the polyphenol content, and the higher the caffeine content (Lin *et al.,* 2003).

2.7. Tea phytochemistry

The chemical composition of tea is highly complex, owing to the abundant presence of different classes of chemical compounds. The primary chemical compounds found in tea are given in Figure 2.4. and the structural formula of some main components is provided in Figure 2.5. The main class

Table 2.2. Different types of tea and its production (Sharangi , 2009)

Types of tea	Process of production
Green tea	Prepared from unfermented leaves
White tea	Buds (before fully opened) and young tea leaves collected followed by steaming and drying of leaves. Minimum amount of processing.
Black tea	Longer fermentation
Oolong tea	Partial fermentation
Pu'erh tea	Proceesing similar to black tea. The only difference is that large leaf variety of tea plant is used which can be picked throughout the year.

of chemical compounds is described below.

2.7.1. Polyphenols

Tea is regarded as the most popular dietary source of polyphenols that accounts for 50–70% of tea water extracts (Yao *et al.,* 2006). Polyphenols provide astringency, the 'drying' sensation that we experience in the mouth after consumption of the tea beverage. A strong cup of tea usually is loaded with 180 to 240 mg of polyphenols.

The polyphenols are flavonoids that are produced biosynthetically in considerable amounts ranging from 0.5% to 1.5% (Vinson *et al.,* 1995). The major abundant flavonoids comprise catechins (flavan-3-ols), (Millin *et al.,*1969) and: (-)-epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC) are the four major catechins predominantly found in green tea. These different structures are very important for their pharmacological usage (Zhao *et al.,* 2001). Tannins are the second major polyphenols present

Table 2.3. Different types of tea and is health benefits

Tea type	Health benefits	Reference
Black tea	Antioxidant and anti-inflammatory, Cardiovascular diseases, Diabetes, GIT cancer (Esophageal, gastric, pancreatic, colon, & colorectal cancer), Other cancers (Prostate, lung, breast, liver & skin cancer), Mental alertness/ memory improvement, Bone health, Gastric emptying, Obesity, Rheumatoid arthritis, Neurological effects, Viral infection, Bacterial infection, Asthma & allergy, Mood & cognitive performance, Dental health.	(Naveed *et al.,* 2018)
Green tea	Antioxidant and hepatoprotective activity, anticancer and anti-mutagenic activity, antimicrobial and antiviral activity, anti-schisosomiasis and antiparasitic activity, cardioprotective activity, antidiabetic and anti-obesity activity, gastrointestinal tract problems relieving activity, neuroprotective activity, anti-inflammatory, analgesic, antipyretic,and anti-allergic activity, skeletomuscular system relieving activity.	(Aboulwafa *et al.,*2019)
Oolong tea	Anti-inflammation,antioxidant activity, anti-obesity, anti-cancer, hypoglycemic effect, prevention of heart disease, antimicrobial activity (*Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas fluorescens*, Salmonella sp. and *Staphylococcus aureus)*	(Weerawatanakorn*e t al.*, 2015)
White tea	Cardioprotective effects, antidiabetic potential, anticarcinogenic and antimutagenic activities, neuroprotective activity, antimicrobial properties, Anti-obesity potential	(Dias, 2013)

in tea products, which are responsible for astringency (Okuda *et al.,*1985). Additionally, the phenolic acids consist of chlorogenic acid, caffeic acid, gallic acid, coumaric acid, and quinic acid ester. The flavanols comprises of kaempferol, myricetin**,**and quercetin (Aboulwafa *et al.,* 2019).

2.7.2. Purine Alkaloids

The second major constituent of tea is caffeine which belongs to the xanthine bases or the purine alkaloids. Other metabolites like theophylline and bromine, are found in smaller amounts.

2.7.3. Triterpenoid Saponins

The triterpenoid saponins comprise floratheasaponin A-F (Yoshikawa *et al.,* 2008), which are predominantly found in higher concentrations in seeds and flowers (Man *et al.,* 2010).

2.7.4. Amino Acids

Arginine, aspartic acid, glutamic acid, glutamine, and serine**,** as well as theanine, accounts for more than 90% of the whole amino acids (1–4% of dry weight) found in the leaves of *C. sinensis* (Horie *et al.,* 1993). Other amino acids like Tryptophan, valine,

Table 2.4. Primary chemical compounds of tea characterized by chromatic methods (Yashin *et al.,* 2015)

Sl No.	Compound name	Main representative elements
1.	Catechins	Flavanols: 12 catechins are indentified, including 8 occurring in significant quantity, i.e., (+)-catechin, (-)- epicatechin, (-)-gallocatechin, (-)-epigallocatechin, (-)-catechin gallate, (-)-epicatechin gallate, (-)-gallocatechin gallate, (-)-epigallocatechin gallate
2.	Oxyaromatic acids	Gallic, caffeic, quinine, chlorogenic, n-coumaric acids
3.	Flavonols	Quercetin, kaempferol, myricetin
4.	Theaflavins	Theaflavin, theaflavin-3-O-gallate, theaflavin-3'-O-gallate, theaflavin-3-3'-O-gallate
5.	Teagallins	Teagallin
6.	Thearubigins	High-molecular weight polymers of catechin gallates with molecular weight from 1000 to 40000 Da
7.	Pigments	Carotenoids and chlorophyll
8.	Alkaloids	Caffeine, theophylline, theobromine
9.	Sugars	Glucose, fructose, saccharose
10.	Amino acids	Isoleucine, leucine, methionine, threonine, phenylalanine,glutamine, asparagine, alanine, serine, proline, histidine, glutamic acid, aspartic acid, theanine
11.	Vitamins	C, α-, β-, γ-, δ-tocopherols, riboflavin
12.	Dibasic acids	Succinic, malic, tartaric, citric, quinic, aspartic, glutamic, oxalic acids
13.	Cations	K+, Na+,Ca2+, Mg2+, NH4 +, Al3+
14.	Metals	Fe, Zn, Cu, Ni, Al
15.	Lignans and triterpenoid saponins	Mixture of many compounds

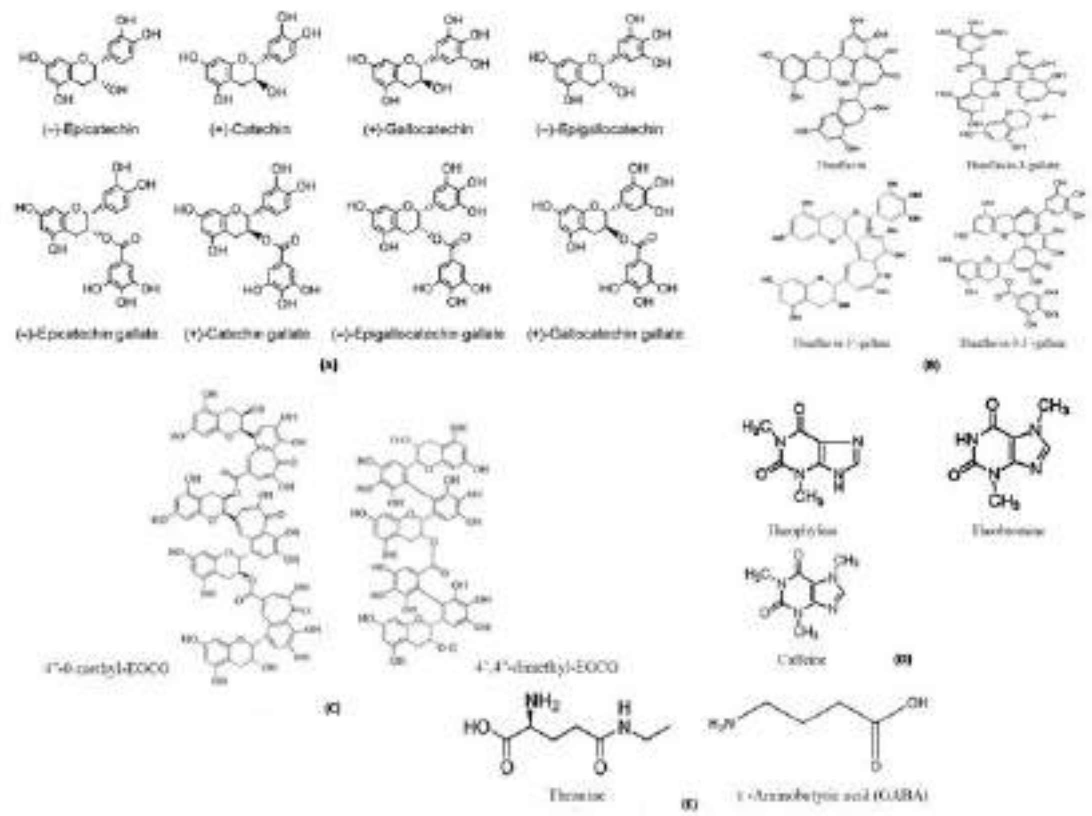

Fig.2.4. Structure of primary chemical compounds of tea (A) Eight major catechins found in tea (B) Tea theoflavins (C) Oligomeric catechins (D) Tea methylxanthins (E) Tea theanine

threonine, lysine, tyrosine, leucine, and lysine are also found. The amino acid attributing to the flavor and exotic taste of green tea i.e., Theanine (5-Nethylglutamine) is the only amino acid existing solely in tea plants and is the major amino acid found in largest amounts in green tea i.e., about 1–2% of the dry weight of leaf (Aboulwafa *et al.,* 2019). The presence of this rare amino acid is also reported (Casimir *et al.,* 1960) in the *Xerocomusbadius* (edible bay boletes mushroom**).**

2.7.5. Other constituents

Tea comprises minerals and traces elements like, Na, Cr, Al, Ca, K, Co, Cu, F, Fe, Mg, Se, Sr, Mo, Ni, P, and Zn, which comprises about 5% of dry weight (Cabrera *et al.,* 2006). About 15 –20% of dry weight is represented by proteins, whose enzymes comprise an important fraction (e.g., carotenoid cleavage enzymes), and 5–7% of dry weight is represented by carbohydrates like sucrose, glucose, fructose, and pectin. The most found lipids are linoleic and α-linolenic acids. Sterols such as stigma sterols are also found in tea. Other important compounds found in tea are vitamins (B, C, and E), chlorophyll pigments (carotenoids), volatile compounds mainly related to tea aroma (aldehydes, alcohols, long-chain aliphatic alcohols). Green tea is enriched with the bioactive compound policosanol, long-chain aliphatic alcohol which has much health-promoting activity (Choi *et al.,* 2016).

2.8. Health benefits of tea

2.8.1. Antioxidant

Tea is enriched with polyphenols like catechins, theaflavins, and thearubigins which are high in antioxidants and beneficial for health. The antioxidant activity of tea polyphenols is due to their ability to scavenge superoxide along with the increased or enhanced activity of some detoxifying enzymes like catalase, glutathione-S-transferase, glutathione peroxidase, quinine reductase, and glutathione reductase present in the small intestine, liver, and lungs. The free radical scavenging activity of EGCG has been confirmed by previous *in vitro* and *in vivo* studies. Among all the antioxidants like vitamins C and E (tocopherol) and carotene, tea catechins are better. The antioxidant properties of tea are very much crucial for human health which aids in the prevention of atherosclerosis and specifically some coronary artery disease (Miura *et al.,* 2001).

2.8.2. Anticancer

Tea contains various antioxidants and phenolic compounds, where some compounds have shown anti-cancer properties in laboratory conditions as stated in earlier reports (Kris-Etherton *et al.,* 2002). Consumption of green tea decreases the frequency of cancer development and imparts reduced risk of several human malignancies and diabetes. The molecular mechanisms of green tea polyphenols and their therapeutic use in cancer are studied previously by Shankar *et al.* (2007) and stated epigallocatechin- 3-gallate (EGCG), a major polyphenol found in green tea, to be widely studied for its chemopreventive and potential anticancer activity. A wide range of mechanisms is outlined by which epigallocatechin gallate (ECGC) and other green and black tea polyphenols inhibit cancer cell survival as outlined previously by Beltz *et al.* (2006). The polyphenols of tea especially found in green tea inhibit angiogenesis and metastasis which arrest the growth and induces apoptosis celebration of various signaling pathways. Some form of skin tumor formation which is cost by different chemical carcinogens or exposure to UV radiation is inhibited by oral or local application of green tea extracts.

This research compelled many other cosmetic and pharmaceutical companies to supplement their skincare products with green tea extracts (Katiyar and Elmets, 2001). Million cases of skin cancer are diagnosed annually across the globe where most of the cases are of non-melanoma associated with chronic exposure to ultraviolet light. The non-melanoma form of skin cancer is very common and is increasing in occurrence therefore the potential of green tea polyphenols, epigallocatechin gallate as a chemopreventive agent for non-melanoma skin cancer was examined where a double-blind randomized and placebo-controlled phase II clinical trial of epigallocatechin gallate in the prevention of non-melanoma skin cancer was conducted (Linden *et al.,* 2003).

2.8.3. Cardiovascular diseases

Hypercholesterolaemia, a condition when the cholesterol level increases in the circulating blood where the concentration of cholesterol rises above 200 mg/ dL and the level of the high-density cholesterol are below 35 mg/ dL. This condition of high cholesterol level is associated with increased risk of cardiovascular disease and the causative factors for this condition are stress or high intake of refined sugar and animal fats. So along with lifestyle changes, some dietary changes need to be made by replacing animal fats with industrially processed vegetable oils along with increased consumption of green tea which is beneficial for the cardiovascular system which ultimately lowers the cholesterol level and prevents clumping of the platelet.

Coronary artery disease is a result of increased oxidative stress and dysfunction of endothelium and black tea antioxidants can reverse the endothelial dysfunction (Duffy *et al.,* 2001). Prior clinical trial suggests a total of five servings of black tea per day which could help in reducing low-density lipoprotein by 11.1% and total cholesterol by 6.5% in moderately hypercholesterolemic adults (Davies *et al.,* 2003).

Tea is believed to have the potential ability to limit cholesterol absorption in the intestine. Therefore, a study was conducted where the effect of tea on blood lipids was studied keeping all other components of the diet constant. The continuous ingestion of green tea extract which is high in catechin aided in the reduction of body fat, SBP, and LDL cholesterol. The study emphasized the role of such catechin enriched extract to decrease obesity and risk of cardiovascular disease (Nagao *et al.,* 2007). Similarly, the other study also emphasized the effects of a beverage rich in catechin to reduce the cardiovascular disease risk in obese children and thus concluded that consumption of tea enriched with catechin decreases obesity and related risk factors of cardiovascular disease without having any side effects (Matsuyama *et al.,* 2008).

2.8.4. Respiratory disorders

Some respiratory diseases like wheezing, shortness of breath, and difficulty breathing arise as a result of some health conditions like chronic bronchitis, emphysema, asthma, and other lung diseases. Theophylline in tea is used to prevent such alleviating symptoms where it relaxes the lungs and opens air passages thus making it easier to breathe (Sharangi, 2008).

2.8.5. Skin Problems

Tea is used as an age-old home remedy for burns, wounds, and swelling. Green tea extract reduces inflammation, stops or slower bleeding, relieves itchy rashes caused by insect bites.

Tannins present in tea have both antiseptic and anti-inflammatory properties. The important antioxidant compound like flavonoids also possesses antiseptic properties. Green tea extract is considered by researchers as a natural sunblock agent. At certain concentrations, the EGCG present in green tea and the other green tea polyphenols showed the possibility for renewed cell division (Hsu *et al.,* 2003).

2.8.6. Digestion

Tea loaded with phytochemicals having bioactivity like antibacterial, antioxidant, antiseptic, and detoxifying properties are very much effective in treating infectious dysentery as well as easing inflammatory bowel disease. Green tea is very much useful as a traditional home remedy for various digestive problems like inflammatory bowel disease (Rahman *et al.,* 2018).

2.8.7. Anti-diabetic

Studies on animal models revealed that green tea may slow or prevent the progression of Type 1 diabetes. The role of polyphenolic components of tea to inhibit a-amylase from human saliva was investigated using *in vitro* studies by Hara and Honda, (1990). The polyphenols present in tea lower the serum glucose by inhibiting the activity of both salivary and intestinal amylase (starch digesting enzyme). This results in the slow breakdown of starch and minimization in the sudden rise of serum glucose.

2.8.8. Arthritis

Tea especially the green tea has anti-inflammatory properties where the compound like EGCG inhibits the production of several molecules in the immune system that leads to inflammation and damage of joints in arthritis patients (http:// www.nerve.in/ news, 25350069280). The green tea antioxidant compounds will thus help to prevent developing arthritis and consumption of at least four cups of green tea can reduce inflammation in patients with arthritis.

2.8.9. Anti-obesity

A metabolic disorder resulting in obesity is caused by an imbalance between intake and expenditure of energy thus putting a person at stake for lifestyle-related disease. Consumption of a tea rich in catechins along with regular exercise helps to activate whole-body energy metabolism and thus reduce obesity induced by diet (Murase *et al.,* 2006).

2.8.10. Cognitive functions with tea consumption

Tea loaded with compounds of pharmaceutical importance protects the brain corresponding to the aging process and is also inversely associated with the occurrence of dementia, Alzheimer's, and Parkinson's diseases. The main catechin polyphenols compound i.e., the EGCG, has shown neuroprotective properties in a wide range of cellular as well as animal models with neurological disorders (Mandel *et al.,* 2008). A cognitive test examined by studying the relation between intake of flavonoid-rich foods and its effect on cognitive function led to the findings of enhanced cognitive abilities in the elderly (Nurk *et al.,* 2009).

2.9. Solvent extraction and bioactivity

Polar solvents like water, ethanol, and acetone were found to extract major phytochemicals groups than non-polar ethyl acetate and chloroform from *Camellia sinensis* and *Psidium guajava* leaves (Geoffrey *et al.,* 2014). Extraction method should ensure complete extraction of the desired compounds of interest without any chemical modification (Zuo *et al.,* 2002). Extraction and determination of biologically active compounds depend upon the type of solvent used where solvents will diffuse into solid plant tissue and solubilize compounds with the same polarity (Tiwari *et al.,* 2011).Aqueous mixtures of ethanol, methanol, and acetone, water are commonly exploited to extract plants (Sun and Ho, 2005). Researchers have reported the use of aqueous methanol, acetone and ethanol, absolute methanol, absolute ethanol, and boiling water for the extraction of polyphenols from green, black and other types of manufactured tea (Labar *et al.,* 2019). Different solvents like water; aqueous ethanol in different extracting time has been employed to extract phenolics from green and white tea (Rusak *et al.,* 2008). Tea components have been analyzed using high-performance liquid chromatography and high-performance capillary electrophoresis (Horie and Kohata, 2000). A comparative analysis of tea catechins and theaflavins has been performed using high-performance liquid chromatography and capillary electrophoresis (Lee and Ong, 2000). Metabolite Profiling and Quality Assessment of Green Tea or *Camellia sinensis* (L.) was done using ^{1}H NMR Spectroscopy and reported the presence of theanine, gallic acid, caffeine, epigallocatechin gallate, and epicatechin gallate in higher levels than epigallocatechin in Longjing teas compared to other teas (Le Gall *et al.,* 2004). Phenolic ingredients were separated by multilayer countercurrent chromatography (MLCCC) and preparative high-performance liquid chromatography (HPLC) was then applied to obtain pure flavonoids and the purity and identity of isolated compounds was confirmed by different NMR experiments, HPLC-diode array detector (DAD), or gas chromatography–mass spectrometry (GC-MS) analysis (Krafczyk and Glomb, 2008).

Studies have been performed to check the antimicrobial properties of polyphenol fractions of tea and also shown purified catechin fractions from green and black tea, and especially ECG and EGCG inhibited growth of many bacterial species and possessed anticarcinogenic properties, proved to be powerful antagonists of human immunodeficiency virus reverse transcriptase, causing 50% inhibition at a concentration ranging from 10 to 20 ng/ml (Hamilton-Miller, 1995). The flavonols, quercetin, kaempferol, and myricetin showed activity against gram -positive bacteria and phytopathogenic fungi (El-Gammal and Mansour, 1986).

2.10. Tea in nanotechnology

Nanostructured noble metals are widely used in a variety of technological applications and a variety of synthetic approaches have been used to suit these demands but nowadays green chemistry concepts have recently sparked renewed interest in the production of noble metal nanoparticles (Moulton *et al.,* 2010). The development of biological nanoparticle synthesis utilizing microorganisms or plant extracts is essential in nanotechnology since it is not harmful to the environment, unlike chemical synthesis which releases toxic chemicals (Loo *et al.,* 2012). Green synthesis methods, which use biological microbes or plant extracts instead of chemicals, have evolved as a simple and effective alternative to chemical synthesis. Green synthesis outperforms chemical approaches in terms of environmental friendliness, cost-effectiveness, and ease of scaling up for large-scale synthesis. Plant extracts can be used to synthesize nanoparticles, which has the advantage over other biological procedures in that it eliminates the time-consuming process of maintaining cell cultures and aseptic environments and can be scaled up for large-scale production (Loo *et al.,* 2012). There are three main steps during the synthesis of metal nanoparticles, (i) solvent selection, (ii) reducing agent selection, and (iii) capping agent selection (Moulton *et al.,* 2010).

There has been a growing interest in finding environmentally friendly, multifunctional materials for the green synthesis of metal nanoparticles. Tea contains polyphenols, which act as both reducing and capping agents during the synthesis of nanoparticles (Huang *et al.,* 2014). Catechins, a flavanol category of polyphenols found in fresh tea leaves, are extremely abundant (approximately 30 percent of the dry leaf weight). Flavonoids and their glycosides, chlorogenic acid, gallic acid, coumarylquinic acid, and theogallin are among the other polyphenols found. Phenolic compounds have a higher antioxidant potential. Hence, they are excellent reducers of metal ions which highly favor the green synthesis of nanoparticles (Senthilkumar and Sivakumar, 2014).

The simple tea leaf broth, as well as the one containing the ethyl acetate extract of tea leaves, was shown to be highly effective in forming stable Au and Ag nanoparticles quickly (Begum *et al.,* 2009). Three different tea extracts, including green, oolong, and black teas, were used to make iron nanoparticles (Fe NPs) among which FeNPs synthesized using green tea extracts was reported to be the best method for decomposing malachite green (MG). This was due to a higher concentration of caffeine/polyphenols in green tea that function as both reducing and capping agents during the formation of Fe NPs (Huang *et al.,* 2014).

The green synthesis of nanoparticles of the noble metals gold (Au) and silver (Ag) and their antimicrobial applications have been studied extensively. However, very few works have been reported on other metal oxide nanoparticles like FeO_2, TiO_2, CuO, AlO, MgO, ZnO (Senthilkumar and Sivakumar, 2014). Silver nanoparticles (AgNPs) are widely used in biomedical sectors because of their powerful antibacterial effect. The biogenic nanoparticles antibacterial activity was demonstrated against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica,* and *Pseudomonas aeruginosa* out of which *Salmonella* enterica was found to be most sensitive against the synthesized AgNPs *(Rolim et al., 2019).*

Because of its antibacterial, UV-blocking, high catalytic, and photochemical capabilities, zinc oxide (ZnO) nanoparticles have gotten a lot of attention (Meruvu *et al.,* 2011). According to Sharma *et al.* (2010), ZnO nanoparticles (ZnO NPs) have antibacterial and antifungal properties even at low concentrations, making them appropriate for thin coating applications. Raghupathi *et al.* (2011) examined the antibacterial action of ZnO NPs and developed antibacterial agents to suppress bacterial infections against a wide spectrum of pathogens. In comparison to manufactured medicines, the antibacterial activity of ZnO NPs synthesized using green tea was better and comparable (Senthilkumar and Sivakumar, 2014).

2.11. Tea in Bioinformatics

Comprehensive codon and amino acid usage profiling have been executed proficiently with the accessibility of a huge number of plant genomes. In plants, an overall length of the coding sequences, level of gene expression, etc., have been reported to be decisive in governing codon usage (Sablok *et al.,* 2011). In *Arabidopsis thaliana* the correlation between gene expression and codon usage signified a distinct impact of tissue-specific gene expressivity to be an imperative factor influencing codon usage bias (Duret and Mouchiroud, 1999). Analyzing expressed sequence tags of *Citrus* species, translational selection, mutational bias, and gene length were found to influence codon usage variation and it was also concluded that positive selection governs codon usage (Xu *et al.,* 2013). Sablok and colleagues (Sablok *et al.,* 2011) in their study of complete chloroplast genomes of species of pooid grass family reported mutational bias to play a major role in codon biology and also showed hydrophobicity and aromaticity of encoded proteins of each species to be other factors of codon bias. Examination of 207 plant genes subdivided into 53 monocot and 154 dicot genes, suggested codon usage to vary between taxonomic groups mainly due to varying use of G+C content at the degenerate third base (Murray *et al.,* 1989).

In a previous study (Zhao *et al.,* 2011), the coding sequences of 134 proteins from the tea plant (*C. sinensis*) were analyzed using the CodonW and CUSP programs, and the frequency of codon usage that encoded amino acids was calculated and compared to four classes of representative organisms: *Homo sapiens* (human), *Saccharomyces cerevisiae* (yeast), *Drosophila melanogaster* (fruit fly) and *Escherichia coli* (bacteria). The protein-coding sequences of cp genes were investigated (Yengkhom *et al.,* 2019) using bioinformatic techniques to identify the patterns of codon use among the cp genes of three tea groups i.e., *Camellia sinensis* var. *sinensis* (Chinese type), Camellia *sinensis* var. *assamica* (Assam type), *and Camellia pubicosta* (wild tea species).■