# Abstract

"Tea", the worldwide renowned beverage is not only popular as a drink but also as a healthy beverage. The brain-stimulating drink is a popular refreshing cup in every household, street, or corner of this world. The presence of phytochemicals with pharmaceutical importance and health benefits has rendered its popularity among consumers, industrialists, and researchers. Green tea and black tea are the most popular of different types of tea. Lots of research works related to tea has been compiled for decades and it is still being compiled since "tea' not only has a complex genome or a complex taxonomy, it is also a rich reservoir of ailments to various diseases which has been already explored or is yet to be explored.

The main objective of this thesis was centralized in the medicinal and molecular profiling of some elite tea clones of Darjeeling and Dooars. The research topic was framed to explore the uncharted areas of research in tea with special reference to the elite tea clones established for Darjeeling and Dooars.

Germplasm characterization is critical for plant breeders to efficiently use and manage gene banks to promote cultivar improvement and increase crop productivity. When compared to biochemical and molecular markers, morphological descriptors are a significant preliminary and cost-effective characterization of genetic resources. It undoubtedly has numerous drawbacks when subjected to the effects of environmental factors or parameters, resulting in constant fluctuations and enhanced plasticity. Herein, we made an effort to collect or collate the morphological data of the selected tea clones. The data is recorded based on the parameters like standard, yield, quality, suitability, resistance and susceptibility towards various pests and diseases, flavor, etc.

Environmental factors such as plant age and phenology influence morphological features, making the use of such descriptors problematic in the identification and discerning of genetic variation. As a result, molecular markers such as RAPD and ISSR were used to investigate the genome directly since molecular markers are less affected by environmental factors, removing the limits of phenotyping.

We employed a total of 46 different decamer primers to study the genetic diversity of 33 tea accessions. 45 of the

46 RAPD primers tested, resulted in distinct and scorable bands. RAPD screening yielded a total of 807 bands, 4 were monomorphic and the rest 803 were polymorphic. The 45 random primers had a polymorphism percentage of 99.50% and a PIC value of 0.41 on average. The number of polymorphic bands ranged from nine (OPA16) to twenty-six (OPA26) (OPB10). A total of 15 ISSR primers was also employed to screen the genetic differentiation among the 33 tea clones. All of the primers amplified distinct and scorable bands which rendered a total of 298 bands were created, all of which were polymorphic with a PIC value of 0.42 and showed 100 percent polymorphism. The polymorphic bands varied from 14 (UBC808) to 28(UBC808) (UBC825). The Dice coefficient of similarity created a similarity matrix with values ranging from 0.581 to 0.844. The highest similarity at a nodal value of 0.84) was deduced from the dendrogram where the clones like P-1258 and RR4/5, C28 T-246, and C29 TV-19 shared high relatedness. The ISSR primers yielded similar results, with the clones clustering into two primary groups and subgroups, and the dendrogram deduced the highest similarity at a nodal value of 0.82, between the clones like K1/1 and B-15/263. The results of RAPD and ISSR profiling were mostly coordinated by grouping into two major groups and several subgroups. Regardless of the sample source, sampling area, or point of selection or collection, the pattern of sub-grouping does not disclose a distinct pattern of grouping.

Apart from utilizing molecular markers like RAPD and ISSR, no comprehensive attempt was carried out to explore the genetic variations within the chloroplast region of *Camellia sinensis*. Hence, an initiative was undertaken to explore the variation within the matk region employing the barcode primer (matK). We determined the DNA barcode of the tea clones employing the matK (maturase K) gene and investigated the variation within the chloroplast region. A BLAST search of the NCBI revealed 24 clones to be 100% identical to *C. sinensis*. The other remaining clones showed 99.29–99.89% identity with *C. sinensis*. Clones like Thurbo 3 (11125) and Thurbo 9 (11126) showed more similarity with *Camellia mairei* with a percent identity value being 99.87% and 99.57%.

Clones like Thurbo 3 (11125) and Thurbo 9 (11126), on the other hand, exhibited a higher proportion of similarity to other *Camellia* species, with identity values as 99.87% and 99.57% respectively, when compared to 99.61% and 99.29% similarity with

*Camellia sinensis*. The unique cluster in the phylogenetic tree demonstrated the relatedness to other *Camellia species*. Our analysis reports a total of 14 variable sites within the matK region, with nine in the high consensus region and five in the low consensus region. This is the first report of barcode analysis of Indian tea clones, in which we successfully used the single locus matK gene to examine variation within the chloroplast region and came to the conclusion that the matK region is not 100% conserved across *Camellia* species.

Phytochemical research in tea has been primarily focused either on processed or packaged tea. Therefore, we aimed at phytochemical extraction from mature fresh tea leaves utilizing extracting solvents with varied polarity. The sole aim of this particular research was not to study the variation of the phytoconstituents among different clones but to screen the potent solvent for extracting phytochemicals from the underutilized mature tea leaves. Different qualitative, quantitative, and antioxidant assays were used to identify potent solvents. Antimicrobial screening and its validation were carried out utilizing GC-MS and *in silico* methods. Acetone extracts were discovered to be the most effective extraction solvent, followed by methanol and ethanol.

The three solvent extracts, i.e., acetone, methanol, and ethanol consistently proved to be the most effective solvents for extraction of phytochemicals like flavonoid, tannin, steroid, diterpenes, terpenoids, coumarin, cardiac glycoside, saponin, and reducing sugar. However, traces of phytochemicals like tannin, cardiac glycoside, and flavonoid were extracted by the less polar solvents benzene, chloroform, and hexane. In-depth antioxidant profiling revealed decent free radical scavenging activity of varied solvent extracts of the mature tea leaf confirming their potent role against oxidative stress. The *in vitro* free radical scavenging assay through DPPH revealed enhanced antioxidant potentiality of acetone extracts with an average inhibition percentage of 79.86% at 200ug/ml followed by methanol (75.31%), and ethanol (73.90%) when compared alongside the standard ascorbic acid (94.82%). Enhance antioxidant activity of acetone extracts was also evident from ferric reducing power assay, total phenol assay with total phenol quantified as 37.77 mg GAE/g. The highest flavonoid content was recorded as 722.94 mg QE/g in methanolic extract although the average value surpassed in acetone extracts.

The potent extracts showed significant inhibition against *S. aureus* with the

Minimum inhibitory concentrations of 4 mg/ml (acetone extracts) and 8 mg/ml, (methanol extracts) respectively. Probable antimicrobial compounds like phenol, 3, 5-bis (1, 1-dimethyl ethyl), caffeine, and Vitamin E were detected by Gas chromatography-mass spectrometry analysis. With a binding affinity of -7.2 kcal/mol to *S. aureus* DNA gyrase, *in silico* findings confirmed phenol as the most powerful antibacterial compound. The potency of a particular solvent in extracting a certain group of chemicals was thus determined through detailed qualitative and quantitative phytochemical profiling. Out of the other bacterial strains tested, acetone and methanol extract showed increased bioactivity against *S.aureus*. The type of chemicals extracted was determined by GC-MS analysis of extracts. Further *in silico* experiments revealed some intriguing details about phenol's ability to bind to *S. aureus* DNA gyrase.

Apart from molecular and phytochemical aspect unchartered areas of tea research was explored in this study. “Tea”, the reservoir of phytochemicals, especially green tea and black tea has been utilized to synthesize metal nanoparticles via the green route of synthesis. However, no comprehensive attempt has been made to explore the underutilized, other forms of tea. Therefore we aimed to synthesize silver and zinc nanoparticles employing the purple tea extract.

The synthesis of silver nanoparticles (AgNPs) was initially monitored from the change in color from colorless to brown and its characterization further affirmed its synthesis. The reaction mixtures containing different molar concentrations of $AgNO_3$ i.e., 1 mM-10 mM produced Surface Plasmon Resonance (SPR) band ranging from 414 nm - 442 nm. The constant increase in SPR band intensity up to 3mM revealed that the increased concentration of $AgNO_3$ also increased the synthesis of AgNPs. The intensity of the SPR band increased up to 3mM following which it decreased. With an increase in $AgNO_3$ concentration, a red sift towards higher wavelengths was noticed from 418 to 442 nm. The photocatalytic effect aided in the rapid synthesis of AgNPs. The sample kept at 30°C (in the sun) produced a sharper SPR band at 430 nm, whereas the other reaction mixture stored at various temperatures showed a blue shift towards lower wavelengths (304nm). XRD analysis clearly showed the crystalline profile of AgNPs showing major peaks of Silver, at three diffraction angles 38.03°, 44.15°, and 64.40° corresponding to the Ag [111], Ag [002], and Ag [220] respectively. The XRD study also determined the size of the synthesized AgNPs as 38.4

nm. The obtained AgNPs were found to be stable only for 24 hours and after that settled at the bottom due to agglomeration.SEM revealed non-spherical and irregular morphology of synthesized AgNPs with a rough surface with the particles size ranging between 10nm to 40nm in size. The synthesized AgNPs showed antimicrobial properties against *Staphylococcus aureus* and *Bacillus subtilis*.

The highly blue-shifted, maximum absorption peak appearing about 273 nm confirmed the nanoscale production of ZnO product although the highest absorption for bulk ZnO usually occurs around 385 nm. The decrease in absorption could be due to nanoparticle agglomeration and settling in the cuvette. XRD analysis of the sample annealed at 400°C revealed the major peaks [100], [002], and [101] of ZnO occurring at the diffraction angles 31.69º and 34.77º and 36.1º respectively. These diffraction angle values for the ZnO peaks are quite similar to the PDF card number 01-080 -0074, showing that pure ZnO nanoparticles were formed. The samples annealed at 400°C clearly show the creation of ZnO nanoparticles, whereas the samples annealed at 100°C exhibit just the [002] peak with low intensity. The nanoparticle's size was estimated to be on the order of 22.4 nm using XRD analysis. The synthesized ZnO was found to be spherical, with an approximate size of 15-25nm, as indicated by SEM. The synthesized ZnO NPs showed antibacterial activity against all the strains studied with predominant inhibition against *Staphylococcus aureus* and *Bacillus subtilis*.

Bioinformatics study on the nuclear genome revealed *C. sinensis* to be AT-rich, where the preference of AT-rich codons over the GC ones was evident. The total codon usage pattern revealed that AT-rich codons with RSCU > 1 coded for 15 amino acids. 29 of the 61 codons were AT-rich, with 22 codons ending in either Adenine or Thymine. Our research revealed the use of AT-rich codons such as AAG, GAA, GAT, AAT, AAA, GTT, GCT, TTG, CTT, TCT, and ATG.

The AT nucleotide compositional constraint was the predominant factor governing the codon usage pattern of *C. sinensis* as evident from a significantly high and positive correlation of Axis 1 of Relative synonymous codon usage (RSCU) with A3 and T3 indices. The pronounced effect of nucleotide compositional constraint was also evident from GC3 versus Nc plots. Apart from that the GC3 versus Nc plot also deduced the role of mutational bias and

translational selection in dictating the codon usage. The impact of translational selection was further intensified by the RSCU based scatter plots where the potentially highly expressed genes (PHX) and the potentially lowly expressed genes (PLX) clustered separately on the extreme opposite ends on the axis of separation. Gene expression level was the other determinant affecting the codon usage signatures as evident from the correlation. The complementary strand-associated genes clustered together in RSCU-based scatter plots of leading and lagging strand-associated genes, indicating the absence of replication-associated mutational pressure. Amino acid usage revealed the highest use of Leucine (L), Serine (S) and the other amino acids used in higher frequencies were Alanine (A), Lysine (K), Glutamic acid (E), Valine (V), and Glycine (G). Strong and significant correlations of the two major axes of RAAU with hydropathicity index GRAVY and aromaticity of the gene products revealed hydrophobicity and aromaticity to govern the amino acid usage signatures of the *C. sinensis* genome. The role of gene expression level in dictating amino acid usage in amino acid usage in all the *Camellia sinensis* was apparent from the high correlation between expression level (CAI) and the two major axes of separation of genes based on RAAU. A significant positive correlation noted between (CAI) and energy cost in the tea genome ($r = .271$, $p<0.01$) indicated the utilization of less energetically costly amino acids in highly expressed genes.

Lifestyle disorders are gradually taking center stage in the global clinical landscape. Increased stress and poor eating habits have increased the risk of diseases such as type 2 diabetes, anxiety, obesity, cardiovascular issues, and so on. Every disorder leads to a chain of disorders. For instance, obesity could lead to diabetes, polycystic ovary syndrome, a higher level of triglycerides which can further add up to hypertension, etc. This chain of linked disorders could act vice versa. Some of these disorders could be treated naturally with functional meals and drinks. Tea use is rapidly increasing in popularity due to its numerous health advantages. Many works have been accomplished in tea as such with specific reference to green tea and black tea. So, the sole aim was to explore the unchartered area of polypharmacology. Purple tea (*Camellia sinensis* var. kitamura) has recently sparked interest as a functional beverage due to its high antioxidant content. To fully escalate the potential of Purple tea (PT), reverse

pharmacology and a target-fishing technique were used. The findings from target-fishing studies have shown PT to be effective against numerous lifestyles disorders such as type 2 diabetes, hyperlipidemia, and coronary heart disease, as well as being important in the treatment of cancer-related complications. Through gene ontology and pathway enrichment analysis, an attempt has also been made to elucidate the mechanism of action of PT against various disorders. It was found to target pathways such as ErbB signaling, Natural killer cell-mediated cytotoxicity, Neurotrophin signaling, PPAR signaling, Insulin signaling, Ras signaling, and others. As a result, we can speculate that PT consumption may protect us from a variety of lifestyle diseases. Additionally, similar studies conducted in under-utilized mature leaf (ML) of tea were mainly found to target the PPAR signaling pathway. ML probably could be effective against various diseases like Type 2 diabetes, obesity, polycystic ovary syndrome, higher level of plasma HDL cholesterol (hypercholesterolemia), high level of triglycerides, Alzheimer's disease, atherosclerosis, insulin resistance, metabolic syndrome, cardiovascular and coronary heart diseases, ovarian cancer, fatty liver, anxiety disorder, hypertension.

Conclusively, this work touches the unexplored multidimensional areas of tea research with special reference to the clones established for Darjeeling and Dooars in a hope that this present endeavor would pave its path for future research for the management, development, and utilization of the most popular health drink.