

Effect of UV Radiation on Therapeutic and Commercial Values of Tea

**THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY (SCIENCE) IN MICROBIOLOGY**



By

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*This thesis is dedicated to my
parents, friends, and relatives, who
have been the pillars of my life
through thick and thin.*

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Abstract

The natural UV radiation varies among seven valleys of Darjeeling (Darjeeling East, Darjeeling West, Kurseong South, Kurseong North, Mirik, Upper Fagu and Teesta Valley) which differs according to the solar zenith angel. There is variation of natural UV irradiance at different altitudes as well as at different seasons (1st flush-March-April, 2nd flush-May-July) of tea harvest at different valleys respectively. Among all the valleys, Kurseong South valley receives maximum UV irradiance during 2nd flush of tea harvest specifically for the highest altitude Castleton tea garden giving rise to best tea with higher qualities (commercial and therapeutic). Assessment of Physiological parameters of tea leaves collected from different gardens of Darjeeling valleys including UV absorbing compounds, Photosynthetic ability corroborates with the natural UV irradiance significantly indicating variation among seven valleys, at different altitude, at different seasons. UV radiation is not only creating oxidative stress here in tea plants of different valleys at different altitudes but also upregulating the secondary active metabolites like flavonoids like catechins, tannins, rutin (flavonoid glycoside) and quercetin attributed towards antioxidant and other quality parameters. No variation was found with enzymatic antioxidants like superoxide dismutase, catalase. Biochemical Characterization of non-enzymatic antioxidants with bioinstrumentation technique like FTIR, Spectrophotometric scan, Spectrofluorimetric scan, TLC, HPLC, LC-MS, GC reveals the upregulation of polyphenols, flavonoid compounds like rutin, Quercetin, epigallocatechin gallate. The variation was prominent among seven valleys of different altitude at different seasons, where the upregulated compounds could be correlated with the corresponding natural UV irradiance. These upregulated compounds can attribute towards the commercial and therapeutic qualities of seven valleys Darjeeling tea. The higher commercial qualities like aroma and colour is correlated with higher UV irradiance in high-altitude grown Kurseong South 2nd flush manufactured tea. That's also the same for therapeutic potential (antioxidant, antimicrobial, anticancer, anti-diabetic, anti-

inflammatory potential) of Kurseong South valley grown 2nd flush tea of high altitude Castleton garden. The antioxidant potential can be statistically correlated with the E-sensor based (E-nose and E-tongue based) indices to development of Therapeutic Index further. Thus UV index (higher UV irradiance) is now the cause to develop higher therapeutic index in high altitude grown Darjeeling tea.

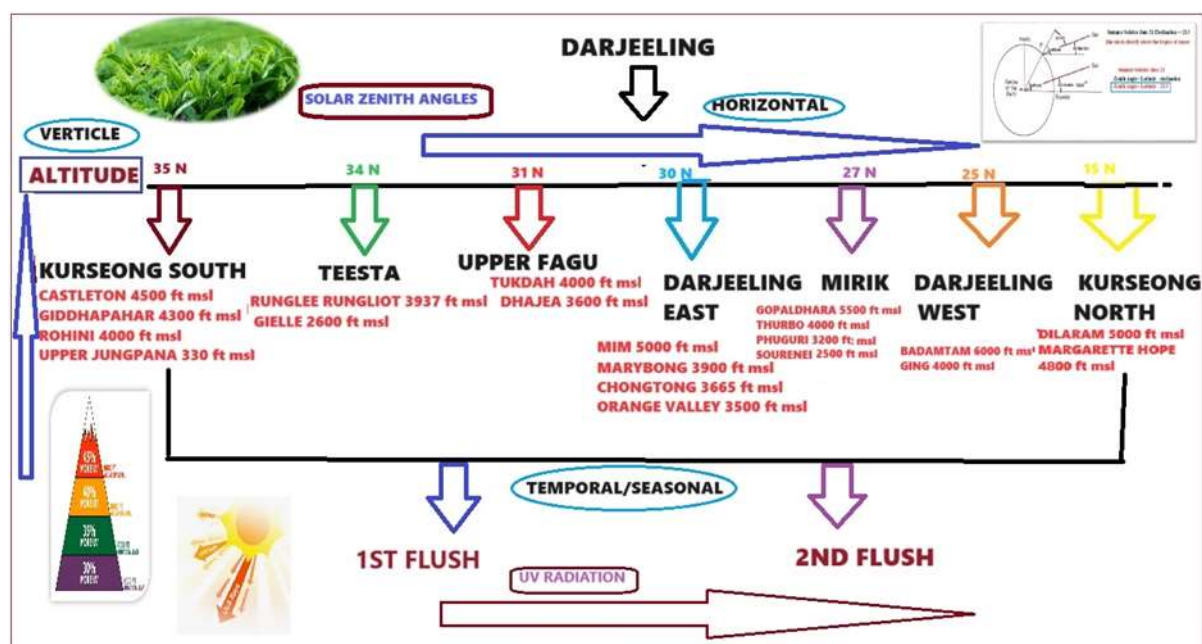
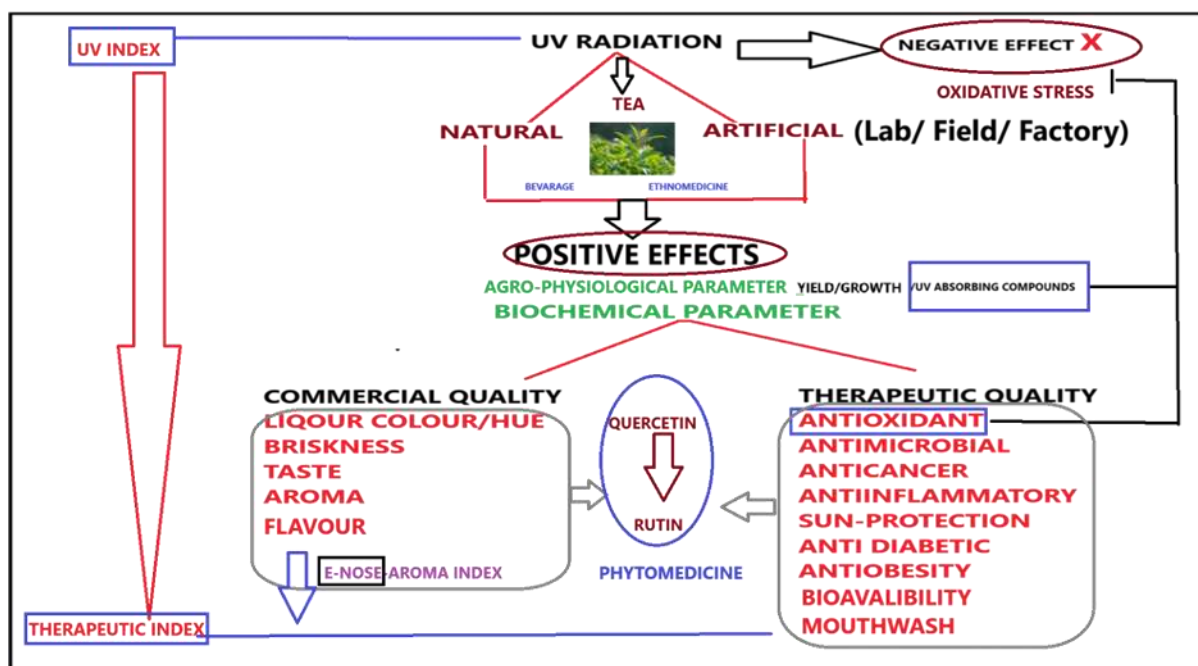
Change in Physiological and Biochemical parameters (commercial and therapeutic potentials) of tea leaves under artificial UV doses in laboratory in one year old potted plants were observed, where the potentials are proportional to the UV doses. Characterization and isolation of active secondary metabolite flavonoid glycoside rutin was mixed with gold nano particle to increase the therapeutic efficiency, which calls for better drug designing.

Change in therapeutic and commercial values of tea during post-harvest manufacturing in laboratory and factory with artificial UV exposure calls for the industrial importance of Indian tea clones. Biochemical (commercial and therapeutic potential) characterization of four differentially manufactured tea (White, Green, Oolong and Black) reveals the variation of active components like rutin, quercetin, epigallocatechin gallate, which might be responsible for differential changes in Physico-chemical, biochemical and therapeutic parameters of under normal ambient UV exposure in aluminium with silica packaging during storage.

Biochemical Characterization (commercial and therapeutic potentials) of different herbal and medicinal tea and correlating the value with E-nose and E-tongue (Nor aroma index and Taster's score) develops therapeutic index for medicinal and herbal tea.

This thesis aims to focus on the quality enhancement of tea at field, laboratory and factory by correlating with the natural or artificial dose of UV radiation.

GRAPHICAL ABSTRACT



Chapter 1

INTRODUCTION

CHAPTER 1

INTRODUCTION

1.1. Background: Why is UV radiation taken as a subject in this thesis?

1.1.1 Global climate change and UV radiation: *Increased level of ambient solar UV (Ultraviolet) radiation—a global threat*

The global trend of depletion of stratospheric ozone resulting from a major enhancement in man-made pollutant chemicals viz. chlorofluorocarbon (CFC) (Western et al., 2024) methyl bromide, hydrobromofluorocarbons (Chipperfield and Bekki, 2023), massive industrialization and accumulation of greenhouse gases like carbon-di-oxide, methane, nitrous oxide (Filonchik et al., 2024) rapid deforestation (Buchadas et al., 2022), burning of fossil fuels (Perera, 2017) and certain agricultural practices like slash and burn agriculture (Bezerra et al., 2024) has increased the penetration of spontaneous solar Ultraviolet (UV) irradiance (through depleted ozone holes of the atmosphere) on the earth's surface (Barnes et al., 2019). Depletion of the protective ozone layer beyond the critical level by atmospheric pollutants that interact photochemically with ozone will promote specifically the transmission of highly injurious UV radiation (Neale et al., 2021). Ultraviolet radiation on the whole, consists of a large range of wavelengths, varying in damage-causing potential: UV-C (280nm-200nm), though damage causing component, is eliminated/filtered by the stratosphere (Tang et al., 2024); UV-A (320nm-400nm) is the least harmful for living organism and slightly affected by the ozone layer; (UV-B (280-320nm) is the most harmful irradiance on earth being the most biologically absorbed and damaging component (Tang et al., 2024). Records have been taken over the years that indicate a significant increase in UV-B irradiance on Earth. (Chatzistergos et al., 2023). The level of spontaneous natural UV radiation on Earth depends on some factors, including cloud cover and aerosols (Sulzberger

et al.,2019), the most prominent and consistent of these being latitude and altitude (Kera et al.,2024)

1.1.2 Factors affecting UV radiation:

The geographical and climatic factors affect UV radiation

Geographical factors include latitude and longitude. Other environmental factors include cloud cover, ozone layer, humidity, rainfall, sunshine hours and slope.

- *The latitude* that determines the zenith angle of the sun rays striking a location is a major factor in determining the extent of UV irradiance at a given location. Consequently, UV irradiance was recorded as high particularly at high- and mid-latitudes (Eleftheratos et al.,2014) i.e., between 15-35° latitude in both the Southern and Northern hemispheres during their respective summer months.
- *An altitude-related* increase in UV intensity occurs due to rarefaction of the atmosphere that absorbs the damaging UV rays (Schmucki et al.,2002). An increase in altitude (that increases air rarefaction) by 1000m has been recorded to enhance UV irradiance by 6-8% (Schmucki et al.,2002)

1.2 Electromagnetic properties and types of UV Radiation:

UV radiation (100-400nm) is about 4.4% of the total electromagnetic spectrum. The electromagnetic spectrum is vast and UV radiation falls under a relatively small portion of it. UVA and UVB are the most active components of solar radiation. Solar ultraviolet (UV) radiation has photons with energy ranging from 3.1 to 12 electron volts (eV). This is close to the minimum energy needed to ionize atoms

1.2.1 1. Natural UV radiation:

Natural UV radiation refers to the ultraviolet radiation emitted from the sun. UV radiation is composed of three components: UVA which has a wavelength (λ) from 320 to 400 nm, UVB has a wavelength from 280 to 320 nm and UVC has a wavelength from 100 to 280 nm. UVC radiation is mostly absorbed by the ozone layer. UVA and UVB rays reach the Earth's surface in sufficient quantities. (Gromkowska-Kępa et al.,2021). Among UVA, UVB, and UVC, UVB radiation is the most effective one which has a significant impact on the biological systems. UVA penetrates deep into the skin, causing aging, skin cancer, and other related diseases. UVB is primarily responsible for sunburn and vitamin D production (Tang *et al.*, 2024). UVC is absorbed by Earth's ozone layer.

1.2.2 Artificial UV radiation:

Artificial UV radiation refers to man-made sources of ultraviolet radiation, which is useful for various purposes, including disinfection and sterilization of different systems to eliminate microorganisms from the systems. Drying of inks and paint coatings for complete adherence to the substrate. Artificial UV radiation is also used in medical treatments (e.g., phototherapy for jaundice) Food processing and preservation of food to increase its shelf-life air and water purification. Scientific research (e.g., spectroscopy), Horticulture and plant growth (Sarkisyan et al.,2022). Different sources of UV radiation include different UV lamps (mercury vapor, LED, and fluorescent) of different UV fluence (in KJ/mol). UV LEDs are light-emitting diodes, Excimer lamps, Pulsed UV lamps, and UV lasers. They have different characteristics: with different wavelengths (varying from 100-400 nm). Intensity is variable according to the source, the spectrum is also broad or narrow depending on the source.

1.3 Effects of UV radiation on various life forms:

UV radiation has a great impact on the biological system. UV rays are not only responsible for photoaging, and sunburn but also have some beneficial effects. UV radiation can be used to treat many diseases like solid tumors and inflammatory diseases (Tang et al, 2024). UV radiation helps all living forms by helping in photosynthesis which is not possible without it. UV radiation shows both positive and negative effects on the various life forms.

Spontaneous/ambient solar UV irradiation causes bio-molecular damage either by--

- (a) direct hits causing linear bond breakage in bio-molecules and membranes (Sugiyama T et al., 2021) or by
- (b) oxidative stress causes cell damage via the production of UV-related reactive oxygen species (ROS) (de Jager TL et al., 2021).

Since UV-C is least penetrating through the ozone layer (Umar et al.,2022) and thus not relevant in causing cellular damage, most studies on ambient UV stress tolerance relate to UV-B and UV-A caused damage in living organisms (Goutam et al.,2022). Of these two, the low-energy waves of UV-A are not very effective in causing significant bio-molecular damage; UV-A radiation is thus not of much importance in the study of damage due to UV radiation (Çavuşoğlu et al.,2022). UV-B radiation that is absorbed by both animal and plant systems (Ir Golovatskaya I F et al.,2023) causes adverse effects including oxidative stress in animals (Roy S 2023) as a well-documented carcinogen (Vanhaelewyn et al.,2020) and in plants constitutes a study of major importance. Apart from the damaging effect, UV radiation can initiate a signalling pathway to combat oxidative injury in the living system (Tang X et al., 2024).

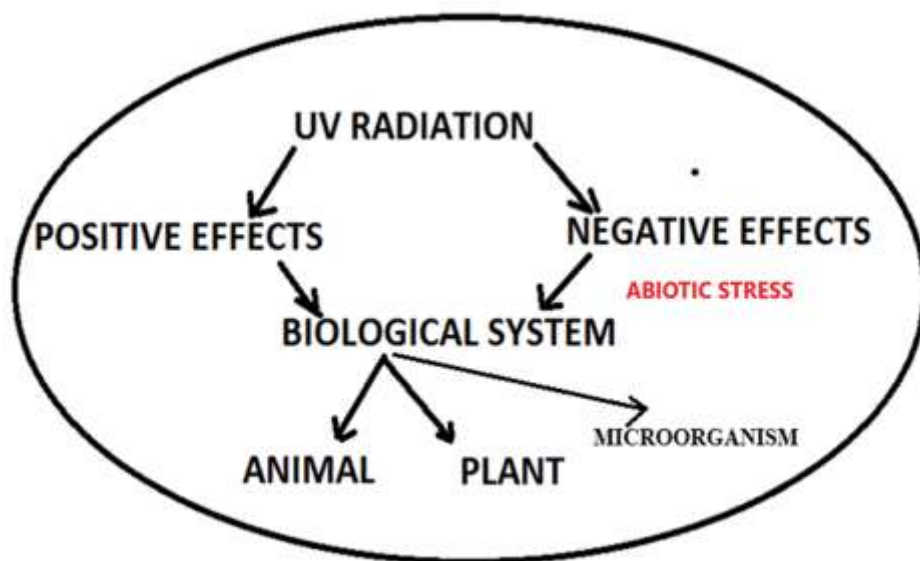


Figure 1.1: Effect of UV radiation on Biological System

1.3.1 Negative effects of UV radiation:

UV radiation can show various negative effects on plant, animal, and microbial systems.

1.3.1 Negative effects of UV radiation on Plant System

UV radiation reduces plant growth and productivity (Rai et al.,2023). UV radiation increases plant susceptibility to pathogens (Murali et al., 2017). UV radiation harms aquatic organisms, particularly phytoplankton (Häder DP et al., 2020). UV radiation disrupts aquatic food chains, impacting ecosystem balance (Mostofa et al., 2019).

Excess exposure to UV radiation can reduce plant growth and biomass production (Ayesha S et al.,2023). UV radiation also causes leaf damage, reducing photosynthetic area (Murali et al., 2017). Decreased photosynthesis affects the plant growth and metabolism. Too much UV radiation can also cause stem elongation, reducing plant stability (Golovatskaya IF et al.,2023). UV radiation decreases photosynthetic rate, reducing plant productivity (Ayesha S et al.,2023)

UV radiation damages photosynthetic pigments thus inhibiting photosynthesis. (Liu et al., 2017). UV radiation induces DNA damage, disrupting plant development (Lingwan Et al.,2024).UV radiation generates reactive oxygen species (ROS), causing oxidative stress (de Jager TL et al .,2017). UV radiation changes hormonal balance, affecting plant growth and development (Mieke de Wit. et al.,2016)

9. Increased exposure to UV radiation weakens plant defense against pathogens (Mmbando, G S et al.,2024). UV radiation increases drought sensitivity, reducing plant water use efficiency (Murali et al., 2017). UV radiation also decreases crop yield due to reduced photosynthesis and increased stress (Liu et al., 2017).

1.3.2 Negative effects of UV radiation on animal Systems

UV radiation increases the risk of neurodegenerative diseases, such as Alzheimer's (Sutherland et al., 2018). UV radiation affects mood and cognitive function (Lam et al., 2015). UV radiation reduces fertility and reproductive success (Feyerabend et al., 2017). UV radiation increases the risk of birth defects and developmental disorders (Kim et al., 2017). UV radiation causes DNA damage, leading to mutations and cancer (Britt, 2004). UV radiation induces to formation of pyrimidine dimers, disrupting DNA replication (Tamura et al., 2018). This whole central dogma is hampered by UV radiation. UV radiation also accelerates the risk of skin cancer. Such as melanoma (ICNIRP, 2018). UV radiation promotes photoaging, leading to wrinkles and age spots (Ortiz AA et al., 2014). UV radiation increases cataract risk (WHO, 2018). UV radiation causes photokeratitis which leads to eye pain and ultimately vision loss (Gao et al., 2017).UV radiation suppresses immune responses, increasing infection risk (Schwarz et al., 2018). UV radiation alters cytokine production, disrupting immune homeostasis (Moyal et al., 2018).

1.3.3 Negative effects of UV radiation on the Microbial System

Almazyad (2022) described the effects of UV radiation of different wavelengths on different kinds of microbes causing various cellular molecular damages including DNA causing inhibition of transcription,

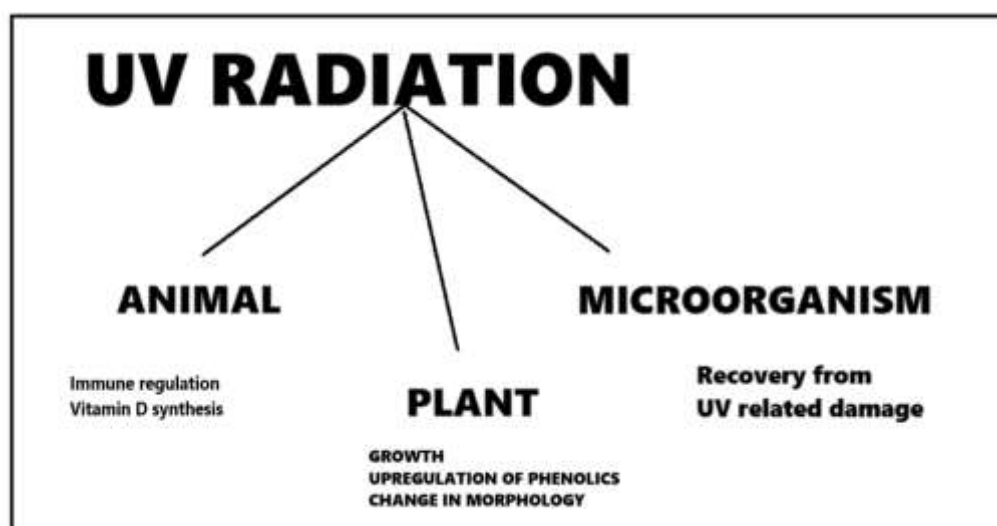


Figure 1.2: Negative effects of UV radiation on various life forms

1.3.4 Positive effects of UV radiation on the biological system:

Along with the positive effects UV radiation can thus show positive effects on various living systems

1.3.2.1 Positive effects of UV radiation on Plant system

Photosynthesis: UV radiation is the only source of energy during photosynthesis, essential for plant growth. UV radiation activates chlorophyll pigment in the leaves of the plants to synthesize food during photosynthesis. (Kataria S et al., 2014). Flavonoid synthesis: UV radiation enhances the production of secondary metabolites such as flavonoids, activating the plant defense mechanism (Lingwan M et al., 2023). Growth regulation: UV radiation also influences plant morphology, promoting optimal growth (Prisca Meyer P et al., 2021). UV

radiation regulates the morphology of plants by stem elongation inhibition that is UV radiation reduces stem elongation, promoting compact growth (Dai Y et al., 2024). It also decreases leaf area, increasing water use efficiency by decreasing transpiration or excessive loss of water from plants. (Murali et al., 2017). UV radiation also stimulates root growth, improving nutrient uptake (Mmbando, G. S. et al., 2024). UV radiation upregulates photosynthetic rate, improving plant productivity UV radiation also regulates photosynthetic pigment composition, optimizing light harvesting property. (Liu et al., 2017). UV radiation induces flavonoid synthesis, enhancing plant defense, and has a potent antioxidant capacity (Miao N et al., 2022). UV radiation also stimulates phenolic compound production, activating plant resistance to pathogens (Murali et al., 2017). UV radiation increases vitamin C and E levels, enhancing plant antioxidant capacity which is one of the major defense systems of plants (Liu et al., 2017). UV radiation regulates auxin distribution, controlling plant growth and development radiation helps in the stimulation of ethylene production, promoting plant defense responses (Lingwan M et al., 2023). UV radiation enhances drought tolerance by regulating stomatal conductance (Murali et al., 2017). UV radiation induces pathogen resistance by stimulating defense-related gene expression (Tsurumoto, T et al., 2022). It improves crop yield by enhancing photosynthetic rate and reducing disease susceptibility (Liu et al., 2017). UV radiation also enhances fruit quality by regulating sugar and acid metabolism (Murali et al., 2017).

1.3.2.2 Positive effects of UV radiation on the animal system

Vitamin D synthesis: UVB radiation initiates vitamin D production, which strengthens bone development, and enhances the immune system (Bikle DD et al., 2021). Circadian rhythm regulation: UV radiation helps to regulate biological clocks (Vandewalle et al., 2013).

Immune system modulation: UV radiation also regulates immune responses, thus preventing autoimmune diseases (Schwarz et al., 2018).

1.3.2.3 Positive effects of UV radiation on the microbial system:

Disinfection: UV radiation kills bacteria, viruses, and fungi, preventing infections (Enwemeka C S et al., 2021). Antibiotic resistance prevention: UV radiation reduces antibiotic resistance development (Mckenzie, 2018).

1.3.3 Application of UV radiation on various fields

UV radiation thus shows benefits for human health ecological aspects and therapeutic applications

1.3.3.1 Application of UV Radiation to Human Health

1. Mood improvement: UV radiation boosts serotonin levels, soothing anxiety attacks and depression (Lam et al., 2015). Skin health: Skin conditions like psoriasis are improved by little exposure to UV radiation. Cancer prevention: UV radiation induces DNA damage, triggering cancer-preventing mechanisms (Schwarz et al., 2018).

1.3.3.2 The positive impacts of UV radiation on the ecological benefits

Water purification: UV radiation helps in the elimination of waterborne pathogens (Adeniyi, A. O et al.,2024). Air quality improvement: UV radiation decomposes pollutants (Chhaya V. Rekhate et al.,2020). Soil health: UV radiation controls soil microbial communities (Silva I et al.,2024)

1.3.3.3 Therapeutic applications of UV radiation:

Phototherapy: UV radiation cures conditions like jaundice and seasonal affective disorders (Wilson B D et al.,2012).UV radiation combined with psoralen helps in the treatment of skin diseases (Schwarz et al., 2018).

1.4 Mode of action UV Radiation-Induced Damage causing oxidative stress at cell-molecular level: combat with antioxidant strategies

UV radiation can show its effects on the cell molecular level either directly hitting the linear bonds of DNA, protein, or fatty acids of lipids or can show the effects by production of reactive oxygen species (ROS) mediated oxidative stress-related damages.

1.4.1 DNA Damage:

UV induces Cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone (6-4) photoproducts (PPs) formation which damages DNA affecting central dogma. (Steurer B et al.,2019). UV-induced DNA damage leads to mutations, deletions, and chromosomal aberrations. UV-induced DNA damage and mutations contribute to skin cancer development (Pfeifer GP et al.,2020)

1.4.2 Protein Damage

UV radiation forms reactive oxygen species (ROS) which results in protein carbonylation. Protein carbonylation causes disruptive proteins whose expression leads to several diseases. (Davies et al., 2016). Not only does oxidation of proteins UV radiation also induces proteolysis and aggregation (Kultz et al., 2017).

1.4.3 Lipid Damage:

Formation of lipid radicals gives rise to oxidative stress ultimately resulting in lipid peroxidation by UV radiation (Girotti et al., 2015). Lipid disruption causes changes in membrane fluidity and permeability ultimately damaging cell integrity. (Wagner et al., 2016).

1.4.4 Cellular Responses

UV also induces apoptosis which is known as programmed cell death. (Gary AS et al., 2020). UV causes cytokine release but too much cytokine release stresses the immune system causing a cytokine storm. (Bernard, J. J et al., 2019). UV also causes cellular aging and senescence (Martic I et al., 2020). UV also induces damage leading to photoaging and skin wrinkles. (Sharma P et al., 2024). UV-induced immune suppression increases infection risk (Bernard, J.J., 2019).

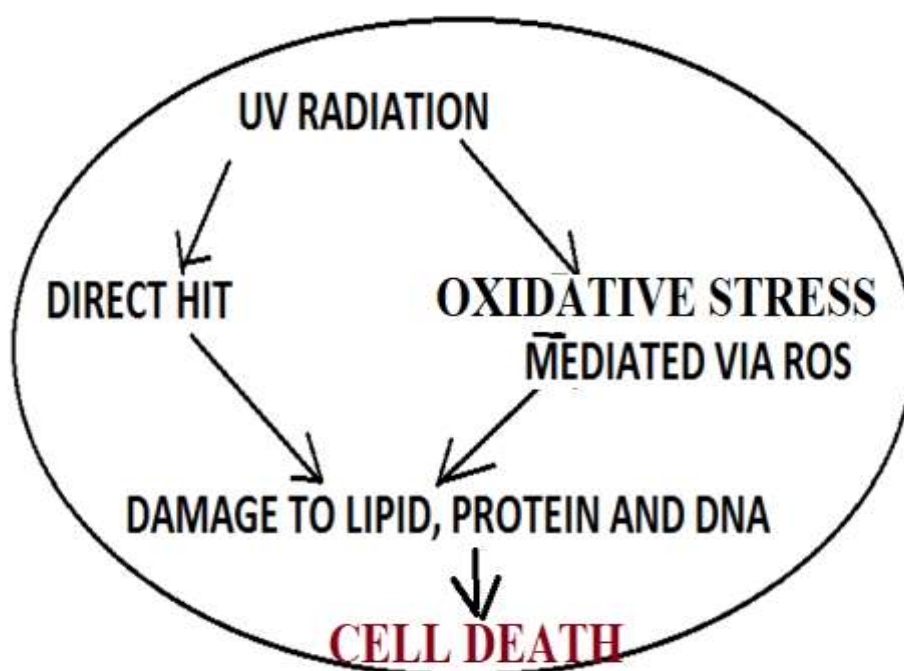


Figure 1.3: Effects of UV radiation on cell molecular system

1.4.5 UV radiation: Abiotic oxidative stress factor in plants: Mechanism of action

Plants, enduring prolonged periods of sunshine that include UV radiation provide particularly interesting material for the study of

(a) incidents of UV caused direct hit damage of linear bonds of bio-molecules and

(b) oxidative stress (the stress caused by ambient solar UV radiation by the generation of free radicals is referred to as photo-oxidative stress-(Borges CV et al., 2023)

(a) UV caused direct hit-related damage of linear bonds of bio-molecules:

The high energy content of UV radiation causes bio-molecular damage by direct impact on most of the linear bonds of bio-molecules in living cells including plant systems (Masumai R et al., 2013) It is pertinent to mention here that for survival on earth (particularly under high ambient UV radiation) plants have evolved protective compounds viz. flavonoids that by their structural stability under high energy serve as UV screens for protection of other cellular bio-molecules (Jadidi, M et al., 2023).

(b) UV caused oxidative stress in plants:

The use of sunlight for photosynthesis and consequent exposure to the ultraviolet portion of solar radiation add to the predicament of oxidative damage caused by UV radiation. Spontaneous natural UV irradiance in the subtropical region at times of the equinox provides an appropriate environment for setting up experiments for understanding the UV effect on plants (Craig W et al., 2014). The effect of ambient UV radiation on plants including oxidative stress was demonstrated by Jadidi, M 2023 working on *Pelargonium graveolens*. So direct hits of UV radiation on biomolecules along with UV-caused oxidative injury are particularly relevant for the study of spontaneous/natural/ambient UV radiation-related effects (damage and concomitant protection) on plant systems.

1.5 Plant Survival Under Natural UV Radiation on Earth: UV Adaptive/UV-Adapted

Trait: UV Acclimation Strategy in Plants:

Plants have adapted many strategies to combat the effects of harmful UV radiation. They have evolved various ways to acclimate to UV radiation which causes DNA, protein damage, and unnecessary oxidative stress to cellular components. Plants adopt some strategies like thickening cuticle which reduces UV penetration. Waxy coatings on leaves reflect UV radiation (Molina I et al., 2023). Trichomes are the hair-like structures on leaves that reduce UV transmission (Wang, X et al., 2021). Leaf folding also reduces UV exposure (Quan M et al., 2021). The thicker bark of the plant protects inner tissues from overexposure to UV radiation (Kumar et al., 2015). Stem hairs reduce UV transmission (Li et al., 2017). Deeper roots reduce UV exposure (Huang et al., 2017). Root hairs increase water uptake, reducing UV stress (Zhang et al., 2018). Petals with UV-reflecting pigments reduce UV radiation (Schulte A N et al., 2019). Flowers orientation are also adapted by plants to avoid direct UV radiation (Creux et al., 2021). Plants to protect themselves from UV radiation produces anthocyanins (pigmentation compounds) act as UV-protective compounds. (Kaur S et al., 2023). UV radiation causes oxidative stress by giving rise to reactive oxygen species thus flavonoids are produced by the plants to protect against UV-induced oxidative stress (Jakubczyk K et al., 2020). The acclimation includes UV stress tolerance as avoidance or migration called phototactic effects, and upregulation of antioxidant enzymes including non-enzymatic and enzymatic antioxidants. The enzymatic antioxidants have linear bonds that are being attacked by UV radiation having high energy content but nonenzymatic antioxidants like flavonoids due to the structure-activity (SAR) relationship show the UV absorption and acclimation strategy. The UV acclimation in plant systems includes the activation of the repair system and programmed cell death, UV-mediated signal transduction pathway, and resynthesis of the expression of new enzymes by gene x environment interaction. Plants have stationary

lifestyles so they must have robust UV adaptive strategies. All these include UV adaptive strategies for plants under natural UV radiation. For survival under natural challenges of ambient UV radiation (including oxidative stress), living cells have developed survival strategies (tolerance strategy) that depend on UV screening efficiency as well as antioxidant potential in plants.

For survival under spontaneous UV stress (for tolerance of this spontaneous UV stress) on earth, plant systems maintain multiple types of **survival strategies** that may be divided into two major categories: Cell protection/survival strategies constitute **macromolecular repair/turnover** by the help of repair enzymes that provide an important survival strategy (tolerance strategy) in living organisms. This is true for hydrated systems as in leaf of plants at different altitudes. But under conditions of reduced activity of repair enzymes such as in dehydrated embryonic axes of mature (orthodox) seed (Ebene L A et al., 2019) they are gradually rendered 'dead' i.e. they become non-viable, if not protected by other survival strategies.

Antioxidants---Oxidative stress-related cell damages occur when the generation of ROS in Having a stationary lifestyle and using sunlight for autotrophy through photosynthesis, plants are exposed to extensive ravages of ambient solar radiation that includes UV radiation (Jager et al 2017). Today's enhancement in UV radiation in earth's atmosphere and associated global climate change thus calls for developing a comprehensive understanding of the bio-molecular events for cell destruction (Goutam et al.,2022) and inherent cell protective/tolerance mechanisms with an aim at undertaking appropriate manipulation of relevant traits in plants (Barnes et al.,2023). This could help to develop plants that would be better suited to survive under extensive/enhanced UV radiation on earth, Mmbando GS et al.,2024 have demonstrated the beneficial effects of UV radiation on field-grown plants. It is pertinent to mention here that ambient/spontaneous UV radiation acts as a signal in the seed system on the mother plant in the field (during maturation-associated dehydration stress) to trigger up

synthesis and accumulation of secondary metabolite viz. flavonoids (Golovatskaya et al.,2023) which in turn help to protect against the ravages of oxidative injury (may also be caused by ambient UV radiation along with heat and humidity) by acting as UV screen as well as an undegraded antioxidant during 'dry' post-harvest storage.

1.5.1 UV avoidance strategy in plants:

Phototropins are proteins that control chloroplast movement in response to ultraviolet and blue light. This is called phototropin-mediated chloroplast movement. UV-B light can directly induce chloroplast movement through phototropins. However, at higher intensities, UV-B can also indirectly affect chloroplast movement by disrupting the actin cytoskeleton, which moves the chloroplasts (Hermanwicz et al., 2019).

1.5.2. UV upregulation of Antioxidant compounds in plants: UV absorbing compounds

It was stated the activity of antioxidant enzymes SOD, APO, guaiacol peroxidase, and GR are enhanced in cucumber cotyledons in response to UV radiation. Activation of antioxidant enzymes by UV radiation has earlier been observed in *Arabidopsis thaliana*, wheat, and cucumber (Vladimir D et al.,2021). The enzymatic antioxidants are the target of the direct and indirect damages incurred by UV radiation having low energy linear bonds but due to the structure-activity relationship, the flavonoid group of compounds having Π to Π^* transition of electrons and phenol quinone tautomerism can act as UV shield and effective antioxidant compounds. One of the varieties of plant UV responses is the synthesis of UV-absorbing flavonoids which constitute an effective non-enzymatic mechanism to reduce the photo inhibitory and photooxidative damage caused by UV stress, either reducing the penetration of incident UV radiation or act as quenchers of reactive oxygen species (ROS) (Valle et al., 2020).

Antioxidants are a system that exceeds the system's ability as antioxidants to neutralize and eliminate them. Antioxidants (both enzymatic and non-enzymatic) are the basic cell protective agents that hold reduced ROS-related bio-molecular damages (Irato P et al.,2021) which are other types of survival strategies against oxidative stress in plants.

Antioxidants are mainly of two types—(i)enzymatic and (ii) non-enzymatic

(i) Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) ((Jomova K et al.,2024). It is pertinent to mention here that activities of enzymatic antioxidants however remain precluded under low moisture content of dry quiescent embryonic axes of orthodox seeds (in plant system) during storage in the natural environment (viz. farmers' seed storage). For survival of seed (i.e. maintenance of seed viability during post-harvest storage of mature (dry) seed, non-enzymatic antioxidants are particularly relevant.

(ii) Non-enzymatic antioxidants include flavonoids, glutathione, and ascorbic acid ((Tahir H et al.,2024). Glutathione and ascorbic acid both having linear bonds may be susceptible to the direct hit of spontaneous UV radiation or oxidative injury and thus cannot be operative during post-harvest dry storage in embryonic axes under normal ambience. Of these, flavonoids only have a structure-activity relationship that remains undegraded by direct hit of spontaneous UV irradiance as well as by oxidative injury.

UV radiation is one of the climatic abiotic factors to cause the generation of oxidative stress in plants causing damage to cell molecules and ultimately causing death and senescence. Oxidative stress can cause lipid peroxidation including the fatty acid oxidation chain initiation, and elongation. The termination step can only be achieved by the presence of an antioxidative system of plants.

Mechanism of action of flavonoids as well as interrelation with UV irradiance:

(i) ACT AS UV SCREEN BY ABSORBING UV RADIATION:

The Flavonoid group of secondary metabolite compounds by having λ_{max} (absorption maxima) at ~UV-B range (i.e. 280-320nm) are capable of UV absorption and can act as UV screen (Lingwan M et al.,2023)

(ii) STRUCTURAL INTEGRITY AGAINST AMBIENT UV IRRADIANCE:

Having a conjugated π electron system in the core nucleus that allows $\pi \leftrightarrow \pi^*$ transition for attenuating higher energy absorbed from UV radiation helps them to be undegraded during UV irradiance-related damage (viz. during dry post-harvest storage). It is pertinent to mention here that the process of absorbing UV radiation and neutralization the high energy via structural attribute is referred to as the photo-oxidative cyclization of flavonoids (Chouhan et al.,2023)

(iii) ACTING AS AN ANTIOXIDANT:

The antioxidant properties of these compounds are related to their abilities to donate electrons and to act as reactive oxygen species scavengers by the formation of stable aroxyl/phenoxy radicals (Vuolo et al., 2019). By going through phenol $\leftrightarrow$ quinone tautomerism (Baldini, L, et al.,2023) in the polyphenolic side chain via delocalization of electrons (Cvitanović A B et al., 2018) it can protect itself from molecular damage by its structure-activity relationship (SAR). Flavonoids are very specific against photo-oxidative stress generated by ambient solar UV radiation (Ferreyra, M. L. F et al., 2021).

Remaining stable under high-energy UV radiation, flavonoids, mitigate **UV-caused damage** in plant cells (Ferreyra MLF et al.,2021) —in terrestrial plants, - Golovatskaya I F et al.,2023 ;

Schulz, E et al 2021—in seeds of Arabidopsis; Gromkowska-Kępa et al.,2021—in barley *Hordeum vulgare* L.; Kantharaj, V et al 2023-- in diverse plant groups, Jan R et al.,2022-- in

rice seed, Rai S et al., 2022-- in Cyanobacteria) and act as the best contender for survival strategy in dehydrated embryonic axes of seed under ambient farmers' storage (Gu et al., 2008, Reddy and Wani, 2007). Thus, along with the mitigation of UV irradiance-related damage by the UV screening attribute flavonoids confer antioxidant activity in many plant systems Dias, M. C et al., 2021; Li et al., 1993 showed that flavonoid-deficient mutants of arabidopsis, in which the expression of flavonoid synthesizing enzyme chalcone synthase (tt4) or chalcone-flavanone isomerase (tt5) is blocked, were hypersensitive to **UV-B radiation**. Conversely, an arabidopsis mutant that is tolerant to **UVB radiation** showed constitutively increased accumulation of flavonoids and other phenolics (Bieza and Lois, 2001). It was mentioned earlier that the dosimetric effect of ambient UV radiation for accumulation of flavonoids in maize by Stapleton and Walbot, 1994. Koopmann and Hahlbrok, 1997 have demonstrated that due to structural integrity, flavonoids remain undegraded under UV radiation stress in parsley leaf. Interestingly, UV radiation while causing cell damage on the one hand is also capable of bringing about cellular protection by (a) signalling cellular mechanism for synthesis and accumulation of UV absorbing sunscreens (act as UV photoreceptor—Apoorva et al., 2021 and the (b) activation of antioxidant defenses (Xue et al., 2022—working with red algae *Neoporphyra haitanensis*. Seed storability is thus dependent on the efficiency of constitutive and UV-induced mechanism of cell protection by the non-enzymatic antioxidants and natural UV screen, both features being attributable to flavonoids. Lepiniec, L et al., 2006 have reported the role of flavonoid metabolism responsible for arabidopsis seed viability.

1.5.3 UV SIGNAL TRANSDUCTION OF REPAIR AND RESYNTHESIS FOR COUNTERING DELETERIOUS EFFECTS OF UV RADIATION IN PLANTS:

Signal transduction in plant systems to upregulate the antioxidant enzymes and repair enzymes (SOS response) was reported by Vanhaelewyn et al, 2020. So deleterious oxidative stress effects via ROS-mediated damage could be encountered by the plant system.

1.5.4 GENOTYPE X-(UV) ENVIRONMENT INTERACTION-BASED TRAIT EXPRESSION IN PLANTS:

Genotype x UV Environment Interaction refers to the interaction between a plant's genetic makeup (genotype) and UV radiation (environment) that influences trait expression. Genotype-by-environment interaction (GEI) is a phenomenon that occurs when the same genotype produces different phenotypes in response to different environments. This happens because the environment affects the expression and function of genes that influence a trait.

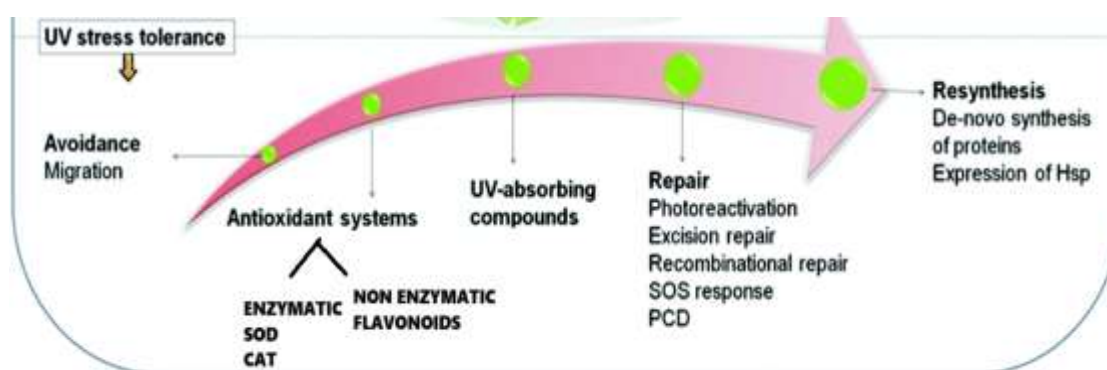


Figure 1.4: UV Acclimation Strategy in Plants

1.6 WHY TEA IS A SUITABLE CANDIDATE FOR RESEARCH?

Tea is a suitable candidate for research for some reasons. Tea is one of the most widely consumed beverages globally, with a rich cultural and economic history. Nutritional value: Tea is rich in antioxidants, polyphenols, and other bioactive compounds with potential health

benefits. Diverse varieties: With over 10,000 known tea cultivars, tea offers a wide range of genetic diversity for study.

1.6.1 COMPLEX PRODUCTION PROCESS:

Tea production involves multiple steps (cultivation, harvesting, processing, and brewing), making it an interesting subject for agricultural, chemical, and sensory research. Potential health benefits: Tea has been linked to various health benefits, such as cardiovascular health, anti-inflammatory effects, and anti-cancer properties.

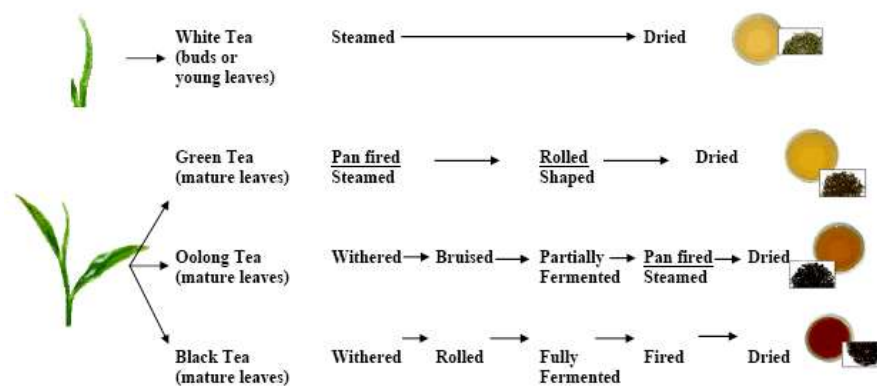


Figure 1.5: Manufacturing process of different types of tea: White tea, Green tea, Oolong tea, and Black tea.

White tea is only withered, Green tea is semi-dried, Oolong tea is partially fermented, and black tea is fully fermented.

1.6.2 ENVIRONMENTAL IMPACT:

Tea cultivation and production have environmental implications, making sustainability research crucial. Economic importance: Tea is a significant crop for many countries, with research potential for improving yield, quality, and marketability. (Chen *et al.*,2023)

1.6.3 RESEARCH SCOPE OF TEA:

Research areas of tea include Plant breeding and genetics, Agricultural practices and sustainability, Phytochemistry and bioactive compounds, Nutrition and health benefits, Sensory evaluation and quality control, Food safety and microbiology, Environmental impact and climate change, Tea processing and technology, Market trends and consumer behavior, Cultural and social studies. Tea's unique combination of cultural, economic, and scientific significance makes it an attractive subject of interdisciplinary research. Besides natural tea, herbal tea, or medicinal tea also has a greater impact on medical research since they are highly available, low in cost, and have little or no side effects.

1.6.4 EFFECTS OF NATURAL AND ARTIFICIAL UV RADIATION ON TEA:

Tea (*Camellia sinensis*) nowadays is one of the most discussed research areas in plant sciences. It is not only the most popular aromatic beverage but is also used as a medicinal drink. (Zhang, et al.,2022). Tea is originated as the medicinal drink in the Yunnan region during the Shang dynasty. An early credible record of tea drinking dates to the 3rd century AD, in a medical text written by Hua Tuo (May *et al.*,2000). The four main types of tea are green, black, white, and oolong tea which differ in the way they are harvested and processed. These teas are produced from the same plant, *Camellia sinensis*. White tea is only withered, green tea is not fermented, oolong tea is partially fermented and black tea is fully fermented. Because of the different processing of tea leaves, the flavour of the tea leaves also changes significantly. Different flushes of tea that are variable seasons of plucking of tea leaves after their harvest, also have different flavours. (Engelhardt *et al.*, 2023). The quality of fresh tea is both genetically and environmentally regulated. (Schuh, C *et al.*, 2018) Different seasonal flushes varied qualities of tea. There is also a positive correlation between UV radiation and the upregulation of secondary metabolites like UV-B absorbing compounds, catechins,

tannins, polyphenols, and total antioxidant values in high-altitude grown tea (Shyam Choudhury *et al.*, 2018). Climatic factors like soil (texture pH mineral content), mist, fog, cloud, humidity, evaporation rate, wind speed, rainfall, and UV rays - all of these affect the growth of plants by interacting with the genes of plants. Even the flavour of leaves both aroma and taste is the outcome of the agro-climatic factors as stated by Shyam Choudhury *et al.*, 2020. Altitude also plays an important factor in the quality of tea and tea plants. Solar zenith angle differs with altitude thereby affecting the overall production of plants, especially tea plants. Tea plants generally grow at altitudes from 1968 ft-6500 ft. From the studies by Shyam Choudhury *et al.*, 2020 it has been found that tea plants growing at an altitude of about 4500 -5000ft.msl are found to be superior in quality. UV rays are one of the important abiotic factors responsible for the overall development of tea plants thereby affecting the biochemical properties as well as inducing the flavour of tea leaves. UV rays can be beneficial and harmful for the plants, especially for tea plants. The flavour of the tea is mainly because of some primary and secondary metabolites like amino acids, organic acids, soluble sugars, and evaporating aromatic compounds. These compounds are formed during carbon assimilation in photosynthesis, glycolysis, and the tricarboxylic acid cycle (TCA cycle). Too much exposure to UV radiation can cause a reduction in photosynthesis which eventually decreases the production of flavour-related compounds in tea. Tea plants at high altitudes are exposed to too much harmful ultraviolet radiation because of ozone layer depletion, and the generation of oxidative stress develops. Changes in UV radiation can be a result of altitudinal variation. Since tea plants are grown at different altitudes they are exposed to different doses of UV radiation. Different doses of UV radiation along with varied agro-climatic parameters can affect the plant physiology, yield, biomass, photosynthetic ability, and morphology (Murali *et al.*, 2017). It can also affect metabolism (nutrient content) including oxidative stress management. Thus, the impact of such agroclimatic parameters

including natural UV radiation on tea-growing gardens needs proper research and documentation. During summer solstice (March to July) tea plants receive maximum UV radiation with optimum solar zenith angle which gives rise to the most aromatic 2nd flush tea indicating the positive influence of UV radiation. Besides, natural UV radiation artificial UV B lamps with UV filters on tea fields or factories ameliorate the negative effects of UV radiation only enhancing the positive effects of the UV radiation. Artificial UV radiation in proper doses helps to upregulate the secondary metabolites in plants which is responsible for the enhancement of flavour aroma and therapeutic potentials of tea plants.

This thesis aims to find the effects of natural and artificial UV radiation on the different altitudes grown in seven valleys of Darjeeling tea having different solar zenith angels. The variation could be manifested in commercial values like aroma, colour, and briskness and the therapeutic values include the antioxidant, antimicrobial, and anticancer potential.

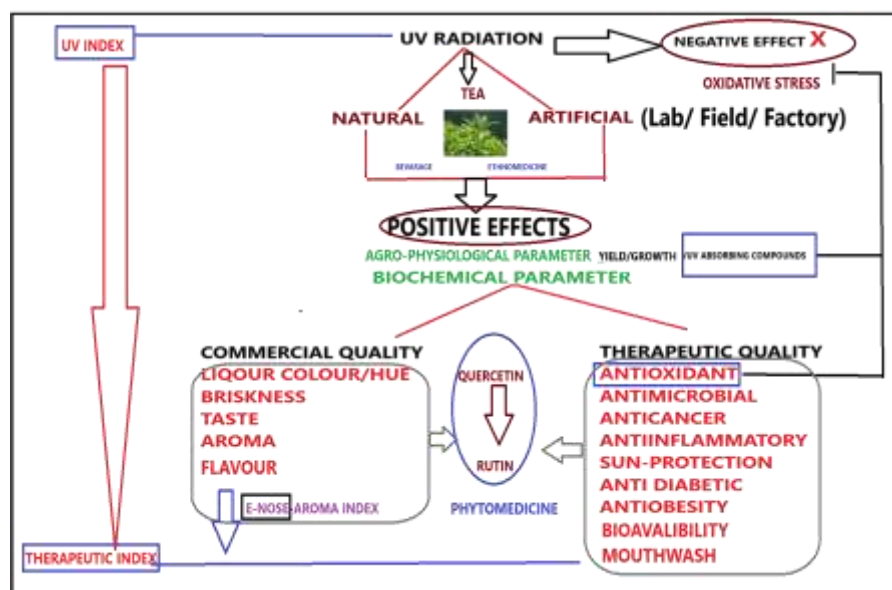


Figure 1.6: Effect of natural and artificial UV radiation on tea plant

1.7 SCOPE OF THESIS:

The key focus of the thesis is to understand the correlation between the natural environmental and biochemical parameters of tea. Natural environmental as well as seasonal parameters

affect the quality of the tea aroma. The processing parameters viz temperature humidity give rise to different quality tea and show different health beneficial effects against UV radiation-related oxidative stress. One of the most important natural factors which determine the quality of the tea is UV radiation. Documentation of natural UV irradiance, prevalent at high altitudes grown tea fields and characterization of their therapeutic as well as commercial value which overall indicate the quality of the tea is done. UV radiation can be mimicked artificially in a laboratory or factory with similar doses to produce the same quality of tea clones. The use of UV screens can ameliorate the damaging effects of natural UV radiation and supplemented UV lamps can upregulate the beneficial active components of tea which gives enormous opportunity for growing tea at non-conventional sites with high aromatic and therapeutic values. The volatile and non-volatile components that affect the therapeutic and commercial values of tea and herbal tea can be easily detected by E-sensor-based techniques like E-Nose and E-Tongue. These values can be statistically related to the medicinal values of teas like antioxidant, antimicrobial, and anti-cancerous properties thereby generating a therapeutic index. Moreover, the therapeutic indices help to authenticate the therapeutic or medicinal values of unknown samples of medicinal and herbal teas based upon the indices derived from E-nose (volatile) and E-tongue (non-volatile) data. These therapeutic indices would help in nano-medicine development starting from the concept of ancient ethnomedicine. Scientific documentation of biochemical characteristics of medicinal tea would help in designing high-quality ethnomedical drugs with these medicinal tea extracts thereby also increasing commercial benefits. New varieties of medicinal plants with better qualities can also be developed with these studies which will not only benefit the basic agronomic environmental studies but will also put a milestone in the medical field.

In brief, this thesis provides a comprehensive and in-depth study of the therapeutic and commercial values of tea and herbal tea utilizing both conventional and sensor-based

approaches. This study will shed new light on the close relationship between UV radiation and therapeutic and commercial values of tea. Thus, opening new avenues in biotechnology, and biomedical industrial fields to explore and potentially develop new engineered nano-medicines.

1.8 OBJECTIVE:

- 1. MEASUREMENT OF AGRO-CLIMATIC PARAMETERS (INCLUDING NATURAL UV RADIATION) OF SEVEN VALLEYS OF DARJEELING TEA GARDENS**
- 2. CHARACTERIZATION AND IDENTIFICATION OF THERAPEUTIC VALUES AND COMMERCIAL POTENTIAL OF DIFFERENT TYPES OF TEA (FRESH AND MANUFACTURED) OF SEVEN VALLEYS OF DARJEELING UNDER NATURAL UV RADIATION**
- 3. DETERMINATION OF THERAPEUTIC AND COMMERCIAL VALUE OF TEA UNDER ARTIFICIAL UV RADIATION AND COMPARISON WITH NATURAL UV FLUENCE**
- 4. CHARACTERIZATION OF DIFFERENT TYPES OF MANUFACTURED TEA (WHITE, GREEN, BLACK, OOLONG) AND COMPARISON OF THEIR SHELF LIFE INTO DIFFERENT PACKAGING UNDER AMBIENT UV RADIATION**
- 5. CHARACTERIZATION OF THERAPEUTIC VALUES OF MEDICINALLY IMPORTANT BEVARAGES AND DEVELOPMENT OF E-SENSOR BASED THERAPEUTIC INDICES OF ALL TEA**

1.9. PLAN OF THESIS:

The plan of the thesis is as follows:

Chapter 1: This chapter provides a brief introduction to the scope and motivation behind the thesis work.

Chapter 2: This chapter provides the literature review.

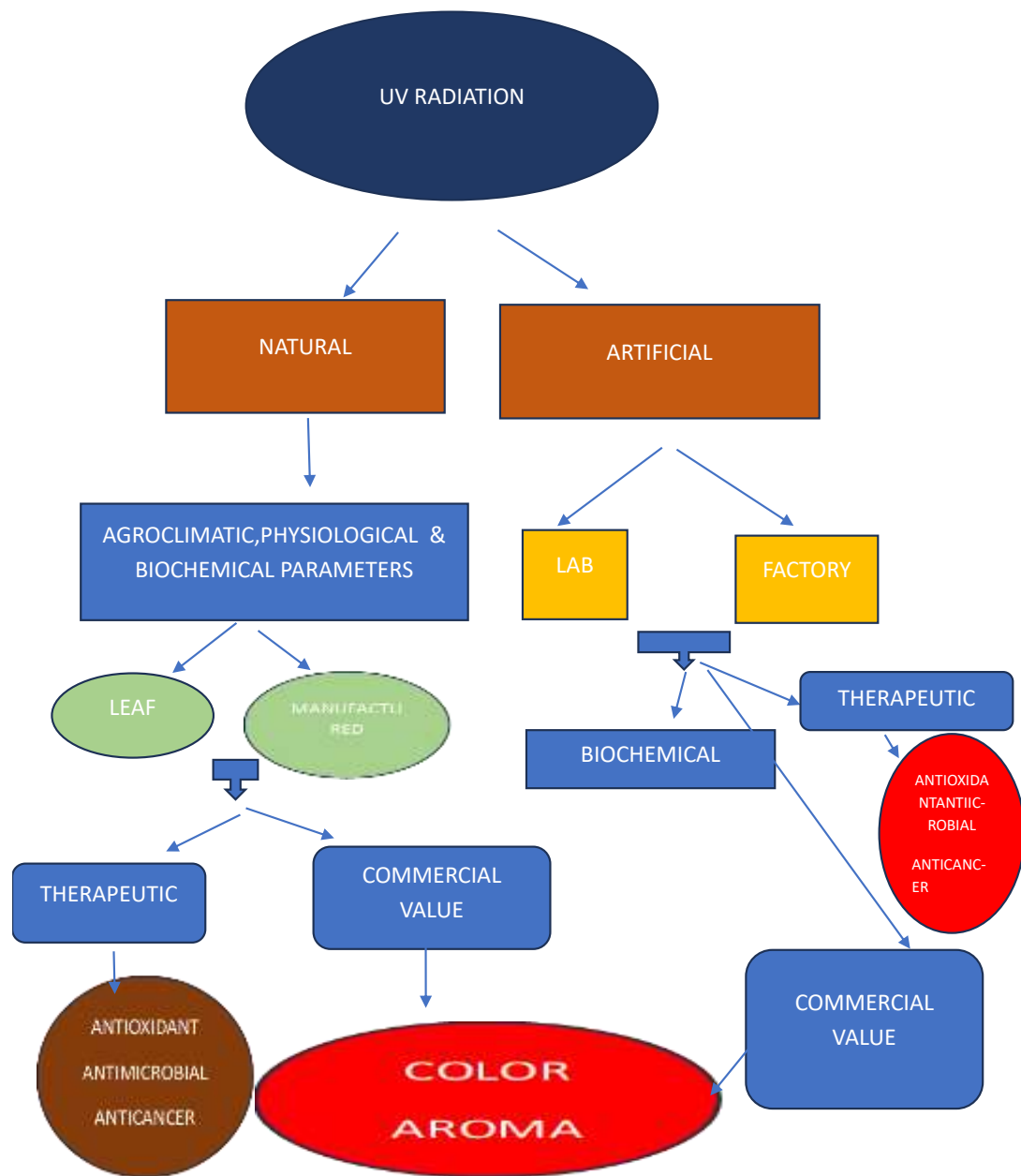
Chapter 3: This chapter provides the materials and methods (elaborate experimental procedures).

Chapter 4: This chapter focuses on the results.

Chapter 5: This chapter provides an insight into the discussion of each objective.

Chapter 6: This chapter summarizes each objective of the thesis and presents the conclusion and future aspects.

SCHEME OF WORK:



Chapter 2

LITERATURE REVIEW

CHAPTER 2

LITERATURE REVIEW

2.1. UV Measurement and UV Index

UV radiation is measured mostly by the UV meters in the uwatt/cm²/sec ratio. The UV index is the measurement of the permissible dose of UV radiation (natural or artificial) that has the potential to cause damage to a living system. UV index can be categorized into five types: 0-4, no risk of UV damage, 4-5, low risk of UV damage, 5-7, medium risk of UV damage, 7-10, high risk of UV damage, more than 10 extreme risk of UV damage. (Heckman CJ et al., 2019). The UV index is dependent on other environmental factors such as cloud cover, latitude, altitude, ozone layer, aerosols, pollutants, etc. (Heckman et al., 2019)

2.1.1. Natural UV radiation

Natural UV radiation refers to the ultraviolet radiation emitted by the sun, which plays a crucial role in various biological and environmental processes. Natural UV radiation is the sole source of energy for the Earth. It has both positive and negative impacts on all life forms.

2.1.2. Artificial UV radiation

Artificial UV radiation refers to man-made sources of ultraviolet radiation used in various applications. Artificial UV radiation can be used in various doses to nullify the negative effects of natural UV radiation. Artificial UV radiation can be used to enhance certain compounds of the plants in the laboratory.

2.2. Effects of UV Radiation on Plant Physiology and Metabolism

UV radiation, particularly UV-B (280–320 nm), significantly impacts plant physiology and metabolism. Exposure to UV radiation induces the production of reactive oxygen species (ROS), which can lead to oxidative stress if not properly managed (Lee & Yang, 2017). In response, plants activate antioxidant defence systems, including enzymes such as superoxide dismutase and catalase, to mitigate ROS damage (Jansen et al., 1998). Additionally, UV exposure frequently triggers the accumulation of secondary metabolites, particularly phenolic compounds, which help protect plant cells from damage. In tea plants, this response leads to an increase in catechins, flavonoids, and other polyphenols, enhancing the plant's UV tolerance and contributing to its therapeutic qualities when consumed (Sang et al., 2011). Flavanols are a subclass of flavonoids that mainly refers to catechins in *Camellia sinensis*, consisting of epigallocatechin Gallate (EGCG), Gallo catechin Gallate (GCG), epigallocatechin (EGC), Gallo catechin (GC), epicatechin Gallate (ECG), catechin Gallate (CG), epicatechin (EC), and catechin (C), which are the main substances affecting tea quality. UV-B radiation affects tea quality by modifying catechins content and catechins metabolism. Zheng et al. (2008) studied the accumulation of catechins in tea leaves affected by UV-B radiation concerning time and irradiation dose rate. The study revealed that a short period of 30 minutes and low irradiation dose rate (below $1.0 \mu\text{mol m}^{-2} \text{s}^{-1}$) of UV-B radiation stimulated accumulation of major catechins such as EGCG and EGC, whereas the accumulation of tea catechins was suppressed by UV-B radiation with high irradiation dose-rate and longer period of radiation. Tea plants are grown at different altitudes and thus face various doses of UV radiation.

2.3. Impact on the Therapeutic Value of Tea

Research has shown that UV radiation can significantly enhance the polyphenol content in tea leaves. Studies indicate that UV-B exposure induces the synthesis of catechins and flavonoids, key compounds responsible for tea's antioxidant properties (Li, Zhang, Sun, & Chen, 2015). Catechins, including epigallocatechin gallate (EGCG), are particularly sensitive to environmental factors, with UV exposure often resulting in elevated levels. This increase in polyphenolic content boosts the antioxidant capacity of tea, contributing to its therapeutic effects, such as reducing inflammation and potentially lowering the risk of chronic diseases (Guan et al., 2018). According to Choudhury et al. (2018), the higher the altitude, the higher the UV irradiance, i.e., the altitudinal heights are directly proportional to the UV irradiance, which is measured by the UV meter. UV radiation in controlled exposure can upregulate the secondary metabolites, which thereby are responsible for enhancing the flavor profile of tea. Total antioxidant and UV-absorbing compounds are highly corroborated with higher UV exposure to high-altitude-grown tea. The greater extent of natural UV dose enhances the production of non-enzymatic antioxidant activities as flavonoids, especially flavonoid glycosides. The flavonoid glycoside, specifically Rutin, serves as an aroma precursor as well as better antimicrobial components. The increased content of such active components in different altitude-grown tea clones was qualitatively and quantitatively measured by Layer Chromatography and UV spectrophotometric scan, respectively. Thus, being exposed to UV radiation, which is harmful to plants, they have evolved cell protective/repair mechanisms (viz. antioxidants) that help them to grow and flourish under natural ambient UV radiation. The positive effect of UV radiation is influenced by the signalling of protective genes conferring cell protection, described by Mustafi et al. (2014). According to a study conducted by Choudhury et al. (2012), tea clones growing at higher altitudes were found to have higher UV-absorbing compounds and thus exhibit higher antioxidant as well as antimicrobial

potential. Ning Lin et al. (2021) reported that ambient UV-B radiation enhances the tea quality by inducing a metabolic flux in flavonoid biosynthesis. It is also determined from the study that the absence of ambient UV radiation is corroborated by the decreased content of flavonoids. In a similar study by Zeyi et al. (2017), UV radiation was found to increase the catechin amino acid flavonoid content, thereby upregulating the astringent flavours of the tea. Accumulation of the UV radiation-induced catechins, flavonoids not only act as UV screens but also have therapeutic potentials like antimicrobial, anti-cancerous, and anti-inflammatory activities. Kapoor et al. (2021) observed that regular intake of green tea catechins induced by UV radiation is potent in reducing erythema inflammation. Yusuf et al. (2007) carried out experiments that determined that green tea polyphenols have a positive effect concerning chemoprevention and have been found to suppress the carcinogenic activity of UV radiation. They protect against damaging effects of UV radiation, such as UV-induced sunburn, UV-induced immunosuppression, and skin photoaging. Green tea polyphenols, when used with traditional sunscreens, further protect the skin against the negative effects of ultraviolet radiation. UV exposure not only increases the total polyphenol content but also alters the specific composition of catechins in tea leaves. For example, UV radiation has been shown to elevate levels of EGCG, which is known for its strong antioxidant, anti-inflammatory, and anticancer properties (San et al., 2004). Alterations in catechin composition due to UV radiation can thus directly impact the therapeutic potential of tea, enhancing its value as a functional beverage with diverse health benefits. The increased phenolic content due to UV radiation directly enhances the antioxidant profile of tea leaves. Antioxidants are crucial for neutralizing free radicals, which are linked to oxidative stress and various health issues, including cardiovascular diseases and cancer (Chen et al., 2008). By promoting higher antioxidant activity, UV radiation effectively increases the health benefits of tea, making it a potent natural source of disease-preventive compounds (Li et al., 2015).

2.4. Impact on the Commercial Value of Tea

2.4.1. Enhancement of Quality Attributes

From a commercial standpoint, the enhancement of polyphenol content through UV exposure positively influences the quality attributes of tea, such as taste and aroma, which are important determinants of consumer preference (Guan et al., 2018). High-antioxidant teas have garnered considerable market interest as health-conscious consumers increasingly seek functional beverages with scientifically backed health benefits. By understanding optimal levels and duration of UV exposure, tea producers can harness this environmental factor to enhance both the therapeutic profile and commercial appeal of their products, potentially yielding economic advantages (Li & Wu, 2016).

2.4.2. Market Preferences for High-Antioxidant Teas

High levels of polyphenols and antioxidants in tea leaves are associated with a superior health profile, making these teas more desirable in the market. The demand for teas with high antioxidant content reflects a broader consumer trend toward functional foods and beverages that offer health benefits beyond basic nutrition (Chen et al., 2008). Thus, teas cultivated with controlled UV exposure to maximize polyphenolic content align well with these market preferences, enhancing their commercial value.

2.4.3. Economic Benefits of Controlled UV Exposure in Cultivation

The potential for controlled UV exposure in tea cultivation offers a promising avenue for improving tea quality. By fine-tuning the UV exposure levels in tea fields, producers can optimize the chemical profile of the tea leaves, leading to higher-quality products (Wang et al., 2000). This approach allows tea growers to target specific therapeutic compounds,

increasing both the perceived and actual value of their tea products in the marketplace (Li et al.,2016).

2.4.4. Potential Downsides and Challenges

- **Overexposure and Degradation of Quality**

While moderate UV exposure can enhance the therapeutic compounds in tea, excessive exposure may lead to detrimental effects. High levels of UV radiation can cause cellular damage, resulting in a decline in leaf quality and potential degradation of key bioactive compounds (Lee et al., 2017). This poses a challenge for tea growers, who must balance the positive effects of UV on bioactive compound synthesis with the potential risks to overall plant health and crop yield. Sustainable cultivation practices that optimize UV exposure are essential for achieving the desired therapeutic and commercial outcomes (Jansen et al., 1998).

- **Balance in Cultivation Practices**

Achieving a balance in UV exposure is crucial for ensuring that the tea plant derives benefits without suffering adverse effects. Controlled UV exposure may vary depending on factors such as plant variety, environmental conditions, and desired tea characteristics (Sang et al., 2011). Cultivation practices must be carefully tailored to avoid overexposure and to achieve a consistent quality that meets market expectations.

2.4.5. Impact on Yield

In addition to quality concerns, high UV levels can also affect tea crop yield. Although increased UV exposure may boost certain beneficial compounds, it can also reduce plant vigor and productivity if not managed appropriately (Lee et al.,2017). Maintaining a balance that promotes both the quality and yield of tea plants remains a challenge for researchers and tea growers alike.

2.5. Future Directions and Implications for the Tea Industry

2.5.1. Innovation in UV Manipulation Techniques

The potential to harness UV radiation to enhance tea's therapeutic and commercial value opens new avenues for innovation in the tea industry. Techniques such as UV-controlled greenhouse environments or selective UV-filtering materials allow for precise manipulation of UV exposure, maximizing the desirable attributes of tea leaves while mitigating risks (Zhou et al., 2019). These technologies could significantly improve the consistency and quality of tea products, providing a competitive edge in the market.

2.5.2. Breeding for UV-Resilient Tea Varieties

Breeding programs focused on developing UV-resilient tea varieties provide a sustainable approach to improving tea's quality and yield. By selecting traits that enhance resilience to UV radiation, researchers can create tea plants that thrive in high-UV environments without compromising quality (Zhou et al., 2019). Such varieties could support sustainable production practices, reduce the need for chemical interventions, and contribute to the economic viability of tea cultivation in regions with high UV levels.

2.5.3. Implications for Tea Product Development

The findings on UV radiation's effect on tea could lead to new tea products marketed for their heightened health benefits. For instance, specialty teas with boosted antioxidant content could be marketed as "UV-enhanced" for consumers seeking functional beverages with superior therapeutic qualities. This differentiation could appeal to health-conscious consumers and drive demand in niche markets, thereby expanding the commercial opportunities for tea producers (Chen et al., 2008).

2.6. Tea Plants' suitability for UV exposure:

• Tea Plant Responses to UV Radiation

Camellia sinensis var. *sinensis*: The variety that exhibits high UV tolerance and flavonoid accumulation, which makes it a suitable candidate for studying the effects of UV radiation on them. (Wargent et al., 2009). Tea plants, when subjected to natural UV radiation, increase flavonoid production, which has a potent antioxidant potential that acts as a major defense system in plants against harmful UV radiation, which carries out unnecessary oxidation of their biomolecules. Thus, tea plants respond optimally to UV radiation for the accumulation of flavonoids. (Wargent et al., 2009). Besides flavonoid production, UV also induces the production of catechins in tea, which thereby improves tea quality, enhancing tea plant growth and yield. (Li et al., 2017). Tea plant also expresses some UV-responsive genes that influence phenolic compound production. The UV intensity of about 10-20W/m² is optimum for the growth and flavonoid production. (Mohanpuria et al., 2017). Tea plants' responses to UV radiation vary depending on environmental conditions. Exploring genetic variation in tea plants in response to UV radiation can scale up UV exposure experiments to commercial tea plantations. Thus, it can be said that UV radiation is one of the key factors that impact tea plants and tea quality.

2.7. Darjeeling Tea

Darjeeling tea originated in the 19th century in the Darjeeling district of West Bengal, India (Sen, 2017). Darjeeling tea is produced in 87 tea gardens covering 17,500 hectares (Tea Board of India, 2020). Production ranges from 10-12 million kg annually (Sen, 2017). Darjeeling is a small district which is located in the extreme north of India in the Himalayan foothills. Darjeeling falls under the state of West Bengal, India. Darjeeling tea is famous and most popular throughout the world for its high quality, based on its aroma, colour, and taste

(flavor) – all attributable to its geographical origin and nearly impossible to replicate. In 2004, Darjeeling tea became the first product in India that was registered under the Act which is Geographical Indications of Goods (Registration and Protection) Act of 1999 (thereafter known as the GI Act). Darjeeling tea is also famous for its therapeutic potential for its anticancer antimicrobial (antibacterial, antifungal, antiparasitic) activities. Darjeeling tea contains high levels of catechins, theaflavins, and thearubigins (Bhattacharya et al., 2018), which confer their unique muscatel flavor. Aromatic compounds include linalool, geraniol, and β -ionone, which impart the aroma of Darjeeling tea, which is a distinctive feature. (Mohanpuria et al., 2017). The mineral content of Darjeeling tea includes potassium, magnesium, and manganese, conferring the therapeutic benefits of the tea. (Ghosh et al., 2015). Darjeeling tea has high antioxidant activity (Bhattacharya et al., 2018). Studies have shown that regular consumption of Darjeeling tea may reduce cardiovascular risk (Ghosh et al., 2015). Darjeeling tea may also play its role in the reduction of inflammation (Mohanpuria et al., 2017). Because of the vast therapeutic values of Darjeeling tea, it could be selected as an ideal clone for cultivation in the study area.

2.8. Documentation of agro-climatic and physiological parameters of tea gardens of Darjeeling:

Singh et al., (2016) elaborately discussed the agroclimatic and physiological factors affecting the Darjeeling tea industry. To discuss physiological factors, they discussed the role of solar radiation on tea. Solar radiation, along with other agroclimatic parameters of Darjeeling tea gardens, such as temperature, rainfall, humidity, condition of the soil, cloud cover, solar zenith angle, and slope, play an important role in the quality enhancement of Darjeeling tea. Agroclimatic parameters like temperature ranging from 12°C to 22°C are optimum for the high-quality Darjeeling tea. (Sen et al., 2017). Optimum rainfall of about 2000mm to 3000mm

also influences the growth of Darjeeling tea. (Ghosh *et al.*, 2015). Bhattacharya *et al.* (2018) demonstrated that the optimum relative humidity for Darjeeling tea ranges from 60% to 80 %. Soil high in organic compounds and a pH of 5.5-6.5 is suitable for Darjeeling tea. (Mondal *et al.*, 2017). Darjeeling tea plants exhibit high photosynthetic rates (10-15 $\mu\text{mol}/\text{m}^2/\text{s}$) (Ghosh *et al.*, 2015). Tea plants show moderate transpiration rates (2-4 $\text{mmol}/\text{m}^2/\text{s}$) (Sen *et al.*, 2017). Stomatal conductance ranges from 0.1 to 0.5 $\text{mol}/\text{m}^2/\text{s}$ (Bhattacharya *et al.*, 2018). Chlorophyll Content: Darjeeling tea leaves contain high chlorophyll content (1.5-2.5 mg/g) (Mondal *et al.*, 2017).

2.9. Biochemical characterization of Darjeeling tea under natural UV radiation:

Therapeutic and Commercial values

Ghosh *et al.*, 2023 demonstrated the therapeutic values of Darjeeling tea in terms of antioxidant and antimicrobial potential. Das *et al.*, 2022 had discussed about the therapeutic bioactive constituents of Darjeeling tea. Choudhury *et al.*, 2018 discussed the upregulation of therapeutic bioactive compounds viz flavonoid glycosides of Darjeeling tea under the influence of natural UV radiation. Hazra *et al.*, 2021 discussed the change in ecophysiological traits and antioxidant properties of Darjeeling tea varieties under natural solar radiation (consisting of UV radiation). The survival strategies of different plants, including tea, were vividly described by Sen Mandi. Muscatel-flavored majestic Darjeeling tea is also eminent to show its therapeutic potential (antioxidant and antibacterial potential— Choudhury *et al.*, 2015). According to the Tea Board of India, the therapeutic value of high altitude-grown Darjeeling tea was reported but lacking documentation. Bhattacharya and Sen Mandi, 2011 had discussed that natural UV radiation upregulates flavonoid glycoside (Rutin) as aroma precursor which is being cleaved by aroma causing enzyme β -D glycosidase to convert it to Quercetin which is being transformed to release volatile aroma components like Linalool

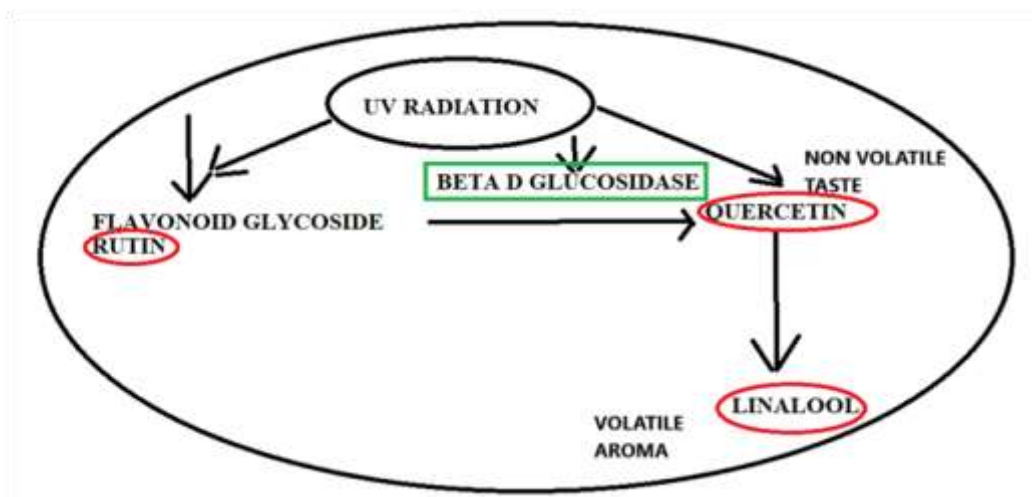


Figure 2.1: Formation of volatile aroma compounds by natural UV radiation

2.10. Biochemical characterization of Darjeeling tea under artificial UV radiation:

Therapeutic and commercial potential

Zheng et al., 2008 discussed the increased levels of catechins under the influence of artificial UV radiation, causing better therapeutic values. Under the influence of artificial UV radiation, there is an increase in tea's flavonoid content (Li et al., 2019). To combat the harmful doses of natural UV radiation, artificial UV radiation in optimum doses can be applied to tea in fields, factories, or laboratories to produce high-quality tea clones. Besides, natural UV radiation artificial UV B lamps with UV filters on tea fields or factories ameliorate the negative effects of UV radiation, only enhancing the positive effects of the UV radiation. Artificial UV radiation can induce an increase in the production of secondary metabolites like flavonoids, which act as defense mechanisms in plants as well as have therapeutic potential. However, the induction by artificial UV radiation is less than that of natural UV radiation because UV radiation, together with other environmental parameters, helps in the production of more secondary metabolites. Very little work has been done in this regard.

2.11. Biochemical (therapeutic and commercial) characterization of different manufactured tea:

The different ways of biochemical characterization of differentially manufactured teas can vary based on the type of tea, the degree of fermentation, and the geographical location where the tea is grown. Saha et al., 2017 had demonstrated the biochemical characterization of white tea. Anand et al., 2014 characterized Darjeeling-grown green tea biochemically. Rachineni, 2023 discussed the characterization of Darjeeling tea with an NMR study. The different stages of differential manufacturing (with different processing parameters. temperature, and humidity) of black and CTC tea give rise to differential quality indices of tea and show differentials of health-beneficial effects of oxidative stress (Emdadi et al., 2009). Flavor/aroma (quality) related molecular characterization (RT- PCR) of black (manufactured) tea was done by Shi et al., 2014 with polyphenol oxidase, β -D glucosidase primers. Ho et al., 2015 demonstrated the profiles of volatile aroma, antioxidant, and antimicrobial components of different types of manufactured tea with different manufacturing conditions (heat, UV radiation). Seran, 2007 demonstrated the upregulation of secondary metabolites in another culture of tea. Tiwari et al., 2005 determined the MIC (minimum inhibitory concentration) of green and black tea extracts containing UV-upregulated flavonoids against pathogenic *S. typhimurium* 1402/84 strain. Gogoi et al., 2016 reported the profiles of polyphenols (antioxidants) aroma components of manufactured black and green tea varieties of different flushes. Bhuyan et al., 2013 made some of the biochemical fingerprinting of manufactured black tea but not correlated with responsible environmental/manufacturing parameters. According to Choudhury et al., 2020 there are four main types of processed tea – Green tea, White tea, Oolong tea, and Black tea. White tea is only dried, green tea is not fermented, Oolong tea is semi- fermented, and Black tea is completely fermented. The active components, Rutin and Quercetin, are present in different

amounts in different types of tea, which are responsible for antioxidant and antimicrobial activity, as determined by Choudhury et al.,2020. Thus, it can be said that during the manufacturing process, the content of such active metabolites is changed. Quercetin content is highest in white tea, green tea, oolong tea, and black tea. These four types of tea have immense therapeutic potential. Studies have shown that tea flavonoids impart antimicrobial properties such as antifungal, antibacterial, antiprotozoal as well as antiviral properties. Among the different tea types white tea shows higher antimicrobial properties than green, oolong, and black tea. Antimicrobial Properties of Tea White tea >Green tea>Oolong tea >Black tea. Green Tea contains EGCG (Epigallocatechin-3-gallate), a type of catechin that bolsters weight loss. Bioactive components in tea, such as theaflavins of black tea, catechins of green tea, and polyphenols, can improve diabetes complications, while oolong tea consumption was associated with a lower risk of impaired glucose tolerance (IGT). Iyer et al.,2019 reported that anti-photoaging, stress resistance, neuroprotective, and autophagy are properties of one of the most widely known functional foods—green tea. Bioactive components of black tea have been attracting much attention to human health. There is a vast literature suggesting the beneficial effects of regular green and black tea intake on the malignancies incidence in humans, including liver, lung, stomach, pancreatic, colon, breast, oral, and prostate cancer. Besides the anticancerous, antimicrobial, and antioxidant activities of tea, various other potential health benefits of tea are recorded. Such therapeutic potential calls for further research on animal and human models with tea extracts. The different stages of differential manufacturing (with different processing parameters, temperature, and humidity) of black and CTC tea give rise to differential quality indices of tea and show differentials ' beneficial effects against oxidative stress (Emdadi et al., 2009).

2.12. Biochemical characterization of herbal and medicinal tea:

Herbal tea, also known as tisane, is a type of tea that is made from the leaves, flowers, roots, or fruits of plants other than the *Camellia sinensis* tea plant (Liu et al.,2023). Uchegbu et al., 2024 discussed the biochemical characterization of some herbal teas. Kumari et al., 2024 discussed the medicinal properties of some Western Himalayan-grown herbal teas and compared them with green and black teas. Herbal teas having medicinal properties are called medicinal tea.

2.13. Development of therapeutic index:

Wasilewski, 2019 discussed the utility of electronic sensors such as E-nose and E-tongue for pharmaceutical analysis. Muller PY,2012 studied the therapeutic index, which is a quantitative measure of the efficiency of the drug or the therapeutic agent such as UV radiation. The therapeutic index is the ratio of the unsafe dose to the safe therapeutic dose. E-nose is an electronic device that mimics the human olfactory senses and thus determines the volatile components by using different sensors. (D'Amico et al. 2015). E-tongue is an electronic device that mimics the human taste buds, thus determining the non-volatile organic matter. (Cosío et al.,2018). The rapid detection of the volatile and nonvolatile components of different types of herbal or medicinal tea gives a score that can be correlated with the antioxidant, antimicrobial, and anticancer data. The correlation helps to derive the therapeutic indices of Medicinal and Herbal teas. (Choudhury et al.,2022) Moreover, thus, it would help to corroborate the therapeutic potentials of unknown samples of medicinal and herbal teas based upon the therapeutic indices derived from the scores generated from E-nose and E-tongue.

2.14. UV index to therapeutic index

UV therapeutic index is determined as the permissible dose of UV radiation, which has its efficiency in many health benefits such as mood improvement, serotonin production, Vitamin D production, and many more. Thus, there is a huge relationship between the UV index and the therapeutic index.

Conclusion:

UV radiation exerts a profound influence on the physiological, biochemical, and commercial characteristics of tea. Controlled UV exposure enhances the polyphenolic content of tea leaves, improving their therapeutic properties and aligning with consumer preferences for high-antioxidant products (Li et al., 2015). However, managing UV exposure is crucial to prevent potential negative effects, such as cellular damage and reduced crop yield. As research continues to shed light on optimal UV exposure levels, the tea industry can harness this knowledge to develop cultivation practices that maximize both the therapeutic and commercial value of tea, meeting the growing demand for health-promoting, high-quality tea products.

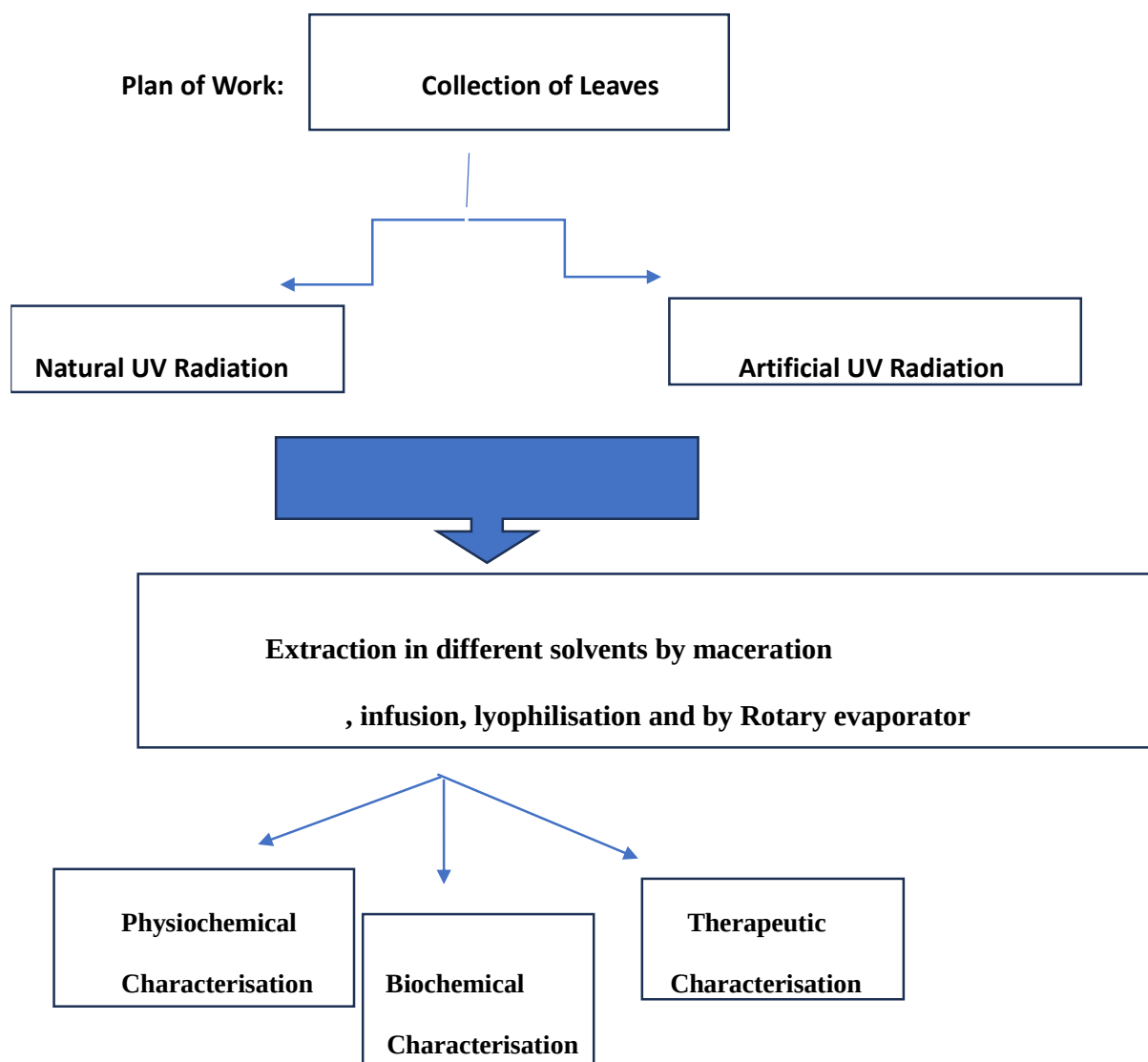
Chapter 3

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CHAPTER 3

MATERIAL AND METHODS



3.1 Collection of the tea leaves:

The fresh leaves (two leaf a bud) and manufactured tea leaves were collected from the gardens of the seven valleys of Darjeeling, West Bengal. After collection, the leaves are kept in zip pouches in ice boxes and brought to Kolkata St. Xavier's College laboratory. The

leaves were collected from different altitudes of seven valleys and at different seasons/flushes (1st flush mid-March to April, 2nd Flush, May-July, autumn flush, September-October).

3.2 Location and site of collection:

The seven valleys of Darjeeling (27.0416°N, 88.266° E)

Seven valleys are as follows:

1. Darjeeling East
2. Darjeeling West
3. Kurseong South
4. Kurseong North
5. Mirik
6. Upper Fagu/Rungbong
7. Teesta



Figure 3.1: This figure shows the location of Darjeeling (27.0416°N, 88.266° E) in India.

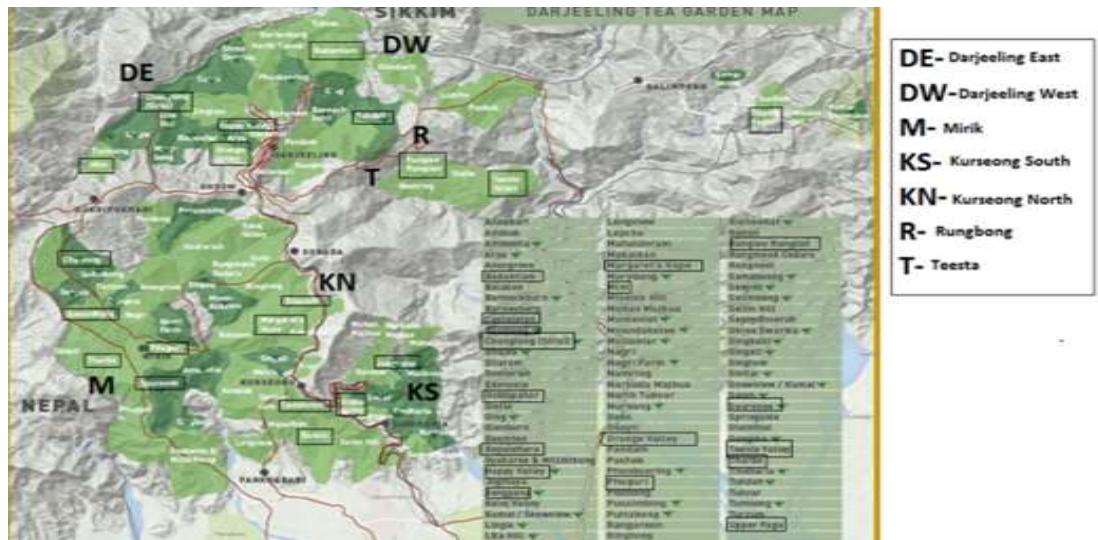


Figure 3.2: This figure shows the road map of the seven valleys of Darjeeling.

The Altitudes of each garden of the seven valleys of Darjeeling:

Darjeeling East Tea Garden	Altitude (ft.msl)
Marybong	3900
Mim	5000
Orange Valley	3500
Chongtong	3665
Darjeeling West Tea Gardens	Altitude (ft.msl)
Badamtam	6000
Ging	3717
Kurseong South Tea Gardens	Altitude (ft.msl)
Castleton	4500
Giddhapahar	4354
Rohini	4400
Upper Jungpana	3300

Kurseong North Tea Gardens	Altitude (ft.msl)
Dilaram	6000
Margarette Hope	4800
Mirik Tea Gardens	Altitude (ft.msl)
sourene	3500
Thurbo	5000
Gopaldhara	6500
Phugiri	3900
Upper Fagu Tea Garden	Altitude (ft.msl)
Tukdah	4000
Dhajia	3600
Teesta Tea Garden	Altitude (ft.msl)
Runglee-Rungliot	5100
Gielle	2600

Table 3.1 shows the altitudes of each garden of the seven valleys of Darjeeling.

3.3. Extraction Process:

For the biochemical characterization of tea, the extract preparation was done using a cold **maceration process** using different solvents such as DMSO, methanol ethanol, water, and acetone. Cold Maceration Process- the tea leaves were weighed and they were ground with a mortar pestle with different solvents. Then the homogenised mixture was centrifuged at 5000 rpm for 10 minutes at 5°C and then the supernatant was collected and stored at -20°C for further use. The concentration is 500mg/20ml which is 25mg/ml. Besides the cold maceration process, the tea leaves were also **infused** with different solvents and kept overnight. The next day the supernatant was collected and stored at -20°C for further use. This modified

maceration technique has several advantages over traditional extraction methods. The direct homogenization and centrifugation steps narrow down the process which reduces the risk of sample degradation and also minimizes the time required for extraction.

Tea leaves of high altitude, low altitude, and different types of manufactured leaves in methanol extract are subjected to higher concentration under a **rotary evaporator**, an instrument used to remove the solvents from samples through evaporation efficiently. It is used to purify the samples or make them more concentrated. Aqueous extracts of tea leaves were subjected to **lyophilization** to vaporize the water molecules from the sample leaving them in the powder form.

The extracted tea leaf samples can now be subjected to a variety of analytical techniques to identify and quantify the specific bioactive compounds present. These compounds may include polyphenols, alkaloids, flavonoids, and other compounds with potential health benefits, such as antioxidant, anti-inflammatory, and anti-cancer properties. Further analysis can help elucidate the unique chemical composition and biological activities of tea leaf extracts from different regions, providing valuable insights into their potential applications for various health-related and medical purposes, including functional foods, nutraceuticals, and pharmaceuticals.

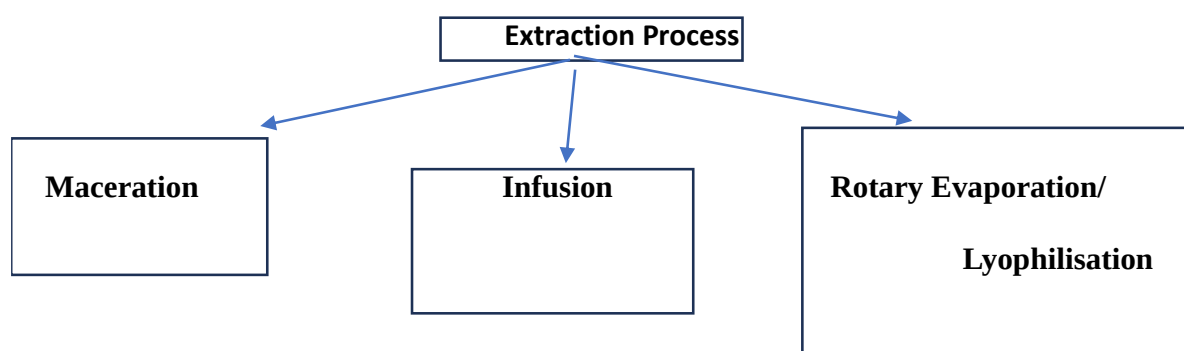


Figure 3.3: Different extraction Process.

3.4 Determination of Agro-climatic Parameters:

Agroclimatic Parameters include External Factors as well as Internal Factors

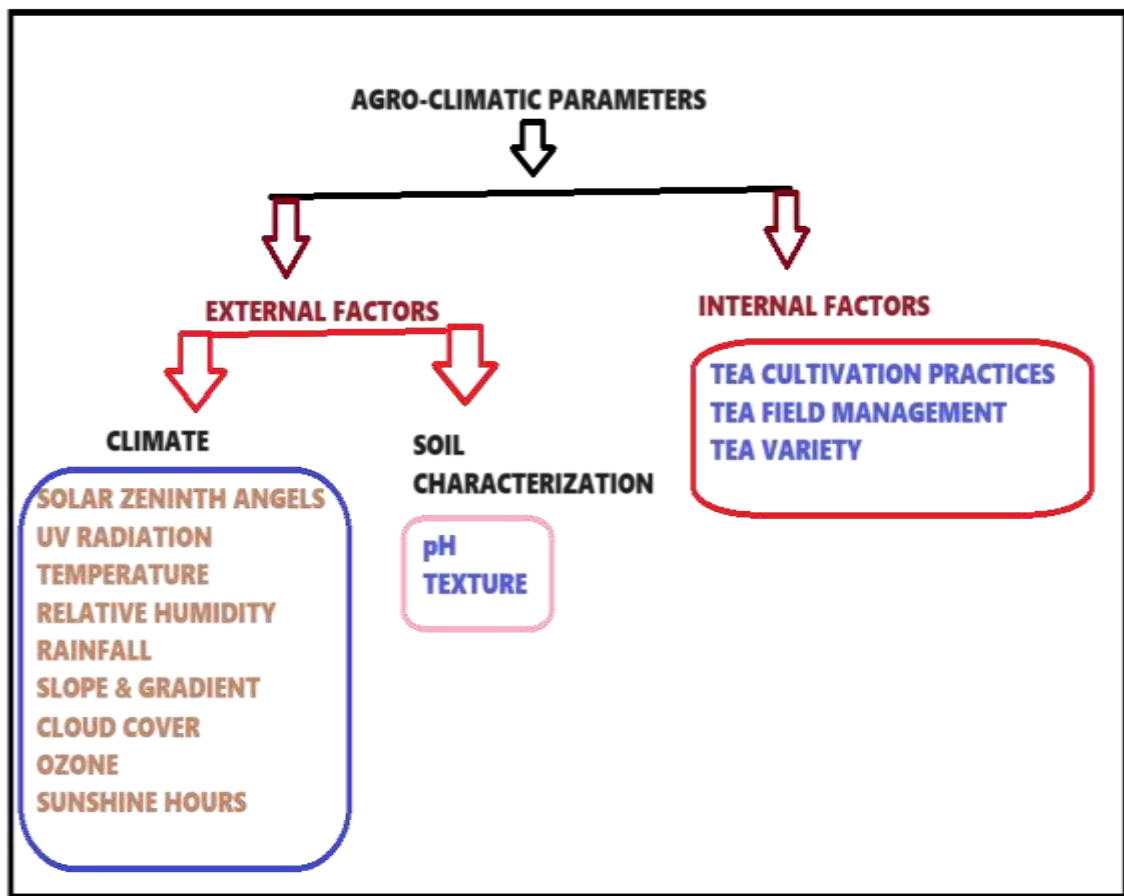


Figure 3.4: Agroclimatic parameters

3.4.1.External Factors:

- ✓ **Climate**-Temperature, Relative humidity, Rainfall, Slope and gradient, Cloud cover, Ozone, Sunshine, Natural UV radiation, Solar Zenith Angles were measured. These factors are measured at Bose Institute, Darjeeling, Mayapuri. Table 4.1
- ✓ **Soil Characterization**-Soil pH, Soil texture.The soil pH is measured with the help of a pH meter. Table 4.1



Figure 3.5: Shows different instruments with which the external factors were measured in Bose Institute, Mayapuri, Darjeeling Campus

- ✓ **Temperature:** Temperature was measured with the help of a thermometer.
- ✓ **Relative Humidity:** It was measured with the help of a hygrometer.
- ✓ **Slope:** The elevation of the land was measured with the help of a clinometer. It measures the distance or rise between two points, and the quotient is multiplied by 100.
- ✓ **Cloud cover:** The cloud cover is measured with the help of ceilometer in Dobson Unit.
- ✓ **Ozone:** It is measured with the help of the Dobson Spectrophotometer. It measures the intensity of sunlight at two wavelengths one at weakly absorbed by ozone, and one strongly absorbed by ozone.
- ✓ **Sunshine:** Sunshine is measured with the help of a pyranometer.

All the parameters are described in Table 4.1

Natural UV radiation is measured using a Lutron UV340A UV light meter in $\mu\text{W}/\text{cm}^2/\text{sec}$ at various altitudes in different gardens in Darjeeling, over different time intervals. Figure 4.1.2



Figure 3.6: UV light meter

Solar Zenith Angels: The angle between the sun's ray and vertical direction is measured for calculating solar zenith angle.

3.4.2 Internal Factors

Tea Cultivation Practices, Field management and Tea variety:

The tea cultivation practices are known from various gardens, including conventional breeding, the application of biofertilizers (such as cow dung), biopesticides, pruning, and grafting. The clonal variety (**AV2**) of the teas was authenticated from DTRDC, Darjeeling Tea Research and Development Centre, Kurseong. Table 4.1.

Variety:

C. sinensis var. *sinensis*, OLD CHINA BUSH (CHESIMA), Amber valley



Figure 3.7: AV-2 ,Tea variety

3.5. Determination of Agro-physiological parameters:

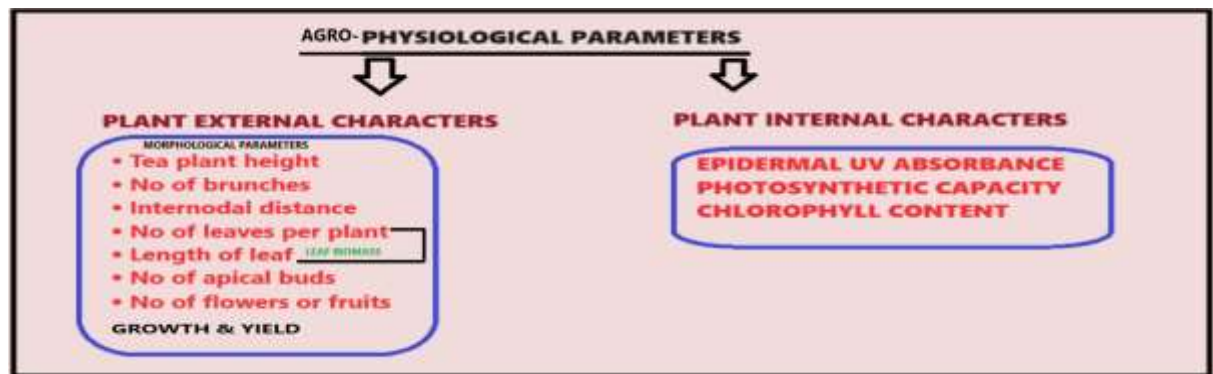


Figure 3.8: Plant Agroclimatic Parameters: External and Internal Characters.

3.5.1 Plant external characters:

Tea plant height, no. of branches, intermodal distance, no. of leaves per plant, length of leaf, no. of apical buds, no of flowers were measured. All are measured with the help of a measuring tape.

3.5.2 Determination of Physiological parameters of fresh leaves of Darjeeling tea gardens: Plant Internal characteristics

3.5.2.1 Epidermal UV absorbance:

It was measured with the help of a Dualex epidermal UV absorbance meter. The transmission ratio of the absorbances absorbed by chlorophyll in far red and near infrared wavelengths is used as a reference.



Figure 3.9: Shows Dualex epidermal UV absorbance meter.

Excitation and emission spectra of intact leaves were measured with the help of Fluorescence microscopy. Cross Section of the leaf was made 85µm thickness. Paradermal images were made by slicing the whole leaf into 1 mm squares before treating it with reagents which results into better infiltration of the reagents into epidermal and mesodermal layer cells. Naturstoff reagent A was mounted with 100mM Potassium Phosphate buffer pH 6.8 1% NaCl buffer solution. Naturstoff reagent A chelates the characteristic fluorescence depending on the hydroxyl group present (according to Goulas et al., 2005) Figure 4.2.1.2b,2c

3.5.2.2 Measurement of UV absorbing compounds:

200mg of each sample were placed in 1.4ml of 99:1 methanol: HCl and allowed to extract for 48hr at -20°C . The extract was centrifuged at 10,000 r.p.m for 10 min. Supernatant was collected and absorbance of supernatant (1ml) was read Spectrophotometrically (UV 2401 PC SHIMADZU, Japan) at 305 nm for determination of total UV absorbing compounds (according to the method of Mazza et al., 2000). The data presented in terms of O.D $_{305\text{nm}}$ /mg fresh wt. Figure 4.2.1.1a

3.5.2.3 Photosynthetic capacity:

The amount of Carbon of CO_2 fixed into carbohydrates during photosynthesis is the photosynthetic capacity and it was measured by keeping leaves in a close chamber and measuring CO_2 over time with Infra Red Gas analyzer (unit $\mu\text{molm}^2/\text{s}$) (Hupp et al., 2021)Figure 4.2.1.3a,3b

3.5.2.4 Measurement of chlorophyll content:

The chlorophyll content was measured according to Prawira-Atmaja et al., 2018. After maceration of the fresh leaves Four hundreds (400) mg sample was mixed with 5 ml of 80% acetone, and was kept in the dark at 4°C for 15 minutes. Then the mixture was then centrifuged at 3,000 rpm, for 15 minutes. The supernatant was collected to a new centrifuge

tube, and was kept in the dark. The same treatment was done twice and the supernatant was combined so that the total volume was made up to 25 ml using 80% Acetone. The mixture was shaken thoroughly and the absorbance of chlorophyll content was measured using spectrophotometry (Varian carry win UV) with acetone 80% as a blank control. Chlorophyll content was calculated using formula:

$$\text{Chlorophyll a (mg/g)} = \frac{[12.7 \times A_{663} - 2.69 \times A_{645}] \times V}{1000 \times W}$$

$$\text{Chlorophyll b (mg/g)} = \frac{[22.9 \times A_{645} - 4.86 \times A_{663}] \times V}{1000 \times W}$$

$$\text{Chlorophyll a + b (mg/g)} = \frac{[8.02 \times A_{663} + 20.20 \times A_{645}] \times V}{1000 \times W}$$

{Where V = volume of the extract (ml); W = Weights of fresh leaves (g); A₆₄₅: absorbance at 645 nm; A₆₆₃: absorbance at 663 nm.}

Figure 4.2.1.4a,4b

3.6 Determination of Biochemical Parameters:

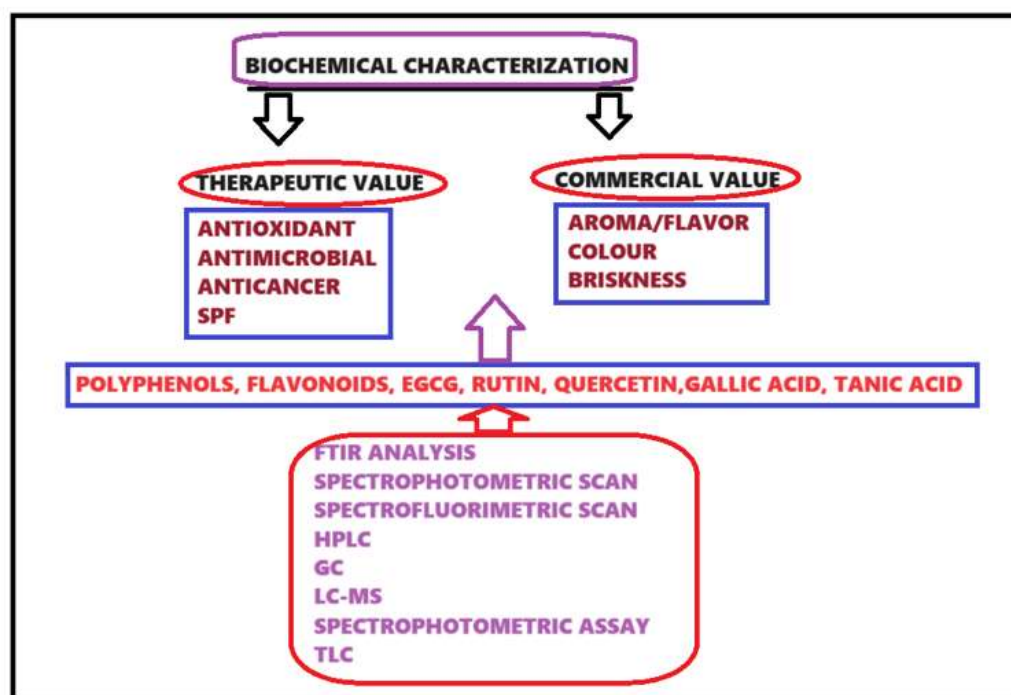


Figure 3.10: Biochemical Characterizations.

For the biochemical characterization of tea (fresh and manufactured), the extract preparation was done by the cold maceration method using different solvents such as DMSO, methanol, ethanol, water, and acetone. The concentration is 500mg/20 ml.

Techniques adopted:

1. **Fourier Transform Infrared spectroscopy (FTIR) analysis**
2. **Spectrophotometric scan**
3. **Spectrofluorimetric scan**
4. **Spectrophotometric assay**
5. **HPLC (High-performance liquid chromatography) analysis**
6. **LC-MS**
7. **Gas chromatography**
8. **Thin layer chromatography analysis**
9. **E-nose, E-tongue assay**

3.6.1 Lipid Peroxidation Assay:

1ml of the tea extract was mixed with 5ml of 0.1% TCA (Trichloroacetic acid) and 0.5% TBA (Thiobarbituric acid). The centrifugation was done then the supernatant was collected and the O.D was measured at 532 nm. The results were represented in terms of concentration of malondialdehyde mM/Mg fresh weight according to Rajinder et al.2020 .Figure 4.3.1.1a,1b

3.6.2 EPR analysis:

Electron Paramagnetic Resonance detects the free radicals with unpaired electrons according to Polovka et al., (2003). 150 mg of embryos from each stock (excised under aseptic conditions) were dried over fresh calcium chloride until the water content was close to 7.5±1% (wet weight basis). The dry embryos were then ground briefly in the porcelain

mortar, sieved through two layers of nylon mesh (pore size approximately 400mm) and finally the powdered material was packed in quartz tubes (3 mm in diameter). The open tubes were then placed in a vacuum desiccator over the calcium chloride at room temperature ($24\pm 1^\circ\text{C}$) overnight. At the room temperature electron paramagnetic resonance (EPR) spectra of the powdered rice embryo samples of each stock were recorded by using a Varian E-112 EPR spectrometer operating at X-band frequencies ($\nu=9.45\text{ GHz}$) having a 100 kHz first modulation and a phase sensitive detection to obtain a field derivative signal. The other stranded parameters are the following: microwave power 15mW, field set 343 mT, receiver gain 5×10^3 , scan time 2 min and modulation amplitude 2mT. 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used as an internal standard ($g=2.0036$). Fig 4a represents representative scan of EPR spectra of different rice varieties and Fig 4b represents peak amplitude of EPR signal (cm/gm dry wt.) at g-value where the maximum absorption is 2.0035. Figure 4.3.2.1a,1b

3.6.3. Enzymatic Antioxidant Assay:

SOD (Superoxide Dismutase) Analysis was done according to Upadhyay and Panda *et al.*, 2013. The O.D was measured at 638.2nm by taking NBT and TEMED.

Catalase Assay: According to Chance and Maehly 1954, in 3 ml reaction mixture 50mM phosphate buffer pH7.0, 15mM H_2O_2 and 2ml extract was taken and the O.D was measured at 240nm. The amount of decline in H_2O_2 determines the catalase activity as catalase converts umole of H_2O_2 equivalence/ mg/min of total protein. Figure 4.3.3.1a

3.6.4. Non-enzymatic antioxidant characterization:

❖ Spectrophotometric scan analysis:

U-2900 Spectrophotometer was used for the spectral scan of tea samples both fresh and manufactured with a specific standard that is quercetin. The spectral scan was run from 500 nm till 200 nm with the scan speed of 400nm/min, sampling interval of 0.5nm and slit width

of 1.50nm. The absorption peaks are around 240nm and 440nm for each of the samples as well for the standard (Atomssa and Ghoslap, 2015) Figure 4.3.4.1a-g

❖ **Spectrofluorimetric analysis:**

F-7000 FL Spectrofluorometer was used for the for different tea samples both fresh and manufactured with a specific standard that is quercetin. 360nm was taken as the excitation wavelength for fluorimetric scan for each of the tea samples. The maximum emission peak of the standard came around 538nm whereas for the tea samples the maximum emission peaks were around 535nm (Khanchi et al., 2007. This trend ensures the presence of Quercetin in different types of tea. Figure 4.3.5.1a-h

❖ **FTIR analysis:**

FTIR (Bruker Apha 11) has been used to determine level of flavonoids in different types of tea leaves (both fresh and manufactured). The spectral scan was done between 200-500nm wavelength. The excitation wavelength for Quercetin was given at 360nm whereas the emission peak for Quercetin was observed at 460nm. The same trend was observed with different types of tea ensuring the presence of Quercetin, Kokalj et al., 2017. Figure 4.3.6.1a-j and Table 4.3.4

❖ **LC –MS:**

It was done from NIPER, Kolkata according to the method of Shevchuk et al., 2018 .Thin layer chromatography-TLC assay was done in silica plate with (by using solvents butanol: acetone: acetic acid-5:5:3 v/v) under UV radiation (UV light 254nm and UV 366 nm) and specific colorimetric agents like Iron chloride and ethanol reagent (Pramiastuti and Joraho, 2020) after that densitometric scan was done (Kamal et al., 2022) Figure 4.3.8.1a-e and Table 4.3.6

❖ GC-MS analysis:

An HP 7890A GC instrument integrated with an Agilent 5975C MSD mass spectrometer. The GC oven temperature was held at 50°C for 5 min, increased to 210°C at a rate of 3°C per min which is maintained for 3 min at 210°C, and subsequently increased to 230°C at 15°C/min. The mass spectrometer conditions were- ionisation energy, 70 eV; ion source temperature, 230°C; quadrupole temperature, 150°C; quadrupole mass spectrometer scan range, 30–500 atomic mass units (amu); solvent delay time, 2.8 min (Lee et al., 2013). Figure 4.3.20 1a,b,c

❖ HPLC Analysis:

To obtain a detailed profile of the polyphenolic compounds present in the extracted tea leaf samples, high-performance liquid chromatography (HPLC) analysis was employed. HPLC is a highly effective analytical technique that allows for the precise separation and quantification of individual components within a complex mixture. A reverse-phase C18 column was employed for the separation of polyphenols. The mobile phase consisted of a gradient of acetonitrile and milli-Q water, with increasing concentrations of acetonitrile over time to elute compounds of varying polarity. The extracted tea leaf samples and standard solutions of tannic acid, gallic acid, quercetin, epigallocatechin gallate (EGCG), and rutin were injected into the HPLC system. The eluted compounds were detected using a UV-visible detector set at 285 nm, a wavelength at which many polyphenols exhibit strong absorbance. The retention times of the peaks in the chromatograms were compared to those of the standards to identify the individual polyphenolic compounds. The peak areas were used to quantify the concentration of each compound based on a calibration curve constructed using the standard solutions. HPLC provides a high-resolution separation of polyphenols, allowing for the identification and quantification of individual compounds even in complex mixtures. This technique is particularly useful for determining the relative abundance of different polyphenols in tea leaves and for comparing the polyphenolic profiles of various tea varieties.

Through the use of HPLC coupled with UV-visible detection, we can gain a comprehensive understanding of the polyphenol content in tea. This analysis enables us to evaluate the potential health advantages related with these compounds. HPLC analysis was conducted using Waters® Empower™ 3 with a C18 column (injection volume 50 µl, duration 15 minutes, Channel name W2489ChA). The eluent was monitored at 285 nm, with Rutin utilized as the reference (Bhattacharya and SenMandi, 2011), Figure 4.3.7.1a-f

❖ Spectrophotometric assay

Total Polyphenol content: The polyphenol content of tea samples both fresh and manufactured was measured with 7%Na₂CO₃ and FC reagent according to Anesini et al., (2008). The % polyphenol content was measured spectrophotometrically at 715nm. Figure 4.3.13.1a-c

Total Flavonoid Content: The flavonoid content of tea samples was measured with 75ul of 5% Na₂CO₃, 150ul of 10% AlCl₃ and 750 ul of NaOH according to Zhisen et al., (1999). The % flavonoid content was measured spectrophotometrically at 510nm. Figure 4.3.14.1a-c

Total catechin content: According to Wallace, 20ul of extracts was added to 100ul of dimethyl cinnamaldehyde reagent (DMAC) reagent and the OD was measured at 640nm and the catechin content was measured with the standard curve. Figure 4.3.12.1a-c

Theaflavin Assay: Tea (9 g) was infused in boiling water (375 g) for 10 min. The infusion was carried out in a Thermos flask with continuous mechanical shaking. After filtration through glasswool, a 10 ml aliquot of the infusion was shaken for 10m in with 10 ml of isobutyl methyl ketone (IBMK) and the two layers allowed to separate. The upper layer (2 ml), 4 ml of ethanol and 2 ml of Flavognost reagent (2% w/v diphenylboric acid-2-aminoethyl ester in ethanol-- were mixed and allowed to stand for 15 min. The absorbance was then measured at 625 nm in Results were calculated on a dry weight basis by the

formula: Theaflavin (u mol/g) = $E_{625} \times 47.9 / (DM/100)$, where E is the OD, optical density, and DM is the dry matter content of the tea sample. Table 4.3.11

Tea colour determination: The O.D. of the extracts were measured at 460nm according to Goodner and Wampler 2008. Table 4.3.12

Tea Hue: The hue of the tea extracts were calculated by multiplying 10 by the ratio of the log of the absorbance at 510nm and 610nm. Table 4.3.12

Rutin and Quercetin Assay: Rutin and quercetin content were measured at 430nm and 405nm respectively according to Mir et al., 2013. Figure 4.3.10.1 and 4.3.11.1a,b

β -DGlucosidase activity: According to Jiang and Li 1999 β -D Glucosidase activity was done with the tea extracts in sodium citrate assay buffer pH 4.0 containing 4mM pNPG and the color developed at 410nm. Figure 4.3.18.1a-c

Linalool Content: According to Bhattacharya and Sen Mandi 2011 tea extract was made with Tris-Cl buffer pH 7.5, 20mg dimethylaniline, 10ml acetyl chloride, 5 ml of acetic acid. It was kept in ice-cold condition for 5 minutes and allowed to stand in room temperature for 30 minutes. Oily phase was separated by a separating funnel and 2g of sodium sulfate was added and filtered. The filtrate was weighed and the percent linalool content was calculated with standard linalool. Figure 4.3.19.1a,b

Assay of Polyphenol oxidase activity : According to Unal et al., 2011, 0.1ml of tea extracts mainly manufactured tea extracts and 0.9ml of Catechol in 200mM phosphate buffer was added and the activity of enzyme was measured at 410nm. Enzyme activity was calculated by Shyam Choudhury et al., 2015. Figure 4.5.6.1

❖ **Thin Layer Chromatography:**

It was done according to Saha et al., 2017 using silica as stationary agent and with Iron Chloride-Ethanol as reagent colouring agent with standard Quercetin and EGCG. For detecting and visualising substances as spots on TLC plate UV chamber consisting of UV

lamps which emit light at wavelengths 254nm and 365nm was used. For identification of components on TLC plate, vanillin was sprayed, which reacts with the compounds and gives colored spots of the components for better analysis. After the development or spotting the colour the spots were quantified with densitometric scanner. Figure 4.3.9.1a,b

E-nose and E-tongue analysis:

The E-nose data were assessed based on Taster's score (T.S) and Nor-aroma index (N.I) following Shyam Choudhury et al., 2021 done from CDAC, Kolkata. Intelligent Electronic sensory technologies to mimic olfactory and gustatory systems to develop comprehensive sensory characteristics like aroma and taste the volatile and non volatile components had been scored with E nose and E tongue. The E-nose system works on a sensor fusion technology array of non-specific Metal Oxide Semiconductor (MOS) Sensors which capture the volatile compounds omitted by tea samples and a digital camera wherein the score is given as Noraroma Index (N.I) with respect to standard black tea samples. The non volatile astringent components are assessed by E-tongue where The voltammetric technique with electrochemical cell with electrodes, reference electrode and counter electrode are there to display Taster's score (T.S) as index of soluble non volatile components for taste.

- ❖ **E-nose analysis:** E-nose also known as Electric nose, detects volatile chemical compounds similar to the human olfactory sense. E-nose comprises some sensors that detect the volatile compounds and bind with them. This binding with the sensor generates electrical properties which are converted to electrical signals. The data or the signal are analysed and they are depicted as Nor-aroma index of the volatile compounds. Figure 4.3.21.1a-c
- ❖ **and E-tongue analysis:** E-tongue also known as Electronic tongue , detects non volatile chemical compounds similar to the human sense of taste. Just like E-nose it

has an array of electrochemical sensors which binds with the non-volatile compounds and the binding as a result generates change in the electrical properties which are converted to electrical signals by the machine itself. The data are depicted as Taster's score for the non-volatile components. Figure 4.3.21.1a-c



Figure 3.11: Shows the AI-based instrument E-Nose



Figure 3.12: Shows the AI-based instrument E-Tongue

3.7 Analysis of the therapeutic Potential of Tea:

3.7.1 Antioxidant potential:

Antioxidant potentials are calculated according to the reduction of 0.004% DPPH and recording the O.D at 517nm according to Shyam Choudhury *et al.*, 2015. Figure 4.3.22.1a-c

3.7.2 Antimicrobial assay:

The antimicrobial assay is done according to Kirby bauer method cup plate method on Mueller-Hinton agar (MHA) plates. Overnight inoculated cultures of *Bacillus subtilis*, *Pseudomonas sp*, *Escherichia coli*, *Salmonella sp*. are poured onto the plates with MHA. Then the plates are allowed to solidify for 25 minutes. After solidification of the agar with a specific organism the wells are bored into the agar with the help of a cork borer. The extracts are then poured into the respective wells. Positive control is the antibiotic Ampicillin (1mg/ml) and the negative control is the solvent in which the extract has been prepared. After the addition of the solutions (extract, positive control, negative control) the plates are incubated for 24 hours at 37°C. The next day the zone of inhibition by the solutions against the respective species are calculated using a ruler according to Radji *et al.*,2013 Figure 4.3.23.1a-u

3.7.3 Anti-fungal assay:

Antifungal activity was checked on antibiotic treated PDA plates with *Aspergillus Sp*. The growth inhibition of the fungus was checked for 3 days as after 3 days there is full growth of *Aspergillus sp*. on PDA plates according to Shivakumar *et al.*, 2023. Figure 4.5,14.1a-c

3.7.4 Anti-Cancer Assay:

MTT assay: The IC₅₀ value of the tea extracts was measured by the MTT or(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay following Abid F., *et al* 2021 .The cell line was HepG2. The data was outsourced from NIPER, Kolkata.
Figure 4.5.15.1

3.7.5 Anti diabetic assay:

***In vitro* inhibition of serum glucose analysed with Kit**

In this method glucose is oxidized to gluconic acid and the O₂ is reduced to H₂O₂ by glucose oxidase. Peroxidase then turns H₂O₂ to nascent [O] which reacts with 4-aminoantipyrine to

form red colored compound, quinoneimine dye which is red in color. The O.D was measured at 540nm. Analysed with Erba Kit. The 500 µl serum was added with 500µl of enzyme mixture and after 5 min incubation the OD was measured . Figure 4.3.27.1

3.7.6 Inhibition of digestive enzyme: Amylase and β-D glucosidase enzyme

The assay is performed according to Li et al. (2015). Inhibition of amylase was done with the tea extracts. The enzyme amylase solution with tea extract and substrate were incubated for ten minutes at 37°C. Then the enzyme activity was measured spectrophotometrically. β-D glucosidase enzyme assay was done according to Jiang and Li.(2020). 50mM of sodium acetate extract buffer pH 5.0 containinh 1mM EDTA ,NaCl, MgSO₄, 1mM PMSF, 1mM sucrose, 2%PVP 5.6% mM βME the tea leaves were extractes centrifuged and the supernatant was assayed in sodium citrate pH4 assay buffer containing 4mM NPG and the color development was measured at 410nm. IC₅₀ was also determined .Figure 4.3.26.1a.

3.7.7 SPF determination: SPF can be calculated using the Mansur equation described below – $SPF = CF \times EE(\lambda) \times I(\lambda) \times Abs(\lambda)$ Where CF correction factor (10), $EE(\lambda)$ = erythmogenic effect of radiation with wavelength λ , $Abs(\lambda)$ = spectrophotometric absorbance values at wavelength λ . The values of $EE \times I(\lambda)$ are constants (Malsawmtluangi et al., 2013

Figure 4.3,29.1a,b

3.7.8 Anti-inflammatory action: Inhibition of Lipoxygenase enzyme:

It is a kit-based assay for the quantification of lipoxygenase enzyme in plant samples using a microreader. LOX oxidises linoleic acid and the oxidation product has the absorption peak at 280nm according to Loncaric. M *et al.*, 2021. Figure 4.3.28.1a

3.7.9: Bioavailability of EGCG in plasma and urine:

This assay was conducted at KPC Medical College under the supervision of Dr. Sujata Mallick. The concentration of EGCG was monitored in plasma and urine for 1-hour interval on a day of 5 healthy men aged between 28 to 40 . Figure 4.3.30.1

3.7.10 Preparation of Gold Nanoparticles: Citrate Method:

At first all the glasswares were washed with Piranha solution which is H_2SO_4 : H_2O_2 (3:1). The glasswares were left for 30 minutes. Then the glasswares were vigorously washed with distilled water and then with MilliQ water. Then the glasswares were dried with a heat gun till the smell of the acid was not there. 100 mM Trisodium citrate was prepared by dissolving 294.1mg trisodium citrate into 10ml of MilliQ water. Then 20mM HAuCl_4 was prepared by dissolving 11.3 mg HAuCl_4 in 1.472ml of MilliQ water. To the freshly cleaned Round Bottom flask 620ul of gold solution was added and to it 50 ml of MilliQ water was added and then the solution was placed in the hot oil bath. The temperature was set to 130°C. The temperature(100°C) was checked with a thermometer. The solution was heated to boiling. It took 10 minutes to reach 100 °C. Then it 600ul of citrate solution was added and the mixture was continuously stirred at 1200 rpm. Approximately, it took 17 minutes to reach the color red wine. After the solution was turned red wine in color it was kept in stirring condition for about 10 minutes without temperature. 150ul of the gold nanoparticle was added into the wells of 96 well plate and the O.D. was observed at 530nm. To 5ml of AuNP, 50ul of PS-40 was added to check whether there was any change in O.D. At every 1-hour interval the O.D. values were checked. The gold nanoparticle solution was then refrigerated for further use. (Liu, C.-Y., & Tseng, W.-L. (2012).



Figure 3.13: Gold nanoparticle

3.8 Artificial UV exposure:

3.8.1 Artificial UV exposure In laboratory with one year old potted plants:



Figure 3.14: One-year-old potted plant from DTR, DC Darjeeling

For exposure to artificial UV radiation different lamps were used. The UV dose of different lamps were measured with a UV meter.

1. Philips UV Lamp Bulb 4-Watt 6 Inch TUV 4-Watt G5 OR Osram Pureitec HNS G5 4 Watt
2. FSL BL UVA lamp T8 for insect killers 15w
3. Philips TUV 30w 1SL/25 T8 (3 feet)
4. Powersun UV l
5. Powersun UV lamp 160w



Figure 3.15: Different artificial UV lamps with different UV fluence

All the lamps were individually taken as tests and as control along with the Control where the lamps were taken but Soda glass filters were given which can cut off the UV-B radiation at 280nm

All Physiological, Biochemical, Commercial and Therapeutic potentials are measured under these UV exposures.

The methods of biochemical and therapeutic potentials are mentioned above. The same procedures of extraction of tea leaves followed by biochemical assay and therapeutic potential assay under natural UV radiation are done with the exposure to artificial UV radiation in the laboratory.



Figure 3.16: Artificial UV exposure in laboratory (St. Xavier's College, Kolkata)

3.8.2 Artificial UV exposure In laboratory to post harvest tea



Figure 3.17: Exposure of artificial UV over post-harvest tea in the laboratory

Two leaf a bud freshly harvested tea was taken in UV chamber and the UV exposure was given at 254nm for the following time intervals upto 300 mins. The control was taken with the same time parameters but kept at dark.

Set	Time
I (Dark)	0min
I (Dark)	30min
I (Dark)	60min
I (Dark)	120min
I (Dark)	180min
I (Dark)	240min
I (Dark)	300min
II (UV exposure)	0min
II (UV exposure)	30min
II (UV exposure)	60min
II (UV exposure)	120min
II (UV exposure)	180min
II (UV exposure)	240min
II (UV exposure)	300min

Table 3.2 : Exposure time of post-harvest tea in laboratory

Then the biochemical analysis was done

3.8.3 Artificial UV exposure In factory to post harvest tea

Artificial UV dose exposure with UV lamp in Castleton factory

UV lamp with the dose standardized in laboratory (maximum 2100 $\mu\text{W}/\text{cm}^2/\text{sec}$) was exposed for different time intervals up to 60 minutes to Castleton tea factory during post harvest manufacturing between drying and sorting. UV exposed leaves were taken for further characterization. Control experiments were conducted without UV exposure.

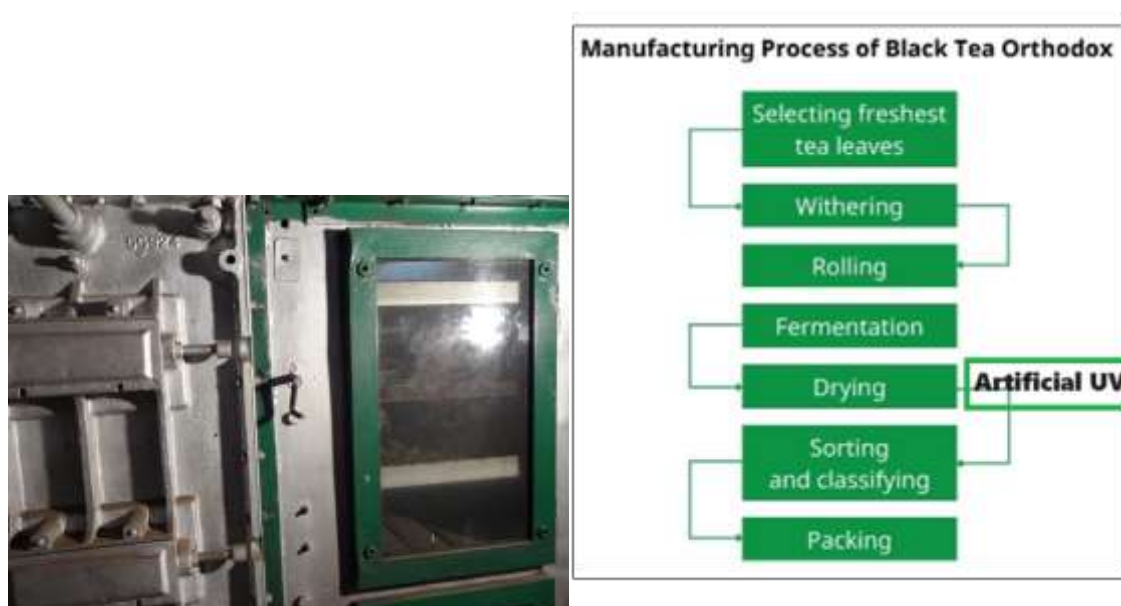


Figure 3.18: Factory set up of artificial UV lamp

Figure 3.18 shows the factory set up of artificial UV lamp in between drying and sorting steps of industrial manufacturing of tea

The exposed tea was taken for biochemical, commercial and therapeutic potential analysis

3.9 Determination of shelf life of different manufactured tea (White, Green, Oolong and Black)

The manufactured tea is stored in Aluminum packaging to determine the shelf life with silica desiccants. The controls were made in aluminum packages without silica (C). They were kept for 18 months.



Figure 3.19: Shows that the manufactured tea are stored in Aluminium packaging with silica desiccants

The Change in Physico-chemical, Biochemical, therapeutic potential values were measured in Aluminium and Silica packaging.

3.9.1 Determination of Physico-chemical parametres in different packaging:

3.9.1.1 Moisture and ash content determination: Moisture content was measured by using a hot air oven at a temperature of 105°C by following Anon.,2000, and ash content was measured according to Srilakshmi,2003, by a muffle furnace at 600°C for 5 to 6 hours. Figure 4.5.22.1 and 4.5.23.1

3.9.2 Crude Protein Content: It was determined by determining the nitrogen content according to Horwitz (2002) Figure 4.5.24.1

3.9.3 Crude Fibre Content: According to Adnan et al., (2013), the %crude fibre contents were determined by digesting samples in 1.2% Sulphuric acid and 1.2% NaOH. Figure 4.5.24.1

3.10. Characterization of Herbal and Medicinal tea:

3.10.1 Materials taken



Figure 3.20: Medicinal Tea



Figure 3.21: Herbal tea

3.10.2 Extraction of herbal and Medicinal tea: The extraction procedure of medicinal tea like Lavender Chamomile white tea, Jasmine Hibiscus green tea, Tulsi green tea and the herbal tea like chamomile tea, lavender tea, Rooibos tea, Hibiscus tea and Blue pea and chamomile tea, Tulsi lemongrass tea, Tulsi ginger tea is same as the true tea. The concentration was taken as 25mg/ml. The maceration was done in ethanol extract and the decoction or infusion was done in aqueous solvents for further biochemical and therapeutic analysis. The tulsi ginger tea and the tulsi lemongrass tea were also taken for validation of the therapeutic indices derived from E-nose e-tongue data with the corresponding antimicrobial and antioxidant data according to Shyam Chowdhury et al., 2022.



Figure 3.22: shows maceration of herbal teas in ethanol solvent.



Figure 3.23: Shows the decoction of herbal teas in aqueous solvent

3.10.3 Biochemical, therapeutic potential analysis were done according to the methods described above.

3.10.4 Determination of Therapeutic index of herbal and medicinal tea

The herbal and medicinal tea were collected and the extraction was done in different solvents by maceration and infusion process. The extracted herbal and medicinal tea were biochemically and therapeutically examined. Antioxidant and antimicrobial data were taken along with the E-sensor based analysis (E-nose and E-tongue).

Herbal tea, tisanes and flowers were collected and their antioxidant antimicrobial tests were done along with the determination of the non-volatile and volatile components with AI based instruments, E-Tongue and E-Nose in terms of Tasters' score and Nor-aroma index respectively. Statistical regression was done with these data of known herbal tea. By comparing with the known samples the therapeutic index of the unknown samples can be determined

3.11 Statistical analysis:

By using Kruskal-Wallis-test, using Chi-Square(df:3) distribution (right-tailed) the p value was determined in every cases to determine the comparison among seven valleys (ANOVA POST HOC analysis) of tea Figure 4.1.6 .

A one-way analysis of variance (ANOVA) was performed to assess the significance of variations among physiological, biochemical, therapeutic parameters with natural and artificial UV radiation.

Regression analysis were done to correlate the therapeutic potentials and E-sensor based data to calculate the therapeutic indices for all tea varieties. With the help of the regression analysis therapeutic index could be developed by taking therapeutic values like antioxidant, antimicrobial, and anticancer values as dependent variables and Nor-aroma index and

Taster's score index as independent variables.

- S.D was also calculated.
- PCA analysis with the heap map was done .Fig 4.3.31.1

All reagents are brought from SRL India.

Chapter 4

RESULTS

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NATURAL UV RADIATION & TEA



RESULTS

OBJECTIVE 1: MEASUREMENT OF AGRO-CLIMATIC PARAMETERS (INCLUDING NATURAL UV RADIATION) OF SEVEN VALLEYS OF DARJEELING TEA GARDENS

EXPERIMENT: 4.1 Measurement of agro-climatic and environmental parameters:

The agro-climatic and environmental parameters were measured annually from 2018 to 2023 at various times of the day.

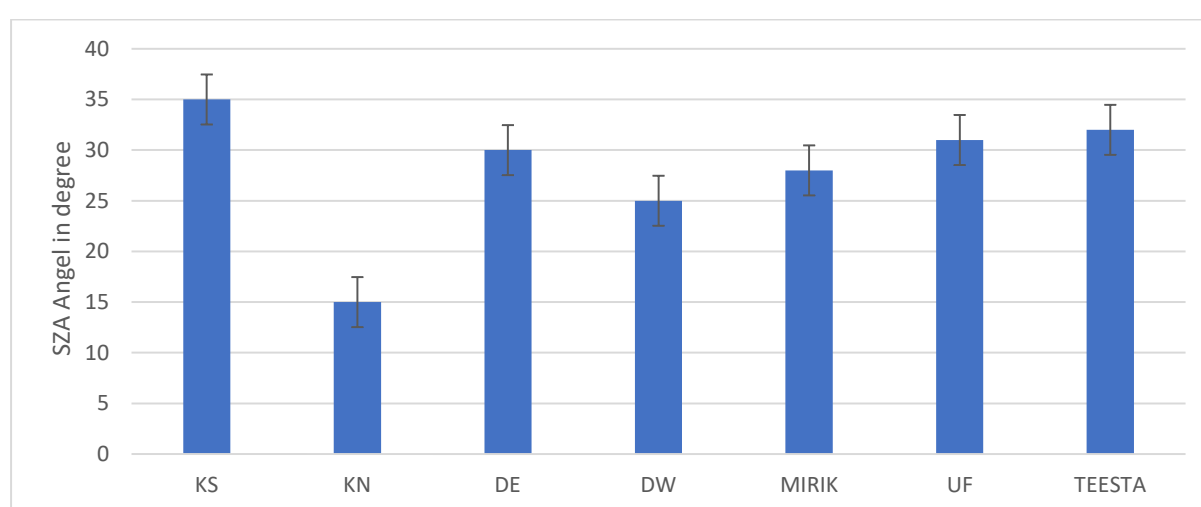


Figure 4.1.1: Variation of solar zenith angle in seven valleys of Darjeeling. S.D=0.5

The solar zenith angle of Kurseong South is about $35^{\circ} \pm 0.5^{\circ}$, which is higher than Teesta Valley at $32^{\circ} \pm 0.5^{\circ}$, Upper Fagu at $31^{\circ} \pm 0.5^{\circ}$, Darjeeling East at $30^{\circ} \pm 0.5^{\circ}$, Mirik at $28^{\circ} \pm 0.5^{\circ}$, Darjeeling West at $25^{\circ} \pm 0.5^{\circ}$, and Kurseong North at $15^{\circ} \pm 0.5^{\circ}$.

Fig 1 shows the variation of UV radiation among seven valleys of Darjeeling, which varies from 15° - $35^{\circ} \pm 0.5^{\circ}$ N and is highest for Kurseong South i.e. $35^{\circ} \pm 0.5^{\circ}$. The values of UV radiation within a narrow range of clearness index values increase almost exponentially with the cosine of the solar zenith angle. Bhattacharya and Sen Mandi, 2010 clearly stated that the variation of UV radiation at high altitudes is due to different solar zenith angles. Each valley has the same solar zenith angle at any altitude so that this solar zenith angle can be taken as a unique marker parameter for geographical and environmental correlation.

EXPERIMENT 4.1.1: MEASUREMENT OF ENVIRONMENTAL PARAMETERS

All environmental parameters of seven valleys

Sl. No.	Environmental Parameters	Kurseong South	Kurseong North	Darjeeling East	Darjeeling West	Mirik	Upper Fagu	Teesta
1.	Geographical Location	26° 52'40"N/88° 16'38"E	26° 52'40"N/88° 16'38"E	27° 2'0"N/88° 16'0"E	27° 2'9.6252"N/88° 15'45.6592"E	26° 53'7.07"N/88° 10'58.06"E	26.9809° N/88.6936° E	27.0124° N/88.4047° E
2.	Rainfall (Average)	3944-4025mm	About 4064mm	About 4000mm	3558mm	About 4000mm	3900mm	4000mm
3.	Condition of Soil	Soil is siliceous and aluminous. Red and yellow soil from gneisses and schist withering. pH-4.5 to 5.5	Soil is siliceous and aluminous. Red and yellow soil from gneisses and schist withering. pH- 5	Sandy, loamy. Reddish in colour pH- 4-5	Sandy and loamy. pH- 4-5	Soil contains sand to clay and is loamy. pH- 5	Red and yellow soil. pH-4.7	Soil is sandy and loamy. pH- 5.5

4.	Temperature (Average)	22.1° C - 17.2° C	25.1° C - 17.2° C	21° C -16° C	19° C -13° C	20° C -17° C	24.1° C - 10.2° C	24.1° C - 20.2° C
5.	Humidity	86%	84%	91%	88%	86%	61%	89%
6.	Sunshine (Average)	7-8 hours/day	7-8 hours/day	7-8 hours/day	7-8 hours/day	7-8 hours/day	8.68 hours/day	7-8 hours/day
7.	Cloud Cover	3-4 octa	4 octa	4 octa	4 octa	3-4 octa	3 octa	3-4 octa
8.	Cultivation and management practices	Conventional breeding, application of biopesticide, biofertilizer, pruning, grafting occasionally	Conventional breeding, application of biopesticide, biofertilizer.	Manual weeding, application of biopesticide, biofertilizer.	application of biopesticide, and biofertilizer.	application of biopesticide, and biofertilizer.	Conventional breeding, of biopesticide, and biofertilizer.	application of biopesticide, and biofertilizer.
9.	Total Columnar Ozone Concentration	24 DU (1DU=2.141 $5 \times 10^4 \text{ug/m}^2$)	24 DU (1DU=2.141 $5 \times 10^4 \text{ug/m}^2$)	40DU (1DU=2.141 $5 \times 10^4 \text{ug/m}^2$)	40DU (1DU=2.141 $5 \times 10^4 \text{ug/m}^2$)	30-34 DU (1DU=2.141 $5 \times 10^4 \text{ug/m}^2$)	22 DU (1DU=2.141 $5 \times 10^4 \text{ug/m}^2$)	34 DU (1DU=2.141 $5 \times 10^4 \text{ug/m}^2$)

	n							
10.	Slope	60° C to 70° C	60° C to 70° C	60° C to 70° C	60° C to 70° C	60° C to 70° C	60° C to 70° C	60° C to 70° C

Table4.1.: shows variation in other environmental parameters of seven valleys of Darjeeling.

There is the correlation between monsoonal circulation and the local topography at the Darjeeling–Bhutan region of Himalaya which receives annual rainfalls (3000–6000 mm) was discussed by Prokop and Walanas (2017). Geological lay-out and the soil, climate interaction was documented in <https://darjeeling.gov.in/economy/>. Assessment of climate change and the thorough impact of agro-climatic parameter variation was documented by Mondal et al., (2024).

EXPERIMENT 4.1.2: MEASUREMENT OF NATURAL UV IRRADIANCE

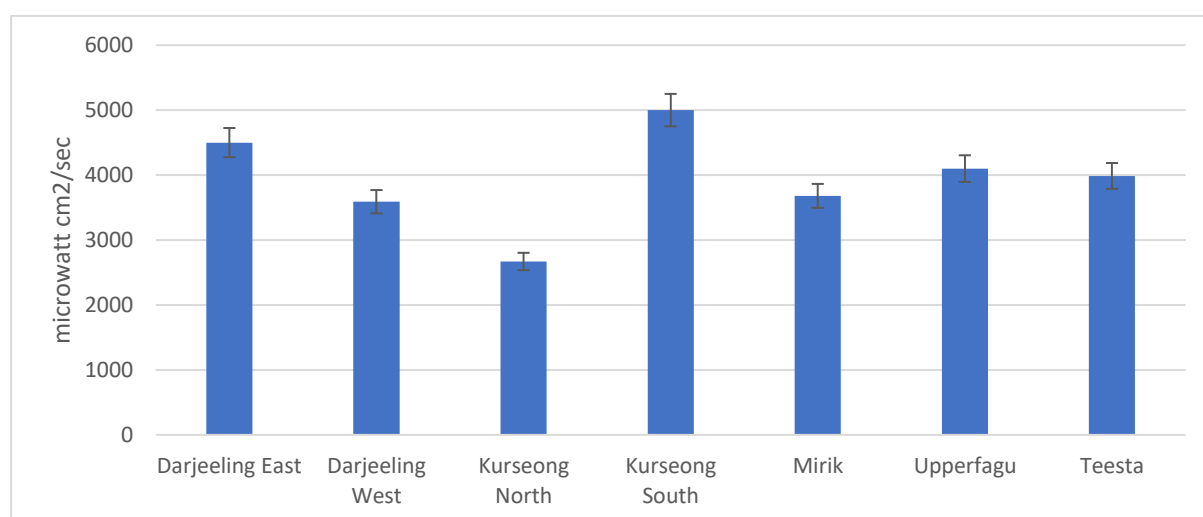


Figure 4.1.2: Variation of natural UV irradiance in seven valleys of Darjeeling.S.D=0.001

Variation of natural UV irradiance in seven valleys of Darjeeling was measured with Lutron UV-340A UV Light Meter (Lux Range 1999 To 19990).The natural UV irradiance of

Kurseong South is about 5000 ± 0.25 microwatt cm^2/sec followed by Darjeeling East (4500 ± 0.001 microwatts cm^2/sec), Upper Fagu (4100 ± 0.001 microwatts cm^2/sec), Teesta (3987 ± 0.001 microwatts cm^2/sec), Mirik (3680 ± 0.001 microwatts cm^2/sec) Darjeeling West (3590 ± 0.001 microwatts cm^2/sec) and Kurseong North (2670 ± 0.001 microwatt cm^2/sec).

Fig 4.1.2 corroborates the difference in natural UV irradiance with the solar zenith angles of the seven valleys of Darjeeling (Fig 4.1.1), stating that Kurseong South is receiving a maximum dose of $5000 \mu \pm 0.25 \text{Watt}/\text{cm}^2/\text{s}$.

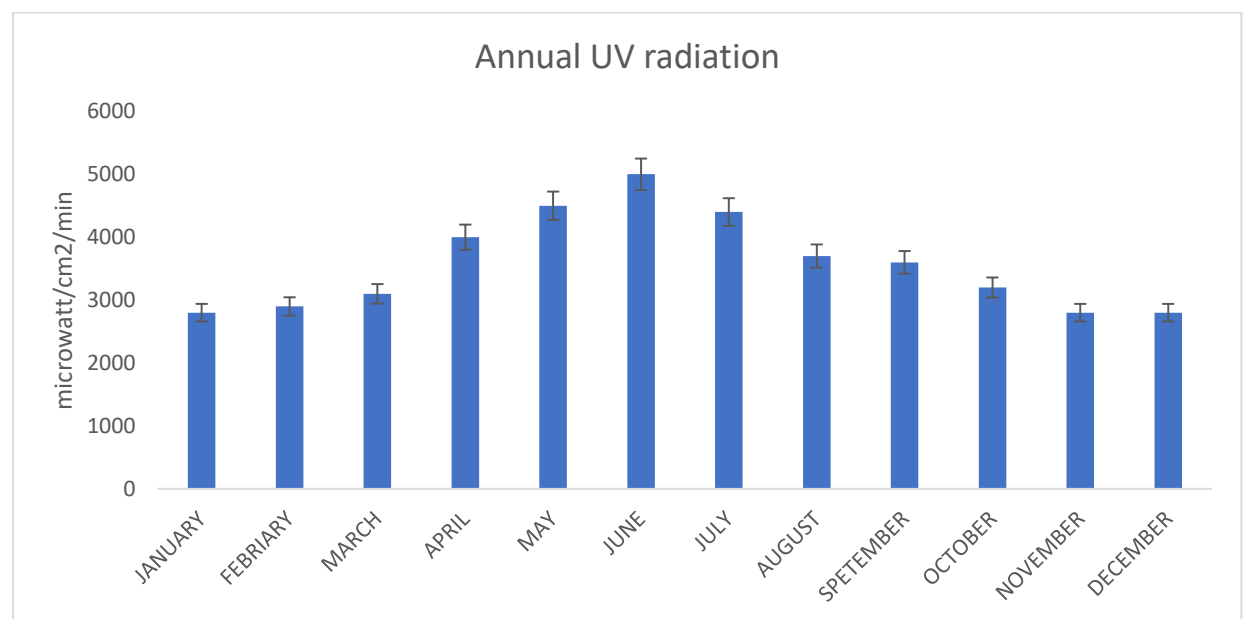


Figure 4.1.3: Variation of annual natural UV irradiance of Kurseong South Valley.S.D=0.001

The annual UV radiation is highest during June which is about 5000 ± 0.001 microwatt/ cm^2/min followed by May at 4500 ± 0.001 microwatt/ cm^2/min , then July at 4400 ± 0.001 microwatt/ cm^2/min , April at 4000 microwatt/ cm^2/min , August at 3700 ± 0.001 microwatt/ cm^2/min , September at 3600 ± 0.001 microwatt/ cm^2/min , October at 3200 ± 0.001 microwatt/ cm^2/min , March at 3100 ± 0.001 microwatt/ cm^2/min , February at 2900 ± 0.001 microwatt/ cm^2/min lastly November, December, and January at 2800 ± 0.001 microwatt/ cm^2/min .

Fig 4.1.3 shows the average annual variation of natural UV irradiance in the Kurseong South valley indicating the highest UV irradiance during the summer solstice during 2nd flush of tea harvest. Here it is pertinent to mention that the 1st flush harvest time is from the end of March to May. Annual variability and the distribution of the variation of UV radiation annually (using Temis data) throughout India, including the Darjeeling district, was mentioned by Bhattacharya et al, 2014

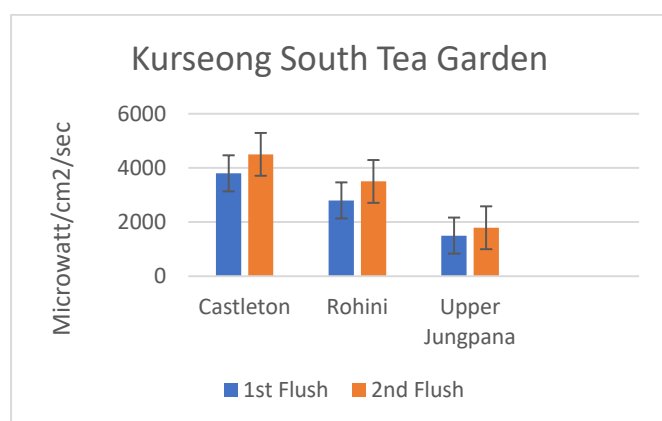


Figure 4.1.4: Variation of natural UV irradiance of different altitude situated gardens of Kurseong South at different flushes (seasonal).S.D=0.001

Castleton at 4500 ft msl altitude receives higher UV irradiation of about 4500 ± 0.001 microwatt/cm²/sec during the 2nd flush than during the first flush which is about 3800 ± 0.001 microwatt/cm²/sec. Rohini at 4400 ft msl altitude receives higher UV irradiation of about 3500 ± 0.001 microwatt/cm²/sec during the second flush and about 2800 ± 0.001 microwatt/cm²/sec during the first flush. Upper Jungpana at about 3300 ft msl receives a higher UV radiation dose of 1790 ± 0.001 microwatt/cm²/sec during the second flush and 1500 microwatt/cm²/sec during the first flush.

It is evident from Fig 4 that Castleton Garden of the 4500 ft msl altitude is receiving the highest UV irradiance during the 2nd flush season. The socio-economic dimension of the Darjeeling tea garden at different altitudes, including the variation of climatic parameters like UV radiation, was discussed by Acharya and Jairu (2020).

Statistical Analysis:

Dep. Variable:	Antiox_Poten	R-squared:	0.972
Model:	OLS	Adj. R-squared:	0.948
Method:	Least Squares	F-statistic:	40.93
Date:	Wed, 17 Jul 2024	Prob (F-statistic):	0.000147
Time:	11:12:22	Log-Likelihood:	-23.693
No. Observations:	12	AIC:	59.39
Df Residuals:	6	BIC:	62.30
Df Model:	5		
Covariance Type:	nonrobust		

	coef	std err	t	P> t	[0.025	0.975]
const	40.4131	16.885	2.393	0.054	-0.903	81.729
Annual_Rainfall	-0.0034	0.007	-0.503	0.633	-0.020	0.013
Annual_relative_humidity	-0.3574	0.245	-1.461	0.194	-0.956	0.241
temperature	-0.0614	0.669	-0.092	0.930	-1.699	1.576
Annual UV Rad	0.0202	0.004	5.693	0.001	0.012	0.029
Productivity	0.1330	7.411	0.018	0.986	-18.002	18.268

Omnibus:	1.915	Durbin-Watson:	2.198
Prob(Omnibus):	0.384	Jarque-Bera (JB):	1.163
Skew:	0.487	Prob(JB):	0.559
Kurtosis:	1.827	Cond. No.	8.81e+04

Figure 4.1.5: Statistical Analysis showing Natural annual UV radiation is the most important factor.

Natural UV radiation is the most significant climatic variant factor among the seven valleys at different altitudes, at different seasons.

Statistical analysis:

Anova Calculator

Data Group 1

35,15,30,25,27,31,33

Data Group 2

5000,2800,4500,3700,3800,4100,4200

Add Group

Reset

Calculate

Test Static F:

232.638

P-Value:

0.00000

Groups Summary

Group 1	Group 2
35	5000
15	2800
30	4500
25	3700
27	3800
31	4100
33	4200

Table 4.1.1 : Statistical data showing that the significant variation among the seven valleys with different solar zenith angles matches the different natural UV irradiance.

Statistical analysis shows (Table 4.1.1) the significance of variation of solar zenith angels among seven valleys of Darjeeling with respecte to differential natural UV irradicance.

Gateva et al., (2021) showed the effect of UV Radiation and other abiotic stress factors on the DNA of different wild plant species grown in three successive seasons in Alpine and Subalpine regions and established that UV radiation shows the most prominent effects among them.

EXPERIMENT 4.2 Measurement of Physiological parameters:

EXPERIMENT 4.2.1 Internal Factors

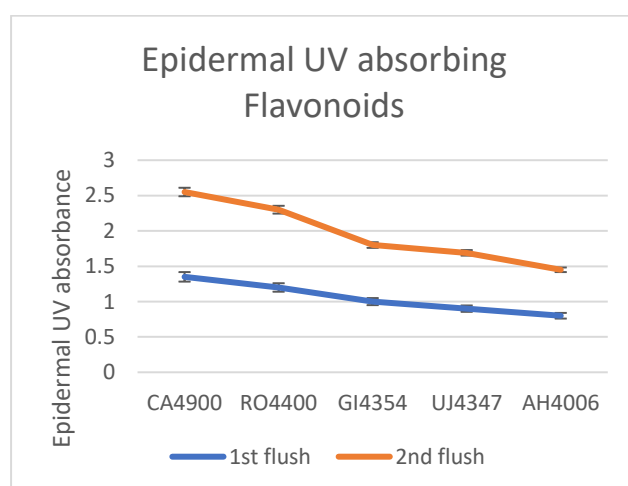


Figure 4.2.1.1a: Variation of UV absorbing compounds in 1st flush and 2nd flush of Kurseong South valley gardens Castleton(CA4900), Rohini(RO4400), Giddhapahar(GI4354), Upper Jungpana (UJ4347) and Ahaldhara (AH4006)S.D=0.01

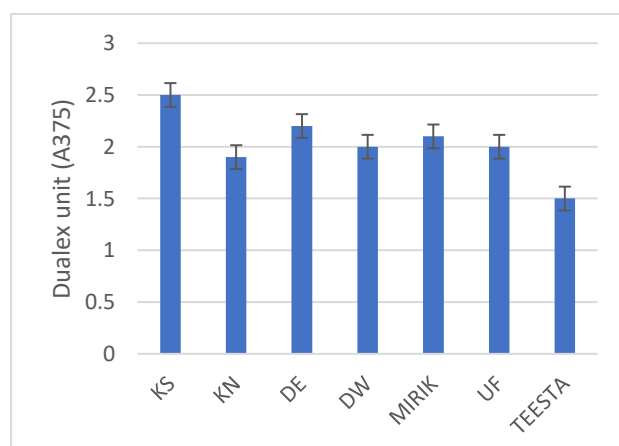


Figure 4.2.1.1b: Histogram showing the epidermal UV absorbance among the seven valleys of Darjeeling S.D=0.1

Fig 4.2.1.1a: Castleton at 4900ft msl contains higher epidermal UV-absorbing flavonoids 2.5 ± 0.01 A.U in 2nd flush than in 1st flush 1.35 ± 0.01 A.U, the same is observed in the case of each of the gardens of Kurseong South valley. Rohini follows Castleton at 4400 ft msl (2.4 ± 0.01 in 2nd flush and 1.2 ± 0.01 in 1st flush) then Giddhapahar at 4354 ft msl (1.9 ± 0.01 A.U in 2nd flush and 1 ± 0.01 A.U in 1st flush) followed by Upper Jungpana at 4347 ft msl (1.79 ± 0.01 in 2nd flush and 0.9 ± 0.01 in 1st flush) and Ahaldhara at 4006 ft msl. (1.5 ± 0.01 A.U in 2nd flush and 0.8 ± 0.01 in 1st flush). The epidermal UV-absorbing flavonoids are proportional to the altitudes.

Fig4.2.1.1b Histogram showing the epidermal UV absorbance among the seven valleys of Darjeeling.

The variation in epidermal UV absorbing compounds viz. flavonoids in seven valleys of Darjeeling (in different altitudes) and different flushes measured by Dualex optical leaf clip meter

Fig 4.2.1. b: Kurseong South shows the highest epidermal UV absorbance among the seven valleys of Darjeeling. Kurseong South Valley 2.5 ± 0.1 AU is followed by Darjeeling East 2.2 ± 0.1 AU, Mirik 2.1 ± 0.1 AU, Darjeeling West 2 ± 0.1 AU, Upper Fagu 2 ± 0.1 AU, Kurseong North 1.9 ± 0.1 AU, and Teesta Valley 1.5 ± 0.1 AU.

Fig4.2.1.1a and 4.2.1.1b showed that the highest epidermal UV absorbance in terms of Dualex unit A345 was found in the case of Kurseong South Valley at the highest altitude 4500 ft msl, Castleton garden during the summer solstice 2nd flush harvest.

Murai et al., (2009) have also studied that in the plant *Plantago asiatica*, higher altitudes show higher UV-absorbing compounds than lower altitudes.

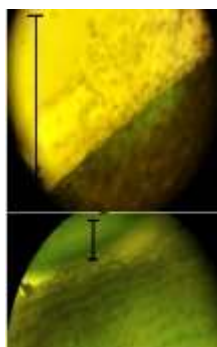


Figure 4.2.1.2a. Histochemical detection of UV-absorbing compounds of leaf section by Fluorescence microscopy.

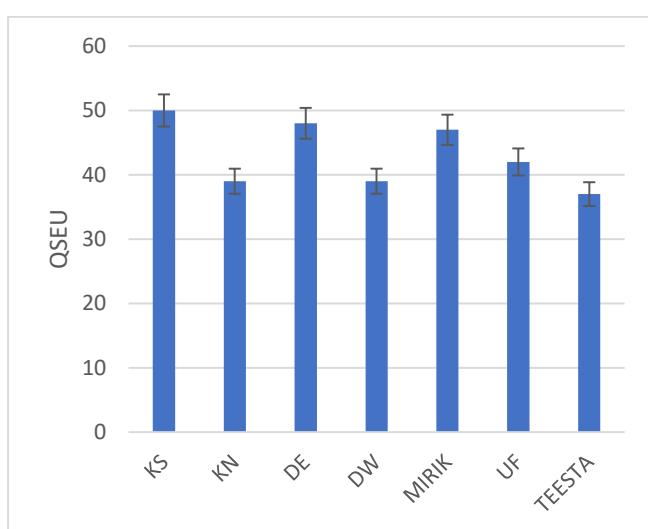


Figure 4.2.1.2b: Variation of epidermal UV-absorbing compounds in seven different valleys of Darjeeling.S.D=0.1

Kurseong South has the highest UV-absorbing compounds (50 ± 0.1 QSEU) among all the seven valleys of Darjeeling. Kurseong South is followed by Darjeeling East (48 ± 0.1 QSEU), then Mirik (47 ± 0.1 QSEU), Upper Fagu (42 ± 0.1 QSEU), Darjeeling West (39 ± 0.1 QSEU), Kurseong North (39 ± 0.1 QSEU), and Teesta (37 ± 0.1 QSEU). Murai et al., (2009) studied the altitudinal variation of UV-absorbing compounds in *Plantago asiatica*

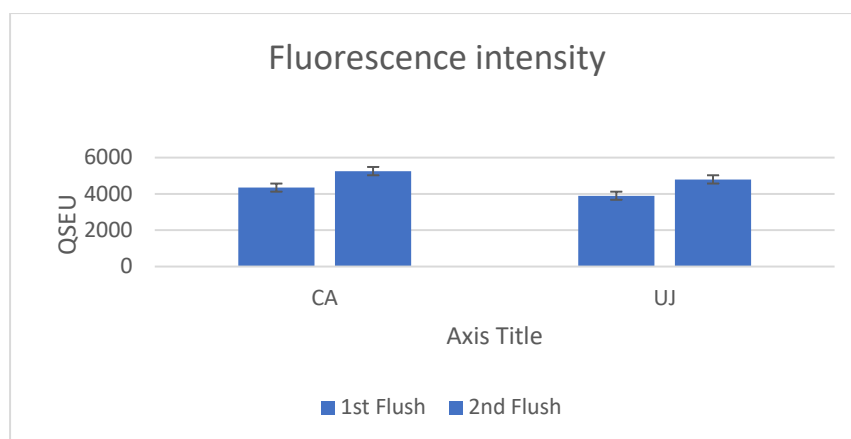


Fig4.2.1.2c: Variation of epidermal UV absorbing compounds in different flushes at different altitudes of Kurseong South Valley(CA- Castleton, UJ-Upper Jungpana)S.D=0.056

Castleton at a higher altitude than Upper Jungpana shows higher fluorescence intensity which is determined in the form of QSEU-n quinine sulfate equivalent units. Excitation and emission spectra of intact leaves were acquired between 220-400 and 400-600 nm, Leaf area- 0.8mm². 1 nmol cm⁻² of quinine sulphate was set to 1000 QSEU-n quinine sulfate equivalent units

Fig 4.2.1.6a,4.2.1.6b and 4.2.1.6c shows that the higher content of UV-absorbing compounds are accumulated in the epidermal layer of high-altitude Kurseong South Garden Castleton during the 2nd flush (5000±0.056 QSEU) and (4300±0.047 QSEU in 1st flush). Low altitude Kurseong South Garden. Upper Jungpana has low levels of UV absorbing compounds (3900±0.010 QSEU in 1st flush and 4800±0.010 QSEU in 2nd flush) than Kurseong South Garden.

Statistical analysis:

Model	Unstandardized Coefficients	Standardized Coefficients	95% confidence interval for B				
	B	Beta	Standard error	t	p	lower bound	upper bound
(Constant)	0.56116		0.0825	6.80172	.021	0.20615	0.91617
UV radiation (uW/cm2/sec)	0.00042	0.99664	0.00002	17.20963	.003	0.00032	0.00053

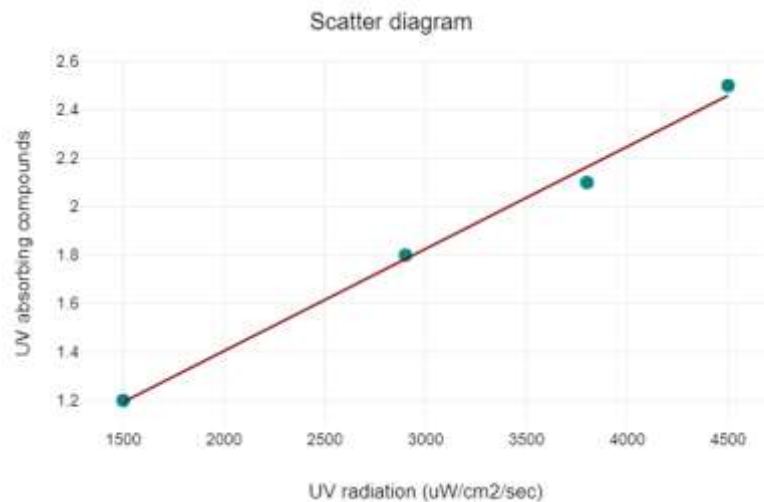


Table 4.1.2 Regression analysis of natural UV irradiance and UV absorbing compounds

Statistical data interprets that there is a significant ($p < 0.003$) positive correlation between UV radiation and UV-absorbing compounds of seven valleys of Darjeeling tea.

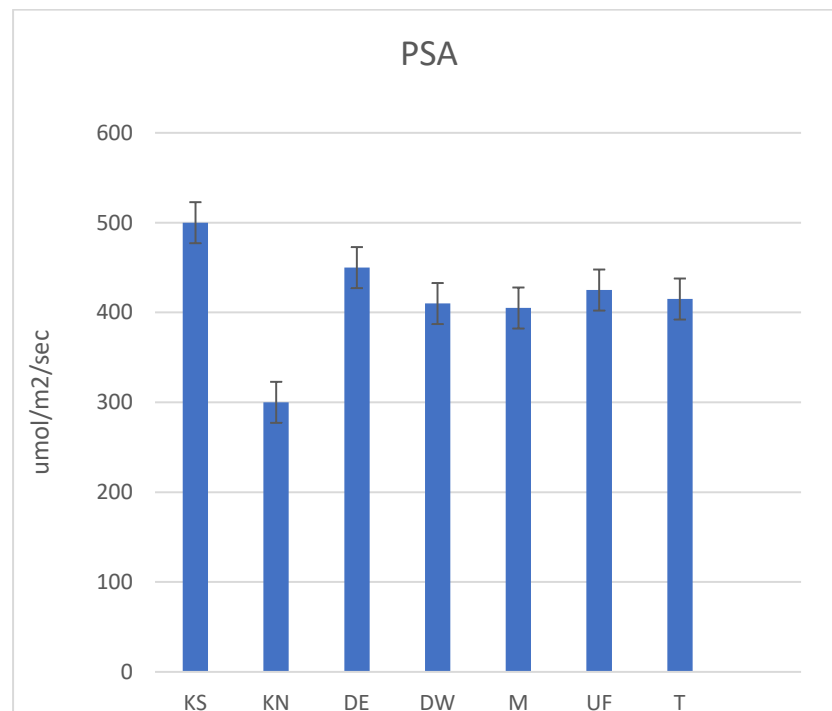


Fig 4.2.1.3a: Variation in Photosynthetic abilities of seven valleys of Darjeeling.S.D=0.01

Fig 4.2.1.3a shows Kurseong South has the highest Photosynthetic abilities($500 \pm 0.01 \mu\text{mol}/\text{m}^2/\text{sec}$) among the seven valleys of Darjeeling. Darjeeling East ($450 \pm 0.01 \mu\text{mol}/\text{m}^2/\text{sec}$) follows Kurseong South. Darjeeling East is followed by Upper Fagu ($425 \pm 0.01 \mu\text{mol}/\text{m}^2/\text{sec}$) then Teesta ($415 \pm 0.01 \mu\text{mol}/\text{m}^2/\text{sec}$) followed by Darjeeling West ($410 \pm 0.01 \mu\text{mol}/\text{m}^2/\text{sec}$) and Kurseong North ($300 \pm 0.01 \mu\text{mol}/\text{m}^2/\text{sec}$) respectively.

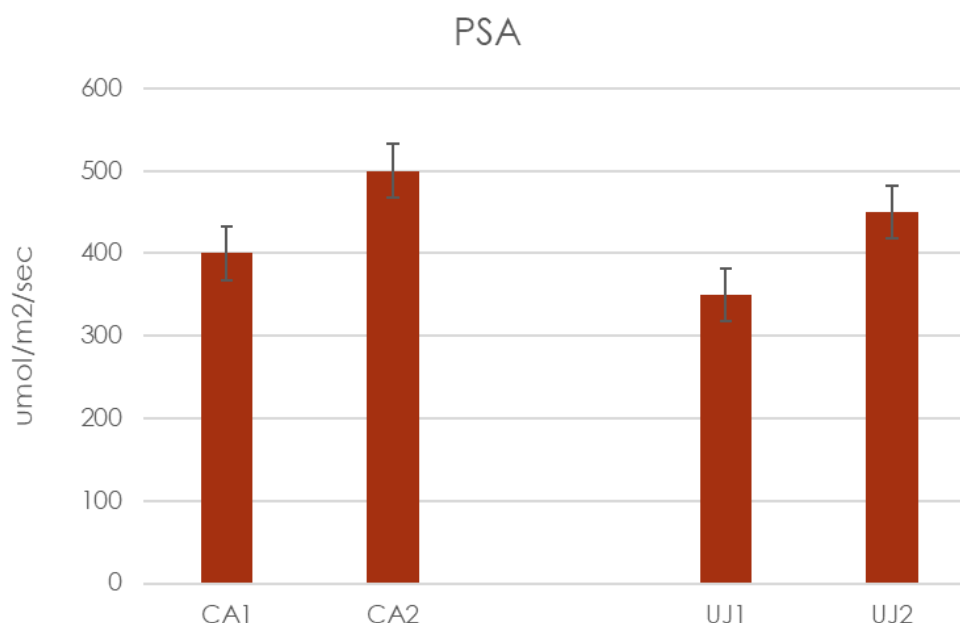


Fig4.2.1.3b: Variation in the Photosynthetic abilities in 1st and 2nd flush of Kurseong South tea gardens, Castleton and Upper Jungpana.S.D=0.01

Fig4.2.1.3b shows the Variation in Photosynthetic abilities of different flushes of different altitudes of Kurseong South Garden. Castleton in Kurseong South has the highest photosynthetic abilities in the second flush ($500 \pm 0.01 \mu\text{mol}/\text{m}^2/\text{sec}$) compared to the first flush ($400 \pm 0.01 \mu\text{mol}/\text{m}^2/\text{sec}$). The same is observed in the case of Upper Jungpana ($350 \pm 0.01 \mu\text{mol}/\text{m}^2/\text{sec}$ in the 1st flush and $450 \pm 0.01 \mu\text{mol}/\text{m}^2/\text{sec}$ in the 2nd flush), which is at a lower altitude than Castleton.

Bresson et al., (2009) stated that altitudinal variations are responsible for photosynthetic abilities in plants.

Statistical analysis:

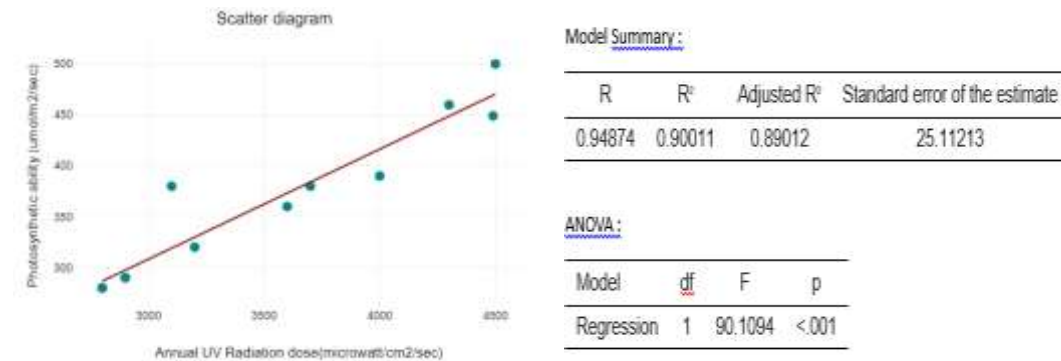


Table 4.1.3 :Statistical data showing correlation between the annual UV radiation and photosynthetic capacity.

Statistical data indicates that there is a significant (<0.001) positive correlation between the annual UV radiation and photosynthetic capacity.

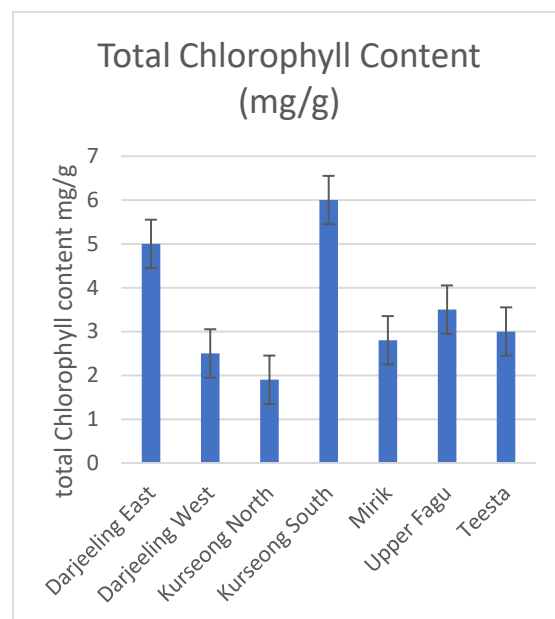


Fig 4.2.1.4a Variation of Chlorophyll content of different valleys of Darjeeling.S.D=0.025

Kurseong South has the highest chlorophyll content(6 ± 0.025 mg/g) among the seven valleys of Darjeeling. Kurseong South is followed by Darjeeling East(5 ± 0.025 mg/g) then Upper Fagu (3.5 ± 0.025 mg/g), Teesta (3 ± 0.025 mg/g), Mirik (2.8 ± 0.025 mg/g), Darjeeling West(2.5 ± 0.25 mg/g) and Kurseong North(1.9 ± 0.25 mg/g).

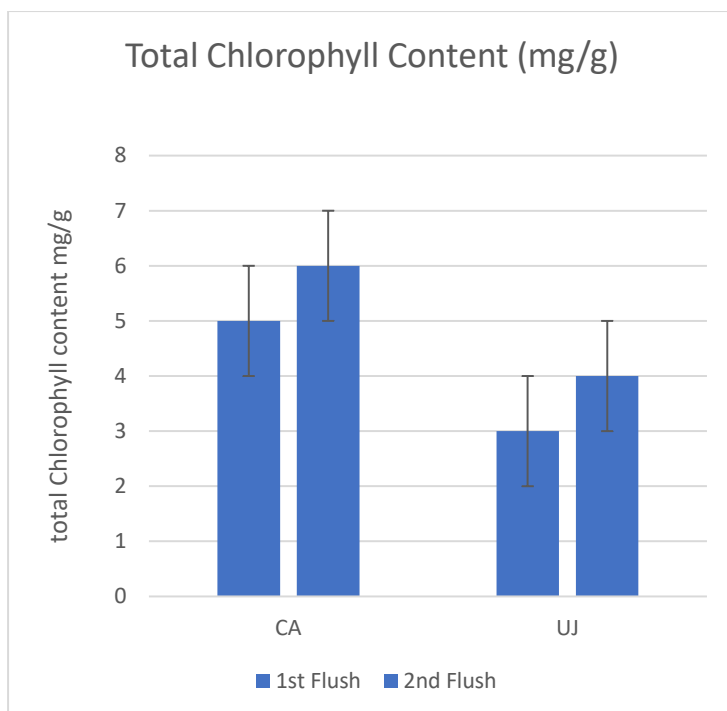


Fig 4.2.1.4b: Variation of chlorophyll content of different altitudes of Kurseong south at different flushes.(CA- Castleton, UJ – Upper Jungpana)S.D=0.025

Castleton has higher chlorophyll content in the second flush ($6 \pm 0.025 \text{ mg/g}$) than in the first flush ($5 \pm 0.025 \text{ mg/g}$). Upper Jungpana at a lower altitude than Castleton shows the same trend, i.e. it has higher chlorophyll content in the second flush ($4 \pm 0.25 \text{ mg/g}$) than in the first flush ($3 \pm 0.025 \text{ mg/g}$).

Figs. 4.2.8a and 4.2.8b show that the variation of chlorophyll content is more prominent in the high altitudes of Kurseong South gardens during the second flush.

Covington et al., (1975) suggested that altitude plays an important role in chlorophyll content in plants.

4.2.2 Physiological Parameters: External Factors

- ☐ Tea plant height-10-15 inches
- ☐ No of brunches-4-6 per tree
- ☐ Internodal distance-4 cm
- ☐ No of leaves per plant-250 approximately

- ☐ Length of leaf-5-10 cm
- ☐ No of apical buds-125 approximately
- ☐ No of flowers or fruits-2-4 together on short branchlets in the leaf axis



Figure 4.2.2.1: External factors were measured with the measuring tape.



Figure 4.2.2.2: Tea plant

OBJECTIVE 2

4.3 Biochemical Characterization: Characterization and identification of therapeutic values (antioxidant, antimicrobial) and commercial potential (aroma) of different types of tea -fresh and manufactured) and comparison among the tea samples of the seven valleys of Darjeeling under natural UV radiation.

Experiment 4.3.1 : Assessment of Reactive oxygen species: Lipid peroxidation study

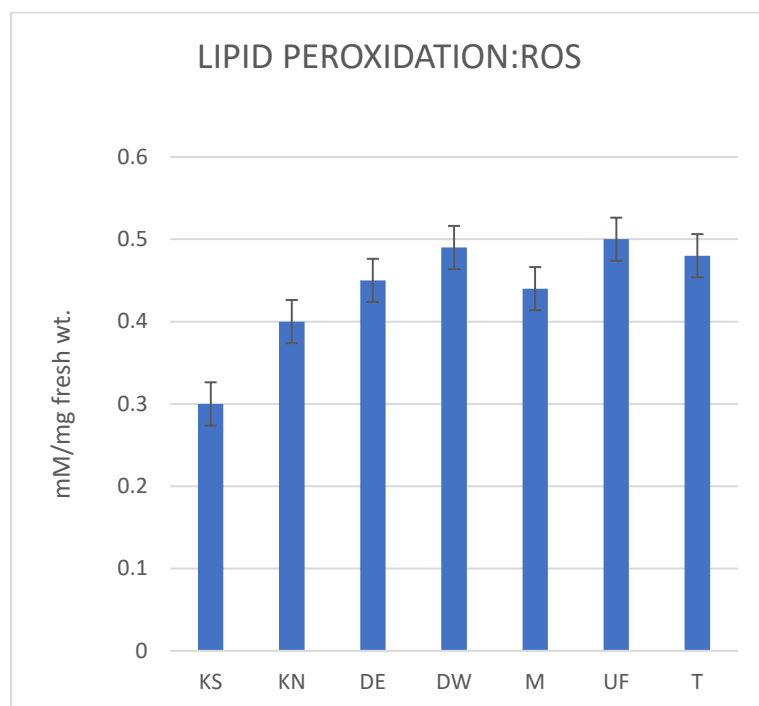


Figure 4.3.1.1a: Lipid Peroxidation of seven different valleys of Darjeeling tea.

S.D=0.001

Fig 4.3.1.1a. shows Kurseong South has the lowest lipid peroxidation(0.3 ± 0.001 mM/mg) among the seven valleys of Darjeeling. The highest lipid peroxidation is observed in Upper Fagu (0.5 ± 0.001 mM/mg) followed by Darjeeling West (0.49 ± 0.001 mM/mg), then Teesta (0.48 ± 0.001 mM/mg), Darjeeling East (0.45 ± 0.001 mM/mg), Mirik (0.44 ± 0.001 mM/mg), and Kurseong North (0.40 ± 0.001 mM/mg).

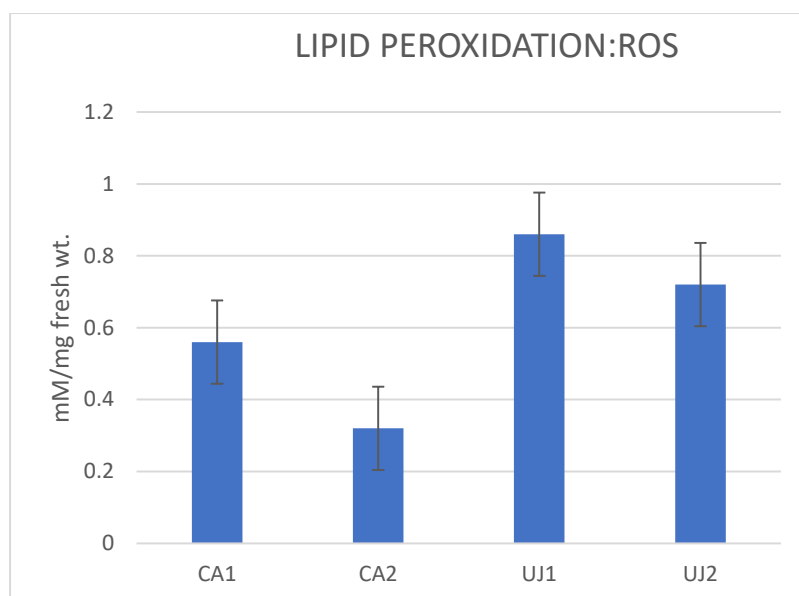


Figure 4.3.1.1b: Lipid Peroxidation of different altitudes at different flushes of Kurseong South gardens. (CA- Castleton, UJ – Upper Jungpana). S.D=0.01

Fig 4.3.1.1b. shows Castleton has the lowest lipid peroxidation activity in the second flush (0.32 ± 0.01 mM/mg) than in the first flush (0.56 ± 0.01 mM/mg). Upper Jungpana has the lowest lipid peroxidation activity (0.72 ± 0.01 mM/mg) than the first flush (0.86 ± 0.01 mM/mg). Dey et al., (2012) discussed the lipid peroxidation inhibition efficacy of tea (*Camellia sinensis*) in India.

Fig 4.3.1.1a and Fig 4.3.1.1b show that the higher free radicals or Reactive oxygen species are present in low altitudes grown tea and higher in 1st flushes, which indicates the absence of some protection factor which are responsible for raising the reactive oxygen species content in terms of Malondialdehyde concentration which is the marker of oxidative stress.

Dey et al., (2012) studied the lipid peroxidation inhibition efficacy of branded tea (*Camellia sinensis*) which are available in India. According to his study inhibition of lipid peroxidation is correlated with high polyphenol content in teas, that is higher the polyphenol content, the lower the lipid peroxidation.

Experiment 4.3.2 :Assay of stable free radicals: EPR study

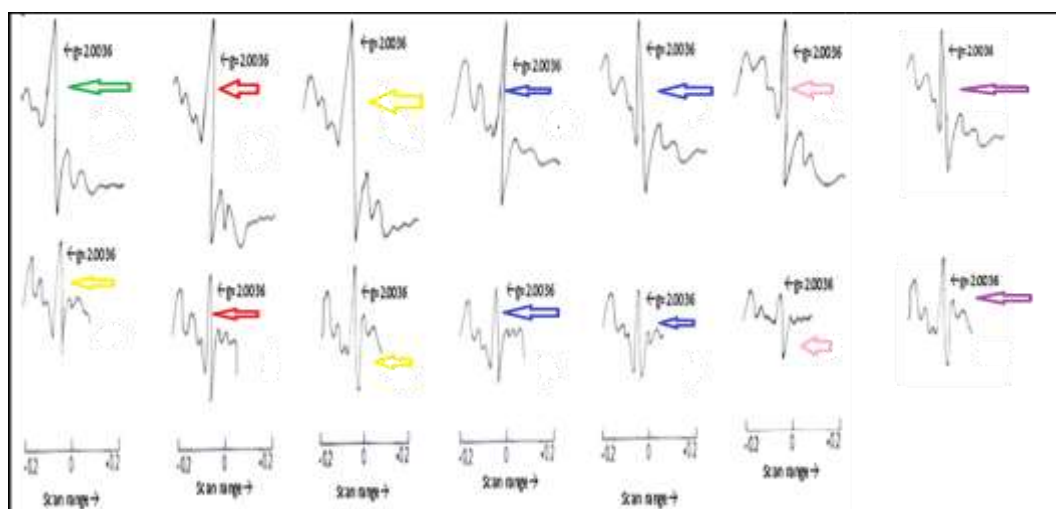


Figure 4.3.2.1a: EPR Analysis of seven different valleys of Darjeeling

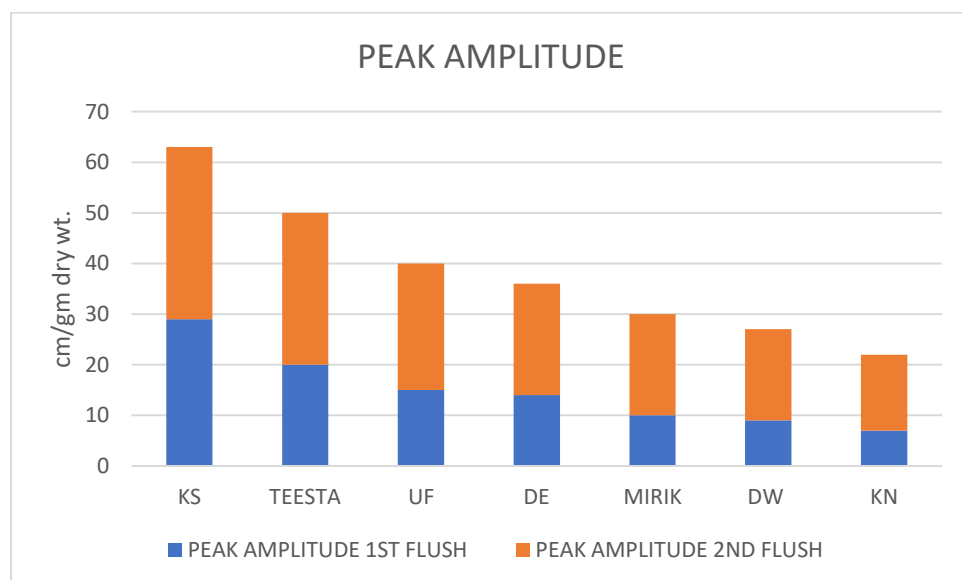


Figure 4.3.2.1b. Variation in peak amplitude in EPR S.D=0.01

Fig 4.3.2.1b indicates the variation in the peak amplitude of the EPR signal at $g=2.0036$, which indicates the stable semiquinone nucleus that produces stable free radicals by saving the system from oxidative stress-related damage (lipid peroxidation, membrane, and macromolecular damage). That is only possible if some flavonoid-based center (having quinone or semiquinone rings) can trap the reactive oxygen species by making them stable. The stable free radicals are being higher at Kurseong South among all the valleys indicating better protection (peak amplitude 62 ± 0.01 cm/gm dry wt.)

Experiment 4.3.3: Assay of enzymatic antioxidants: Superoxide dismutase and Catalase

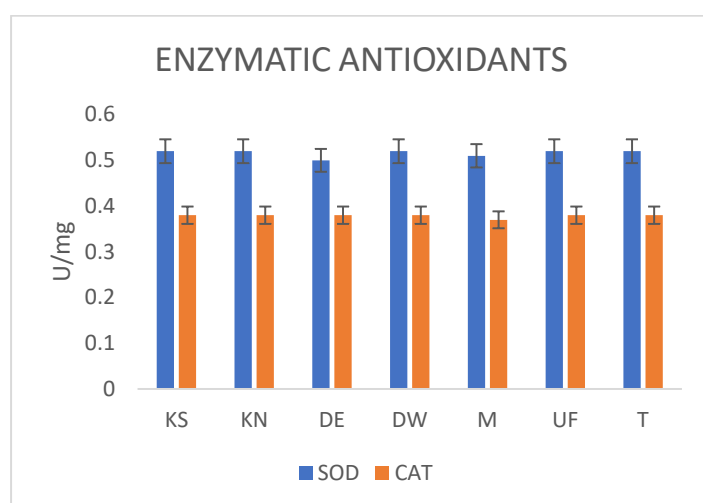


Figure 4.3.3.1a: Spectrophotometric analysis of Superoxide dismutase(SOD) and Catalase(CAT) of different valleys of Darjeeling. S.D=0.01

Fig 4.3.3.1a. All the gardens have almost the same content of Catalase, which is $0.52\text{U}/\pm 0.01\text{mg}$.

TEA TYPE	SOD U/mg of total protein
CA ₁	5.9 ± 0.01
CA ₂	6.2 ± 0.01
uj ₁	5.5 ± 0.01
UJ ₂	5.6 ± 0.01

Table 4.3.1: Variation in SOD activities in different altitudes of Kurseong South gardens Castleton and Upper Jungpana at different flushes .S.D=0.01

Table 4.3.2: Variation in SOD activities in different altitudes of Kurseong garden which is ranging from 5.5 ± 0.01 - $6.2 \pm 0.01\text{U}/\text{mg}$.

Fig4.3.311a and Table 4.3.2 show that there is no such variation in the enzymatic antioxidants like SOD and CAT in different valleys of Darjeeling irrespective of altitudes and seasonal flushes.

4.3.4 Characterization of non enzymatic antioxidant: Flavonoids, Polyphenols, catechins

Experiment 4.3.4.1: Spectrophotometric scan in UV range 200-400nm

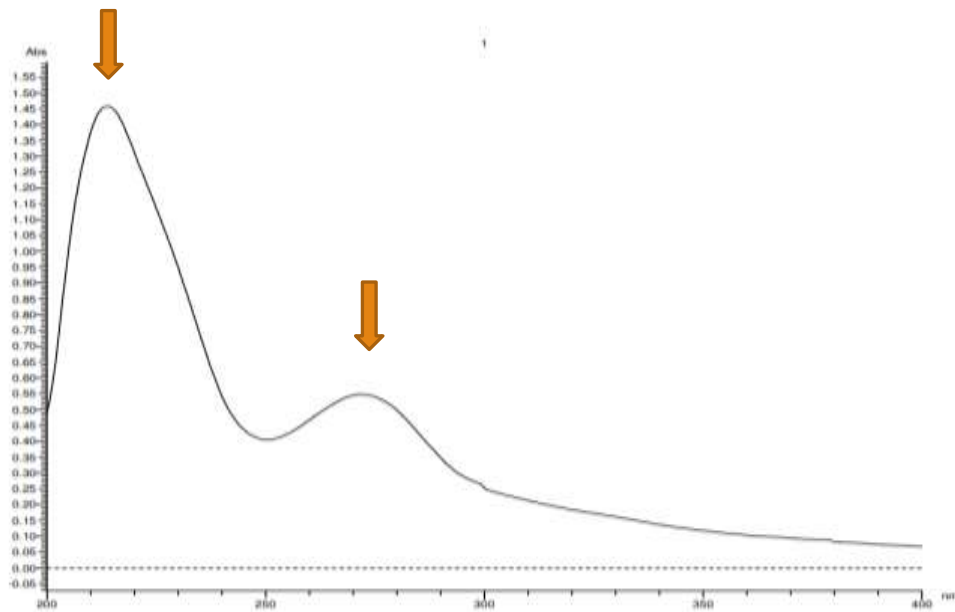


Figure 4.3.4.1a: Spectral scan of low-altitude grown tea (Kurseong South Upper Jungpana-1st flush)

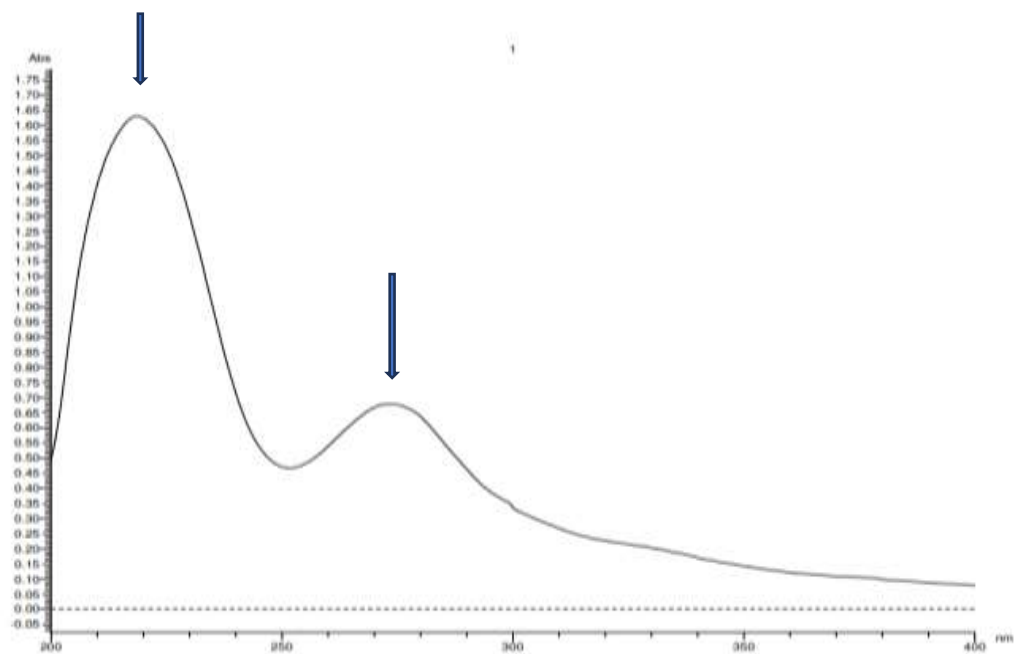


Figure 4.3.4.1b: Spectral scan of high altitude grown tea (Kurseong South Castleton-1st flush)

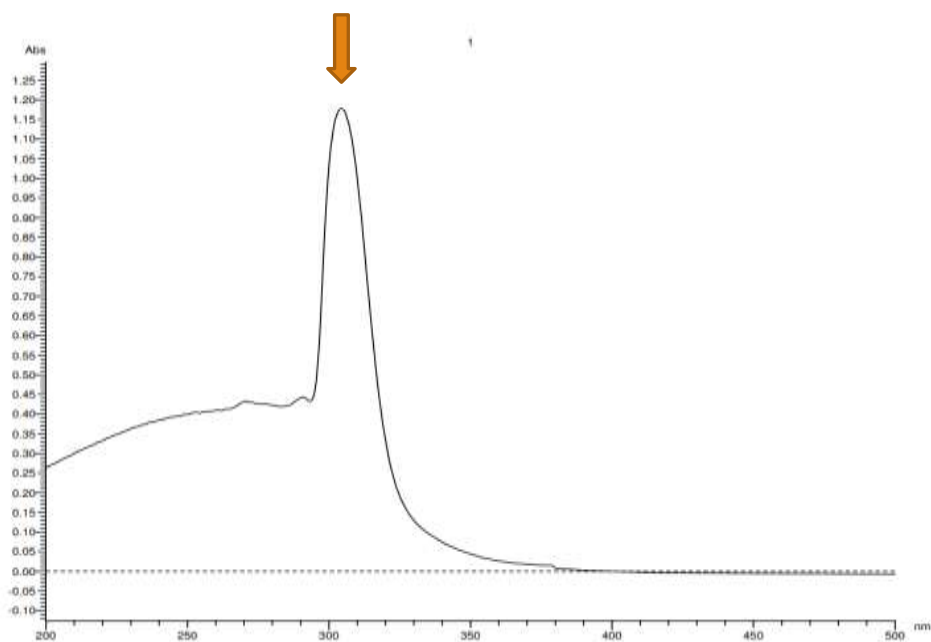


Figure 4.3.4.1c.: A Spectral scan of Epigallocatechin gallate (Standard)

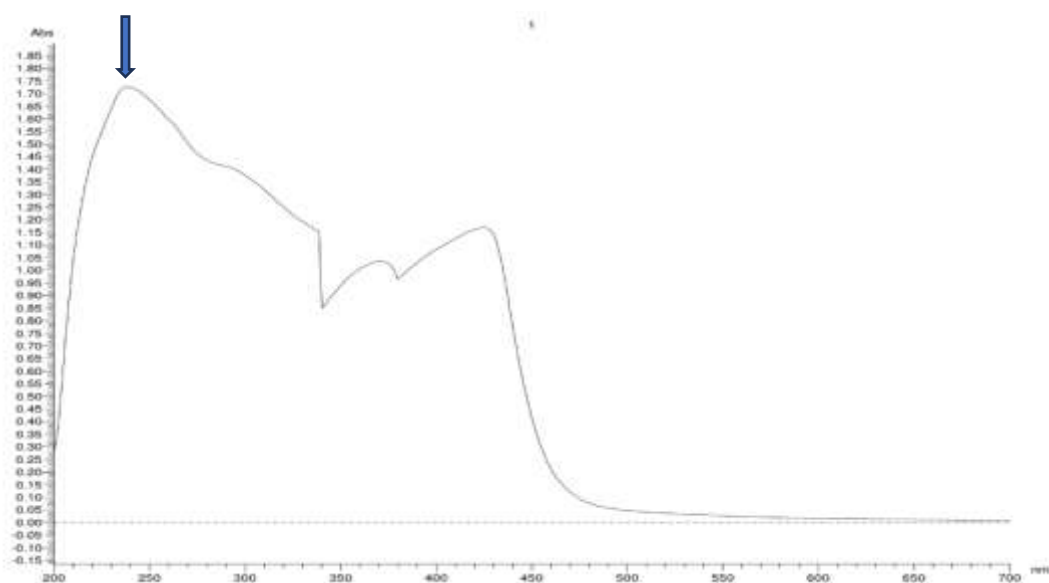


Figure 4.3.4.1d: Spectral scan of Quercetin(Standard)

Fig 4.3.4.1a shows the spectral scan of low altitude. The absorbance of low altitude is at 220nm and 280nm is about 0.55 and 1.62 respectively. The concentration is about 0.32 ± 0.01 mg/ml

Fig 4.3.4.1b. Spectral scan of high altitude. The absorbance of high altitude is at 220nm and 280nm is about 0.65 and 1.62 respectively. The concentration is about 0.38 ± 0.01 mg/ml

Fig 4.3.4.1c. A Spectral scan of Epigallocatechin gallate. The absorbance of EGCG at 320 is 1.18. The concentration is 1 ± 0.01 mg/ml.

Fig 4.3.4.1d. Spectral scan of Quercetin. The absorbance of Quercetin at 220 is 1.70. The concentration is 1 ± 0.01 mg/ml.

These figures show that the remarkable presence of active metabolite Quercetin, specifically in the higher content in the high altitude of Kurseong South.

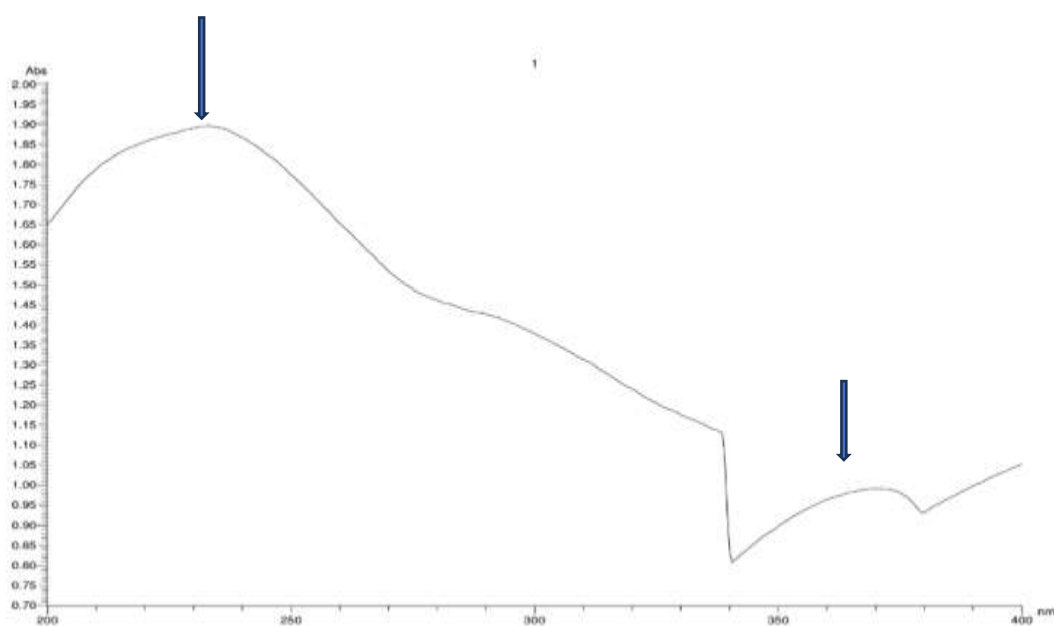


Figure 4.3.4.1e. A Spectral scan of high-altitude grown tea (Kurseong South Castleton-2nd flush)

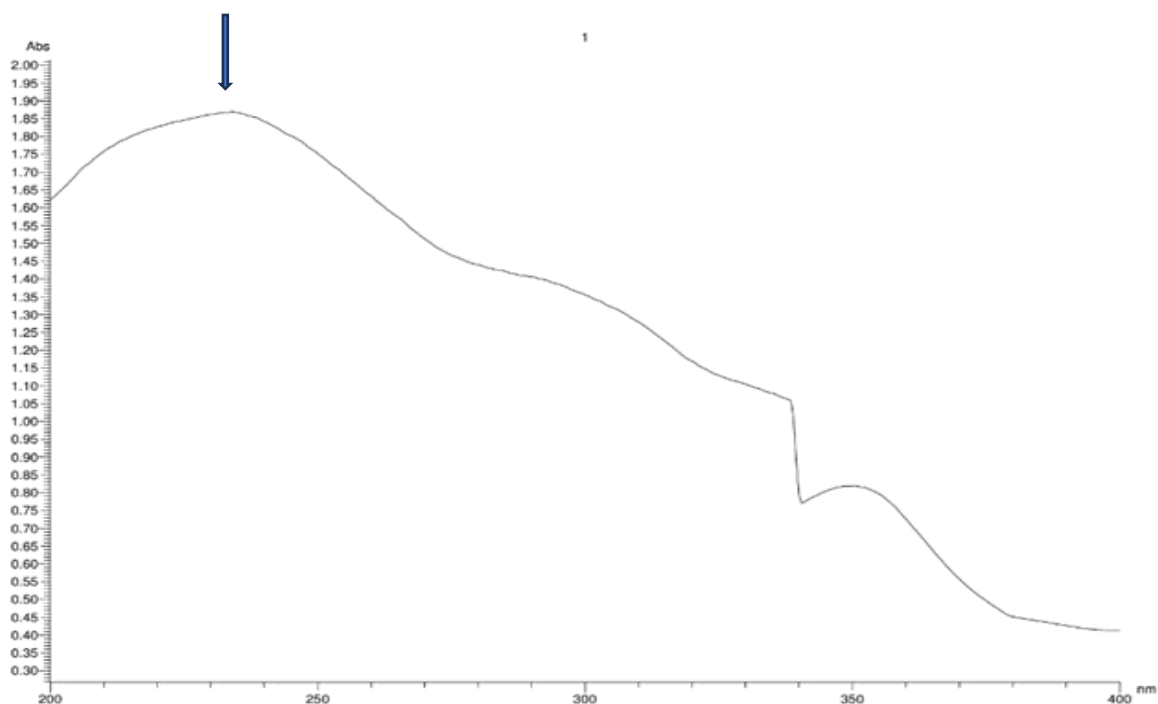


Figure 4.3.4.1f. Spectral scan of Rutin

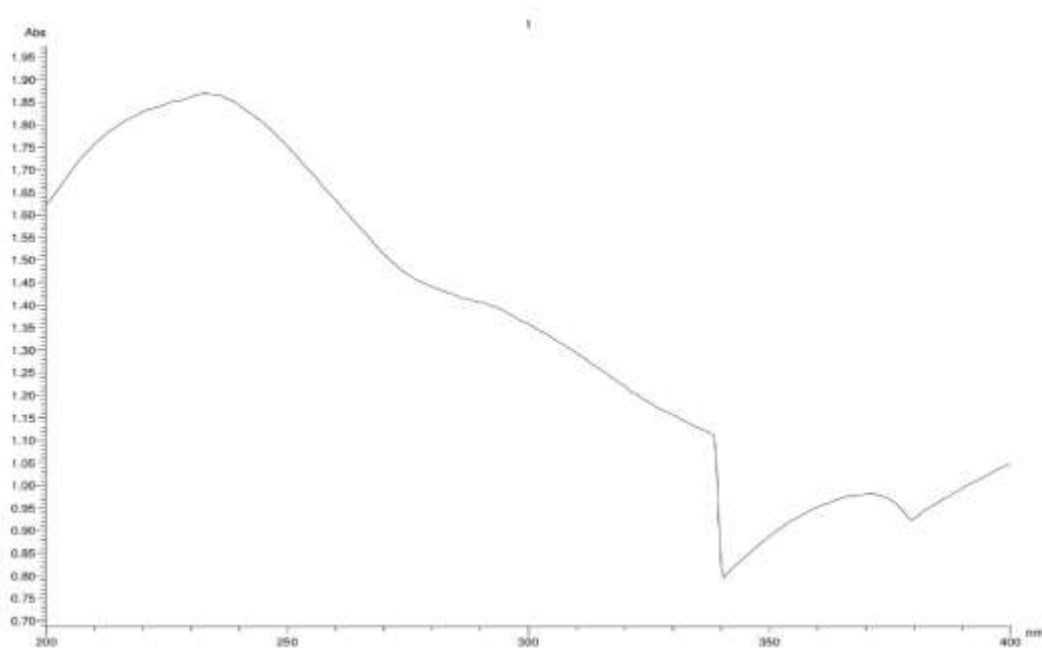


Figure 4.3.4.1g. Spectral scan of low altitude grown tea (Kurseong South Upper Jungpana-2nd flush)

Fig 4.3.4.1e. A Spectral scan of high-altitude grown tea (Kurseong South Castleton-2nd flush). The absorbance at 230nm is 1.82. The concentration is about 0.98 ± 0.01 mg/ml.

Fig 4.3.4.1f. Spectral scan of Rutin. The absorbance at 230nm is 1.85. The concentration is about 1 ± 0.01 mg/ml.

Fig 4.3.4.1g. Spectral scan of low-altitude grown tea. The absorbance at 230nm is 1.80. The concentration is about 0.97 ± 0.01 mg/ml. This shows that the content of Rutin is more in high-altitude 2nd flush than in low-altitude 2nd flush.

Fig 4.3.4.1 a, b, e.f, g shows that the remarkable presence of active metabolite by comparing with standards like Quercetin, Rutin, and Epigallocatechin Gallate, specifically higher content in the 2nd flush of high altitude of Kurseong South.

Experiment 4.3.4.2: Spectrofluorimetric scan

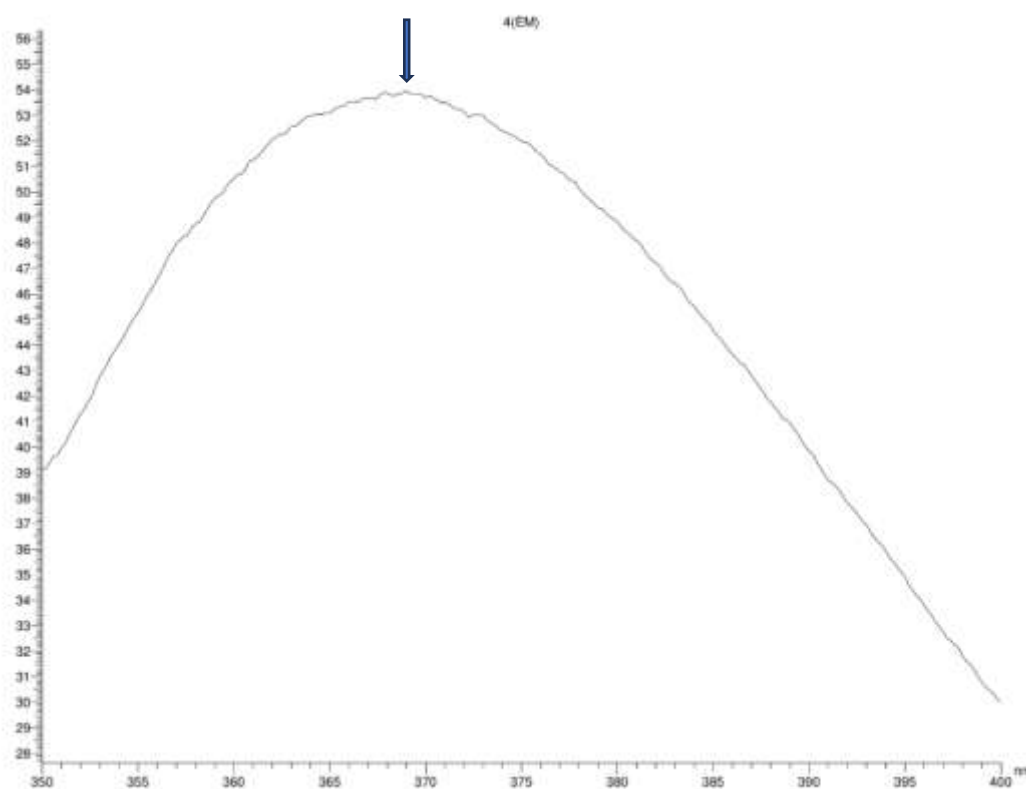


Figure 4.3.5.1a. Spectrofluorometric scan of Epigallocatechin Gallate (Standard)

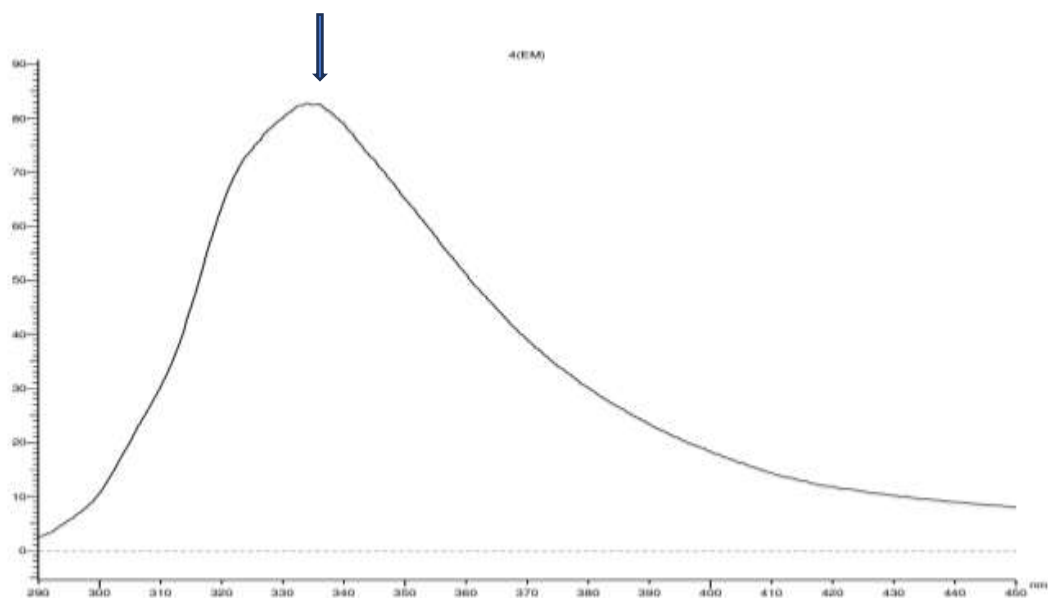


Figure 4.3.5.1b Spectrofluorometric scan of High altitude of Kurseong South valley (Castleton)

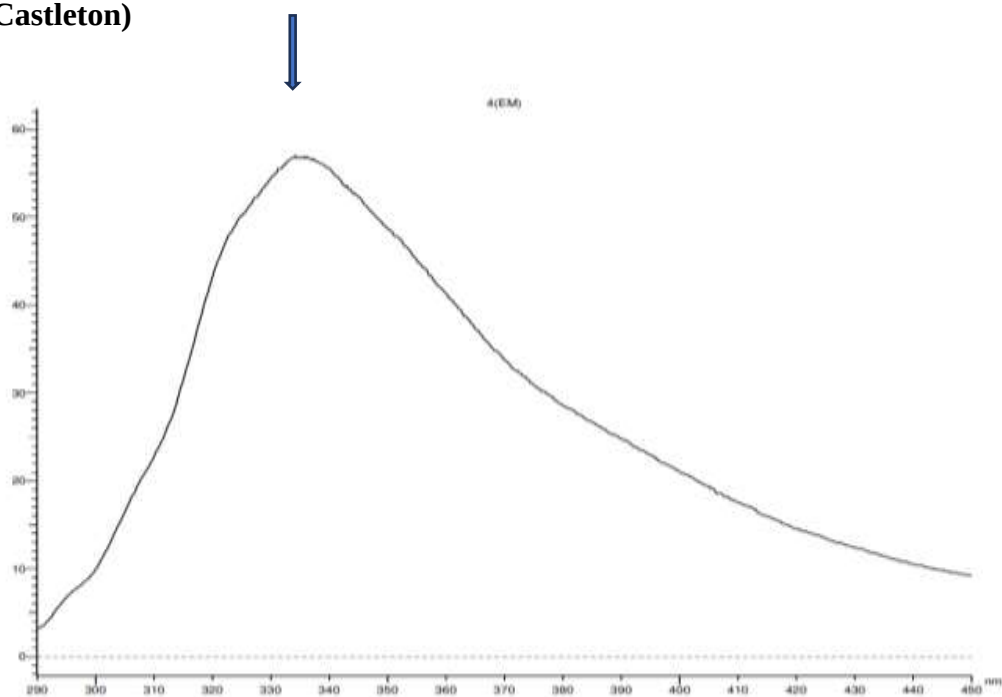


Fig 4.3.5.1c Spectrofluorometric scan of Low altitude of Kurseong South valley (Upper Jungapana)

Fig 4.3.5.1a Spectrofluorometric scan of Epigallocatechin Gallate. The fluorescence intensity is about 54 a.u, and the concentration is about 1 ± 0.1 mg/ml.

Fig 4.3.5.1b Spectrofluorometric scan of High altitude. The fluorescence intensity is about 84 a.u and the concentration is about 1.544 ± 0.2 mg/ml.

Fig 4.3.5.1c Spectrofluorometric scan of Low altitude. The fluorescence intensity is about 58 a.u and the concentration is about 1.073 ± 0.9 mg/ml.

Samples	Fluorescence intensity a.u	Concentration. Mg/ml
HIGH ALTITUDE	84	1.554 ± 0.2
LOW ALTITUDE	58	1.073 ± 0.9
EGCG	54	1 ± 0.1 mg/ml

Table 4.3.2 : Fluorescence Intensity and concentration of different tea with standard EGCG.

Fig 4.3.5.1. a, b, c and Table 4.3.2 show that the remarkable presence of active metabolite by comparing with standard Epigallocatechin Gallate, specifically higher content in high altitude of Kurseong South.

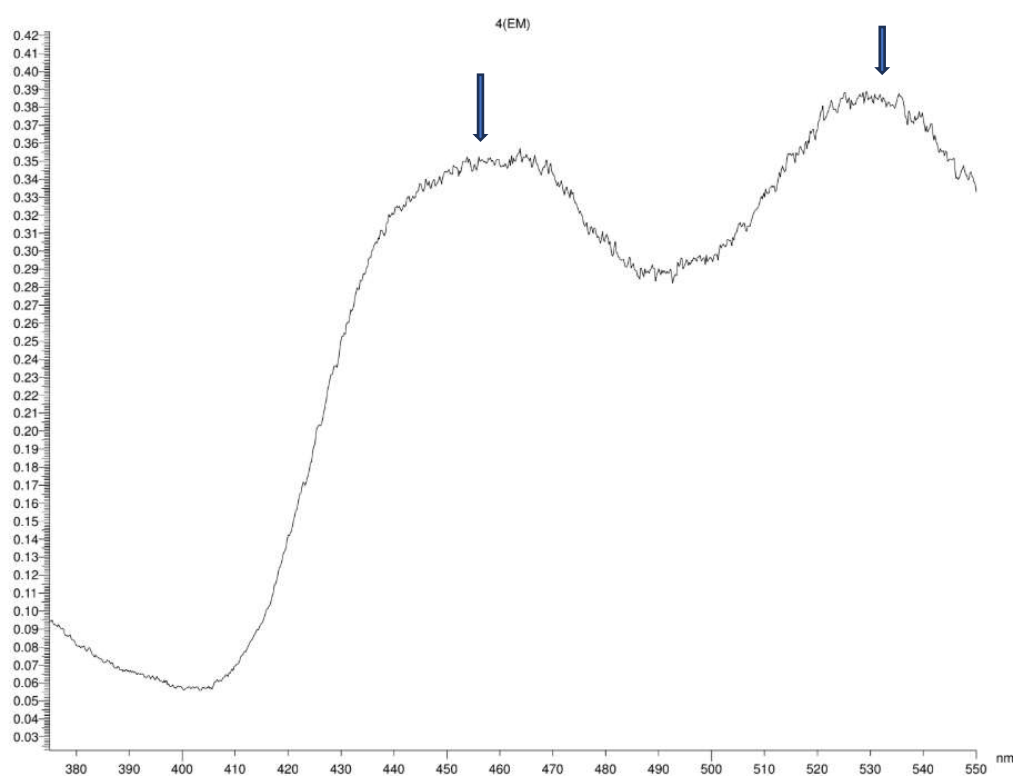


Figure 4.3.5.1d: Spectrofluorometric scan of Quercetin (Standard)

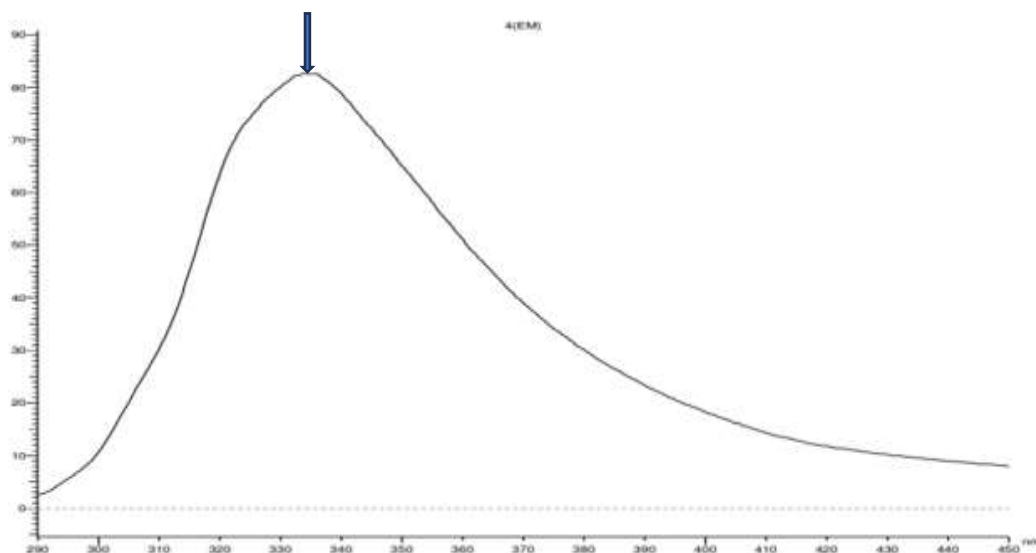


Figure 4.3.5.1e Spectrofluorometric scan of High altitude garden of Kurseong South valley (Castleton)

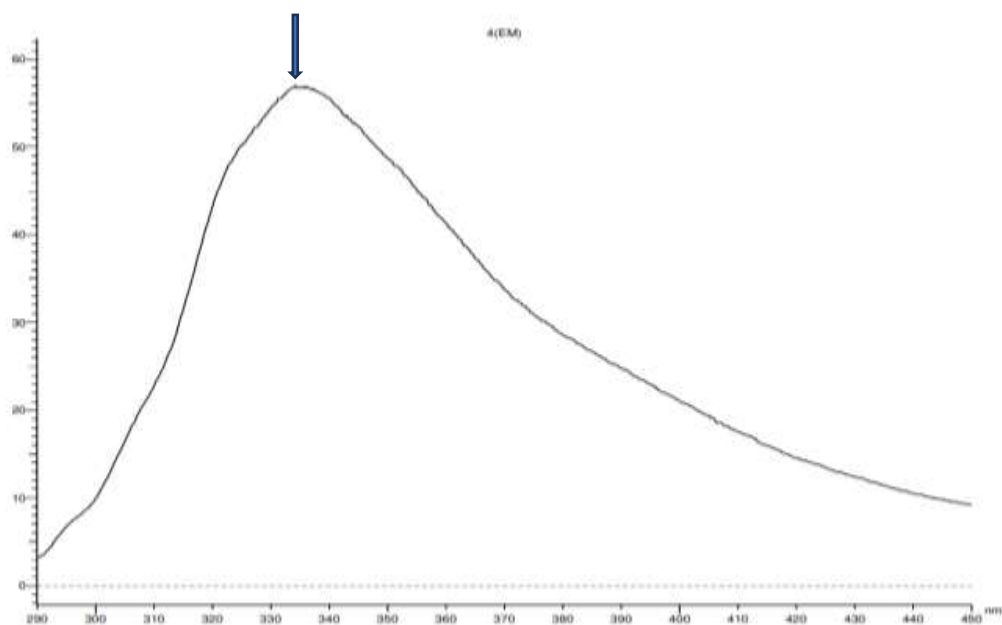


Fig 4.3.5.1f Spectrofluorometric scan of Low altitude garden of Kurseong South valley (Upper Jungpana)

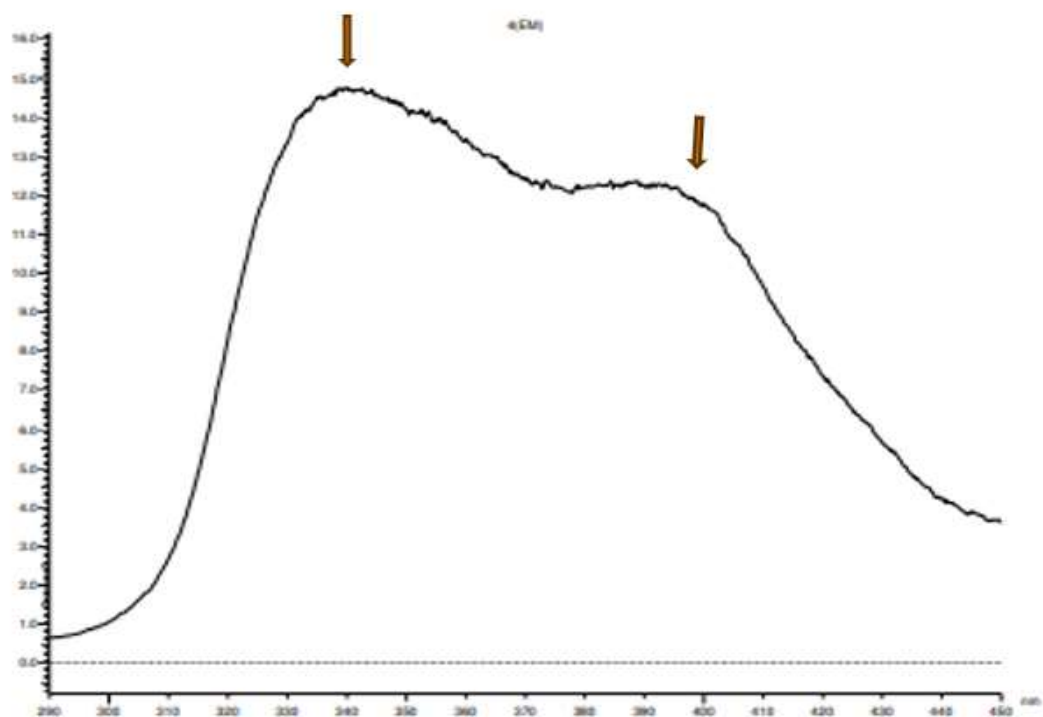


Figure 4.3.5.1 g: Spectrofluorometric scan of High altitude 2ND flush (Kurseong South garden, Castleton)

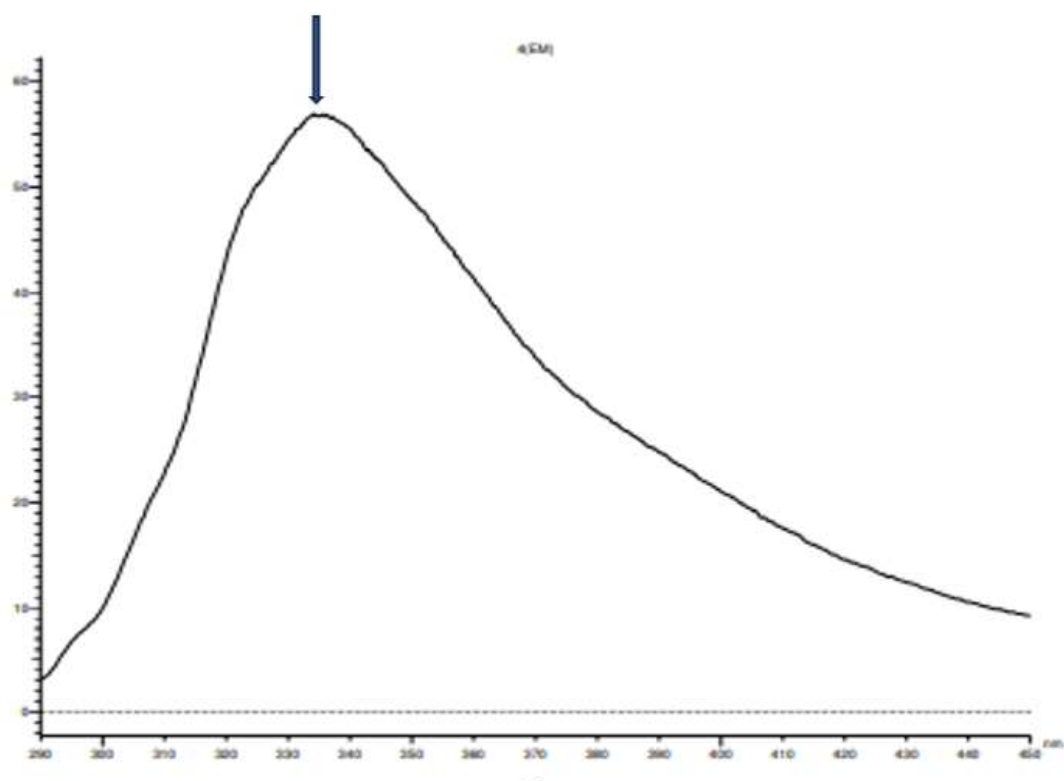


Figure 4.3.5.1h: Spectrofluorometric scan of High-Altitude 1st flush (Kurseong South garden Castleton)

Fig 4.3.5.1d Spectrofluorometric scan of Quercetin. The fluorescence intensity of Quercetin at 450 and 530 nm is about 34 and 38 a.u respectively. The concentration is about 1mg/ml.

Fig 4.3.5.1 e Spectrofluorometric scan of High Altitude. Fluorescence intensity of high altitude at 335nm is about 80 a.u.

Fig 4.3.5.1f Spectrofluorometric scan of Low Altitude. Fluorescence intensity of high altitude at 335nm is about 79 a.u.

Fig 4.3.5.1g Spectrofluorometric scan of High-Altitude 2nd Flush. The fluorescence intensity of Quercetin at 450 and 530 nm is about 14 and 12 a.u, respectively. The concentration is about 0.411±0.001 mg/ml and 0.315±0.002 respectively.

Fig 4.3.5.1h Spectrofluorometric scan of High-Altitude 1st flush. Fluorescence intensity of high altitude at 335nm is about 79 a.u.

Samples	Fluorescence intensity a.u	Concentration mg/ml
	450nm	
Quercetin	34	1
High Altitude 2nd flush	14	0.411±0.001
Samples	Fluorescence intensity a.u	Concentration mg/ml
	530nm	
Quercetin	38	1
High altitude 2nd flush	12	0.315±0.002

Table 4.3.3: Fluorescence Intensity and concentration of different tea with standard Quercetin.

Fig. 4. 3.5.1a, b, e, d, e and Table 4.3.3 show that the remarkable presence of active metabolite by comparing with standard like Quercetin specifically higher content in the 2nd flush of high altitude of Kurseong South.

Experiment 4.3.4.3: FTIR analysis

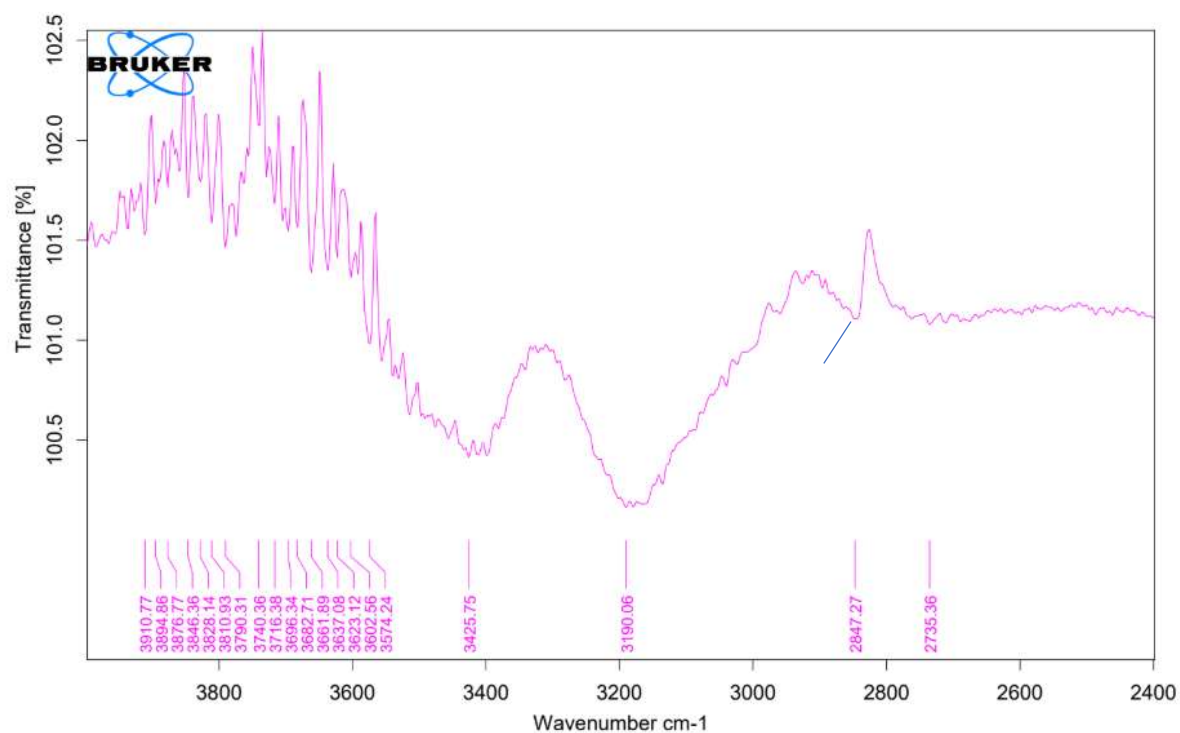


Figure 4.3.6.1a: FTIR Transmittance peaks of standard Epigallocatechin gallate

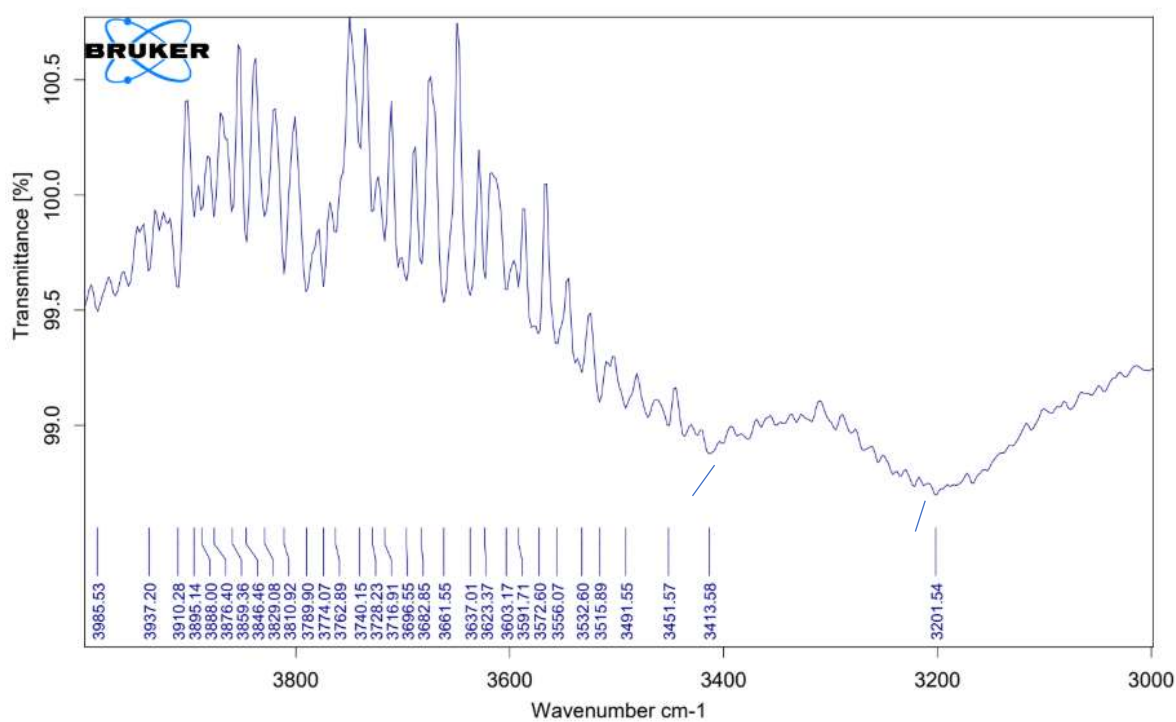


Figure 4.3.6.1 b shows the FTIR Transmittance peaks of standard Quercetin

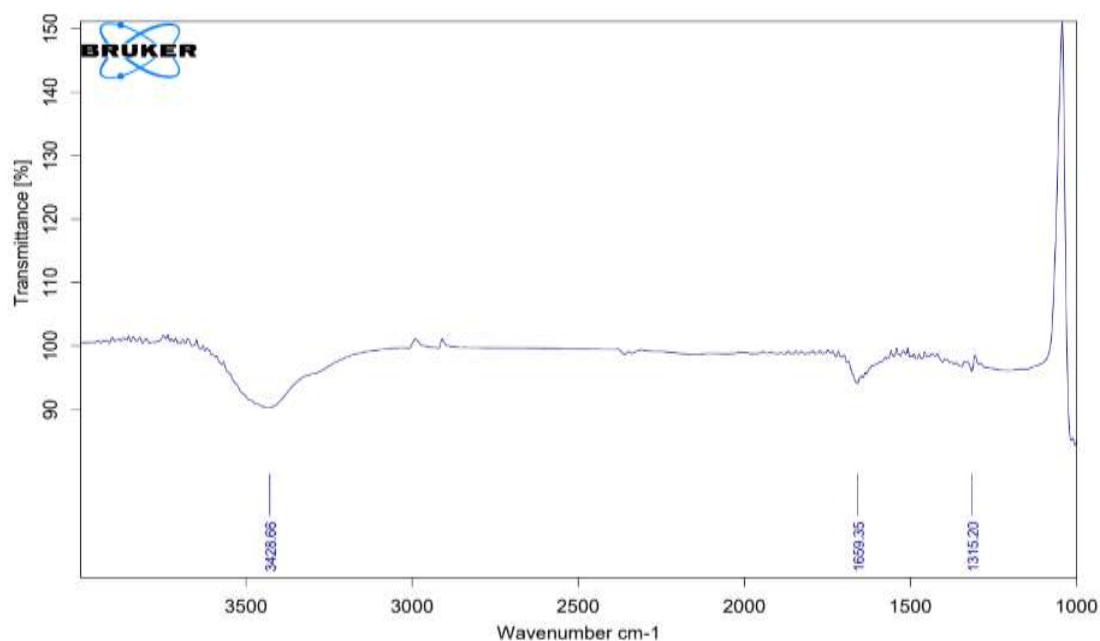


Figure 4.3.6.1 c shows the presence of transmittance peaks of the standards Epigallocatechin gallate and Quercetin in the High altitude Kurseong South tea garden Castleton.

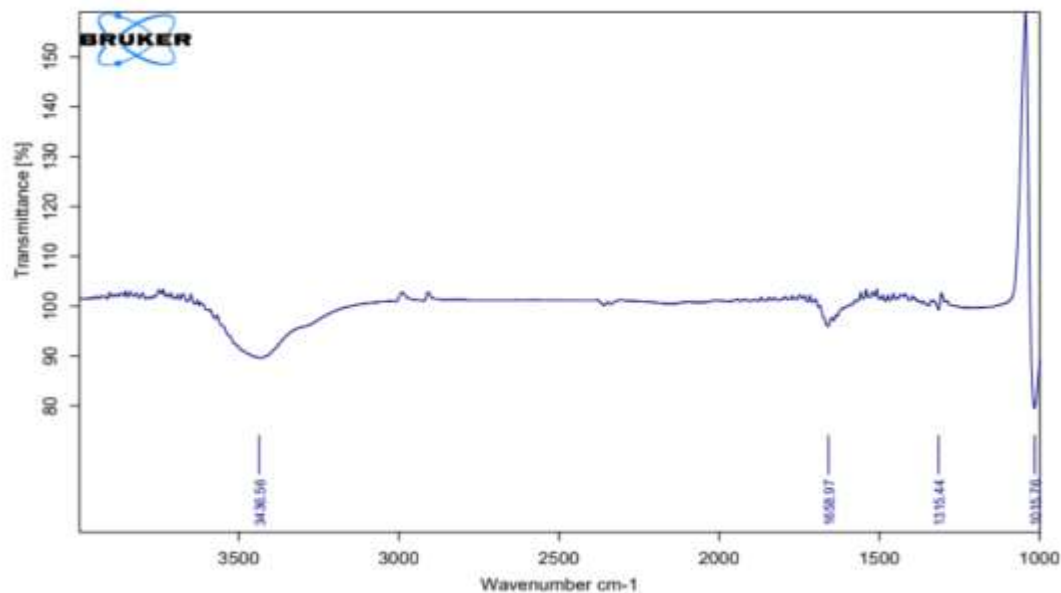


Figure 4.3.6.1d: shows the presence of transmittance peaks of the standards Epigallocatechin gallate and Quercetin in Low altitude Kurseong South tea Garden Upper Jungpana.

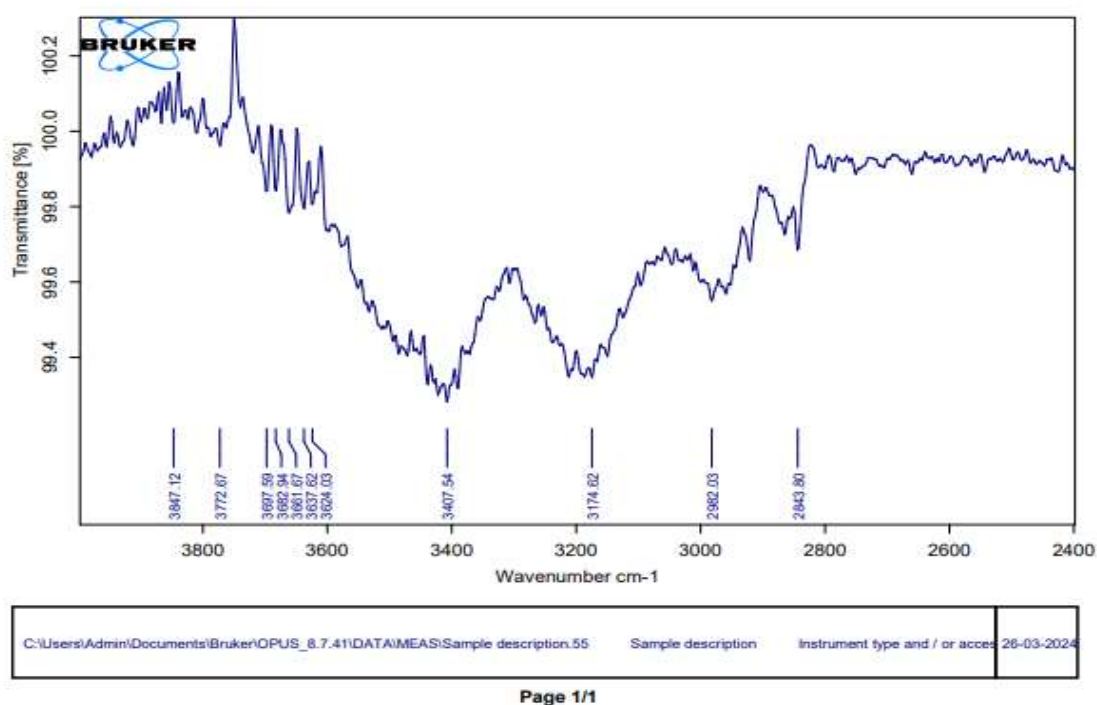


Figure4.3.6.1e: shows the transmittance peaks of standards in high altitude of Kurseong South

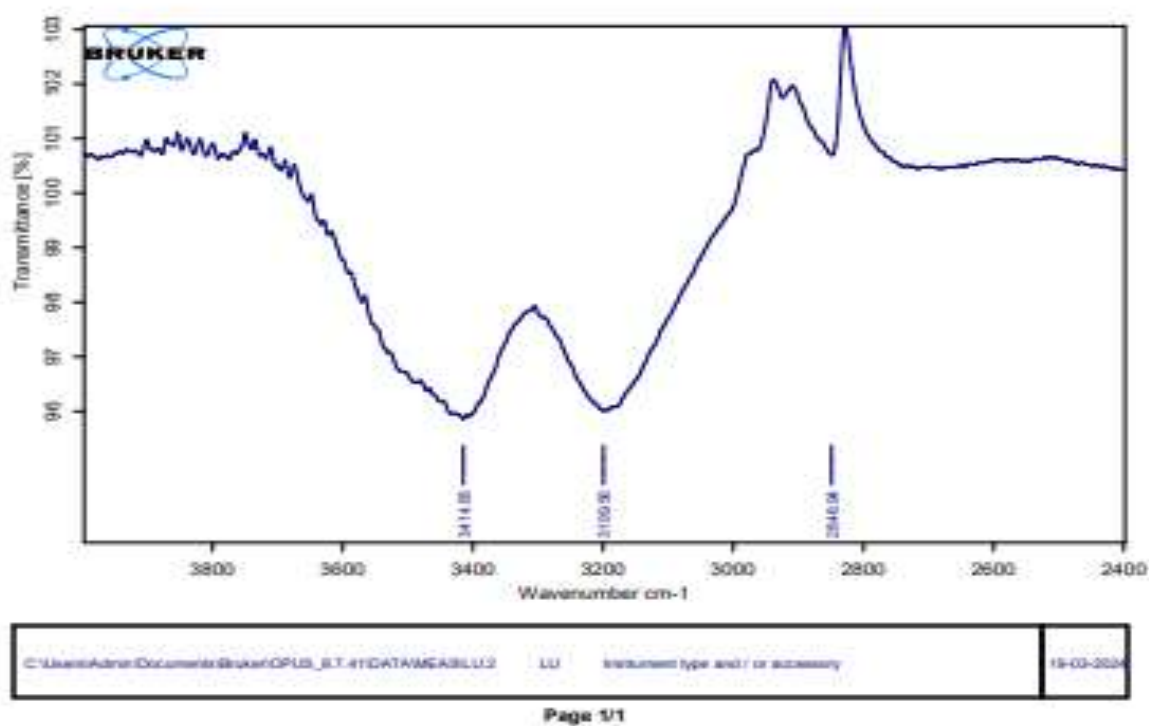


Figure 4.3.6.1f: show the transmittance peaks of standards in low altitudes of Kurseong South.

Fig 4.3.6.1e indicates there is the presence of many more peaks at high altitudes than at low altitude.

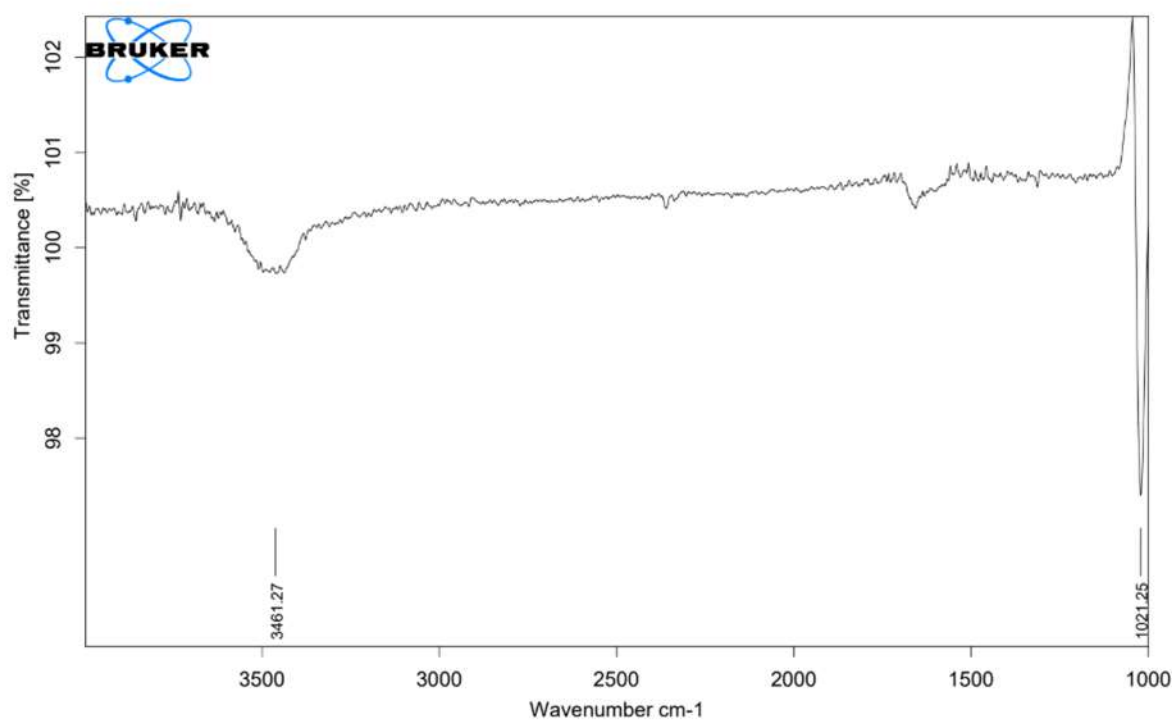


Figure 4.3.6.1g: shows the FTIR Transmittance peaks of the standard Rutin

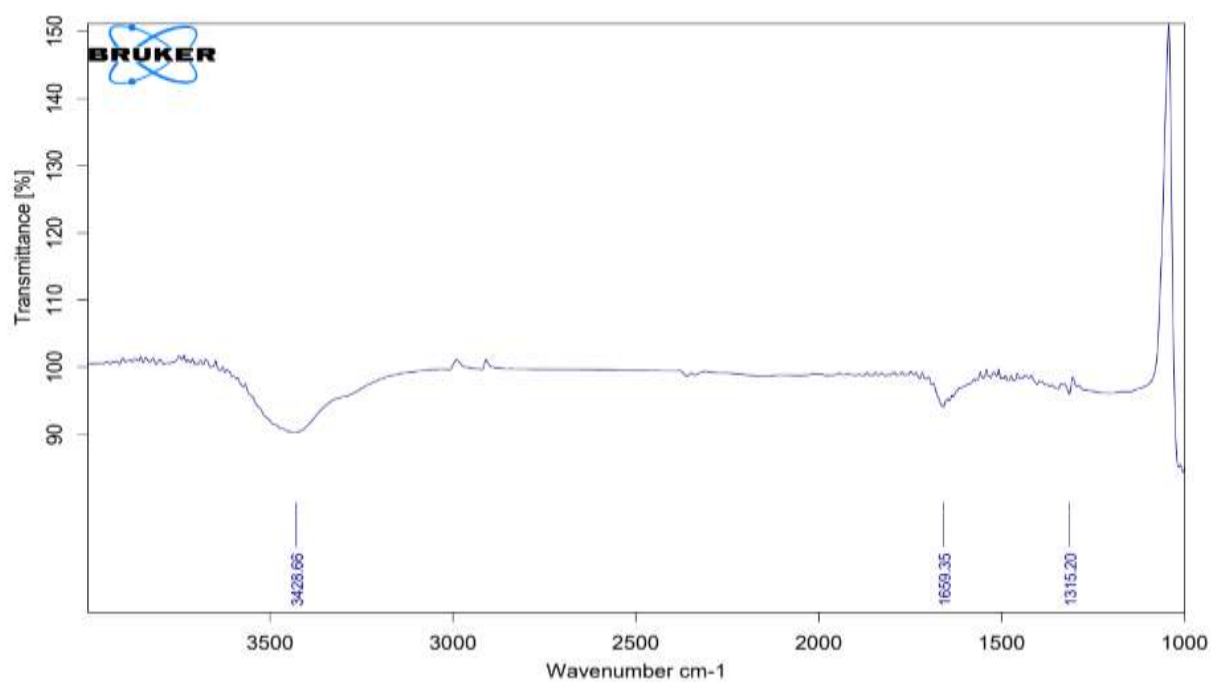


Figure 4.3.6.1h: shows the FTIR Transmittance peaks of High-altitude 1st flush (HA1) (Kurseong South tea garden Castleton)

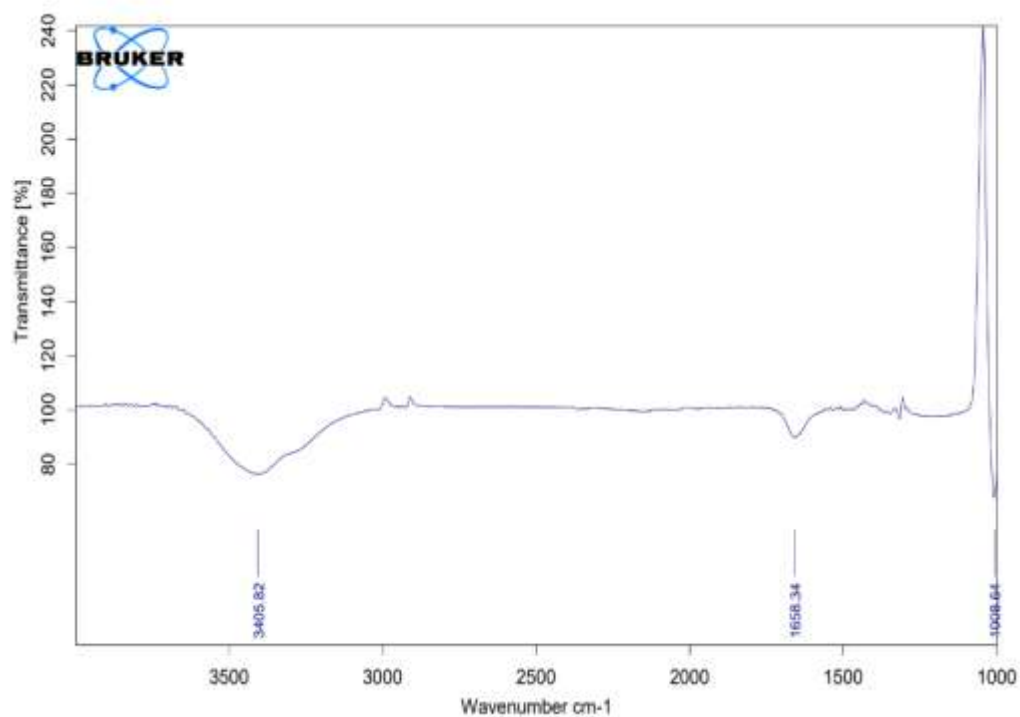


Figure 4.3.6.1i: shows the FTIR Transmittance peaks of High-altitude 2nd flush (HA2)(Kurseong South tea garden Castleton)

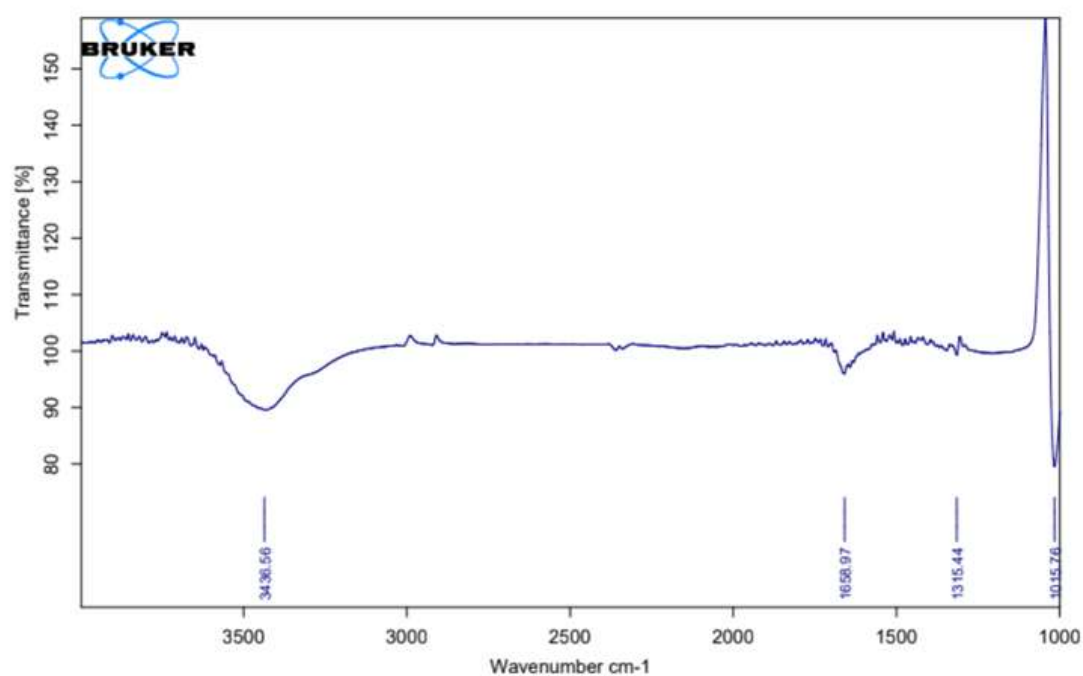


Figure 4.3.6.1j: shows the FTIR Transmittance peaks of Low Altitude (LA)(Kurseong South tea garden Upper Jungpana)

Fig 4.3.6.1g shows the FTIR Transmittance peaks of standard Rutin. The stretching of Rutin at 1021.25 cm⁻¹ is about 99.8% and the bending of Rutin at 3461.27 cm⁻¹ is about 97%. The concentration is 1mg/ml.

Fig 4.3.6.1h shows the FTIR Transmittance peaks of High-altitude 1st flush. The stretching of High-altitude 1st flush at 1008.64 cm⁻¹ is about 70 % and the bending of Rutin at 3405.92 cm⁻¹ is about 80 %. The concentration is about 1.4±0.01mg/ml.

Fig 4.3.6.1i shows the FTIR Transmittance peaks of High-altitude 2nd flush. The stretching of High-altitude 2nd flush at 1000 cm⁻¹ is about 80 % and the bending of Rutin at 3428 cm⁻¹ is about 91 %. The concentration is about 1.55±0.01mg/ml.

Fig 4.3.6.1j shows the FTIR Transmittance peaks of low altitude. The stretching of low altitude at 1013 cm⁻¹ is about 90 % and the bending of Rutin at 3436 cm⁻¹ is about 90 %. The concentration is about 1.1±0.01mg/ml.

SAMPLE	STRETCHING cm ⁻¹	BENDING cm ⁻¹	CONCENTRATION
RUTIN	1021.25-99.8%	3461.27-97%	1mg/ml
LA	1013-90%	3436-90%	1.1±0.01mg/ml
HA1	1008.64-70%	3405.92-80%	1.4±0.01mg/ml
HA2	1000-80%	3428-91%	1.55±0.01mg/ml

Table 4.3.4 shows the presence of phenolic group stretching and bending at different wave numbers.S.D=0.01

The table 4.3.4 shows the presence of phenolic group stretching and bending at different wave numbers the respective concentrations of Rutin at high-altitude flushes and low altitudes.2nd Flush Kurseong South contains more concentrations of Rutin than the 1st flush and low altitude.

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Experiment : 4.3.4.4: HPLC Study

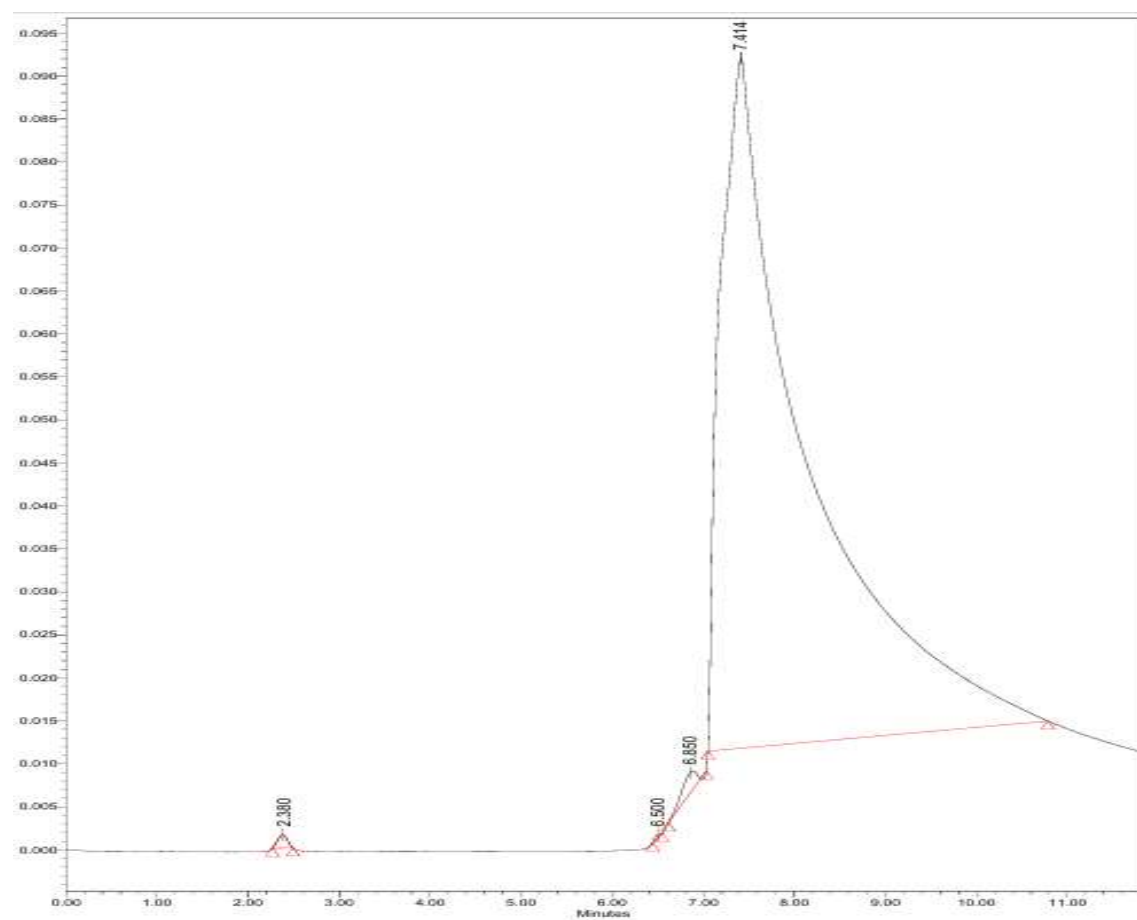
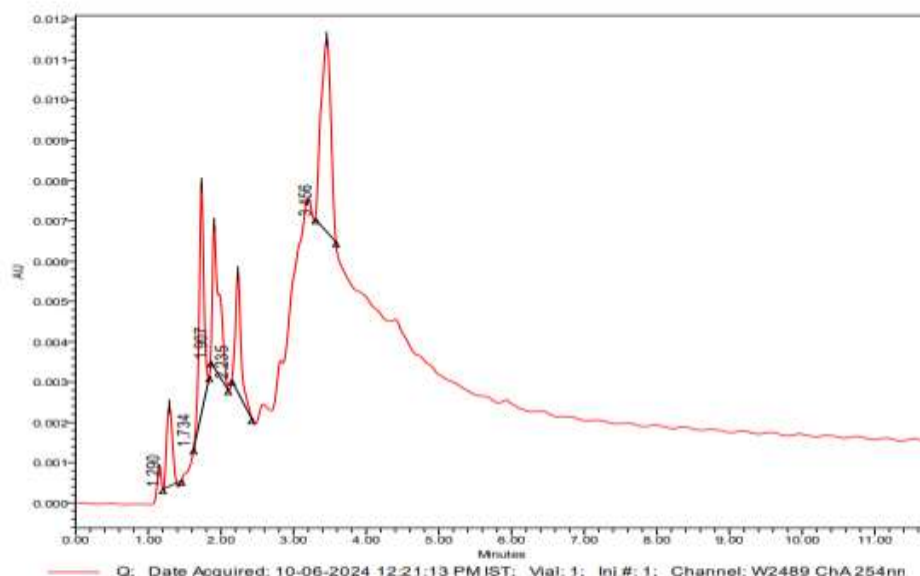


Figure 4.3.7.1a shows the retention peak of standard Rutin



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Figure 4.3.7.1b shows the retention peak of standard QUERCETIN

Fig 4.3.7.1a and 4.3.7.1b show the retention time in minutes of standards Rutin and Quercetin respectively.

Fig 4.3.7.1a shows the retention time in minutes of the standard Rutin. The absorbance (AU) of the retention times of Rutin for 7.414, 2.380, 6.500 minutes are 0.095, 0.006, 0.010 respectively. The concentration of Rutin is 1mg/ml.

Fig 4.3.7.21b shows the retention time in minutes of the standard Quercetin. The absorbance (AU) of the retention times of Quercetin for 1.290, 1.734, 1.907, 1.235, 3.456 minutes are 0.001, 0.002, 0.008, 0.0075 and 0.011 respectively. The concentration of Quercetin is 1mg/ml.

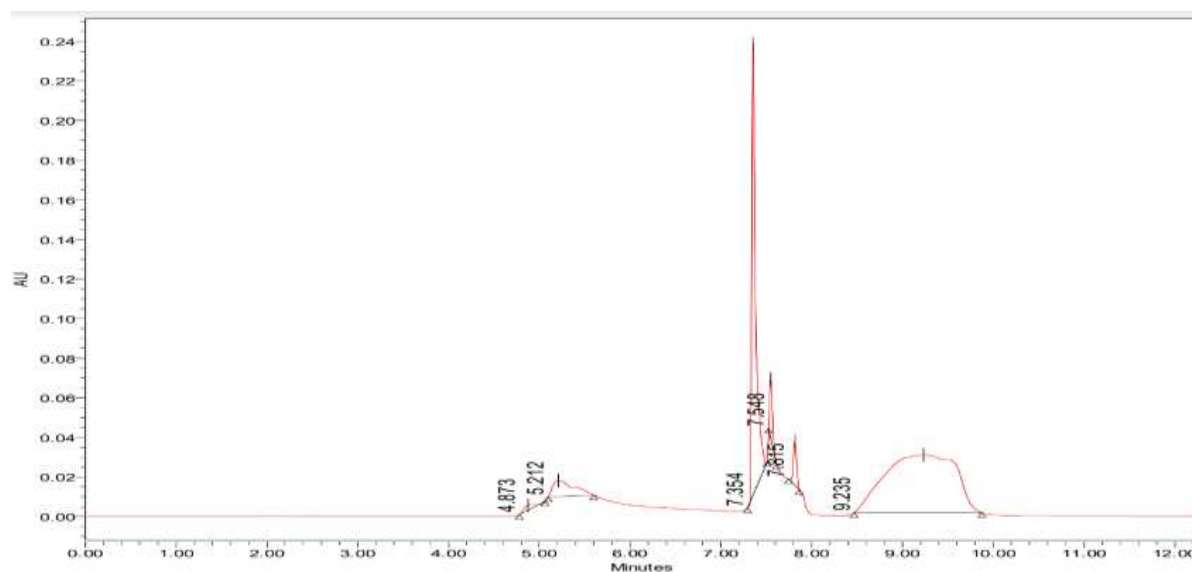


Figure 4.3.7.1c shows the retention peak of High Altitude(HA) (Kurseong South tea garden Castleton)

Fig 4.3.7.1c shows the retention time in minutes of standard Rutin in High altitude Kurseong South Gardens. The absorbance (AU) of the retention times of Quercetin for 4.873,5.212,7.354,7.548,7.815,9.235 minutes are 0.0,0.02,0.0,0.24,0.09,0.04 and 0.00 respectively.

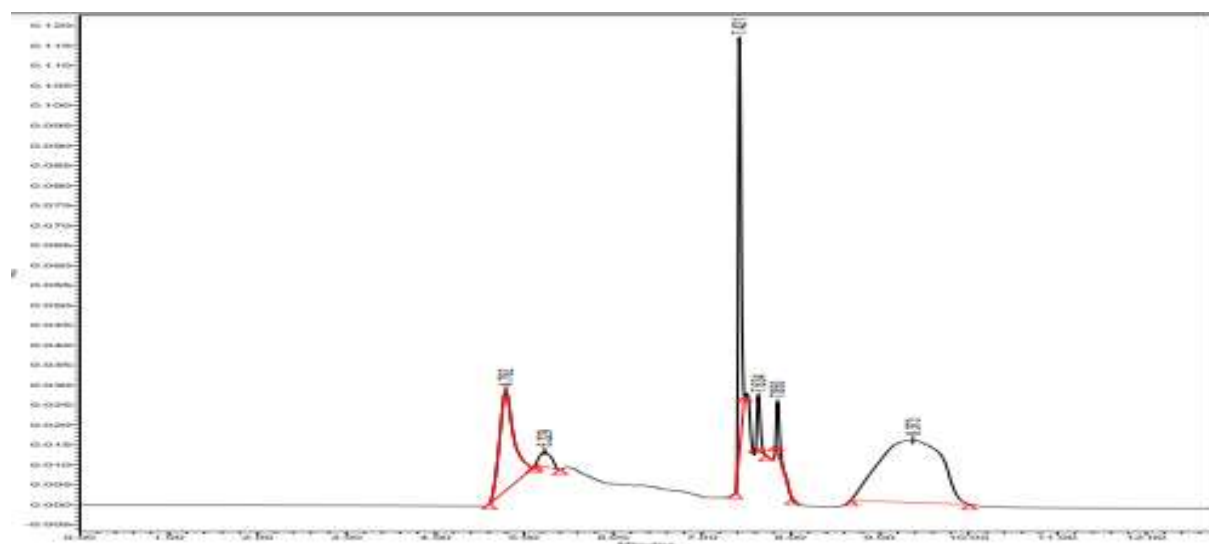


Figure 4.3.7.1d shows the retention peak of Low Altitude(LA) (Kurseong South tea garden (Upper Jungpana)

Fig 4.3.7.1d shows the retention time in minutes of standard Rutin in the Low altitude Kurseong South Gardens. The absorbance (AU) of the retention times of Low altitude for 7.548 minutes is 0.12. The concentration of Rutin in low altitude is $1.26 \pm 0.25 \text{ mg/ml}$.

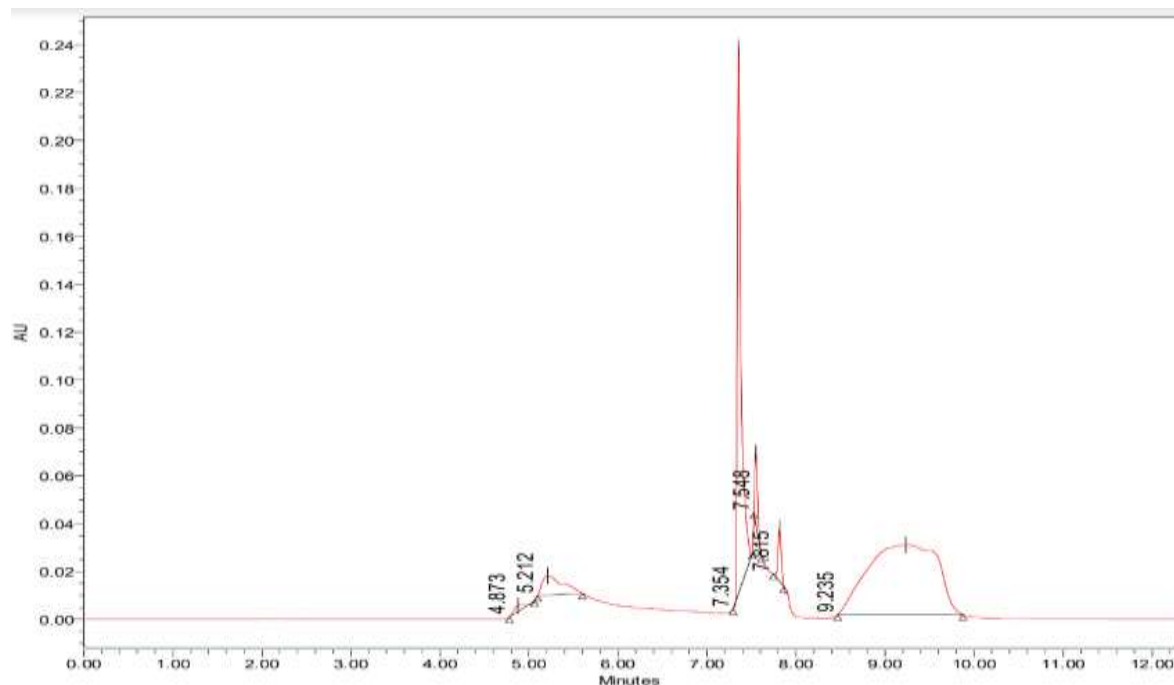


Figure 4.3.7.1 e shows the retention peak of High Altitude 1ST FLUSH (Kurseong South tea garden Castleton)

Fig 4.3.7.1 e shows the presence of Rutin as the retention peak of Rutin matches with that of the 1st flush.

The absorbance (AU) of the retention times of high-altitude 1st flush for 7.548 minutes is 0.20. The concentration Rutin in high altitude 1st flush is $2.52 \pm 0.2 \text{ mg/ml}$.

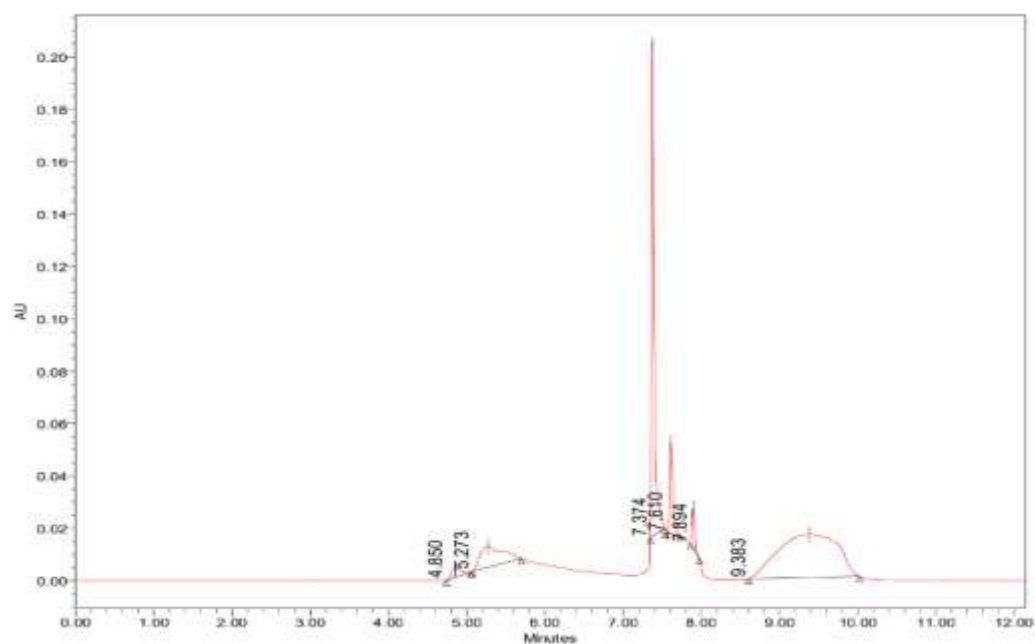


Figure 4.3.7.1f shows the retention peak of High Altitude 2nd flush(Kurseong South tea garden Castleton)

Fig. 4.3.7.1f shows the presence of Rutin as the retention peak of Rutin matches with that of the 2nd flush. The absorbance (AU) of the retention times of high-altitude 2nd flush for 7.548 minutes is 0.24. The concentration of Rutin in high-altitude 2nd flush is 2.1 ± 0.32 mg/ml.

SAMPLE	RETENTION TIME (Min)	ABSORBANCE AU	CONCENTRATION mg/ml
RUTIN	7.414	0.095	1
	2.380	0.006	
	6.500	0.010	
QUERCETIN	1.290	0.001	1
	1.734	0.002	
	1.907	0.008	
	1.235	0.0075	
	3.456	0.011	
HA	7.548	2 nd flush = 0.24	2.1 ± 0.32
		1 st flush = 0.20	2.52 ± 0.2
LA	7.548	0.12	1.26 ± 0.25

Table 4.3.5 shows the retention time of Standards and concentrations in 1st flush and 2nd flush of Kurseong South tea gardens

Table 4.3.5a shows that the high altitude (HA-Castleton) tea clones of Kurseong South is showing higher amount of Rutin (almost equivalent retention time-7.414 and 7.548min) content during second flush by comparing with the standard

Experiment 4.3.4.5:LCMS Study

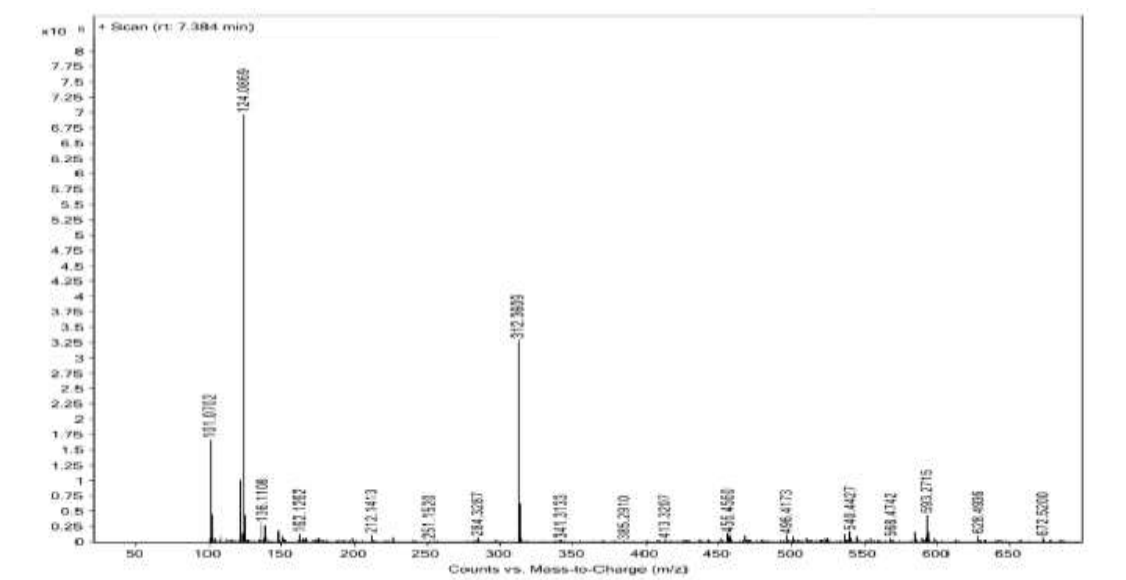


Figure 4.3.8. 1a LCMS study of High altitude (Kurseong South tea garden Castleton)

This figure shows the m/z peaks at 312.8 and 312.5 for high-altitude.

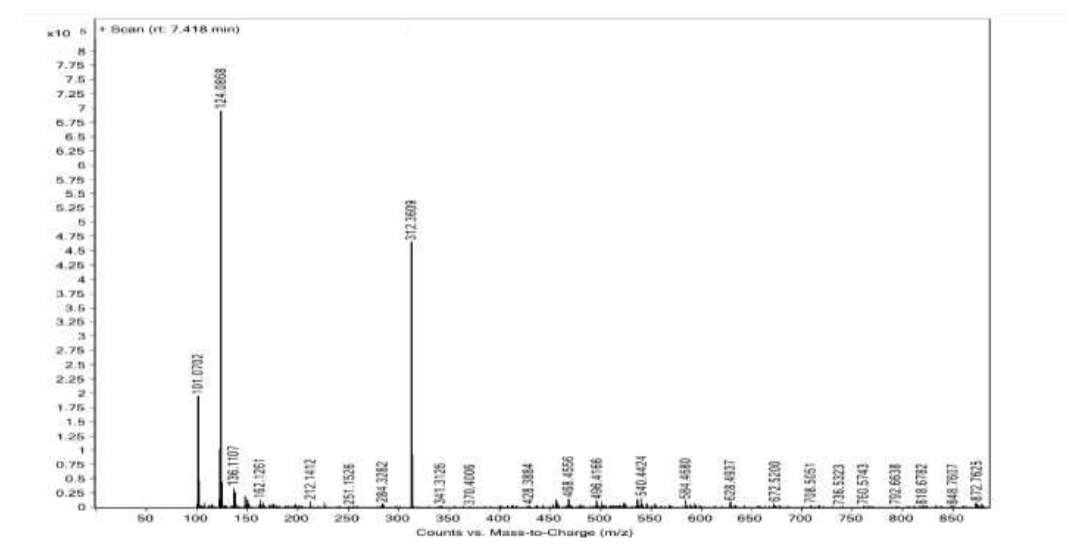


Figure 4.3.8.1b LCMS study of Low altitude(LA)(Kurseong South tea garden Upper Jungpana)

This figure shows the m/z peak at 312.36 for low altitude. The concentration of Rutin is about $1.35 \pm 0.003 \text{ mg/ml}$

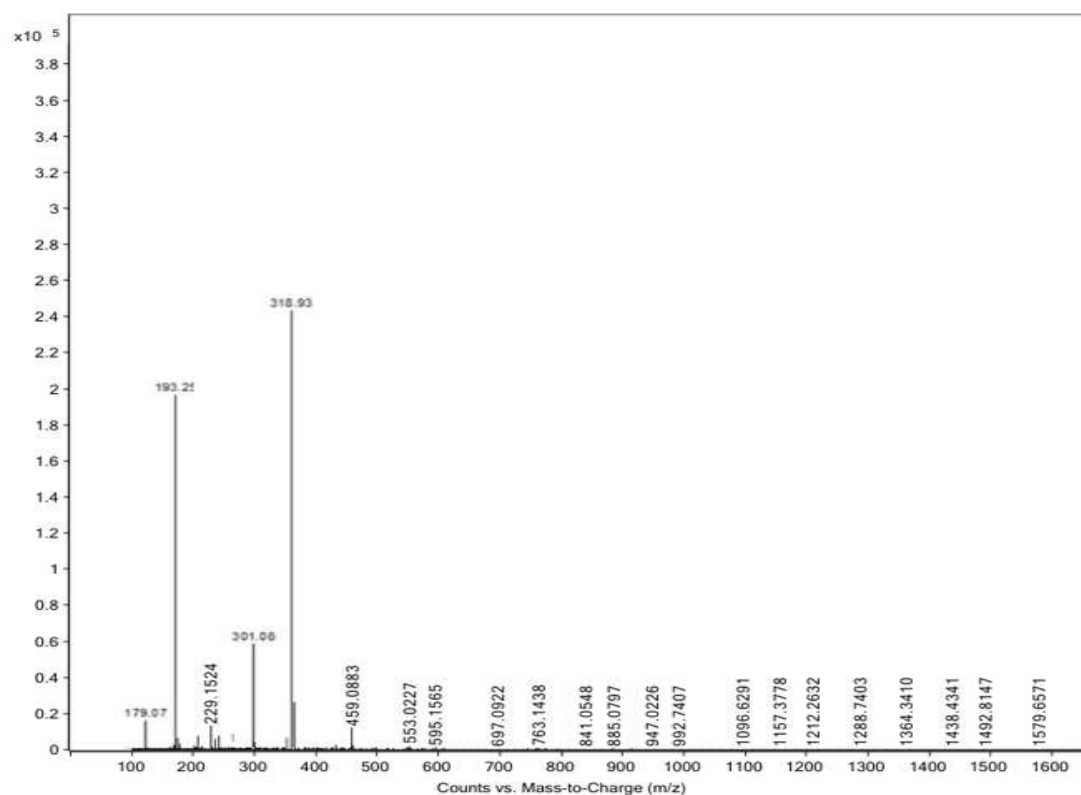


Figure 4.3.8. 1c: LCMS study of standard RUTIN

Fig 4.3.8.1c: shows the m/z peak at 312.3609 for the standard Rutin. The concentration of Rutin is 1 mg/ml

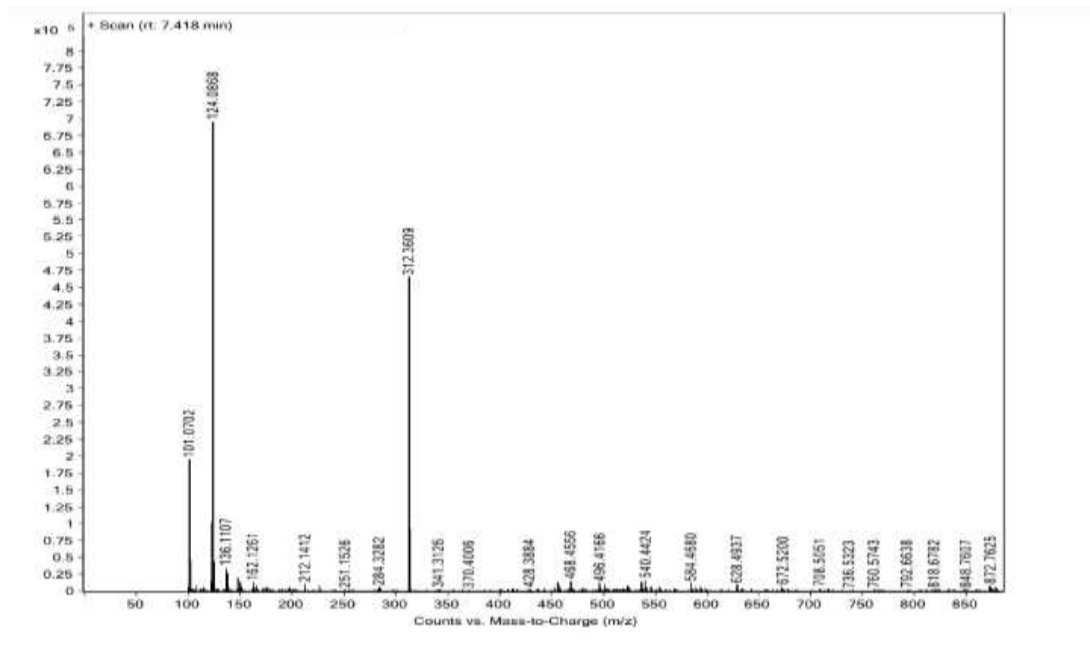


Figure 4.3.8.1d: LCMS study of High Altitude 2ND FLUSH(HA2) Kurseong South tea garden Castleton

This figure shows the m/z peak at 312.8 for high altitude 2nd flush. The concentration of Rutin is about 1.85±0.001 mg/ml.

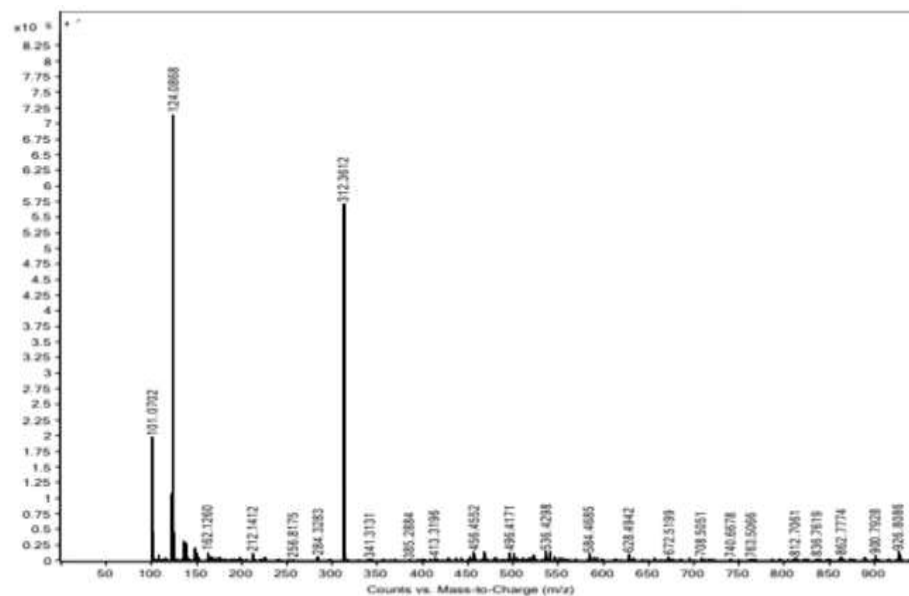


Figure 4.3.8.1e: LCMS study of High altitude 1ST FLUSH (HA1)Kurseong South tea garden Castleton

This figure Fig 4.3.8.1e shows the m/z peak at 312.5 for high altitude 1st flush. The concentration of Rutin is about 0.499 ± 0.005 mg/ml. Fig 4.3.22 a, b, d, e shows the presence of m/z peak, which corroborates with the m/z peak of Rutin.

Sample	m/z	$\times 10^5$	CONCENTRATION mg/ml
RUTIN	312.3609	2.4	1
HA2	312.8	4.75	1.85 ± 0.001
HA1	312.5	1.2	0.499 ± 0.005
LA	312.36	3.25	1.35 ± 0.003

Table 4.3.6 LCMS analysis table

Table 4.3.13 indicates the m/z ratio peak and respective concentrations. High altitude 2nd Flush contains more Rutin than the low altitude of Kurseong South valley. Yashin et al., (2015) have examined different chemical compositions in tea with respect to HPLC and LCMS methods.

Experiment 4.3.4.6 :TLC analysis

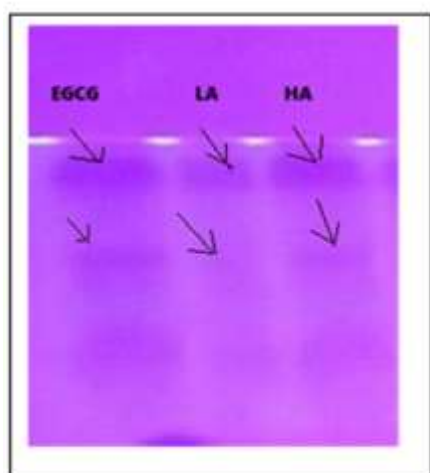


Figure 4.3.9.1a: TLC of High altitude(Kurseong South tea garden Castleton), Low altitude(Kurseong South tea garden Upper Jungpana) with standards EGCG observed under UV radiation at 254 nm

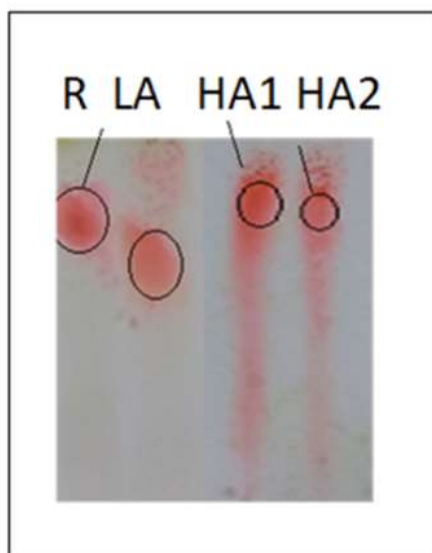


Figure 4.3.9.1b: TLC of High altitude 1st flush and 2nd flush(Kurseong South tea garden Castleton), , Low altitude(Kurseong South tea garden Upper Jungpana) with standard Rutin observed by Vanillin: HCl spraying

Sample	R _f	Densitometric scan
EGCG	UV 254 nm=0.15	
RUTIN	Colorimetric=0.73	100%
HA1	UV=0.15 Colorimetric=0.72	64%
HA2	Colorimetric=0.72	131%
LA	Colorimetric=0.69	53%

Table 4.3.7 shows the retention factor and the densitometric scan for standards EGCG, Rutin and high-altitude 1st and 2nd flush and low altitude.

Thin layer chromatography analysis Fig 4.3.9.22a and Fig 4.3.9.22b show that the presence of spots corresponding to the standards EGCG and Rutin under UV radiation at 254 nm and colour development spray with Vanillin: HCl. shows the retention factor 0.15 at 254nm.

Rutin shows the retention factor 0.73 whereas high altitude 2nd flush and 1st flush shows 0.72 as retention factor. Low-altitude shows 0.69 as the retention factor. Ligor et al.,2008 have studied the flavonoid contents of tea and Rooibos extracts with respect to TLC methodology.

Experiment 4.3.4.7: Spectrophotometric assay of Rutin Content

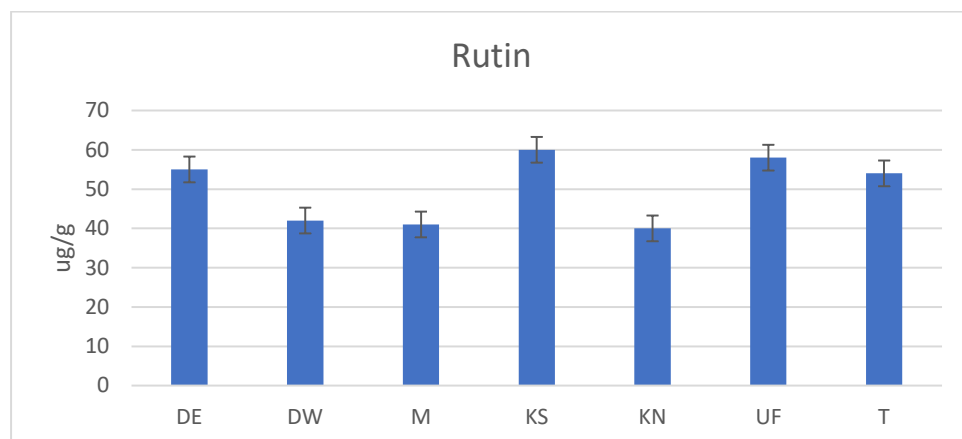


Figure 4.3.10. 1.shows variation in the Rutin Content among the 7 valleys of Darjeeling.S.D=0.01

The Rutin content is highest in the case of Kurseong South Garden.

Kurseong South valley shows a higher Rutin content of about 60ug/g±0.01, followed by Upper Fagu 58±0.01ug/g/g, Darjeeling East 55 ug/g±0.01, Teesta 54±0.01ug/g, Darjeeling West 42ug/g±0.01, Mirik 42ug/g±0.01, and Kurseong North 40±0.01ug/g/g.

Experiment 4.3.4.8. Spectrophotometric assay of Quercetin

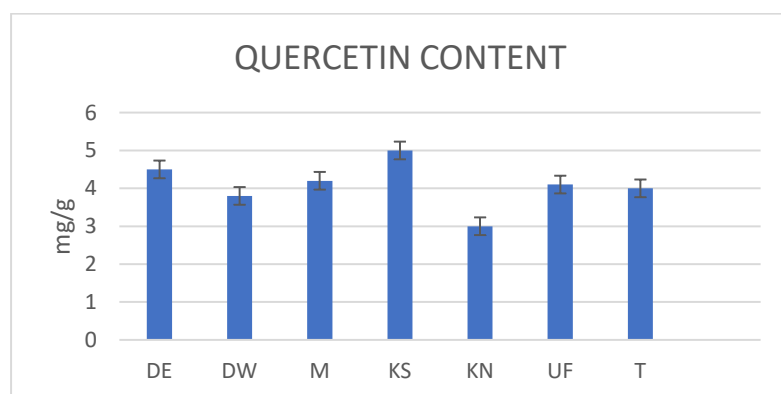


Figure 4.3.11.1a shows variation in the Quercetin Content among the 7 valleys of Darjeeling. S.D=0.001

The Quercetin content is highest in the case of Kurseong South Garden. Kurseong South valley shows a higher Rutin content of about 5mg/g, followed by Mirik 4.2 ± 0.001 mg/g, Upper Fagu 4.1 ± 0.001 mg/g, Darjeeling East 4.5 ± 0.001 mg/g, Teesta 4 ± 0.001 mg/g, Darjeeling West 3.8 ± 0.001 mg/g, and Kurseong North 3 ± 0.001 mg/g. Anand David et al., (2016) have studied the importance of the bioactive flavonoid, Quercetin.

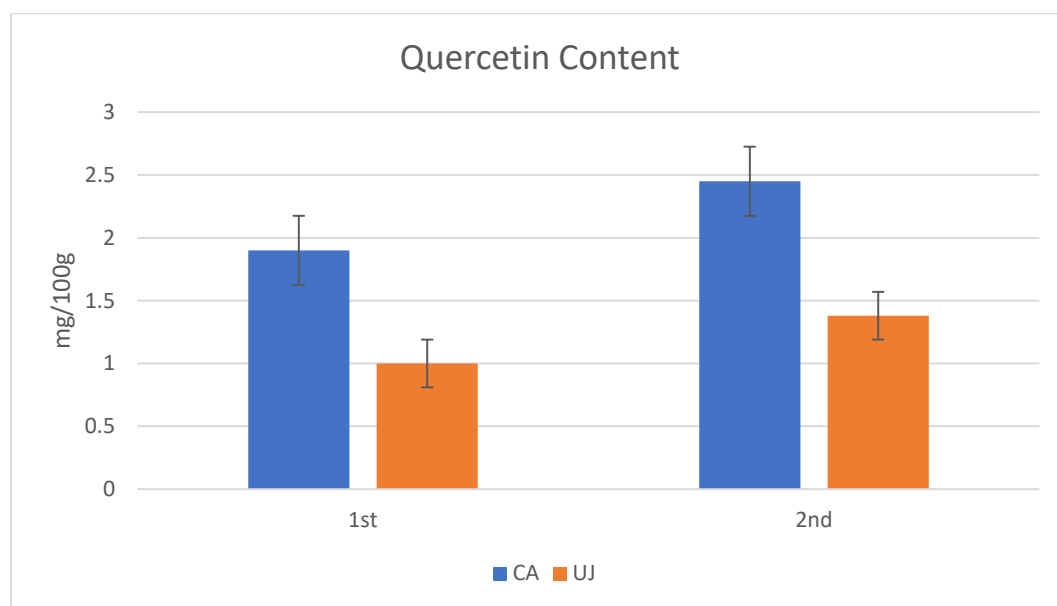


Figure 4.3.11.1b shows Quercetin content in Kurseong South tea garden Castleton and Upper Jungpana in 1st flush and 2nd flush. S.D=0.025

Castleton 2nd flush shows higher quercetin content than the low altitude Upper Jungpana.

Experiment 4.3.4.9. Spectrophotometric assay of total Catechin content

Castleton at higher altitude than Upper Jungpana shows more Quercetin content in the 2nd flush that is about 2.45 ± 0.025 mg/100g than in the first flush which is about 1.9 ± 0.025 mg/100g. Upper Jungpana shows more Quercetin content in 2nd flush (1.38 ± 0.025 mg/100g) than in first flush (1 ± 0.025 mg/100g)

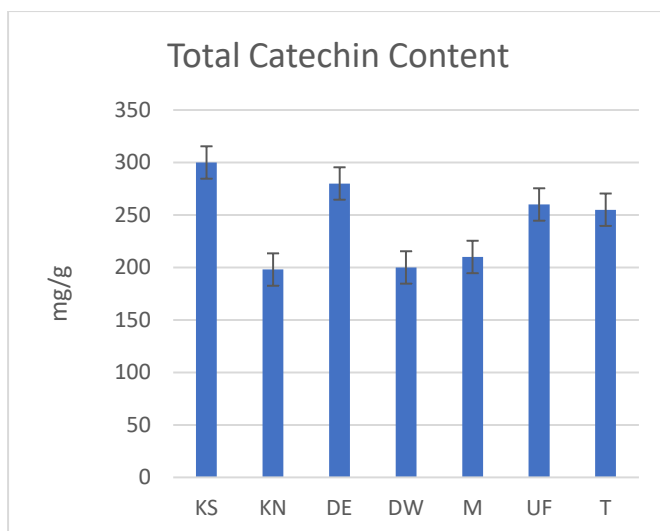


Figure 4.3.12.1a: Total Catechin content of seven valleys of Darjeeling. S.D=0.01

Figure 4.3.12.1a: Kurseong South has the highest catechin content of about 300±0.01 mg/g followed by Darjeeling East (280±0.01 mg/g), Upper Fagu (260±0.01 mg/g), Teesta (255±0.01 mg/g), Mirik (210±0.01 mg/g) Darjeeling West (200±0.01 mg/g) and Kurseong North (198±0.01 mg/g).

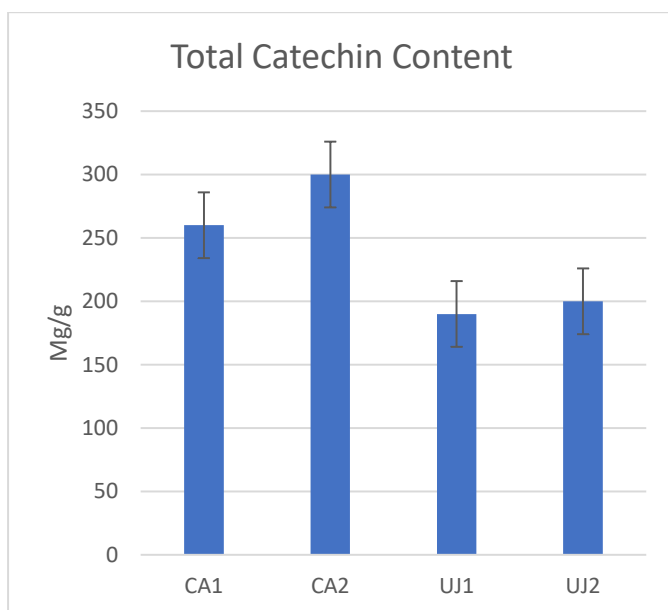


Fig 4.3.12.1b: Total Catechin content of Kurseong South tea gardens Castleton and Upper Jungpana in different flushes. S.D=0.01

Castleton has the highest flavonoid content 300mg/g in the second flush followed by in the first flush 260±0.01 mg/g. Upper Jungpana shows a similar trend in that it has a higher

flavonoid content of about 200 ± 0.01 mg/g in 2nd flush than in the first flush of 190 ± 0.01 mg/g.

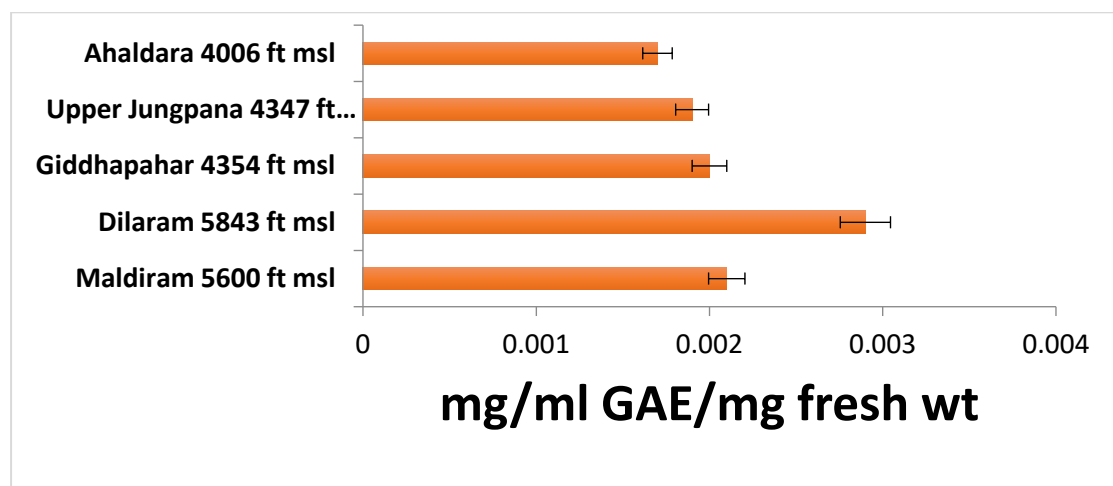


Figure 4.3.12.1c: Total catechin content of different gardens situated at different altitudes of Kurseong South valley. S.D=0.001

This figure shows total catechin content in different altitudes. Dilaram is at a highest altitude than other gardens showing more catechin content than the other low-altitude gardens.

Dilaram at the highest altitude has more catechin content of about 0.0029 ± 0.001 mg/mlGAE/mg fresh weight than other lower altitude gardens. Dilaram is followed by Maldiram (0.0021 ± 0.001 mg/mlGAE/mg fresh weight), Giddhapahar (0.002 ± 0.001 mg/mlGAE/mg fresh weight), Jungpana (0.0019 ± 0.001 mg/mlGAE/mg fresh weight) and Ahaldhara (0.0017 ± 0.001 mg/mlGAE/mg fresh weight). Chen et al.,2009 studied catechin content in oolong tea in different altitudes and seasons.

Experiment 4.3.4.10. Total Polyphenol Content

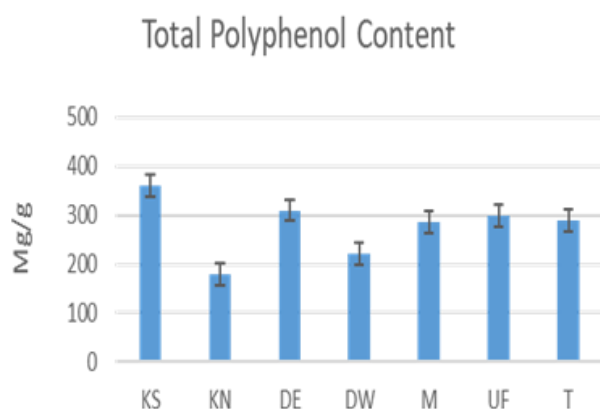


Figure 4.3.13.1a: Total Polyphenolic Content of seven valleys of Darjeeling.S.D=0.01

Fig 4.3.13.1a: Kurseong South has the highest polyphenol content of about 350 ± 0.01 mg/g followed by Darjeeling East (300 ± 0.01 mg/g), Upper Fagu (290 ± 0.01 mg/g), Teesta (285 ± 0.01 mg/g), Mirik (270 ± 0.01 mg/g) Darjeeling West (210 ± 0.01 mg/g) and Kurseong North (198 ± 0.01 mg/g).

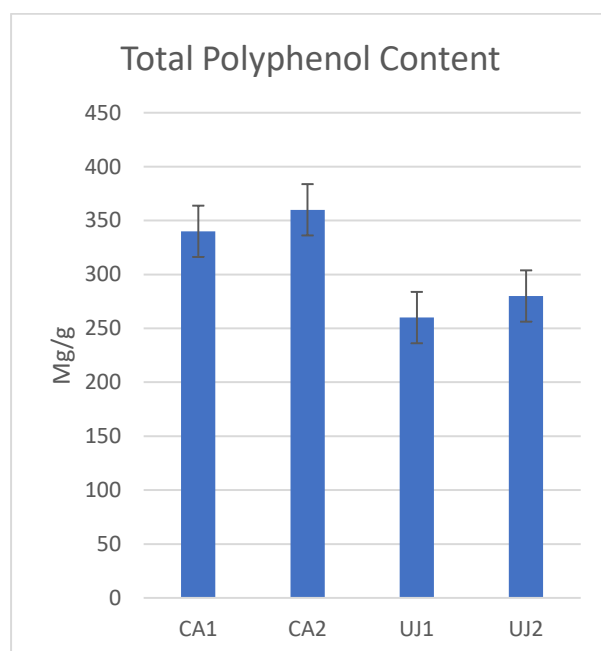


Figure 4.3.13.1b: Total Polyphenolic Content of Kurseong South tea gardens Castleton and Upper Jungpana in different flushes.S.D=0.01

Castleton has the highest polyphenol content 360 ± 0.01 mg/g in the second flush followed by in the first flush 340 ± 0.01 mg/g. Upper Jungpana shows a similar trend in that it has a higher polyphenol content of about 200 ± 0.01 mg/g than in the first flush of 190 ± 0.01 mg/g. Sharma et al., (2020) have also stated clearly that seasonal flushes influence the polyphenol content in tea leaves.

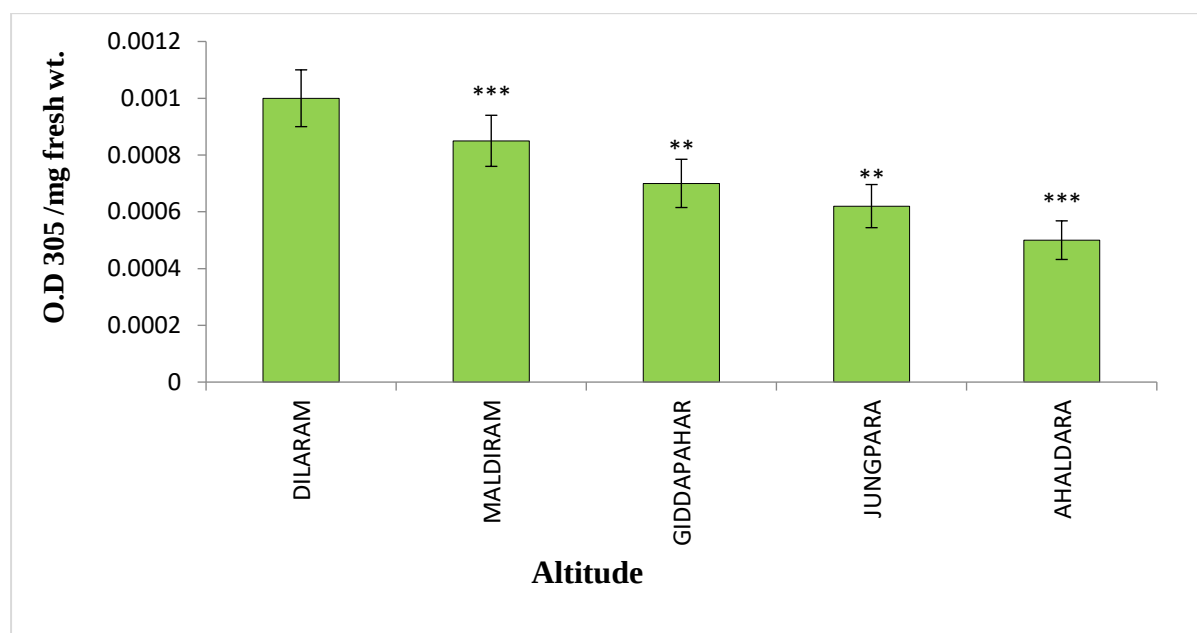


Figure 4.3.13.1c Total polyphenol content of different gardens situated at different altitudes of Kurseong South valley. S.D=0.001

This figure shows total polyphenol content in different altitudes. Dilaram is at a highest altitude than other gardens showing more tannin content than the other low-altitude gardens.

Dilaram at the highest altitude has more catechin content of about 0.001 ± 0.001 O.D 305/mg fresh weight than other lower altitude gardens. Dilaram is followed by Maldiram (0.00085 ± 0.001 O.D 305/mg fresh weight), Giddhapahar (0.0007 ± 0.001 O.D 305/mg fresh weight), Jungpana (0.00062 ± 0.001 O.D 305/mg fresh weight) and Ahaldhara (0.0005 ± 0.001 O.D 305/mg fresh weight). Suleiman et al., (2020) have examined altitudinal variation in polyphenol content of Acacia and Ziziphus honey.

Statistical analysis:

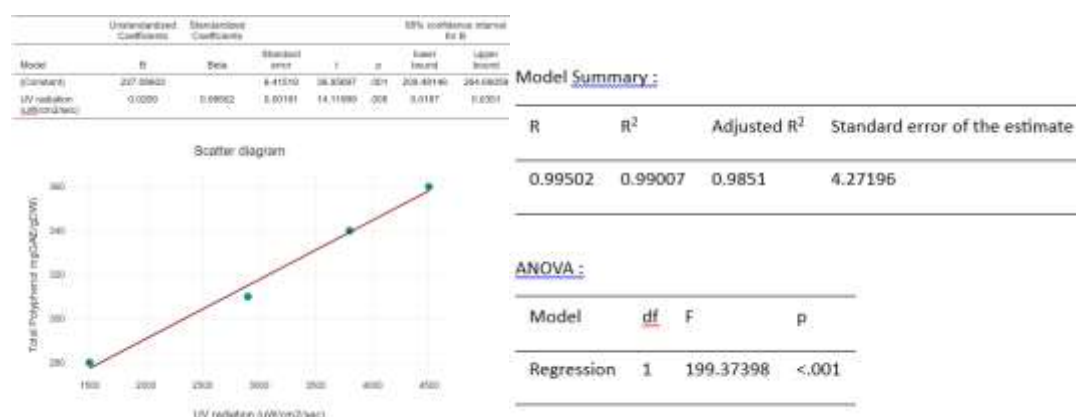


Table 4.3.8: Statistical analysis between the annual UV radiation and total polyphenol content. The data interprets that there is a significant (<0.001) positive correlation between the annual UV radiation and total polyphenol content.

Experiment 4.3.4.11: Spectrophotometric analysis of total Flavonoid Content

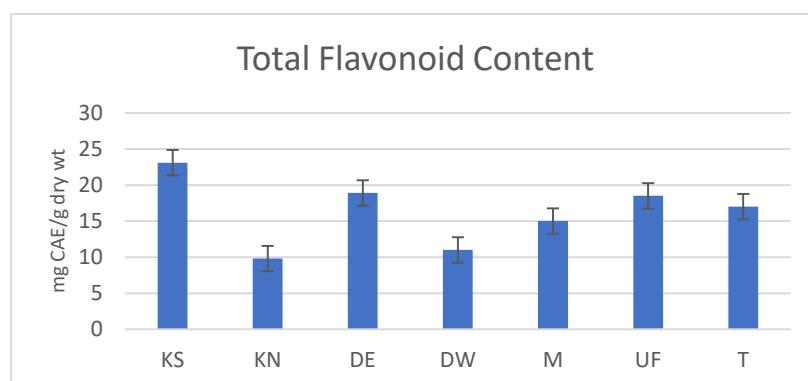


Figure 4.3.14.1a: Total Flavonoid Content of seven valleys of Darjeeling.S.D=0.001

4.3.14.1a: Kurseong South has the highest flavonoid content of about 23.1 mg/g followed by Darjeeling East (18.9±0.001 mg/g), Upper Fagu (18.5±0.001 mg/g), Teesta (17±0.001 mg/g), Mirik (15±0.001 mg/g) Darjeeling West (11±0.001 mg/g) and Kurseong North (9.8±0.001 mg/g).

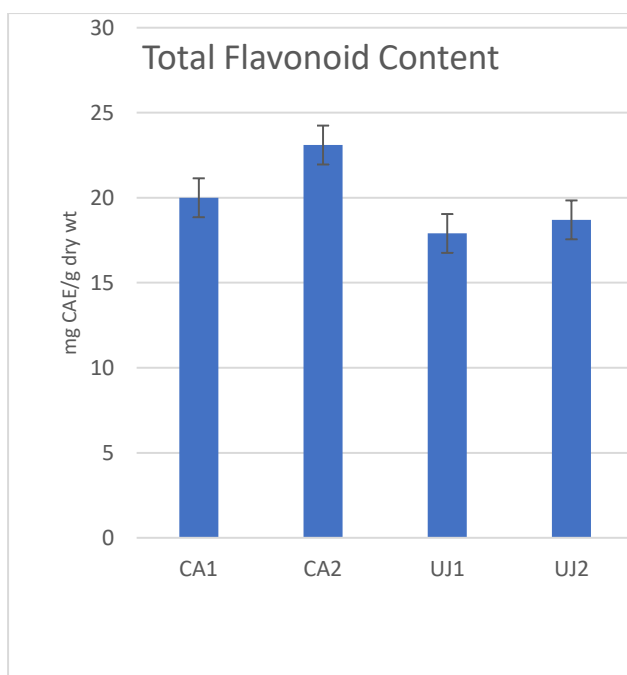


Figure 4.3.14.1b: Total Flavonoid Content of Castleton and Upper Jungpana in different flushes.S.D=0.001

4.3.14.1b: Castleton has the highest flavonoid content 23.1 ± 0.001 mg/g in the second flush followed by in the first flush 20 ± 0.001 mg/g. Upper Jungpana shows a similar trend in that it has a higher flavonoid content of about 18.7 ± 0.001 mg/g in the 2nd flush than in the first flush 17.9 ± 0.001 mg/g.

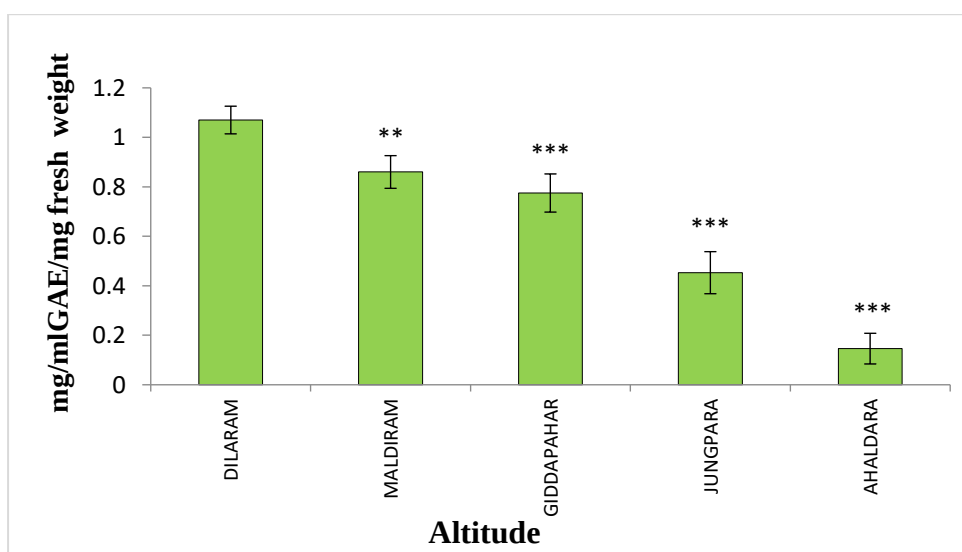


Figure 4.3.14.1c Total flavonoid content of different gardens situated at different altitudes of Kurseong South valley.S.D=0.05

This figure shows total flavonoid content in different altitudes. Dilaram is at the highest altitude, showing more flavonoid content than other low-altitude gardens.

Dilaram at the highest altitude has more flavonoids of about 1.07 ± 0.05 mg/mlGAE/mg fresh weight than other lower altitude gardens. Dilaram is followed by Maldiram (0.86 ± 0.05 mg/mlGAE/mg fresh weight), Giddhapahar (0.775 ± 0.05 mg/mlGAE/mg fresh weight), Jungpana (0.453 ± 0.05 mg/mlGAE/mg fresh weight) and Ahaldhara (0.146 ± 0.05 mg/mlGAE/mg fresh weight). Pandey et al., 2018 studied about the altitudinal variation of flavonoid content in *Thalictrum foliolosum* and their antimicrobial and antioxidant activities.

Statistical analysis

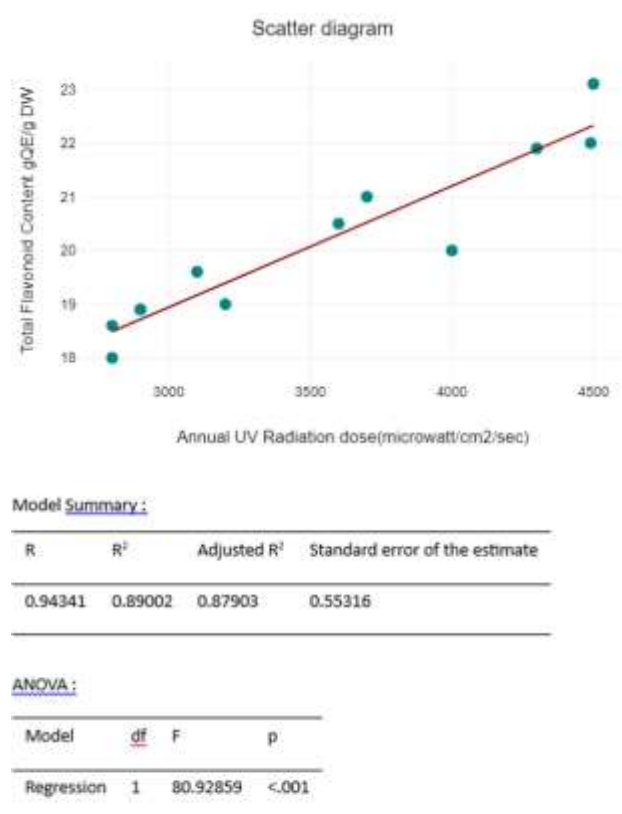


Table 4.3.9: Statistical data between the annual UV radiation and total flavonoid content.

It interprets that there is significant (<0.001) positive correlation between the annual UV radiation and total flavonoid content.

Variables Entered/Removed ^a			
Model	Variables Entered	Variables Removed	Method
1	UV radiation (uW/cm2/s), Temperature (°C), Relative Humidity (%), Rainfall (mm) ^a		Enter
a. Dependent Variable: Production (MKg)			
b. All requested variables entered.			

Model Summary				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.1000 ^a	.1000		
a. Predictors: (Constant), UV radiation (uW/cm2/s), Temperature (°C), Relative Humidity (%), Rainfall (mm)				

ANOVA ^a						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.160	4	0.290		.871 ^b
	Residual	0.000	0			
	Total	1.160	4			
a. Dependent Variable: Flavonoids (mg QE/g)						
b. Predictors: (Constant), UV radiation (uW/cm2/s), Temperature (°C), Relative Humidity (%), Rainfall (mm)						

Coefficients ^a					
Model		Unstandardized Coefficients		Standardized Coefficients	Sig.
		B	Std. Error	Beta	
1	(Constant)	225.384	0.000		
	Rainfall (mm)	-0.059	0.000	-.6788	
	Relative Humidity (%)	0.183	0.000	0.537	
	Temperature (°C)	-3.089	0.000	-.5132	
	UV radiation (uW/cm2/s)	7.005	0.000	.5504	
a. Dependent Variable: Flavonoids (mg QE/g)					

Table 4.3.10: Statistical Data between the annual UV radiation and total flavonoid content .

The statistical data indicates that there is significant correlation between the annual UV radiation and total flavonoid content among all other agroclimatic parameters by the β factor which is 7.005 for annual UV radiation.

ANOVA POST HOC ANALYSIS:

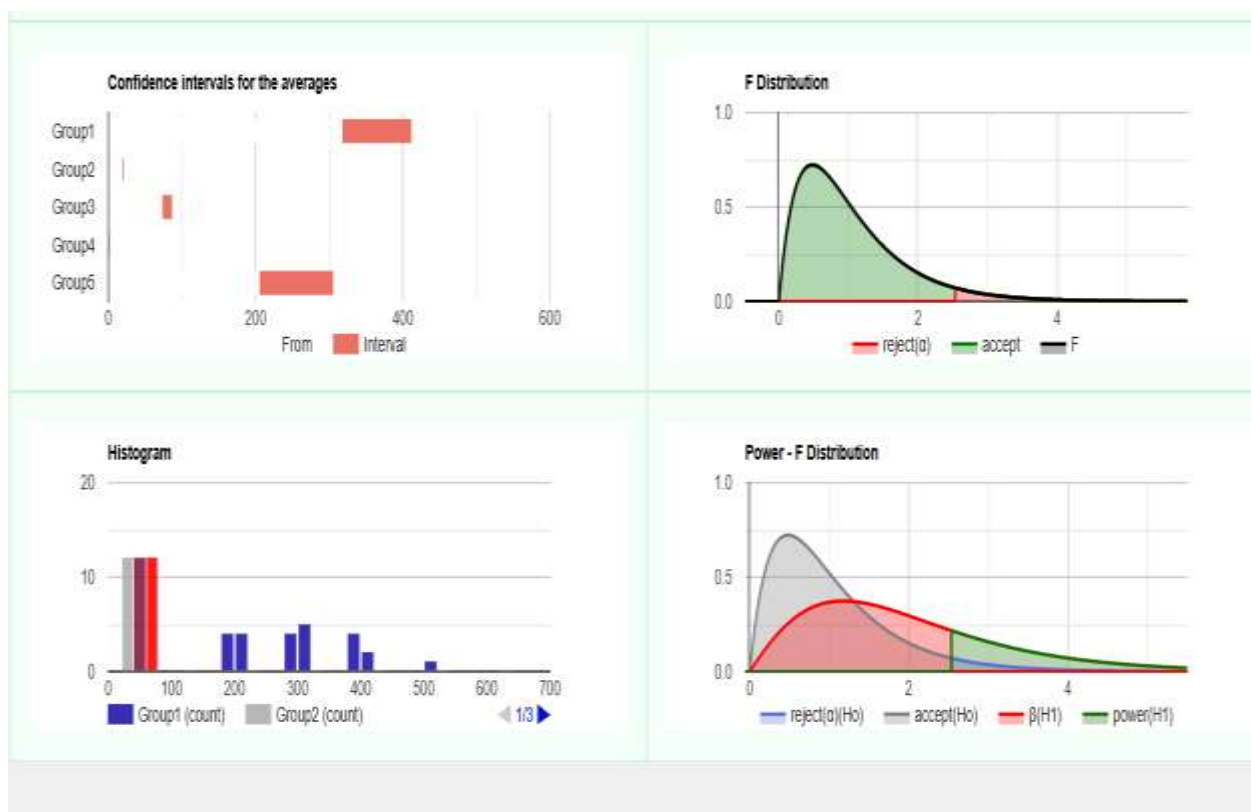


Figure 4.3.15: Anova Post Hoc Analysis

ASSAY OF COMMERCIAL QUALITIES: COLOUR/HUE, AROMA, BRISKNESS

The commercial qualities have been characterized with both leaves and manufactured Black tea of different flushes and different altitudinal gardens of seven valleys of Darjeeling

Experiment 4.3.4.12: Spectrophotometric assay of Theaflavin:

GARDEN	TF% 1 st /2 nd flush
CA1, CA2	20±0.01, 24±0.01
G1, G2	15.2±0.01, 18.5±0.01
UJ1, UJ2	15±0.01, 17.5±0.01

Table 4.3.11 shows variation in theaflavin content in the high altitude and low altitude 1st flush and 2nd flush of Kurseong South tea garden .S.D=0.01

Castleton(CA) at a higher altitude than Giddhapahar(G) and Upper Jungpana(UJ) shows high theaflavin content in the 2nd flush with manufactured black tea

Castleton 2nd flush shows $24 \pm 0.01\%$ TF, whereas 1st flush shows $20 \pm 0.01\%$ TF Giddhapahar 2nd flush shows $18 \pm 0.01\%$ TF, whereas 1st flush shows $15.2 \pm 0.01\%$ TF. Upper Jungpana in 2nd flush shows $17.5 \pm 0.01\%$ TF whereas 1st flush shows $15 \pm 0.01\%$ TF. Zhao et al.,2024 have studied health promoting factors of theaflavin in black tea.

Experiment 4.3.4.13: Tannin content of manufactured tea of different altitudes of Kurseong South garden

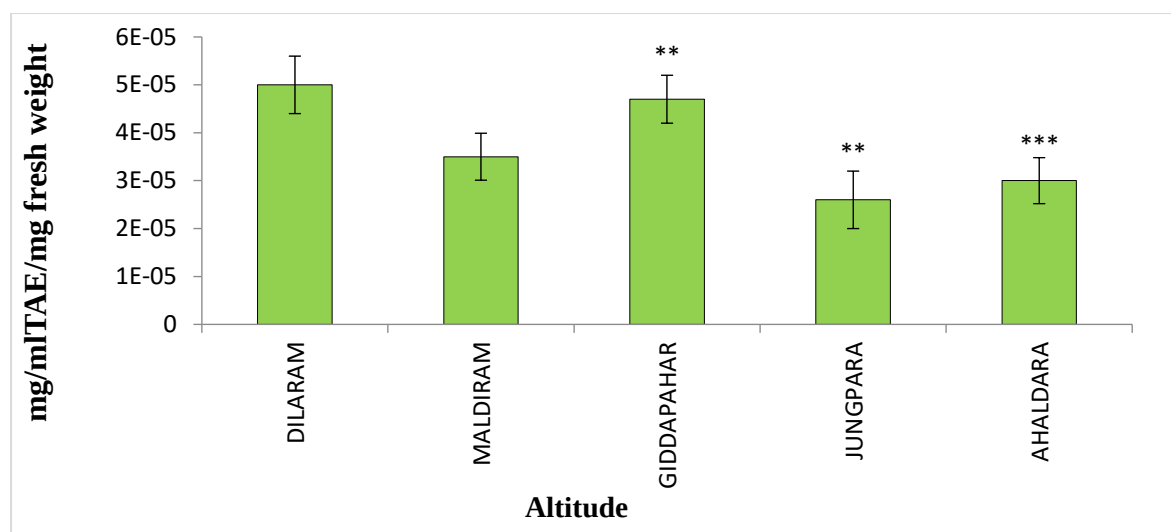


Figure 4.3.16 1: Total tannin content in different altitudes of Kurseong South valley

This figure shows total tannin content in different altitudes. Dilaram is at a higher altitude than other gardens showing more tannin content than the other low-altitude gardens.

Dilaram at the highest altitude has more tannin content of about $5E-05$ mg/mlTAE/mg fresh weight than other lower altitude gardens. Dilaram is followed by, Giddhapahar ($4.7E-05$ mg/mlTAE/mg fresh weight), Maldiram ($3.5E-05$ mg/mlTAE/mg fresh weight) Ahaldhara ($3E-05$ mg/mlTAE/mg fresh weight) and Jungpana ($2.6E-05$ mg/mlTAE/mg fresh weight).

Dong et al., (2021) observed a difference in tannin content in *Rhodiola crenulata* concerning the altitudinal variation.

Experiment 4.3.4.14: Assay of Briskness/Hue and Colour

Tea samples	Color	Hue
1st Flush	0.74±0.02; 0.52±0.01	2.87±0.03;
CA, UJ		2.63±0.02
2nd Flush CA, UJ	0.93±0.01	4.3±0.01;
	0.84±0.01	3.9±0.005
Autumn Flush	0.86±0.01	3.0±0.05;
CA, UJ	0.75±0.03	2.3±0.01

Table 4.3.12 shows variation in color and hue in the high altitude and low altitude 1st flush and 2nd flush of Kurseong South tea garden .

Castleton(CA) at a higher altitude than Upper Jungpana(UJ) shows more color and hue content in the 2nd flush.

Castleton(CA) shows 0.74±0.02, 0.93±0.01, and 0.86±0.01 color in the 1st 2nd flush and Autumn flush respectively. Whereas it shows 2.87±0.03 4.3±0.01hue and 3.0±0.05 in the 1st . 2nd flush and Autumn flush, respectively.

Upper Jungpana(UJ) shows 0.52±0.01, 0.84±0.01, and 0.75±0.03 color in the 1st 2nd flush, and Autumn flush, respectively whereas it shows 2.63±0.02, 3.9±0.005, and 2.3±0.01 hue in the 1st , 2nd flush, and Autumn flush respectively. Jin et al.,2013 have studied different flushes with respect to the color of tea with manufactured black tea

Experiment 4.3.4.15: Assay of Aroma enzyme: Spectrophotometric assay of β -glucosidase activity

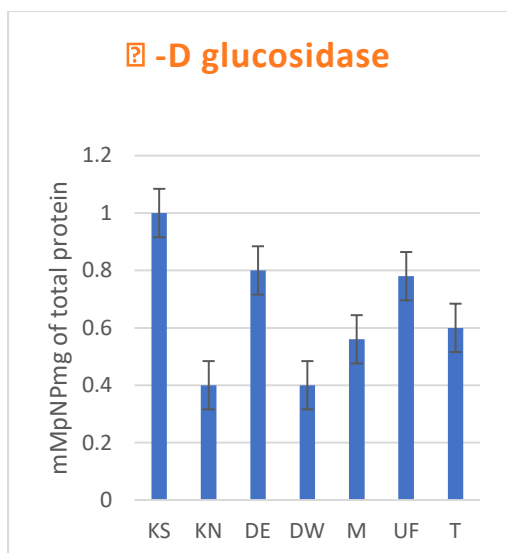


Figure 4.3.18.1a shows variation in β -glucosidase activity of fresh leaves among the seven valleys of Darjeeling. S.D=0.05

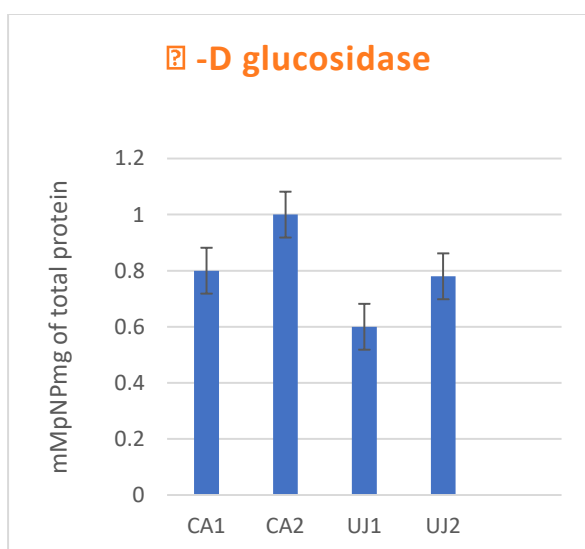


Figure 4.3.18.1 b shows variation β -glucosidase activity in Kurseong South tea gardens Castleton and Upper Jungpana 1st flush and 2nd flush fresh leaves. S.D=0.05

Fig 4.3.18.1a shows that Kurseong South Valley shows a higher β -D glucosidase content of about 1 ± 0.05 mMpNP/mg of total protein, followed by Darjeeling East 0.8 ± 0.05 mMpNP/mg of total protein, Upper Fagu 0.78 ± 0.05 mMpNP/mg of total protein, Teesta 0.6 ± 0.05 mMpNP/mg of total protein, Mirik 0.56 ± 0.05 mMpNP/mg of total protein Darjeeling West

0.4±0.05 mMpNP/mg of total protein and Kurseong North 0.4±0.05 mMpNP/mg of total protein

Castleton 2nd flush shows higher β -glucosidase activity than the low-altitude Upper Jungpana.

Castleton at higher altitude than Upper Jungpana shows more β -glucosidase activity in the 2nd flush, that is about 1 mMpNP/mg of total protein, than in the first flush which is about 0.8±0.05 mMpNP/mg of total protein. Upper Jungpana shows more β -glucosidase activity in 2nd flush (0.781±0.05 mMpNP/mg of total protein) than in first flush (0.6 ±0.05 mMpNP/mg of total protein). Erkanli et al., (2023) have studied the diversity of β -glucosidase enzymes.

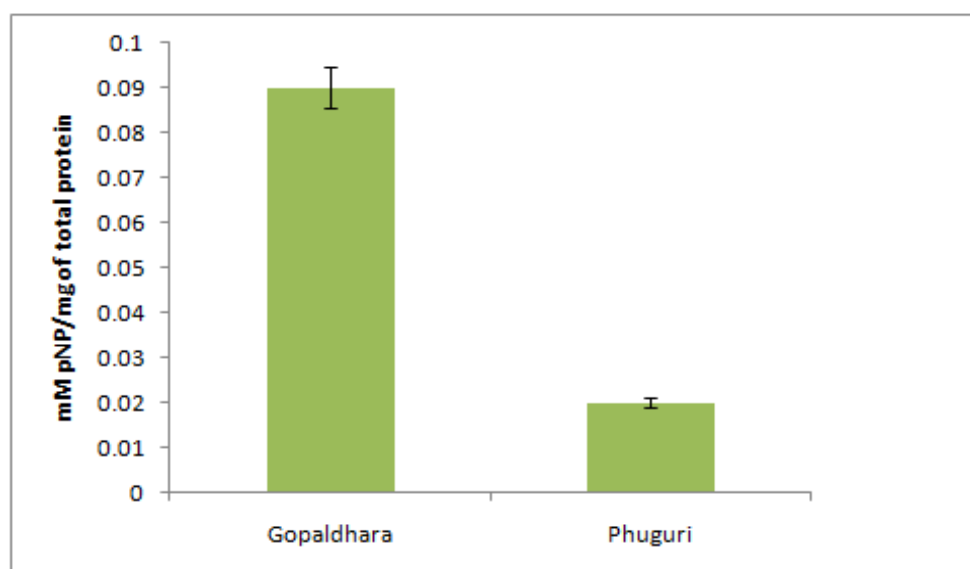


Figure 4.3.18.1 c shows variation in β -glucosidase activity in high altitude and low altitude of Mirik valley tea garden. S.D=0.001

Gopaldhara at higher altitude than Phuguri shows more β -glucosidase activity.

Gopaldhara, at a higher altitude than Phuguri, shows more β -glucosidase activity, about 0.9±0.001 mMpNP/mg of total protein, whereas Phuguri shows less, about 0.3±0.001 mMpNP/mg of total protein.

Siddhanta et al.,(2013) have examined the altitudinal variation of β -glucosidase activity in tea.

Experiment 4.3.4.16: Volatile aroma component linalool: Assay of Linalool

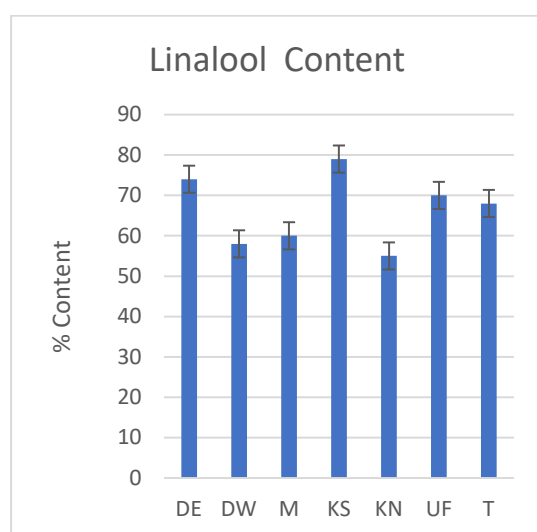


Figure 4.3.19.1a A variation in linalool content among the seven valleys of fresh Darjeeling tea leaves.S.D=0.001

Fig 4.3.19.1a : Kurseong South shows the highest linalool content.

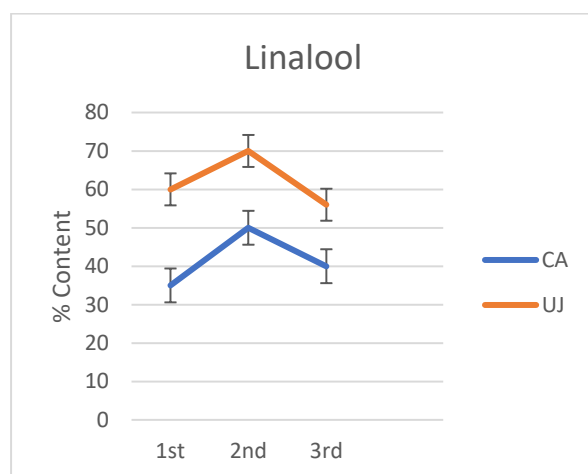


Figure 4.3.19.1b variation in linalool content in Kurseong South tea gardens Castleton(CA) and Upper Jungpana(UJ) 1st flush , 2nd flush and 3rd flush.S.D=0.001

Kurseong South Valley shows the highest linalool content of about 79 ± 0.001 %, followed by Darjeeling East at 74 ± 0.001 %, Upper Fagu at 70 ± 0.001 %, Teesta at 68 ± 0.001 %, Mirik at 60 ± 0.001 %, Darjeeling West at 58 ± 0.001 % and Kurseong North at 55 ± 0.001 %.

. Castleton 2nd flush shows higher linalool content than the low-altitude Upper Jungpana.

Castleton in the 2nd flush shows more content of linalool of about 70% than the 1st flush which is about 60%. Gupta et al., (2011) have shown altitudinal variation of linalool content in the leaves of *Zanthoxylum alatum* Roxb.

Experiment 4.3.4.17: Assessment of volatile aroma causing components: Gas Chromatography

The gas chromatography was done with manufactured tea of high and low altitude of Kurseong South garden of second flush.

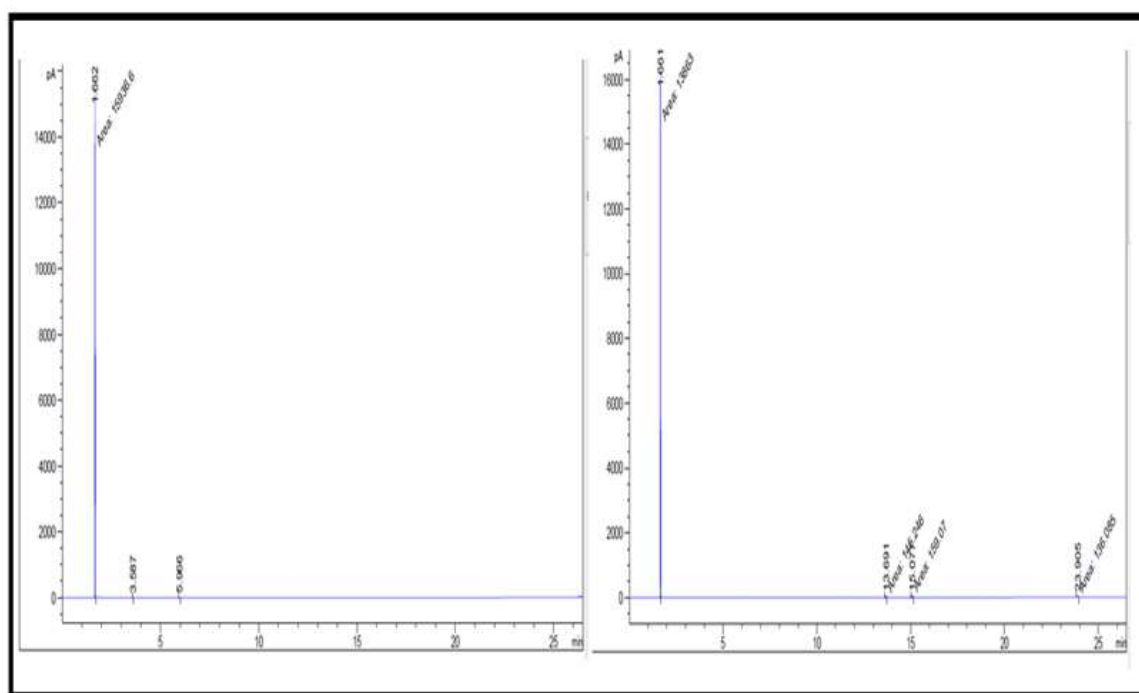


Figure 4.3.20.1a: shows the Gas Chromatography of high and low-altitude of Kurseong South tea Garden.

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.662	MM	0.0171	1.59366e4	1.54967e4	99.98710
2	3.587	BV	0.0199	4.64272e-1	3.52554e-1	0.00291
3	5.966	BB	0.0290	1.59111	8.25933e-1	0.00998
Totals :				1.59386e4	1.54979e4	

Table 4.3.13a :Peak area % of the high and low altitude of Kurseong South tea garden

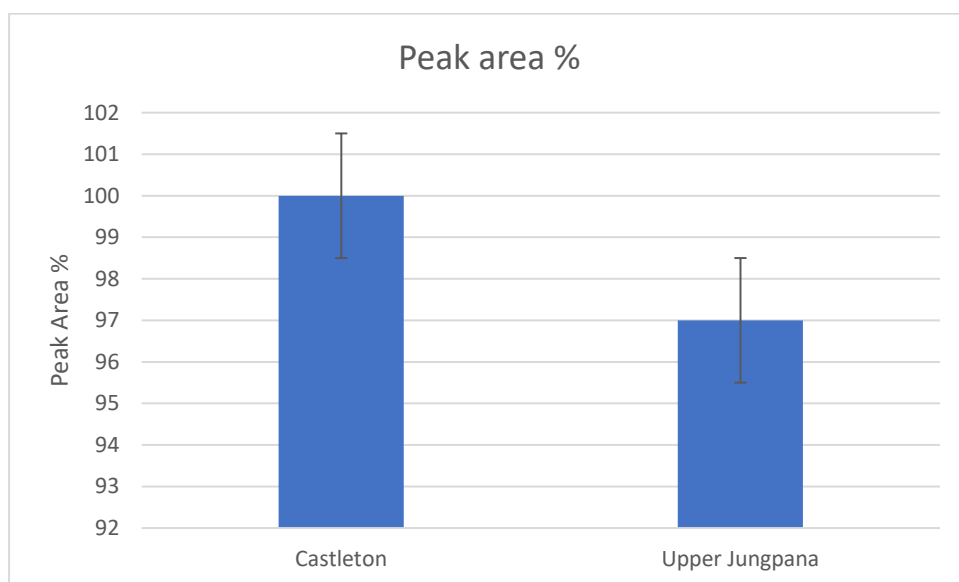


Figure 4.3.20.1b Peak area % of Kurseong South tea garden Castleton and Upper Jungpana.S.D=0.02

Table 4.3.17a and Figure 4.3.20.1b show peak area % of the high and low altitude. Peak area of the high altitude is higher than the low altitude.

The peak area of Castleton is about 100 ± 0.02 and that of Upper Jungpana is about 97 ± 0.02 .

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.661	MM	0.0143	1.38630e4	1.61970e4	96.92101
2	13.691	MM	0.0577	145.24640	41.95574	1.01546
3	15.077	MM	0.0549	159.07043	48.25845	1.11211
4	23.905	MM	0.0543	136.08499	41.78861	0.95141
Totals :				1.43034e4	1.63290e4	

Table 4.3.13b:Peak amplitude of high and low altitude

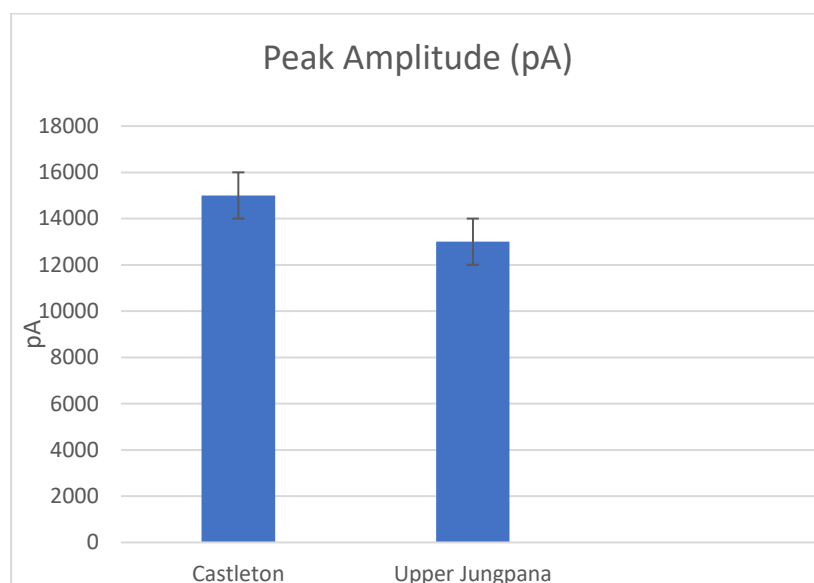


Fig 4.3.20.1c Peak amplitude of Kurseong South tea gardens Castleton and Upper Jungpana.S.D=0.001

Table 4.3.17b and Fig 4.3.20.1c shows peak amplitude of high and low altitude. Peak amplitude of high altitude is higher than the low altitude.

The peak amplitude of Castleton is about 15000 ± 0.001 pA and that of Upper Jungpana is about 13000 ± 0.001 pA

Experiment 4.3.4.18 : E-sensor based analysis of tea: E-nose and E-tongue scores/Indices

The E-nose (total volatile componets) and E-tongue (total non volatile components) were monitored with corresponding manufactured black tea of different gardens at different flushes of Darjeeling seven valleys tea to determine commercial qaility.

Tea Types	T.S	N.I
Kurseong South	2.2±0.01	19.2±0.01
Darjeeling East	2.16±0.01	17±0.01
Teesta	2.16±0.01	12±0.01
Mirik	2±0.01	11±0.01
Darjeeling West	1.93±0.01	10±0.01
Kurseong North	1.73±0.01	9±0.01
KS2	2.2±0.01	19.2±0.01
KS1	1.98±0.01	16.5±0.01
Upper Fagu	2±0.01	12±0.01

Table 4.3.14 shows the Taster's score and Nor-aroma index of tea in different valleys of Darjeeling. S.D=0.01

The E-senosr baseed analysis with E-nose and E-tongue In both cases, Kurseong South is showing the highest Taster's score and Nor-aroma index.

The taster's score of Kurseong South is about 2.2±0.01 whereas the nor-aroma index is about 19.2±0.01. The taster's score of Darjeeling East is about 2.16±0.01 whereas the nor-aroma index is about 17±0.01 . The taster's score of Teesta is about 2.16±0.01 whereas the nor-aroma index is about 12±0.01 . The taster's score of Mirik is about 2±0.01 whereas the nor-aroma index is about 11±0.01 . The taster's score of Darjeeling West is about 1.93±0.01 whereas the nor-aroma index is about 10±0.01 . The taster's score of Kurseong North is about

1.73±0.01 whereas the nor-aroma index is about 9±0.01 .The taster's score of Kurseong South 1st flush is about 2.2±0.01 whereas the nor-aroma index is about 19.2.The taster's score of Kurseong South 2nd flush is about 1.98±0.01 whereas the nor-aroma index is about 16.5±0.01 .The taster's score of Upper Fagu is about 2±0.01 whereas the nor-aroma index is about 12±0.01 .

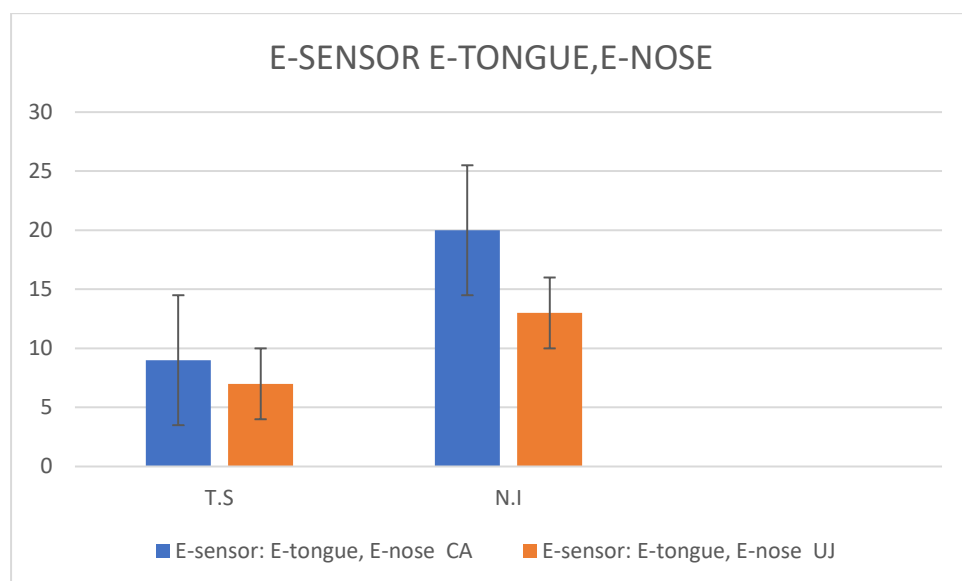


Figure 4.3.21.1a: The taster's score and Nor-aroma index of Kurseong South tea gardens Castleton (high altitude) and Upper Jungpana (low altitude) S.D=0.0001

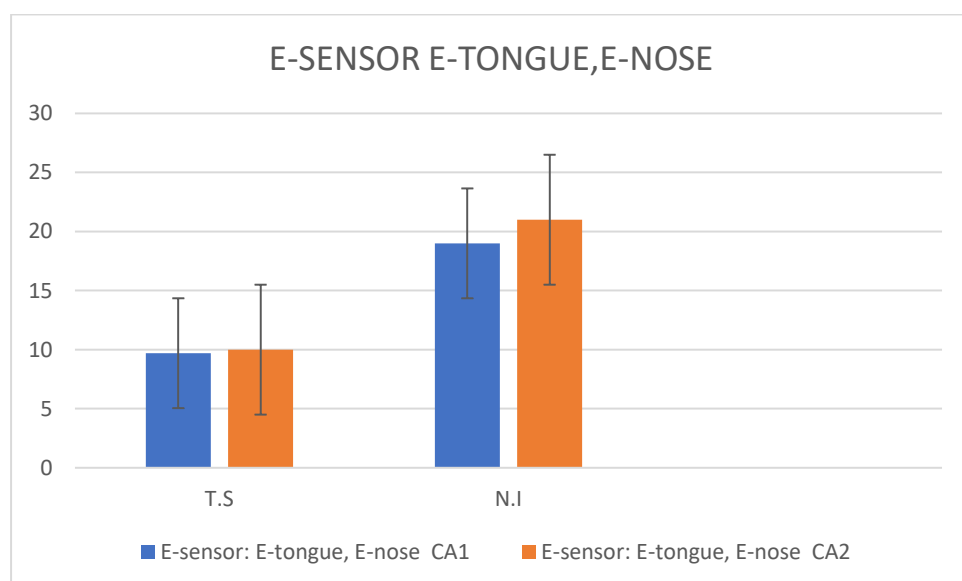


Figure 4.3.21.1b: The taster's score and Nor-aroma index of Kurseong South tea gardens Castleton 1st flush and 2nd flush. S.D=0.0001

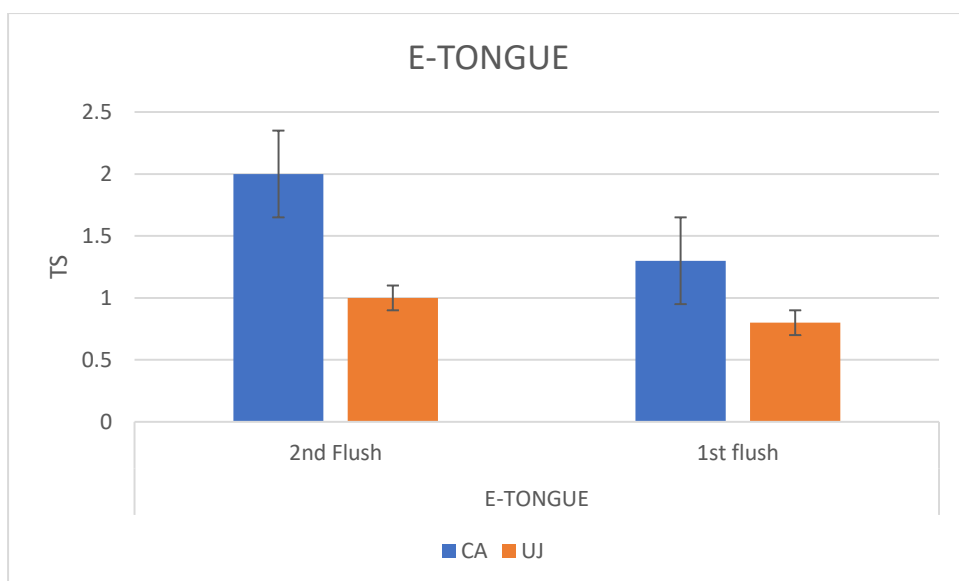


Figure 4.3.21.1c: The taster's score of Kurseong South tea gardens, Castleton(high altitude) and Upper Jungpana (low altitude) during 1st flush and 2nd flush.S.D=0.0001

Fig 4.3.21.1a shows that the Taster's score and Nor-aroma index of tea in high and low altitude 1st and 2nd flush. In all cases, high altitude Castleton 2nd flush is having higher aroma index and taster's score.

Fig 4.3.21.1b and c show that the Taster's score and Nor-aroma index of tea in high and low altitude 1st and 2nd flush. In all cases, high altitude Castleton 2nd flush is having higher aroma index and taster's score.

Fig 4.3.21.1a: The taster's score of Castleton and Upper Jungpana (low altitude) is about 9 ± 0.0001 and 7 ± 0.0001 , respectively. The nor-aroma index of Castleton and Upper Jungpana (low altitude) is about 20 ± 0.0001 and 13 ± 0.0001 respectively.

Fig 4.3.21.1b:: The taster's score of Castleton 1st flush and 2nd flush is about 10 ± 0.0001 and 9.7 ± 0.0001 respectively. The nor-aroma index of Castleton 1st flush and 2nd flush is about 19 ± 0.0001 and 21 ± 0.0001 respectively.

Fig 4.3.21.1c: The taster's score of Castleton and Upper Jungpana (low altitude) during 1st flush is about 2 and 1 respectively. The taster's score of Castleton and Upper Jungpana (low altitude) during the 2nd flush is about 1.3 and 0.8 respectively.

❖ ASSAY OF THERAPEUTIC QUALITIES: ANTIOXIDANT, ANTIMICROBIAL, ANTICANCER< ANTI INFLAMMATORY, ANTIDIABETIC POTENTIAL AND SUN PROTECTION FACTOR, BIOAVAILABILITY

Experiment 4.3.4.19. Assay of Antioxidant Potential

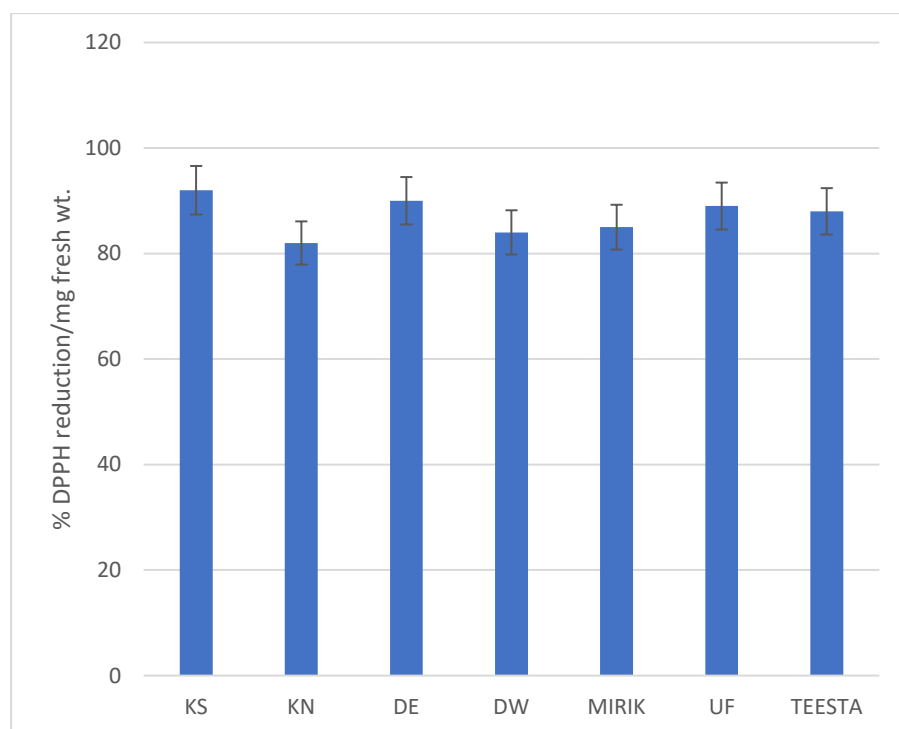


Figure 4.3.22. 1a Variation in antioxidant potential among seven valleys of Darjeeling.S.D=0.01

Among the seven valleys of Darjeeling Kurseong South is having the highest antioxidant potential that is about 92±0.01 % followed by Darjeeling East (90±0.01 %),Upper Fagu (89±0.01 %),Teesta (88±0.01 %),Mirik (85±0.01 %) Darjeeling West (84±0.01 %) and lastly Kurseong North (82±0.01 %).

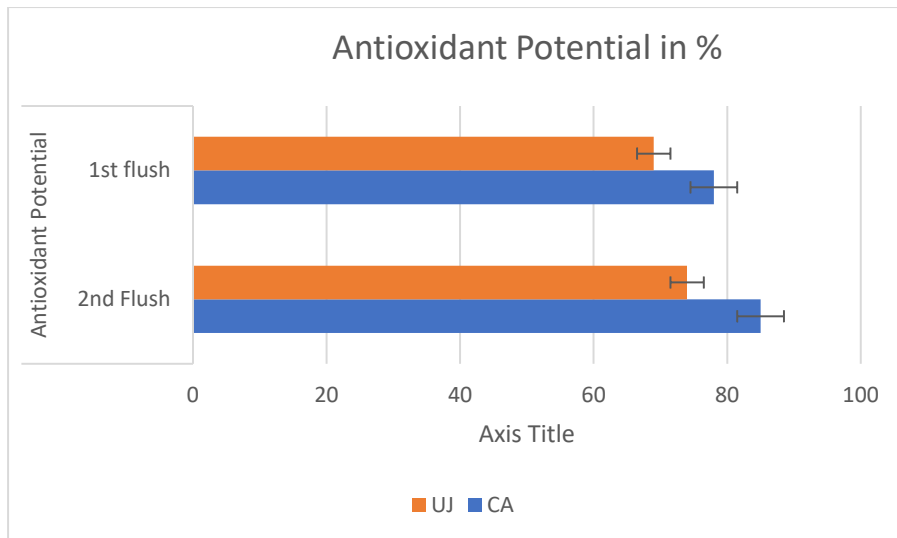


Figure 4.3.22.1b Antioxidant Potential of different altitudes of Kurseong South valley gardens Castleton (CA high altitude) and Upper Jungpana (UJ low altitude) 1st flush and 2nd Flush.S.D=0.01

Fig 4.3.22.1b shows that the antioxidant potential of high altitude Castleton 1st and 2nd flush have higher antioxidant potential than low altitude Upper Jungpana 1st and 2nd flush of Kurseong South valley. Kurseong South valley garden, Castleton, in its 2nd flush, has the highest antioxidant potential, which is 85 ± 0.01 %.

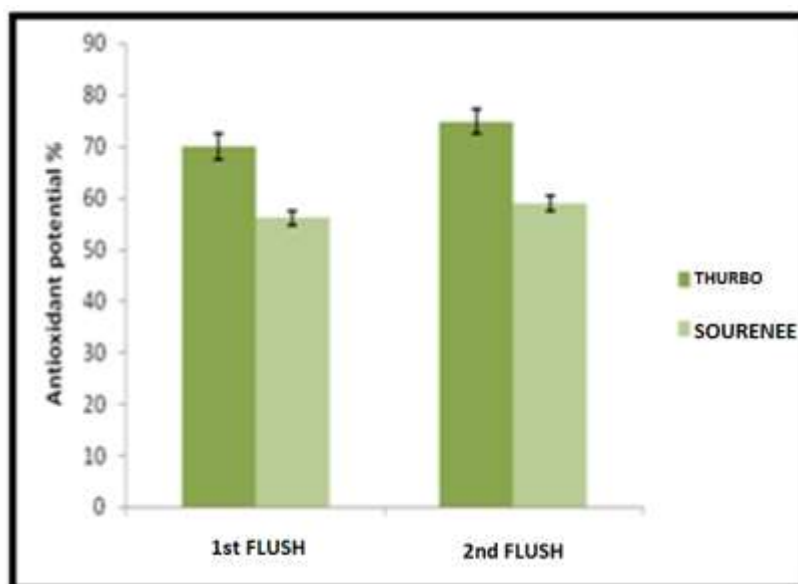


Figure 4.3.22.1c Antioxidant Potential of different altitudes of Mirik valley gardens Thurbo and Sourene 1st flush and 2nd Flush.S.D=0.001

Fig 4.3.22.1c show the antioxidant potential of high altitude Thurbo 1st and 2nd flush and low altitude Sourennee 1st and 2nd flush of Mirik valley.

Thurbo at higher altitudes than Sourennee shows higher antioxidant potential than Sourennee whereas 2nd flush shows more antioxidant potential in both altitudes. Thurbo during 2nd flush shows 76±0.001% antioxidant activity whereas Sourennee shows an antioxidant activity of about 62±0.001% during the second flush. Thurbo during 1st flush shows 57% antioxidant activity whereas Sourennee shows antioxidant activity of about 58 % during the first flush. De et al.,2023 have examined the antioxidant properties of bioactive components of different types of Darjeeling tea during the 1st flush.

Statistical analysis

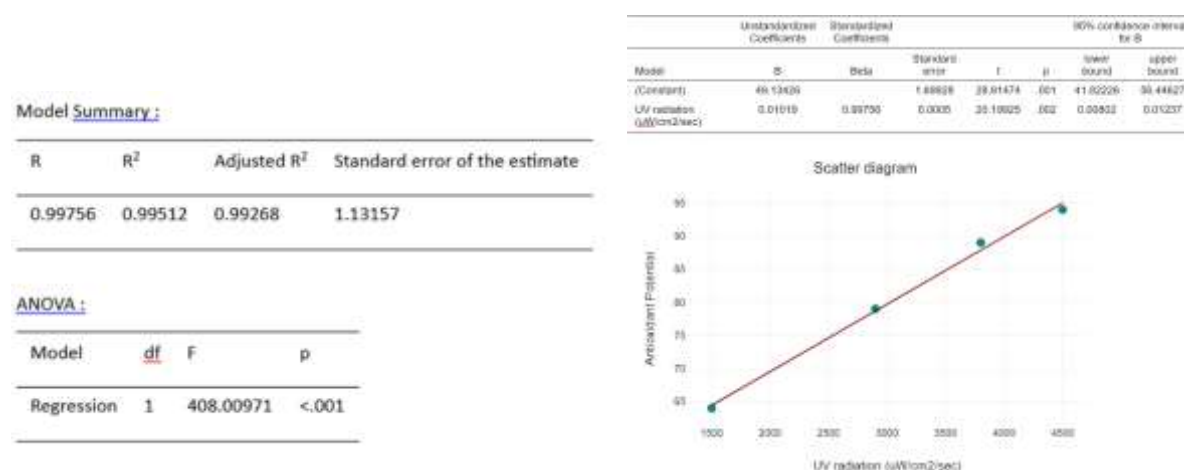


Table 4.3.15 Statistical analysis interprets that there is a significant positive correlation between the annual UV radiation and antioxidant potential.(p<.001).

Experiment 4.3.4.20: Assay of antimicrobial potential

The antimicrobial potentials were assessed by agar cup plate methods against different bacterial cultures mentioned in Materials methods (Section 3.4.2)

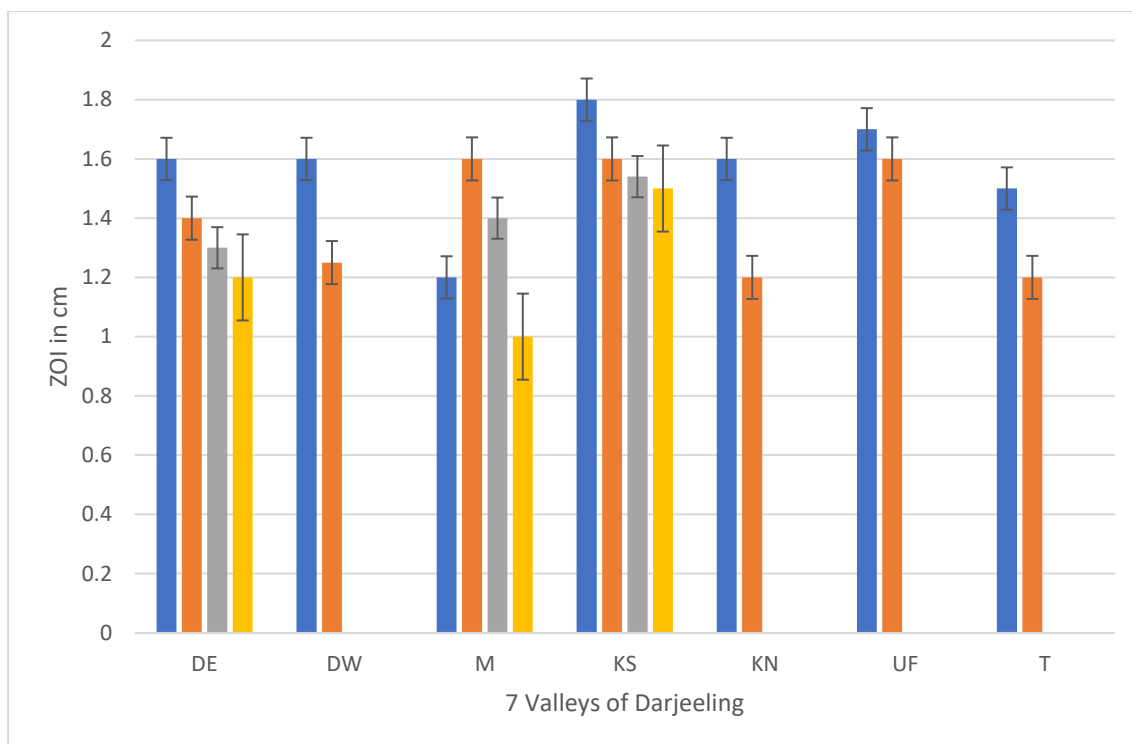


Figure 4.3.23.1a : Antimicrobial Potential of the Seven Valleys of Darjeeling.S.D=0.01

DE= Darjeeling East, MI=Mim (1), MA= Marybong (2), CH=Chongtong (3), OV=Orange valley (4), DW= Darjeeling West, BA=Badamtam (1), GI=Ging (2), M=Mirik, GO=Gopaldhara (1), TH=Thurbo (2), PH=Phuguri (3), SO=Sourennee (4), KS=Kurseong South, CA=Castleton (1), RO=Rohini (2), GI=Giddhhapahar (3), UJ=Upper Jungpana (4), H=Ahaldara (5), KN=Kurseong North, DI=Dilaram (1), MH=Margaret Hope (2), UF=Upper Fagu, TU=Tukdah (1), SU=Sungma (2), T=Teesta, RR=Rungle Runghot (1), GI=Gielle (2)

Kurseong South valleys shows more antimicrobial potential than other valleys of Darjeeling.



Figure 4.3.23.1b:showing zone of inhibition of Darjeeling East tea gardens against *E.coli*



Figure 4.3.23.1c: Zone of inhibition produced by different methanolic extracts of tea of Darjeeling East valley against *Bacillus* sp

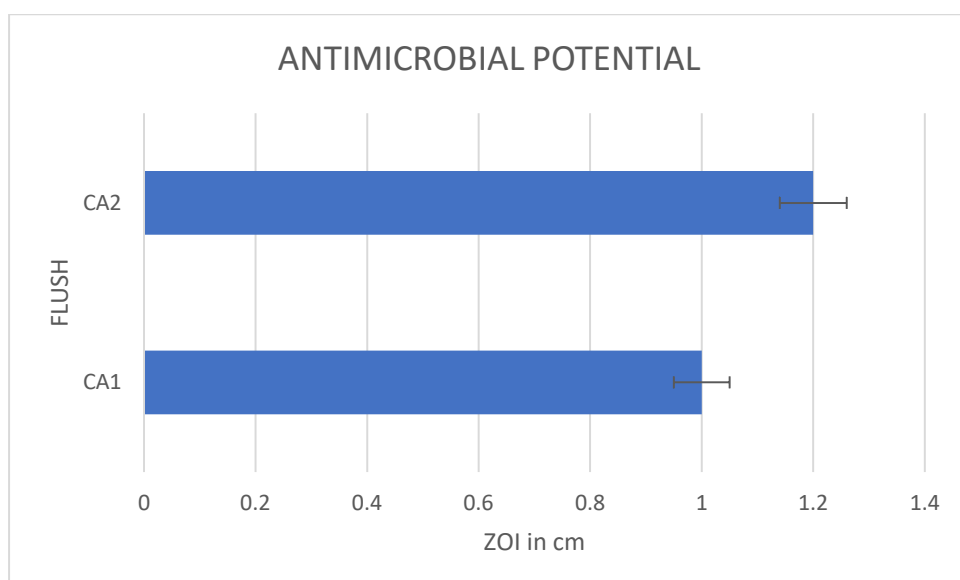


Figure 4.3.23.1d: shows the Zone of Inhibition in Kurseong South tea garden Castleton 2nd and 1st flush. S.D=0.01

Castleton 2nd flush is showing higher zone of inhibition than the 1st flush. Castleton during 2nd flush shows more zone of inhibition that is about 1.2±0.01 cm, and Castleton during the first flush shows less zone of inhibition of about 1 ±0.01cm.



Figure 4.3.23.1e Antimicrobial zone of inhibition of Kurseong South tea garden situated at high and low altitude against *Salmonella* sp.



Figure 4.3.23.1f: Antimicrobial zone inhibition of Kurseong South tea garden high and low altitude against *E.coli*

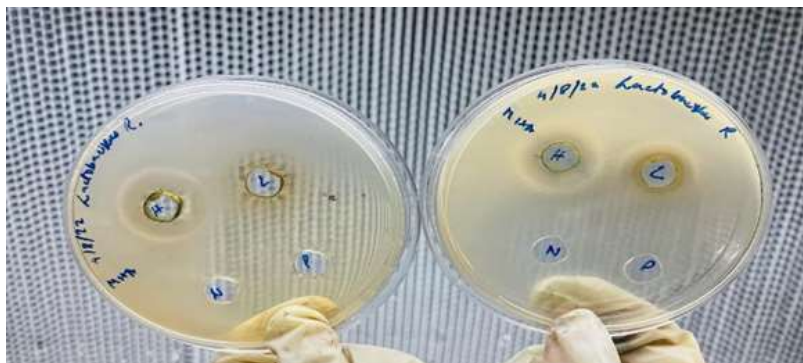


Figure 4.3.23.1g:Antimicrobial zone of inhibition of Kurseong South tea garden high and low altitude against *Lactobacillus* sp



Figure 4.3.23.1h :Antimicrobial zone of inhibition of Kurseong South tea garden high and low altitude against *Staphylococcus sp*

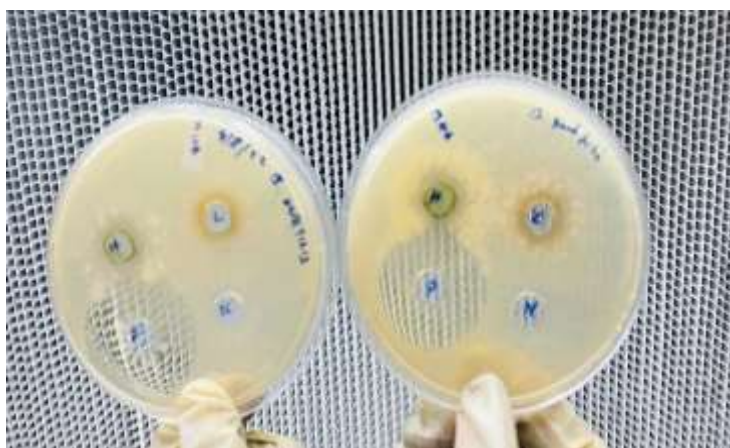


Figure 4.3.23.1i: Antimicrobial zone of inhibition of Kurseong South tea garden situated at high and low altitude against *Bacillus sp*



Figure 4.3.23.1j :Antimicrobial zone of inhibition of Kurseong South tea garden situated at high and low altitude against *Pseudomonas sp*



Figure 4.3.23.1k:Antimicrobial zone of inhibition of Kurseong South tea garden situated at high and low altitude against *Bacillus clausii*



Figure 4.3.23.1l :Antimicrobial zone of inhibition of Kurseong South tea garden situated at high and low altitude against *Bacillus cereus*

Sample	ZOI against <i>E.coli</i>	ZOI against <i>Bacillus cereus</i>	ZOI against <i>Staphylococcus aureus</i>	ZOI against <i>Bacillus clausii</i>	ZOI against <i>Salmonella</i> sp.	ZOI against <i>Bacillus subtilis</i>	ZOI against <i>Pseudomonas</i> sp.	ZOI against <i>Lactobacillus</i> sp.
Ampicillin positive control 5mg/ml	9±0.01 mm	21±0.01 mm	9±0.01cm	11±0.01 mm	9±0.01 mm	21±0.01 mm	22±0.01m	21±0.01m
-ve control	-	-	-	-	-	-	-	-
High altitude(Castl	11±0.02 mm	6±0.02 mm	11±0.02 mm	13±0.02 mm	6±0.02 mm	11±0.01 mm	11±0.01m	13±0.02 mm

eton)								
Low Altitude (Upper Jungpana)	4±0.02 mm	1±0.01m m	8±0.01mm	7±0.01m m	5±0.01 mm	5±0.01m m	5±0.01m m	10±0.02 mm

Table 4.3.16 Altitudinal variation of antimicrobial Potential of tea against various organisms.S.D=0.01 for positive control.S.D=0.02 for high altitude. S.D=0.01 for low altitude.

Table 4.3.22 shows the zone of inhibition of high and low altitude tea against different organisms.High altitude tea shows

11±0.02mm,6±0.02mm,11±0.02mm,11±0.02mm,13±0.02mm,6±0.02mm,11±0.01mm, and 13±0.02 mm zone of inhibition *against E. coli, Bacillus cereus, Staphylococcus aureus, Pseudomonas sp., Bacillus clausii, Salmonella sp, Bacillus subtilis, and Lactobacillus sp,* respectively. Low-altitude tea shows

4±0.02mm,1±0.02mm,8±0.02mm,5±0.02mm,7±0.02mm,5±0.02mm,5±0.01mm, and 10±0.02mm zone of inhibition *against E. coli, Bacillus cereus, Staphylococcus aureus, Pseudomonas sp., Bacillus clausii, Salmonella sp, Bacillus subtilis, and Lactobacillus sp,* respectively.

Ampicillin positive control shows

9±0.02mm,21±0.02mm,9±0.02mm,22±0.02mm,11±0.02mm,9±0.02mm,21±0.01mm, and 2 1±0.02mm zone of inhibition *against E. coli, Bacillus cereus, Staphylococcus aureus, Pseudomonas sp., Bacillus clausii, Salmonella sp, Bacillus subtilis, and Lactobacillus sp,* respectively. Negative control shows no zone of inhibition in each case.

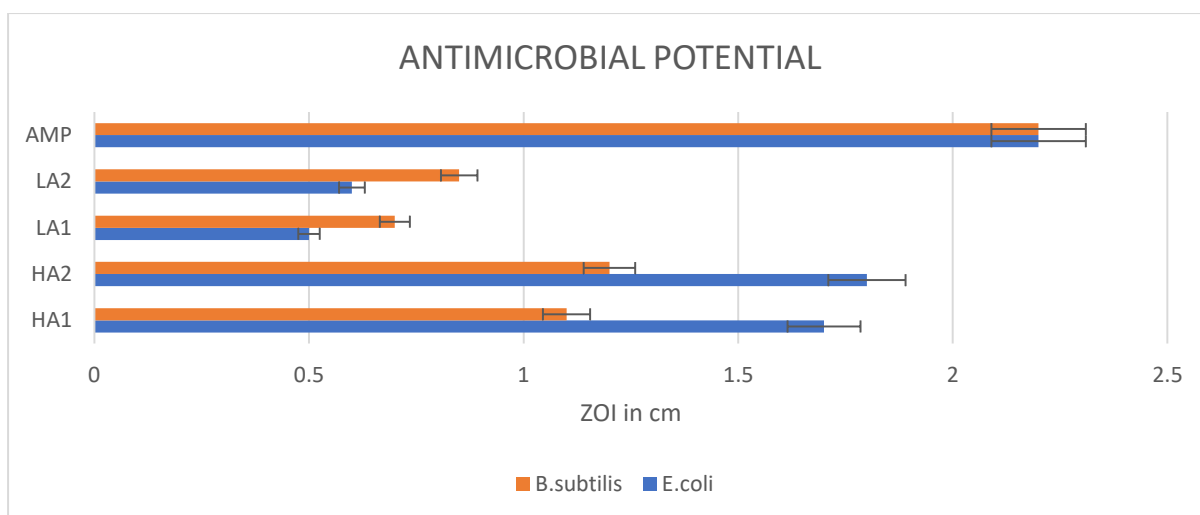


Figure 4.3.23.1m: Antimicrobial potential of high altitude during 1st flush and 2nd flush, and low altitude garden during 2nd flush, Kurseong south tea valley against *B.subtilis* and *E.coli*.S.D=0.02

Antimicrobial potential of high altitude during 1st flush and 2nd flush, and low altitude garden during 2nd flush, Kurseong south tea valley. Ampicillin, the positive control, yields a 2.2 ± 0.02 cm zone of inhibition against *Bacillus subtilis* and *E. coli*. Low altitude garden during 2nd flush (Upper Jungpana shows 0.85 ± 0.01 cm and 0.6 ± 0.01 cm against *Bacillus subtilis* and *E. coli*, respectively. High altitude garden during 2nd flush (Castleton) shows 1.8 ± 0.01 cm and 1.2 ± 0.01 cm against *Bacillus subtilis* and *E. coli*, respectively. High altitude garden during 1st flush (Castleton) shows 0.7 ± 0.01 cm and 0.5 ± 0.01 cm against *Bacillus subtilis* and *E. coli*, respectively. Thus indicating that high altitude garden Castleton shows more antimicrobial potential in 2nd flush than the low altitude tea garden Upper Jumngpana.



Figure 4.3.23.1n:Antimicrobial zone of inhibition of manufactured tea (High altitude and Low altitude Kurseong valley tea) against *Staphylococcus sp*

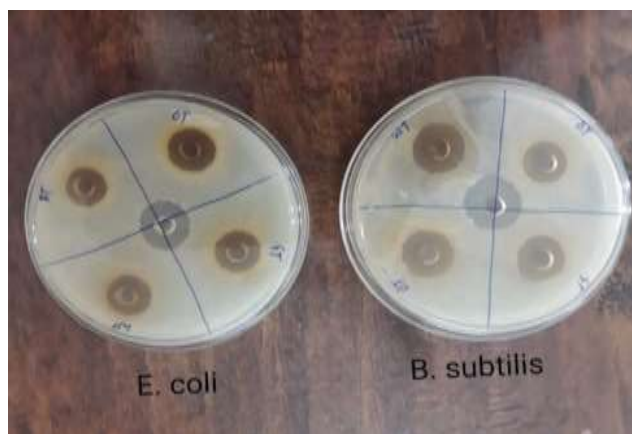


Figure 4.3.23.1o:Antimicrobial zone of inhibition of manufactured tea against *Bacillus sp* and *E.coli*

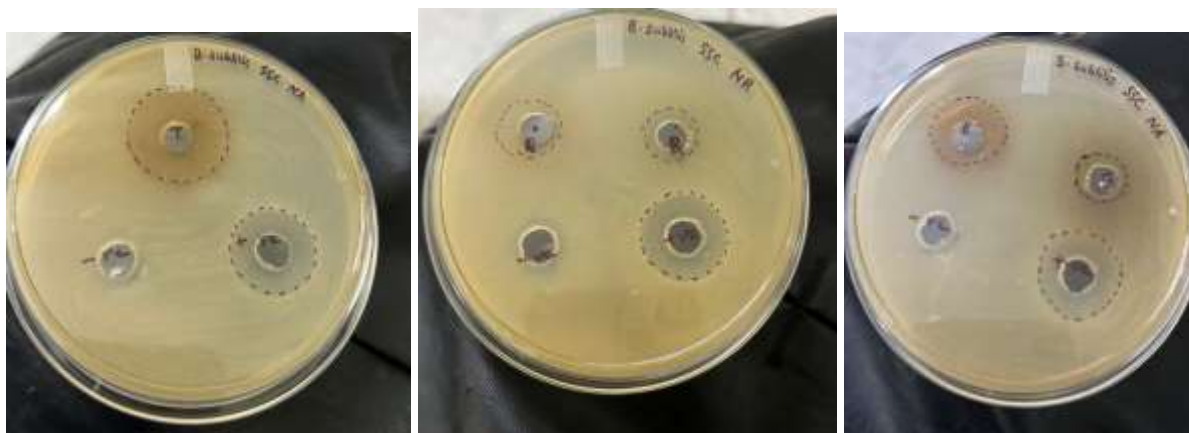


Fig 4.3.23.1p,q and r Antimicrobial zone of inhibition of standards against *B.subtilis*

Sl no.	Standards	ZOI against <i>B.subtilis</i> in cm
1.	Quercetin	0.6±0.01
2.	Rutin	0.8±0.02
3.	EGCG	1.2±0.01
4.	Tannic Acid	1.7±0.02
5.	Gallic Acid	0.6±0.02
6.	Positive control Ampicillin	1.3±0.01
7.	Negative Control	0

Table 4.3.17a shows the zone of inhibition of different standards against *Bacillus* sp.S.D=0.02

Quercetin shows a 0.6±0.01cm zone of inhibition against *Bacillus* sp. EGCG shows a 1.2±0.02 cm zone of inhibition against *Bacillus* sp. Tannic acid shows 1.7±0.02 cm zone of inhibition against *Bacillus* sp. Gallic acid shows 0.6±0.02cm zone of inhibition against *Bacillus* sp.

Positive control shows 1.3±0.01 cm zone of inhibition against *Bacillus* sp. Negative control shows no zone of inhibition.



Figure 4.3.23.1.s, t and u shows the antimicrobial zone of inhibition of standards against *E.coli*.

Sl no.	Standards	ZOI against <i>E.coli</i> in cm
1.	Quercetin	0.6±0.01
2.	Rutin	0.6±0.02
3.	EGCG	0.5±0.01
4.	Tannic Acid	1.3±0.02
5.	Gallic Acid	0.4±0.01

6.	Positive control Ampicillin	2.3±0.02
7.	Negative Control	0

Table 4.3.17b shows the zone of inhibition of different standard flavonoids against *E.coli*. S.D=0.02

Quercetin shows 0.6±0.01cm zone of inhibition against *E.coli*. Rutin shows 0.6±0.02 cm zone of inhibition against *E.coli*. EGCG shows a 5±0.02 cm zone of inhibition against *E.coli*. Tannic acid shows 1.3±0.02 cm zone of inhibition against *E.coli*. Gallic acid shows 0.4±0.02cm zone of inhibition against *E.coli*. Positive control shows 2.3±0.01 cm zone of inhibition against *E.coli*. Negative control shows no zone of inhibition.

Statistical analysis:

	Unstandardized Coefficients	Standardized Coefficients	85% confidence interval for B				
Model	B	Beta	Standard error	t	p	lower bound	upper bound
(Constant)	0.63969		0.26084	2.4524	.134	-0.48271	1.76209
UV radiation (uW/cm2/sec)	0.00031	0.9427	0.00008	3.99585	.057	-0.00002	0.00064

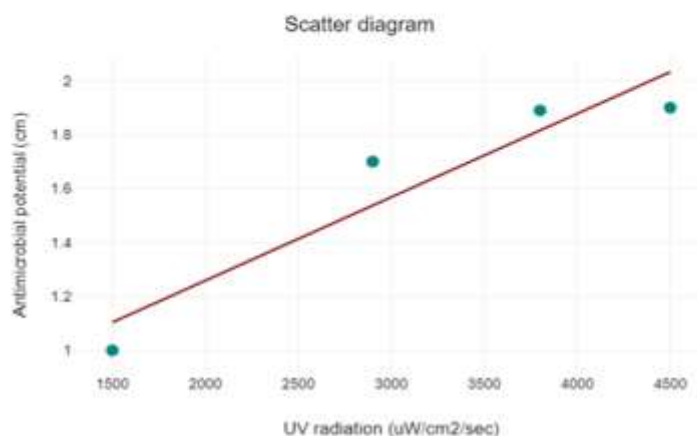


Table 4.3.18 :Statistical analysis interprets that there is a significant positive correlation between the annual UV radiation and antimicrobial potential. ($p<.057$).

XValues	YValues
0.6	19.96
0.6	10.66
0.5	12.68
0.5	17.13
0.85	5.46
0.6	6.63
0.5	5.7
0.75	17.7
0.69	16.5
0.65	14.3
0.67	13.9
0.68	12
M: 0.6325	
M: 12.7183	
Estimate	Estimated Y
1.1	11.3705

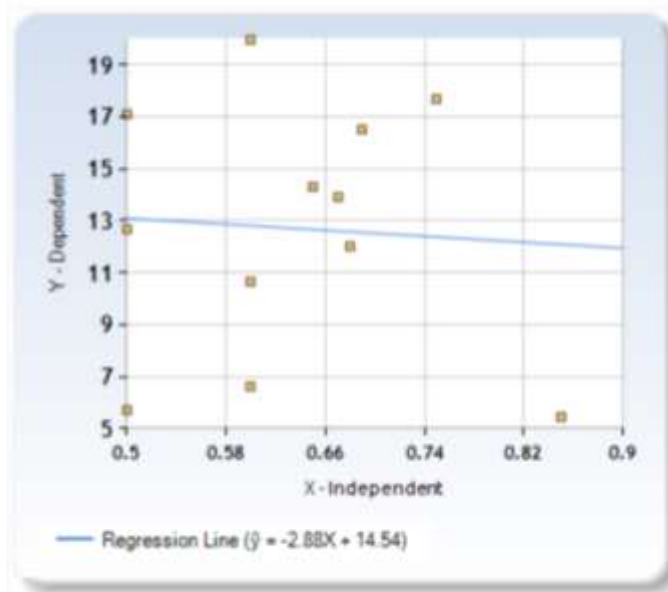


Table 4.3.19 : Regression analysis of antimicrobial potential and E-nose data (Nor aroma indices)

Table 4.3.19 Regression analysis represents that antimicrobial potential (Zone of inhibition) and E.nose data can be related with regression analysis and from the regression analysis the therapeutic index can be determined.

Experiment 4.3.4.21 Anticancer assay (cell viability assay)

Anticancer assay was done with different manufactured and fresh leaf extract of different altitudinal gardens of Darjeeling seven valleys at different seasons against HepG2 cell line and IC 50 was calculated

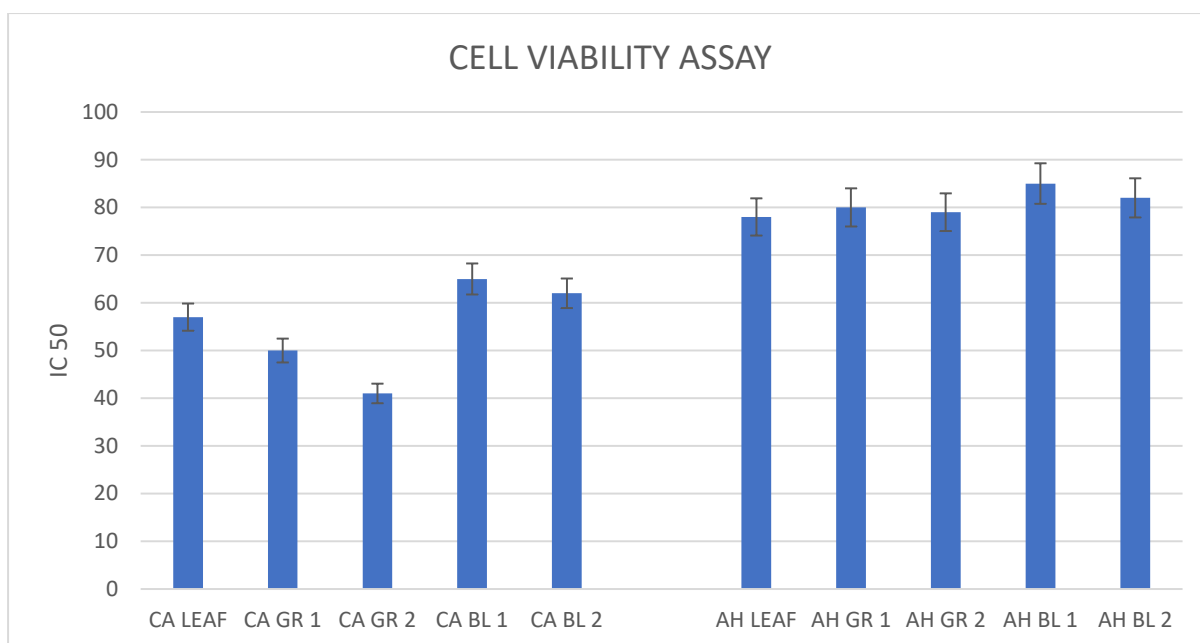


Figure 4.3.24.1 shows the IC₅₀ of different types of tea of high and low altitudes and their flushes S.D=0.001.(CA- Castleton, GR1 – Green leaf 1st flush, GR2 – Green leaf 2nd flush, BL1 – Black tea 1st flush, BL2 – Black tea 2nd flush.AH – Ahaladhara.

High altitude 2nd flush green tea shows the least IC₅₀ than others. The low IC₅₀ indicates the high potential of the drug. Castleton leaf, Castleton Green tea 1st flush. Castleton Green tea 2nd flush, Castleton Black tea 1st flush, Castleton Black tea 2nd flush shows IC₅₀ 57±0.001,50±0.001,41±0.001,65±0.001,62±0.001 respectively.

Ahaldhara leaf, Ahaldhara Green tea 1st flush. Ahaldhara Green tea 2nd flush, Ahaldhara Black tea 1st flush, Ahaldhara Black tea 2nd flush shows IC₅₀ 78±0.001,80±0.001,79±0.001,85±0.001,82±0.001 respectively. IC₅₀ of higher altitudes is less than the IC₅₀ of lower altitudes.

Statistical Analysis

Model	B	Beta	Standard error	t	p	lower bound	upper bound
(Constant)	96.6728		8.16946	11.83344	.007	61.51961	131.82599
UV radiation (uW/cm2/sec)	-0.01336	-0.96857	0.00243	-5.50707	.031	-0.0238	-0.00292

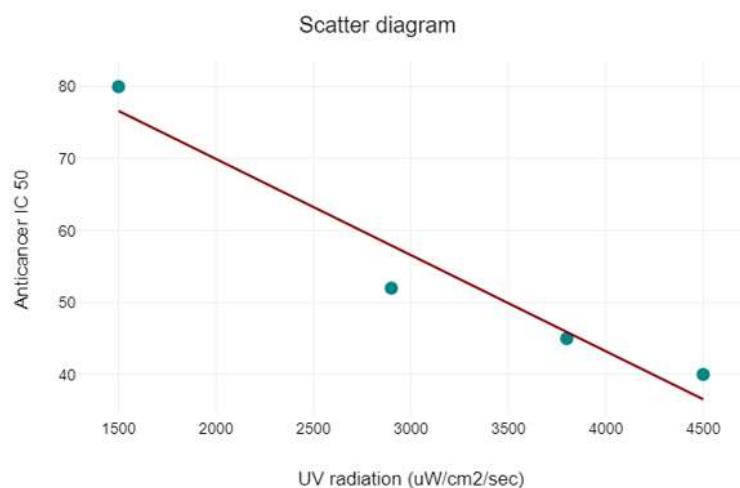


Table 4.3.20 :Statistical analysis

Table 4.3.20 shows there is a negative correlation($p < 0.310$) between IC50 and UV radiation. The lower IC50 value indicates high potential of the drug having higher therapeutic potential.

Experiment 4.3.4.22.: Anti-diabetic assay: Inhibition of Amylase

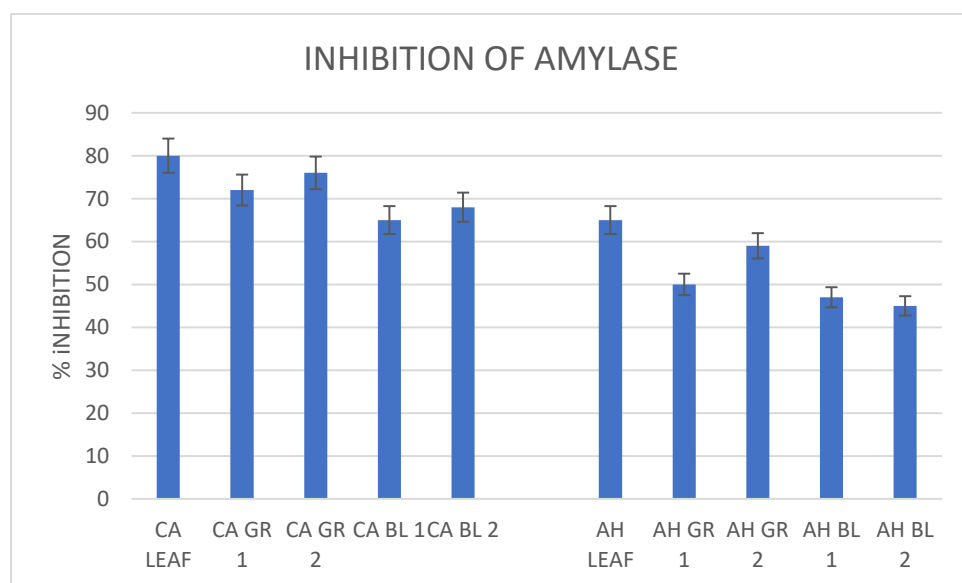


Figure 4.3.25.1 shows the inhibition of amylase in different types of tea of high and low altitudes and different flushes.S.D=0.01

Castleton fresh leaf shows the highest inhibition of amylase, while black leaf low-altitude Ahaldhara, shows the lowest.

Castleton leaf, Castleton Green tea 1st flush .Castleton Green tea 2nd flush, Castleton Black tea 1st flush, Castleton Black tea 2nd flush show %inhibition of amylase of about $80\pm0.01, 72\pm0.01, 76\pm0.01, 65\pm0.01, 68\pm0.01$ respectively.

Ahaldhar leaf, Ahaldhara Green tea 1st flush. Ahaladhara Green tea 2nd flush, Ahaldhara black tea 1st flush, Ahaldhara Black tea 2nd flush show %inhibition of amylase of about $65\pm0.01, 50\pm0.01, 59\pm0.01, 47\pm0.01, 45\pm0.01$ respectively.

High altitude tea leaves show higher inhibition of amylase than the low altitude tea leaves.

Castleton leaf shows the highest inhibition of amylase which is about 80 ± 0.01 % whereas low altitude Ahaldhara black tea leaf shows lowest which is about $45\pm0.05\%$. Tamil et al.,2010 have studied about α -amylase inhibitory activity of an Indian medicinal plant, *Phyllanthus amarus*.

Experiment 4.3.4.23.: Anti-diabetic assay: Inhibition of β -D Glucosidase

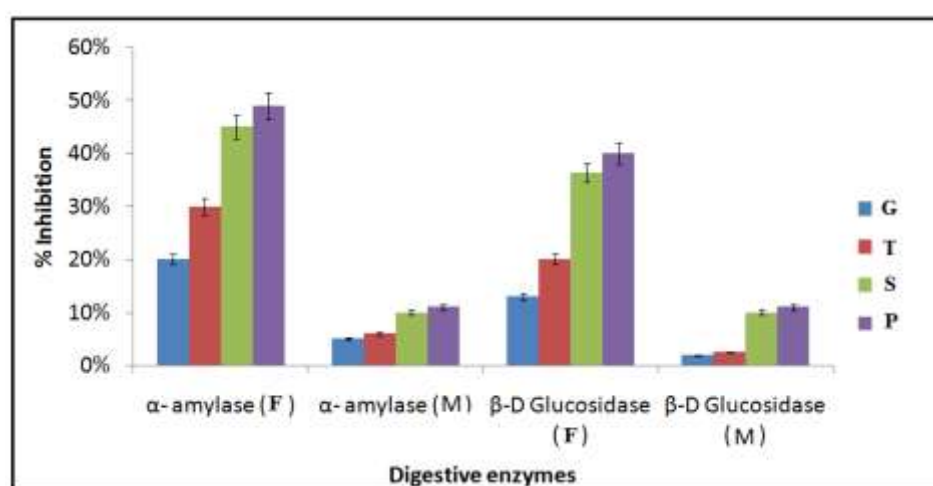


Figure 4.3.26.1a shows variations in α -amylase and β -D Glucosidase among different types of tea.S.D=0.002

Phugiri, at a higher altitude, shows a higher α -amylase and β -D Glucosidase content in both fresh and manufactured tea leaves than other gardens. Phugiri is followed by Sourene, Turbo, and lastly Giddhapahar. The percentages of α -amylase in fresh leaves in Phugiri, Sourene, Turbo, and Giddhapahar are 48 ± 0.002 , 45 ± 0.002 , 30 ± 0.002 , and 19 ± 0.002 , respectively. The percentages of α -amylase in manufactured leaves in Phugiri, Sourene, Turbo, and Giddhapahar are 10 ± 0.002 , 9 ± 0.002 , 5 ± 0.002 , and 3 ± 0.002 , respectively. The percentages of β -D Glucosidase content in fresh leaves in Phugiri, Sourene, Turbo, and Giddhapahar are 40 ± 0.002 , 35 ± 0.002 , 20 ± 0.002 , and 10 ± 0.002 , respectively.

The percentages of β -D Glucosidase content in manufactured leaves in Phugiri, Sourene, Turbo, and Giddhapahar are 10 ± 0.002 , 8 ± 0.002 , 2 ± 0.002 , and 1 ± 0.002 , respectively.

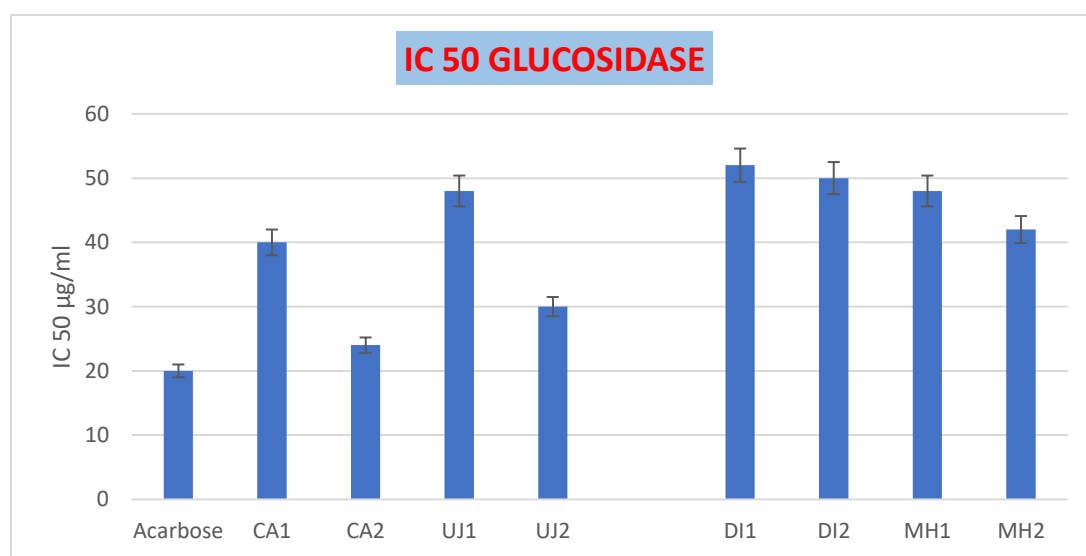


Figure 4.3.26.1b : IC 50 values against β -D Glucosidase inhibition. S.D=0.001

IC 50 ($\mu\text{g/ml}$) is lowest for Kurseong South Castleton garden second flush (CA2) by taking acarbose as control

Experiment 4.3.4.24: Antidiabetic action: Inhibition of serum glucose (estimated with Kit)

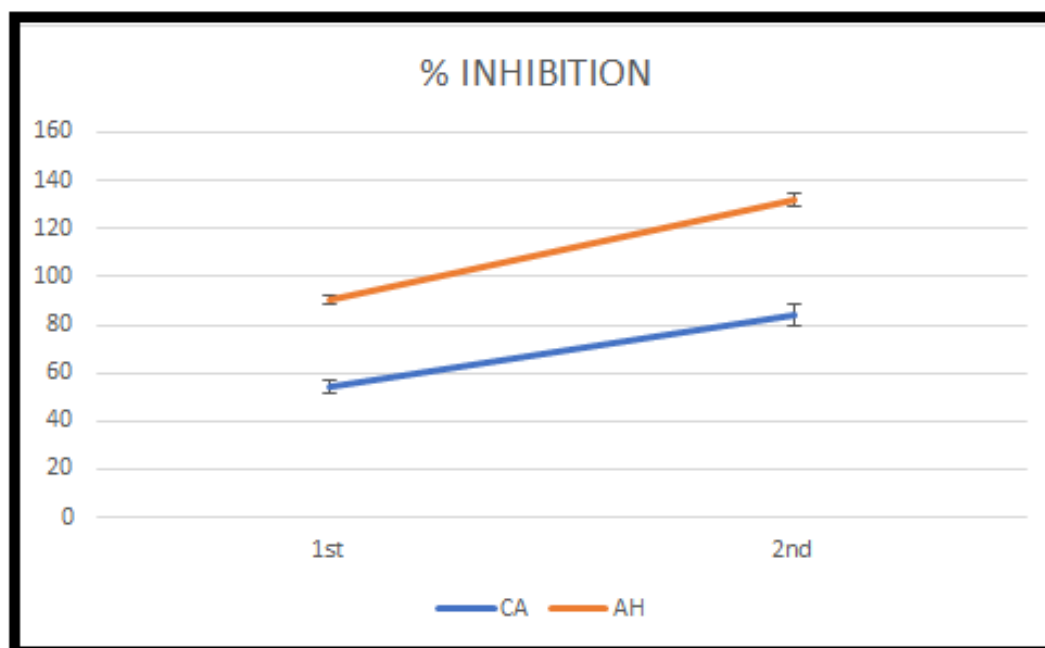


Figure 4.3.27.1 shows Lowering of blood glucose in both high and low altitude flushes. S.D=0.001

Castleton 2nd flush is having lowest %inhibition of glucose. % inhibition of glucose in Castleton 1st flush and 2nd flush are about 59±0.001 and 82±0.001, respectively.

% inhibition of glucose in Ahaldhara 1st flush and 2nd flush are about 95±0.001 and 130±0.001, respectively

Lowering of the blood glucose level is much less in high altitude 2nd flush than in low altitude first flush and second flush. Shehadeh et al., (2021) have summarised that plant secondary metabolites can lower blood glucose levels.

Experiment 4.3.4.25: Anti-inflammatory Assay: Inhibition of Lipooxygenase activity

Anti inflammatory action of different altitude grown different seasonal tea of seven valleys of Darjeeling have been taken for analysis to inhibit LOX lipooxygenase *in vitro*

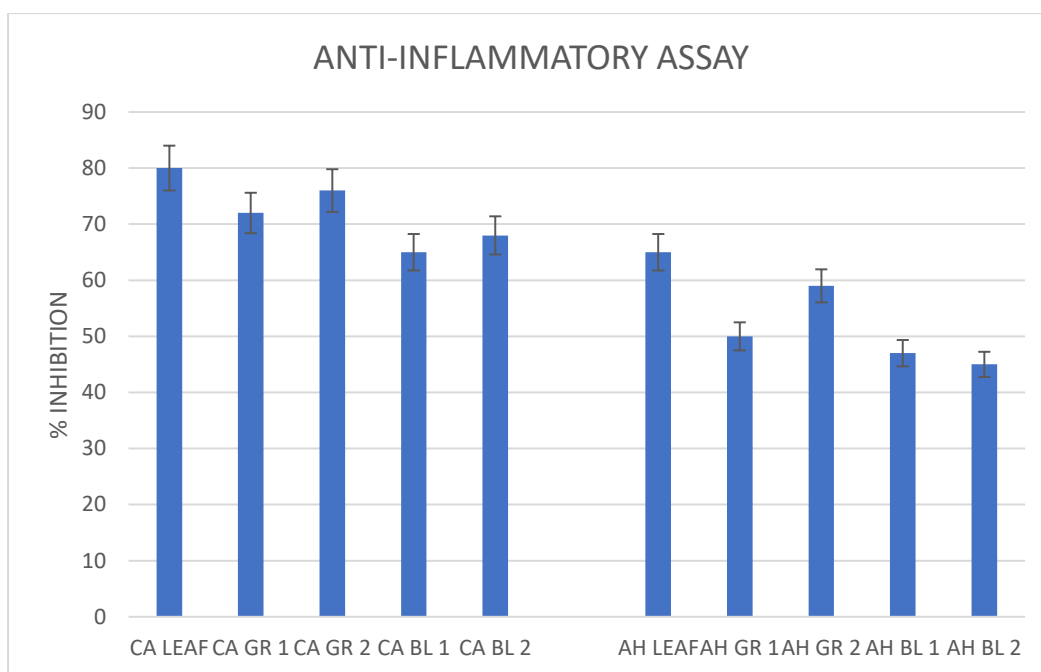


Fig 4.3.28.1a: shows inhibition of anti-inflammation in different types of tea of high and low altitudes as well as in different flushes. S.D=0.01

Castleton fresh leaf shows the highest inhibition of anti-inflammation while the lowest is shown by black leaf low altitude Ahaldhara. % inhibition of Castleton leaf, Castleton Green tea 1st flush, Castleton Green tea 2nd flush, Castleton Black tea 1st flush, Castleton Black tea 2nd flush are about 80 ± 0.01 , 72 ± 0.01 , 76 ± 0.01 , 65 ± 0.01 and 68 ± 0.01 respectively.

Ahaldhar leaf, Ahaldhara Green tea 1st flush, Ahaladhara Green tea 2nd flush, Ahaldhara black tea 1st flush, Ahaldhara Black tea 2nd flush show % inhibition of about 65 ± 0.01 , 50 ± 0.01 , 59 ± 0.01 , 47 ± 0.01 and 45 ± 0.01 , respectively. Truong et al., (2022) have documented that the tea polyphenols have anti-inflammatory activities.

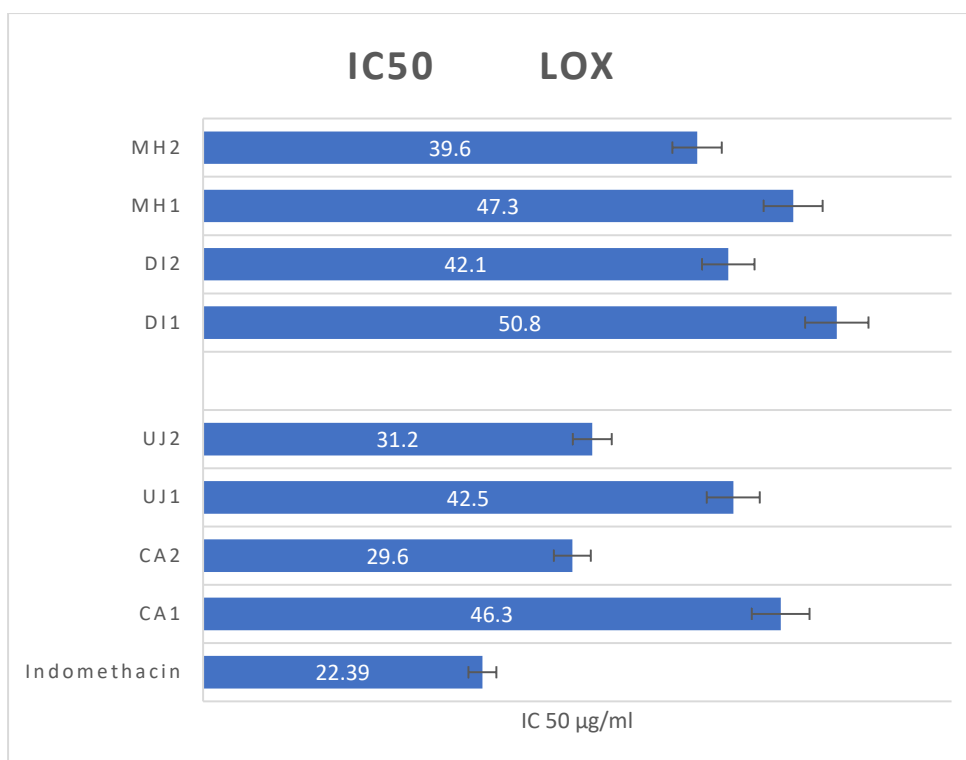


Figure 4.3.28.1b: IC 50 values of Lipooxygenase inhibition by different tea samples.S.D=0.3

IC₅₀ values are lowest for Castleton second flush tea (29.6±.3) by taking Indomethacin as control

Experiment 4.3.4.26: Sun Protection Factor Assay

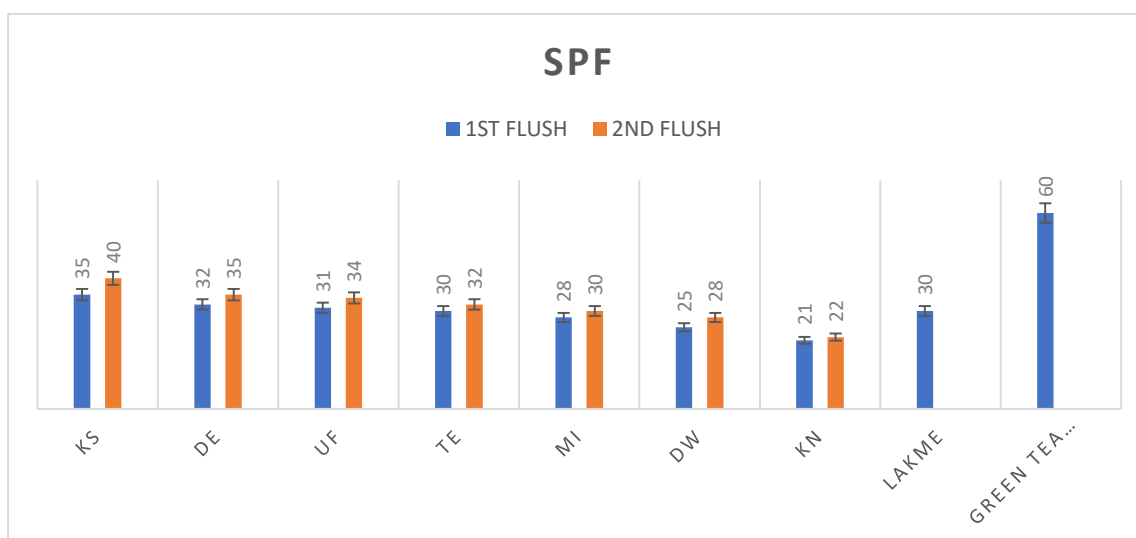


Figure 4.3.29.1a:Sun protection factor assessed of different altitude and seasons of different valleys of Darjeeling.S.D=0.1

The SPF value is highest for Kurseong South second flush tea (40 ± 0.1) among seven valleys

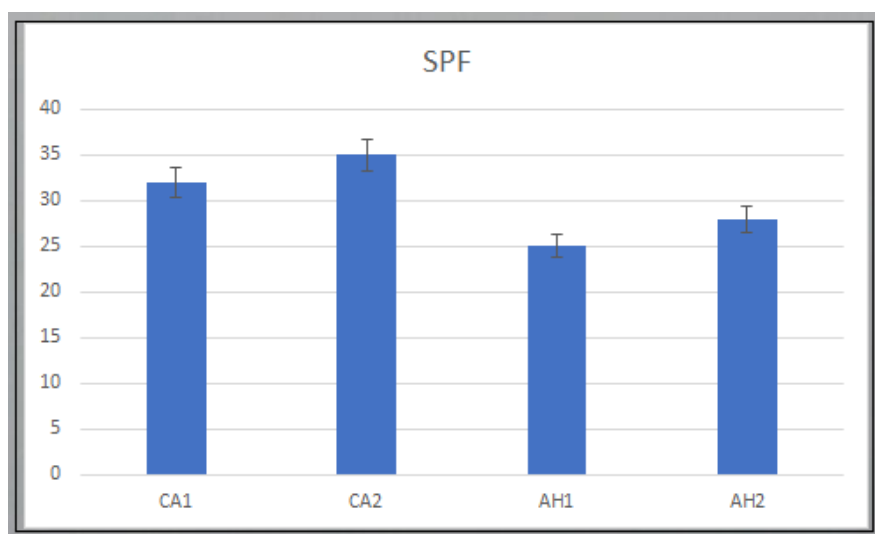


Figure 4.3.29.1b: shows SPF data of different types of tea of high and low altitude 1st and 2nd flush of Kurseong South Valley gardens. S.D=0.002

Castleton 2nd flush is showing highest SPF than Ahaldhara, which is at low altitude. Castleton 1st flush and 2nd flush shows SPF about 33 ± 0.002 and 35 ± 0.002 , respectively. Ahaldhara 1st flush and 2nd flush shows SPF about 25 ± 0.002 and 27 ± 0.002 , respectively.

Experiment 4.3.4.27.: Bioavailability of EGCG in plasma and urine

TEA TYPE	INITIAL DOSE	PLASMA CONC. PEAK TIME	URINE CONC./24 HR
High altitude	110 mg/ml EGCG	119 $\pm$ 1 ng/ml EGCG-1.6 hr	1920 $\pm$ 100 μ g/ml
Low altitude	110mg/ml EGCG	89 $\pm$ 1 ng/ml EGCG-1.6 hr	3000 $\pm$ 100 μ g/ml

Figure 4.3.30.1 shows the bioavailability of EGCG in plasma and urine. S.D=1(Plasma), S.D=100(urine)

In high altitude concentration of EGCG is more than in the low-altitude whereas in low-altitude the EGCG content is more in urine after 24 hours than in high-altitude.

In high-altitude the concentration of EGCG is about $119 \pm 1 \text{ ng/ml}$ in plasma and $1920 \pm 100 \text{ ug/ml}$ in urine. In low-altitude the concentration of EGCG is about $89 \pm 1 \text{ ng/ml}$ in plasma and $3000 \pm 100 \text{ ug/ml}$ in urine.

Experiment 4.3.4.28 PCA Analysis:

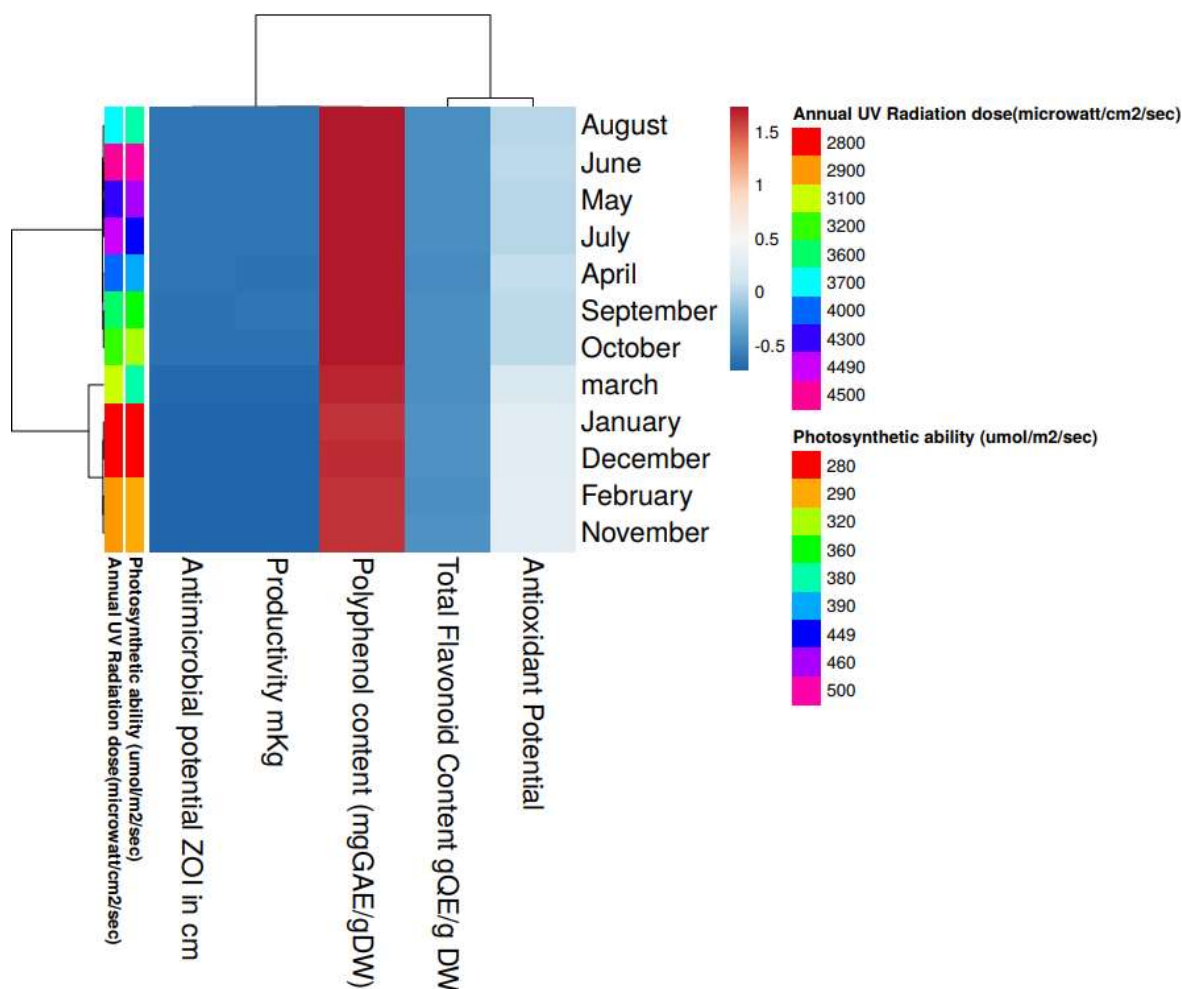


Figure 4.3.31.1 PCA analysis of natural UV radiation and all the physiological, biochemical, therapeutic potential of Kurseong South valley Castleton garden annually.

Experiment 4.3.4.29: Semi-purification of the active components by different extraction methods and by different purification methods:

Kurseong south Castleton 2nd flush fresh tea leaves before extraction is 8gm was taken and the methanol added to it is 100 ml for maceration. After extraction in a rotary evaporator weight of the sample becomes 0.33 g. So the recovery percentage by rotary evaporation is

4.125% and by Lyophilization the same starting material gives 0.22 g so the recovery percentage is 2.75%

The same has been applied for low altitude Kurseong South Upper jungpana tea where the recovery percentage is 3.75% and 1.98% respectively with respect to 8 gm crude extract in 100ml



Figure 4.3.32.1 a: showing antimicrobial potential with semi purified and crude extract tea against *E.coli*



Figure 4.3.32.1b showing antimicrobial potential with semi purified and crude extract tea against *Bacillus sp.*

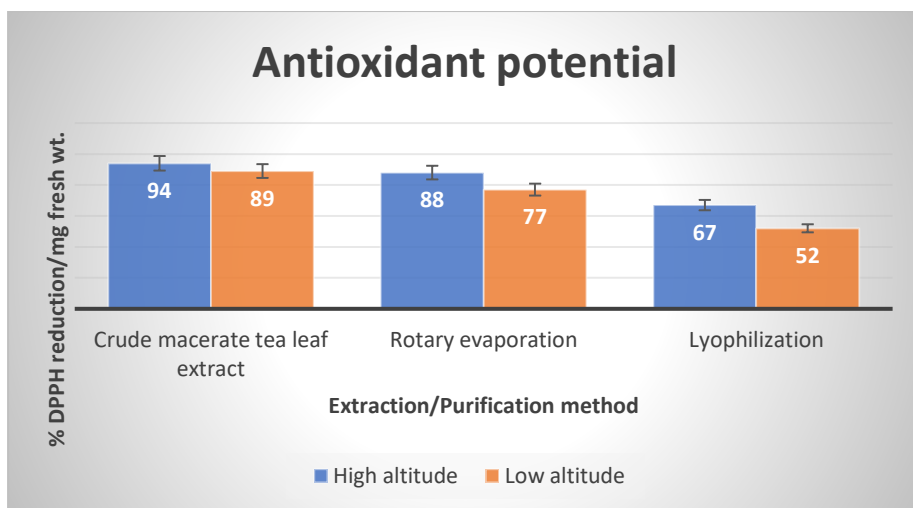


Figure4.3.32.1c :Antioxidant potential of differentially semi purified tea of different altitudes of Kurseong South tea gardens.S.D=0.1

Fig shows the highest antioxidant potentials of macerated crude extracts of high altitude grown Kurseong South tea ($94 \pm 0.1\%$ DPPH reduction/mg fresh wt.), but rotary evaporation shows higher antioxidant potential than lyophilization ($88 \pm 0.5\%$ % DPPH reduction/mg fresh wt) of the same tea.

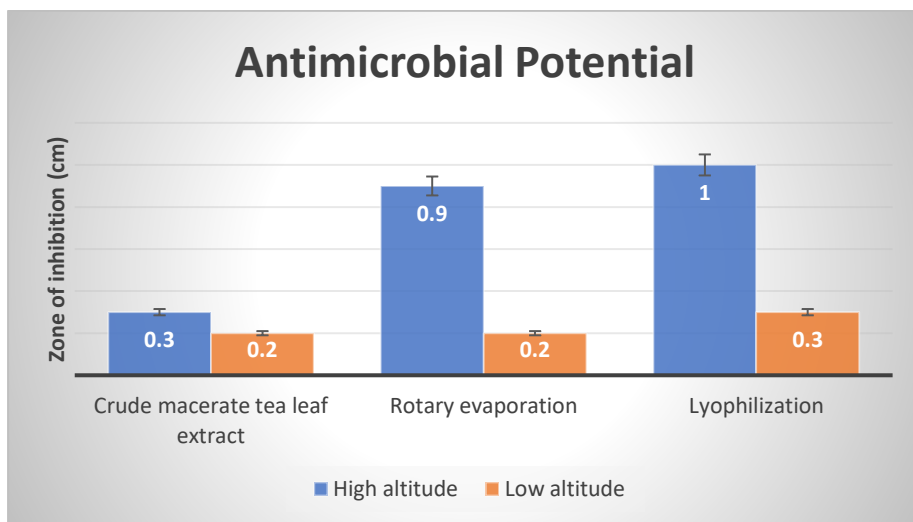
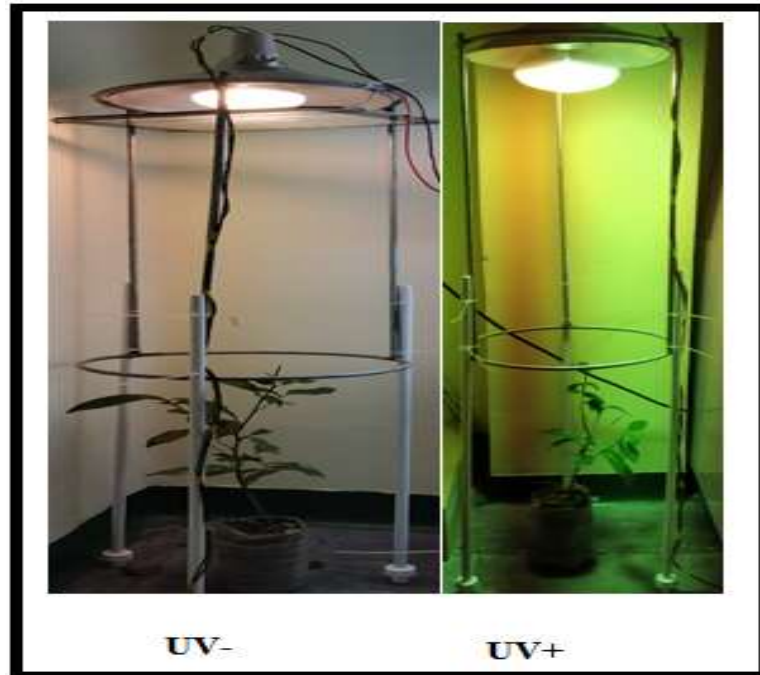


Fig4.3.32.1d Antimicrobial potential of differentially semi purified tea of different altitudes of Kurseong South tea gardens.S.D=0.01

Fig shows the higher antimicrobial potential (1 ± 0.01 cm zone of inhibition) in lyophilized semi purified high altitude tea which calls for further characterization.

ARTIFICIAL UV RADIATION & TEA



OBJECTIVE 3:

Determination of therapeutic (antioxidant, antimicrobial potential) and commercial value (aroma) of tea under artificial UV radiation and comparison with natural UV fluence.

❖ Artificial UV radiation exposure in laboratory

Experiment 4.4.1: Collection of tea plant from DTR and DC, Kurseong and acclimatization at St. Xavier's College, Kolkata.



Figure 4.4.1.1 Collection of 1 year old potted plant from DTR and DC Darjeeling, Kurseong

One year old potted plant of tea was collected from DTR and DC Kurseong, and kept for seven days in St. Xavier's college tissue culture lab for seven days

Experiment 4.4.2: Artificial UV exposure in laboratory to one year old potted plant with differential artificial doses of UV radiation

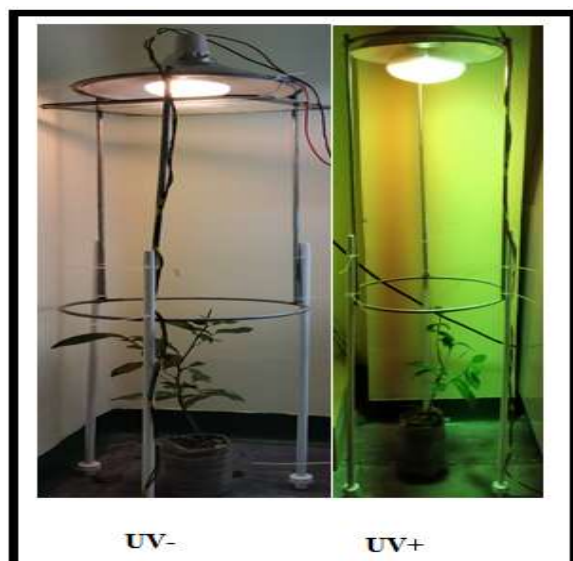


Figure 4.4.2.1a. Set up of artificial UV radiation in the laboratory (With control and UV lamps)

The artificial UV lamps were given to the test plants and control was created by putting soda glass which can cut off the UV-B radiation at 280nm. Table designates different dose of artificial UV radiation. The biochemical (therapeutic and commercial potential) characterizations were done with the leaves collected upto six months.

Artificial UV treatment:

UV fluence ($\mu\text{W}/\text{cm}^2/\text{s}$)	Sign
0	UV0
51	UV1
175	UV2
370	UV3
1037	UV4
2100	UV5

Figure 4.4.2.1b: Different artificial UV lamps of different UV doses

Experiment 4.4.3: Percent increase in Polyphenol content:

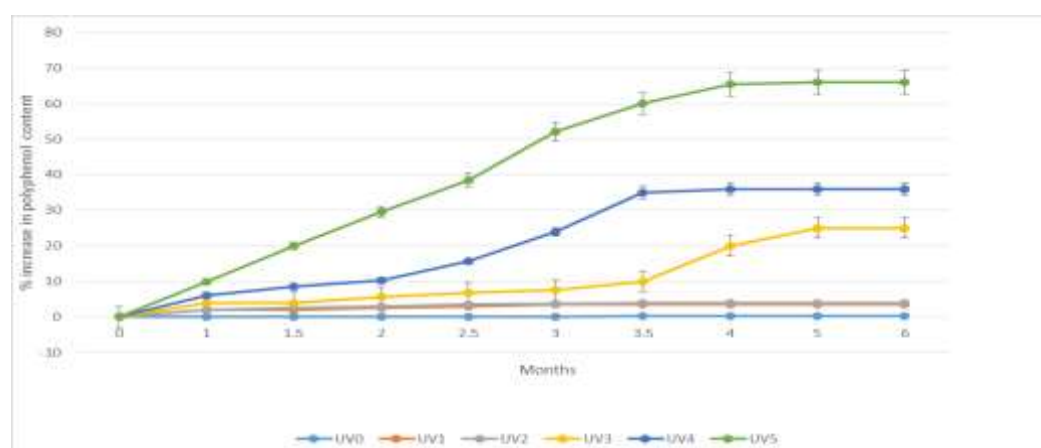


Figure 4.4.3.1: Percent increase in polyphenol content under different artificial UV exposures.S.D=0.001

Percent increase in polyphenol content shows that with $2100 \mu\text{W cm}^2/\text{s}$ artificial UV exposure there is highest % increase in polyphenol content and it is maximum up to 4 months ($70\% \pm 0.001$). When there is no exposure of UV radiation the % content of polyphenol is zero or negligible but there is the increase in % content of polyphenol when the exposure of artificial UV radiation is from $51 \text{uwatt}/\text{cm}^2/\text{sec}$ till $2100 \text{uwatt}/\text{cm}^2/\text{sec}$. There is no increase in polyphenol content when there is no exposure of artificial UV that is control with $0 \mu\text{W cm}^2/\text{s}$. There is slight increase in polyphenol content when the plant is exposed to $51 \mu\text{W cm}^2/\text{s}$ artificial UV and it is constant till the 6th month. $20\% \pm 0.001$ increase in polyphenol content is there from the 4th month when the exposure is about $370 \mu\text{W cm}^2/\text{s}$. In the 5th month with this exposure there is percentage increase in polyphenol content of about $25 \% \pm 0.001$ and it gets constant after that with the same exposure of artificial UV radiation. With $1037 \mu\text{W cm}^2/\text{s}$ artificial UV radiation exposure the percentage increase in polyphenol content is about $15\% \pm 0.001$ and it is getting increased till 4th month which is about $35\% \pm 0.001$ and is getting constant after 4th month. The highest % increase occurred with the maximum dose of $2100 \mu\text{W cm}^2/\text{s}$ artificial UV exposure.

Experiment 4.4.4: Assay of antioxidant potential:

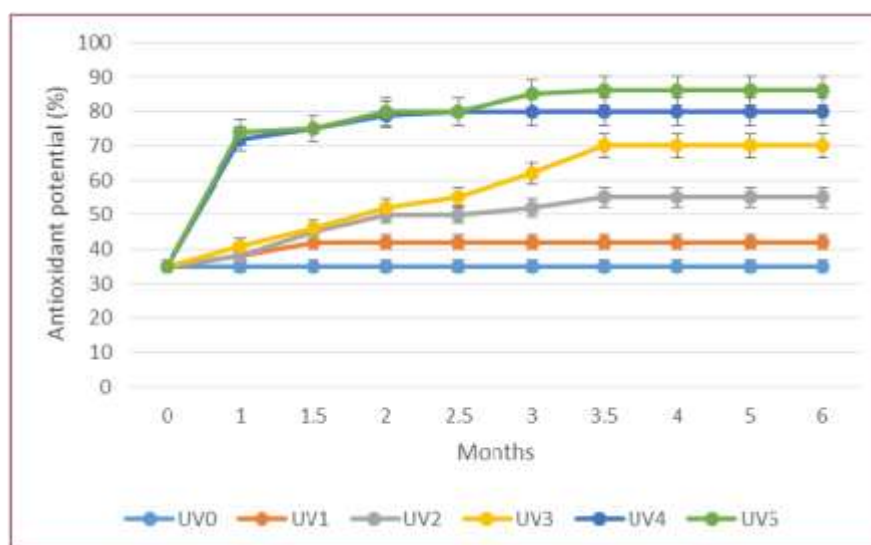


Figure: 4.4.4.1 Antioxidant potential in tea under artificial UV irradiance. S.D=0.001

Fig 4.4.4 Antioxidant potential of tea under artificial UV exposure shows that the highest potential is found with UV5 that is $2100 \mu\text{W cm}^2/\text{s}$ that is $85\% \pm 0.001$ but after 3 months no change is visible. Antioxidant potential % of tea under $1037 \mu\text{W cm}^2/\text{s}$ artificial UV exposure shows $80\% \pm 0.001$ but after 3 months no change is visible. Antioxidant potential % of tea under $370 \mu\text{W cm}^2/\text{s}$ artificial UV exposure shows $70\% \pm 0.001$ in the third month but after 3 months no change is visible. Antioxidant potential % of tea under $175 \mu\text{W cm}^2/\text{s}$ artificial UV exposure shows $60\% \pm 0.001$ in 3.5 month and no change is visible after that. Antioxidant potential % of tea under $51 \mu\text{W cm}^2/\text{s}$ artificial UV exposure shows $40\% \pm 0.001$ in 1.5 month and no change is visible after that. With $0 \mu\text{W cm}^2/\text{s}$ there is no increase in antioxidant potential.

Experiment 4.4.5 Antimicrobial potential assay:



Figure 4.4.5.1a: Antimicrobial zone of inhibition of tea under 1037uwatt/cm²/sec and 2100uwatt/cm²/sec dose of artificial UV radiation against *E.coli*



Figure 4.4.5.1b. Antimicrobial zone of inhibition of tea under 175uwatt/cm²/sec and 370uwatt/cm²/sec dose of artificial UV radiation against *E.coli*



Figure 4.4.5.1c: Antimicrobial zone of inhibition of tea under 51uwatt/cm²/sec and 175uwatt/cm²/sec dose of artificial UV radiation against *E.coli*

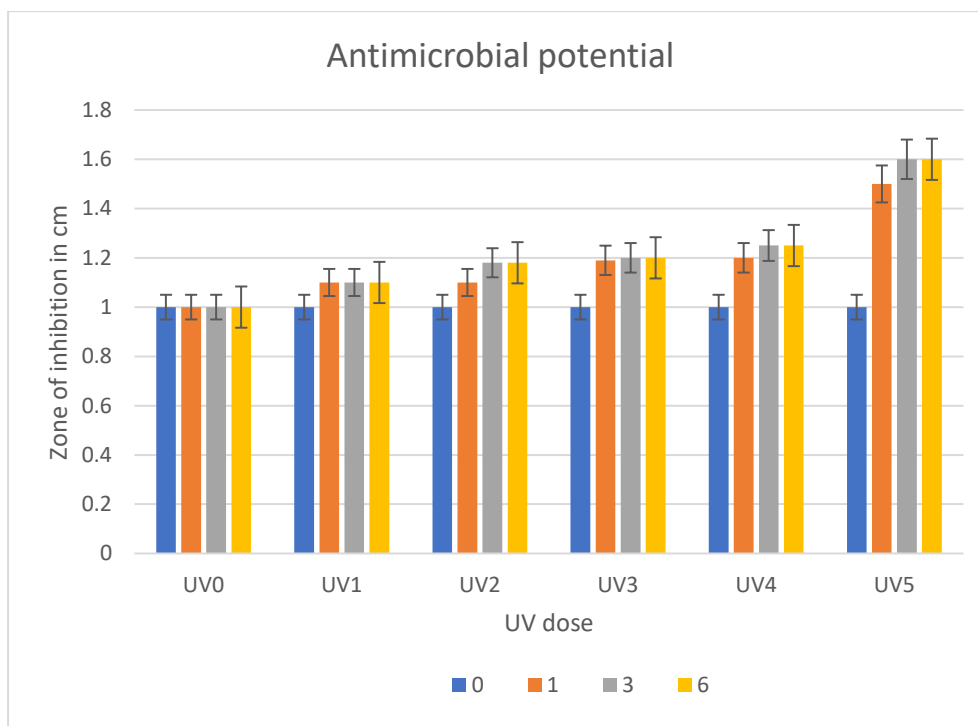


Fig 4.4.5.1d: Histogram of zone of inhibition of antimicrobial potential of tea under different artificial UV exposure.S.D=0.01

Fig 4.4. 5a, 5b, 5c, 5d shows the differential zone of inhibition of tea under differential UV exposure doses.

Highest zone of inhibition is found in case of $2100\mu\text{W cm}^2/\text{s}$ that is about $1.6\pm0.01\text{cm}$ during the 3rd month and after that there is no change till 6th month. With $1037\mu\text{W cm}^2/\text{s}$ the highest zone is about $1.25\pm0.01\text{cm}$ during the 3rd month and after that there is no change till 6th month. With $370\mu\text{W cm}^2/\text{s}$ the highest zone is about $1.2\pm0.01\text{cm}$ during the 1st month and after that there is no change till 6th month. With $175\mu\text{W cm}^2/\text{s}$ the highest zone is about $1.1\pm0.01\text{cm}$ during 1st month and after that there is increase of antimicrobial potential of about $1.18\pm0.01\text{cm}$ till 6th month. With $51\mu\text{W cm}^2/\text{s}$ the highest zone is about $1.1\pm0.01\text{cm}$ during the 1st month and after that there is no change till 6th month. With $0\mu\text{W cm}^2/\text{s}$ the highest zone is about $1\pm0.01\text{cm}$ during the 0th month and after that there is no change till 6th month.

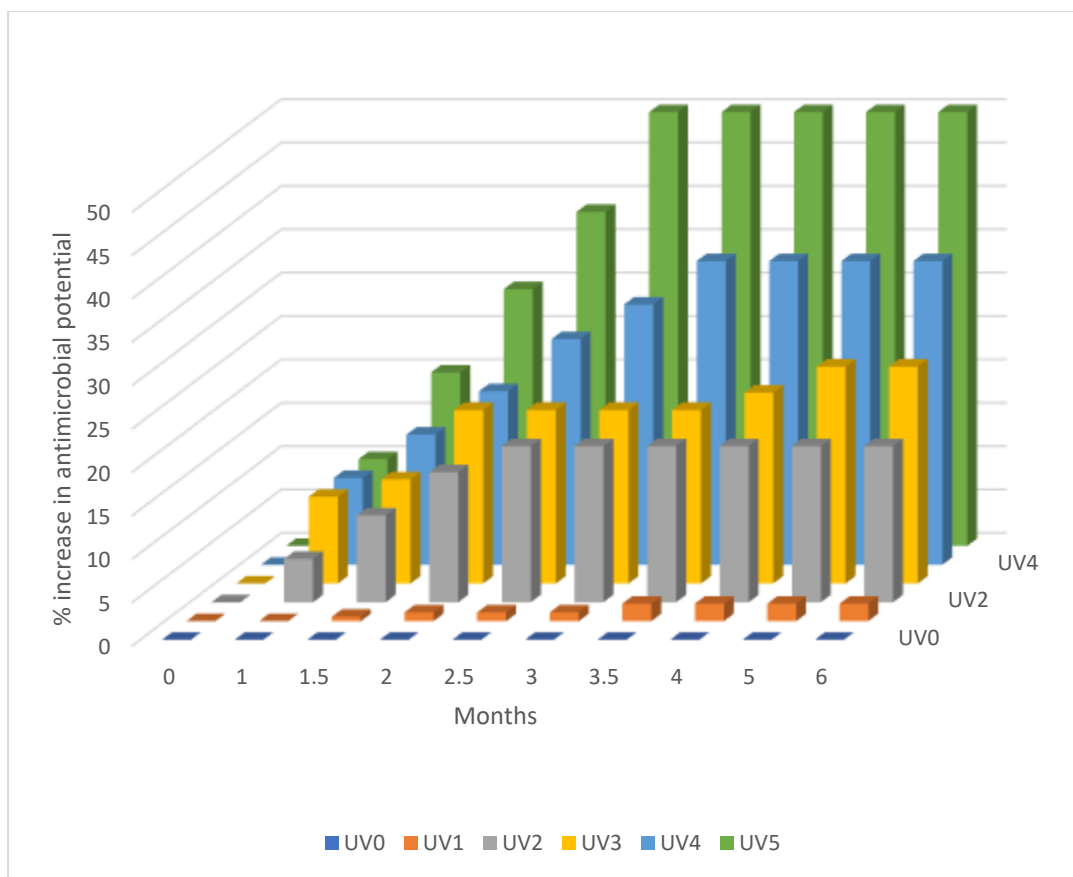


Figure 4.4.5.1e: Percent increase in antimicrobial potential of tea under UV exposure

Fig 4.4.60 shows that the artificial UV exposure UV 5($2100\mu\text{W cm}^2/\text{s}$) shows highest % increase in antimicrobial potential which is about 60% but the increase is observed upto 3.5 months. During the exposure of $0\mu\text{W cm}^2/\text{s}$ and $51\mu\text{W cm}^2/\text{s}$ there is no increase in antimicrobial potential. During the exposure of $175\mu\text{W cm}^2/\text{s}$ there is a slight increase of about 10% in the 1.5 months in antimicrobial potential. The antimicrobial potential increased to nearly 20% during the exposure of $370\mu\text{W cm}^2/\text{s}$ and is constant till 5th month. The antimicrobial potential increased to nearly 35% from the 3.5 month and there is no change after that.

Experiment 4.4.6: Percent increase in UV absorbing compounds:

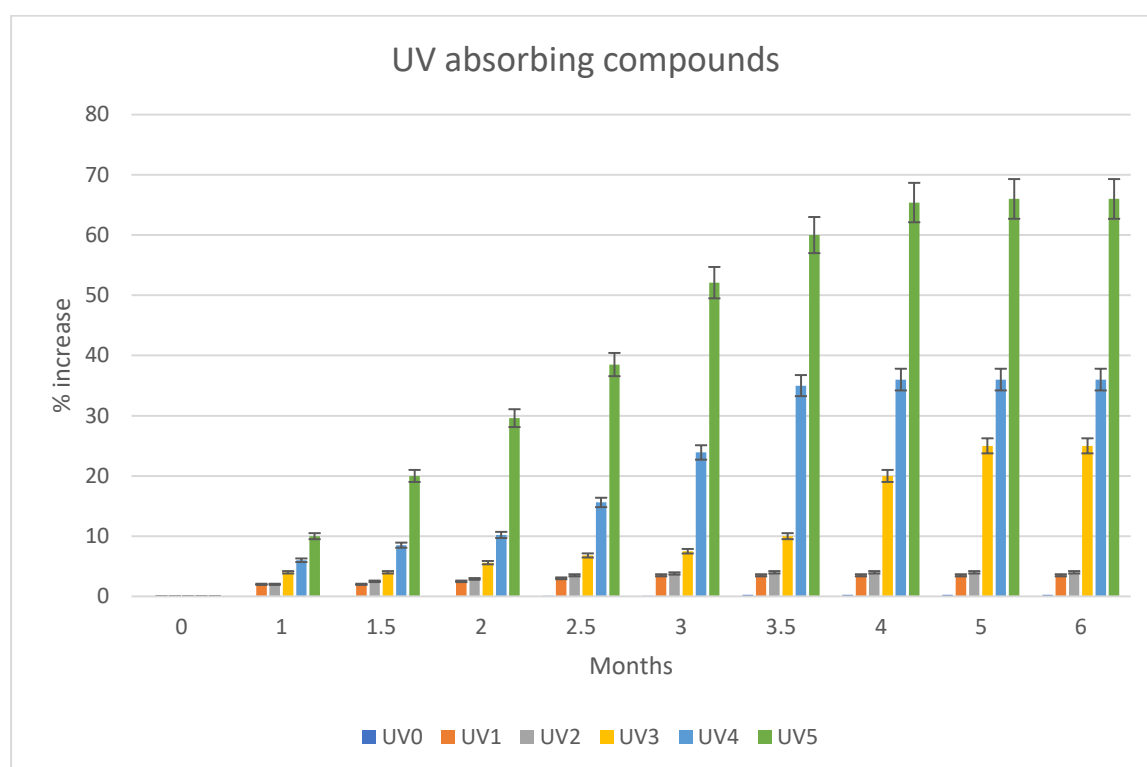


Figure 4.4.6.1: Percent increase in UV absorbing compounds

Fig 4.4.6.1 shows % increase in UV absorbing compounds are highest with UV5 artificial UV treatment but no change is observed upto 4 months. There is no increase in percentage of UV absorbing compounds during the exposure of $0\mu\text{W cm}^2/\text{s}$ and $51\mu\text{W cm}^2/\text{s}$ till 6th month. During the exposure of $175\mu\text{W cm}^2/\text{s}$ there is the increase in UV absorbing compounds from 1st month that is about $4\pm 0.001\%$ and increase is observed till 5th month which is about $25\pm 0.001\%$.

Experiment 4.4.7. Physiological, biochemical (commercial and therapeutic) parameters

UV irradiance $\mu\text{W}/\text{cm}^2/\text{sec}$	Chlorophyll content $\mu\text{mol}/\text{m}^2$	Photosynthetic ability $\mu\text{mol}/\text{m}^2/\text{sec}$	Epidermal UV absorbing compounds AU	Antioxidant Potential (in %)	Antimicrobial Potential cm	Flavonoid content mg/g
2100	2.5 ± 0.002	250 ± 0.2	1.2 ± 0.001	62 ± 0.02	0.78 ± 0.002	21 ± 0.02
1037	2 ± 0.005	200 ± 0.5	1.1 ± 0.002	45 ± 0.01	0.75 ± 0.006	20 ± 0.05
370	0.62 ± 0.001	110 ± 0.3	0.8 ± 0.002	32 ± 0.01	0.36 ± 0.001	15 ± 0.01
175	0.34 ± 0.001	89 ± 0.6	0.6 ± 0.005	22 ± 0.03	0.21 ± 0.003	8 ± 0.002
51	0.089 ± 0.0001	65 ± 0.2	0.22 ± 0.004	18 ± 0.01	0.1 ± 0.0015	5 ± 0.002

Table 4.4.21 shows the variation in physiological parameters like photosynthetic ability, chlorophyll content, epidermal UV absorbing compounds of tea and biochemical and therapeutic parameters under artificial UV exposure. S.D mentioned in table

This ensures that the higher values are obtained with higher UV exposure. At $51 \mu\text{W}/\text{cm}^2/\text{sec}$ UV exposure, the chlorophyll content, photosynthetic ability, epidermal UV-absorbing compound, antioxidant potential, antimicrobial potential, and flavonoid contents are $0.089 \pm 0.0001 \mu\text{mol}/\text{m}^2$, $65 \pm 0.2 \mu\text{mol}/\text{m}^2/\text{sec}$, $0.22 \pm 0.004 \text{AU}$, $18 \pm 0.01\%$, $0.1 \pm 0.0015 \text{ cm}$, $5 \pm 0.002 \text{ mg/g}$, respectively. At $175 \mu\text{Watt}/\text{cm}^2/\text{sec}$ UV exposure, the chlorophyll content, photosynthetic ability, Epidermal UV absorbing compound, antioxidant potential, antimicrobial potential and flavonoid contents are $0.034 \pm 0.001 \mu\text{mole}/\text{m}^2$, $89 \pm 0.6 \mu\text{mol}/\text{m}^2/\text{sec}$, $0.6 \pm 0.005 \text{AU}$, $22 \pm 0.03\%$, $0.21 \pm 0.003 \text{ cm}$, $8 \pm 0.002 \text{ mg/g}$ respectively. At $370 \mu\text{Watt}/\text{cm}^2/\text{sec}$ UV exposure the chlorophyll content, photosynthetic ability, Epidermal UV absorbing compound, antioxidant potential, antimicrobial potential and flavonoid contents are $0.62 \pm 0.001 \mu\text{mole}/\text{m}^2$, $110 \pm 0.3 \mu\text{mol}/\text{m}^2/\text{sec}$, $0.8 \pm 0.002 \text{AU}$, $32 \pm 0.01\%$, $0.36 \pm 0.001 \text{ cm}$, $15 \pm 0.01 \text{ mg/g}$ respectively. At $1037 \mu\text{Watt}/\text{cm}^2/\text{sec}$ UV exposure the chlorophyll content, photosynthetic ability, Epidermal UV absorbing compound, antioxidant potential, antimicrobial potential and flavonoid contents are $2 \mu\text{mole}/\text{m}^2$,

250±0.2µmol/m²/sec, 1.1±0.002AU, 45±0.01%, 0.75±0.006 cm,20±0.05mg/g. At 2100 µWatt/cm²/sec UV exposure, the chlorophyll content, photosynthetic ability, Epidermal UV absorbing compound, antioxidant potential, antimicrobial potential and flavonoid contents are 2.5±0.002umole/m², 250±0.2µmol/m²/sec, 1.2±0.001AU, 62±0.02%, 0.78±0.002 cm,21±0.02mg/g respectively.

Statistical analysis:

Result Details				
Source	SS	df	MS	
Between-treatments	2013186.5066	4	503296.6267	<i>F</i> = 3.47734
Within-treatments	2894720.2251	20	144736.0113	
Total	4907906.7317	24		

The *F*ratio value is 3.47734. The *p*-value is .025992. The result is significant at *p* < .05.

Table 4.4.22: The statistical analysis of UV treatment shows significant correlation with the increased biochemical and therapeutic parameter

Experiment 4.4.8: HPLC chromatogram of UV-treated and untreated tea

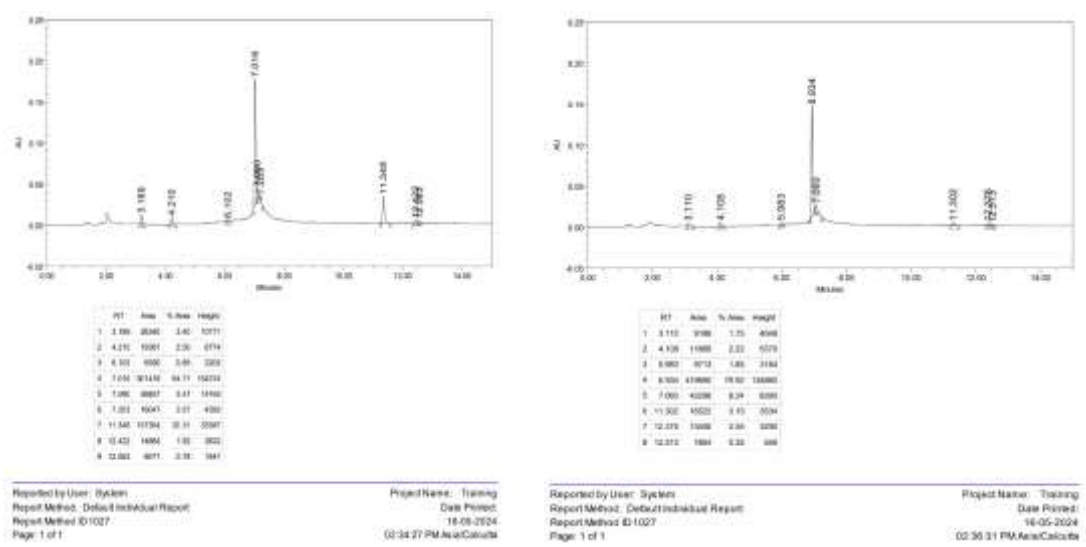


Figure 4.4.7.1 a: HPLC chromatogram of UV-treated and untreated tea

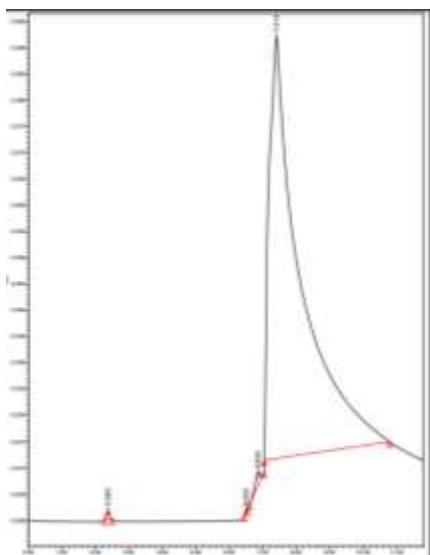


Figure 4.4.7.1b HPLC chromatogram of standard Rutin

By comparing with the standard Rutin (peak at retention time 7.01 min), the peak intensity was measured in a UV-treated sample, which was isolated, purified, mixed with gold nanoparticles and further checked for antimicrobial and antioxidant property.

.Experiment 4.4.9:Antioxidant property of tea eluent, gold nanoparticles and mixture of gold nanoparticles and tea eluent.

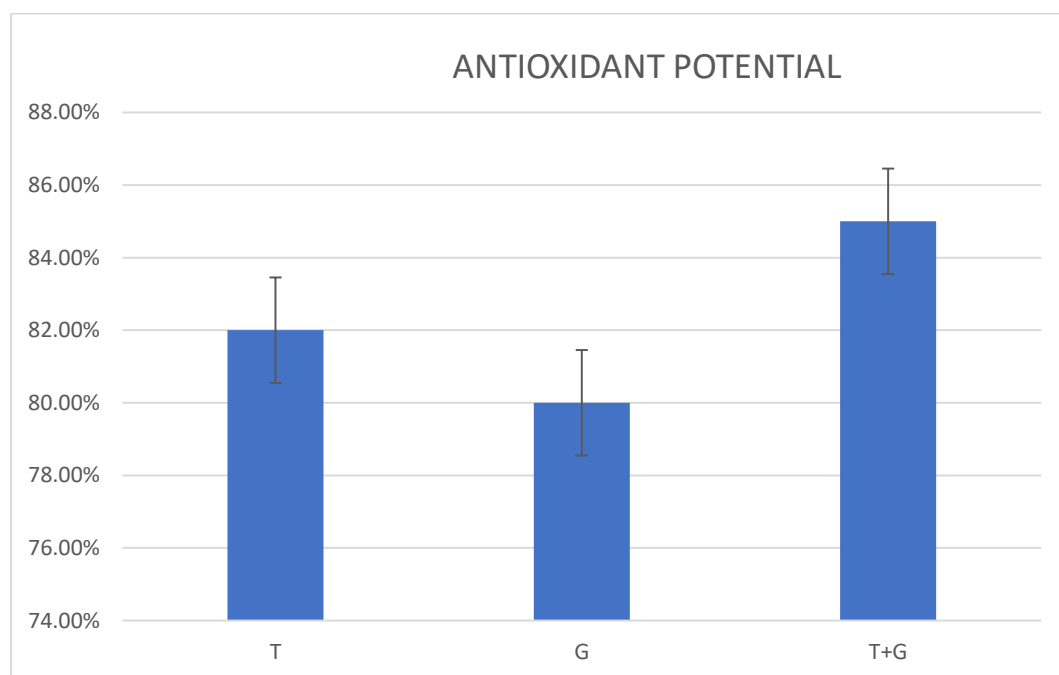


Figure 4.4.8.1 shows the antioxidant potential of the Purified fraction, Gold nanoparticle, and Purified fraction with the Gold nanoparticle. S.D=0.05

The purified sample with gold nanoparticles shows higher antioxidant potential than the purified fraction and gold nanoparticles themselves.

The purified sample shows 82 ± 0.05 antioxidant potential, the gold nanoparticle shows 80 ± 0.05 % antioxidant potential whereas the purified sample with gold nanoparticle shows $85\pm0.05\%$ antioxidant potential.

Experiment 4.4.10 :Antimicrobial assay with purified and gold nanoparticle



Figure 4.4.9.1 shows antimicrobial potential of Purified fraction, Gold nanoparticle and Purified fraction with the Gold nanoparticle.

Purified fraction with the Gold nanoparticle shows a higher zone of inhibition than the purified Fraction itself against *E.coli* .

Purified fraction upregulated with exposure of $2100\text{uw}/\text{cm}^2/\text{sec}$ and Gold nanoparticle shows Higher zone of inhibition than the purified fraction against *E.coli*

Experiment 4.4.11: Post-harvest leaf exposure to artificial UV radiation in laboratory

UV chamber



Figure 4.4.10.1: Artificial UV exposure to post harvest tea leaves

Two leaf a bud tea sample collected from Kurseong South Castleton garden of 2nd flush was kept in UV chamber with artificial UV light at 254nm wavelength (UV dose) and exposed for the following time and taken for characterization

Set	Time
I (Dark)	0min
I (Dark)	30min
I (Dark)	60min
I (Dark)	120min
I (Dark)	180min
I (Dark)	240min
I (Dark)	300min
II (UV exposure)	0min
II (UV exposure)	30min
II (UV exposure)	60min
II (UV exposure)	120min
II (UV exposure)	180min
II (UV exposure)	240min
II (UV exposure)	300min

Table 4.4.23:Time of artificial UV exposure

Experiment4.4.12: Assay of volatile component: Linalool assay

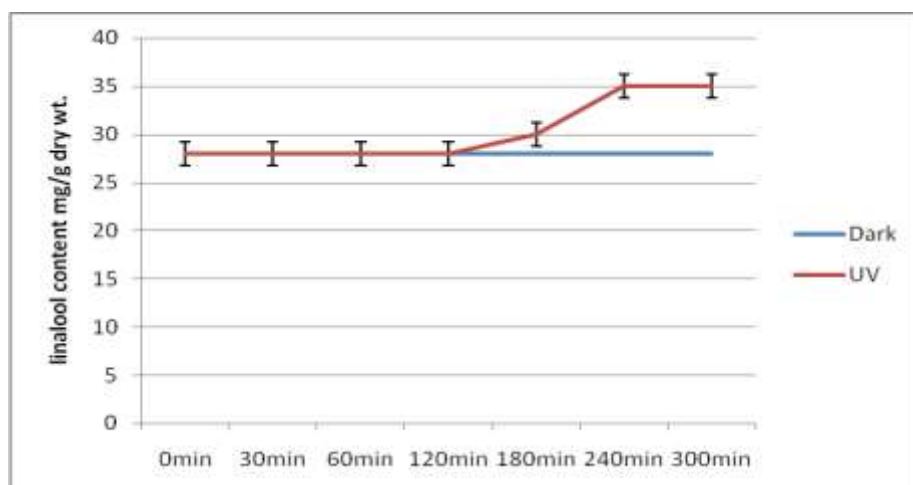


Figure 4.4.11.1 Linalool content in UV-exposed post-harvest tea

Fig 4.4.12.1 shows that the higher linalool content (responsible for volatile aroma components) in post-harvest UV-exposed tea but saturated level is observed after 240 min of exposure

Experiment4.4.13: Artificial UV dose exposure with UV lamp in Castleton factory

UV lamp with the dose standardized in laboratory (maximum 2100 $\mu\text{W}/\text{cm}^2/\text{sec}$) was exposed for different time intervals upto 60 minutes to Castleton tea factory during post harvest manufacturing between drying and sorting. UV exposed leaves were taken for further characterization. Control experiments were conducted without UV exposure



Figure 4.4.12.1: Factory set up of artificial UV lamp

Fig 4.4.66 shows the factory set up of artificial UV lamp in between drying and sorting steps of industrial manufacturing of tea

Experiment 4.4.14:Antimicrobial Assay



Figure 4.4.13.1a Plates to show the antimicrobial potential of UV exposed post harvest tea in factory against *E.coli*

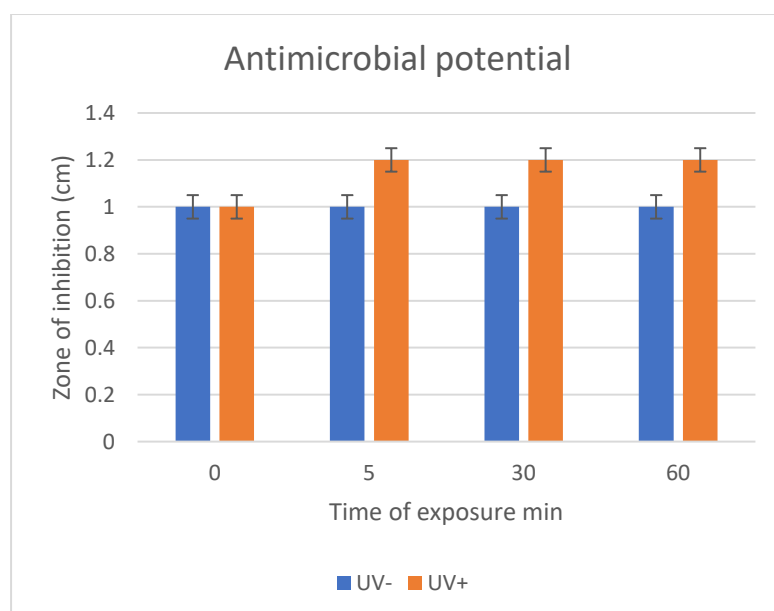


Figure 4.4.13.1b. Histogram of zone of inhibition (antimicrobial potential) of differentially UV exposed post harvest tea in factory during manufacturing.S.D=0.01

Fig 4.4.14.1a and 1b show that the upto 30 min of exposure the antimicrobial potential is increased but after that no change is observed that means the 30 minutes exposure of UV exposure during post harvest manufacture of tea the therapeutic potential like antimicrobial potential is increased.During the exposure to artificial UV radiation there is the increase in antimicrobial potential of about 1.2 ± 0.01 cm from 5minutes and is constant till 60 minutes.But there is no increase in antimicrobial potential in the absence of UV radiation.

Experiment 4.4.15:Antioxidant Assay

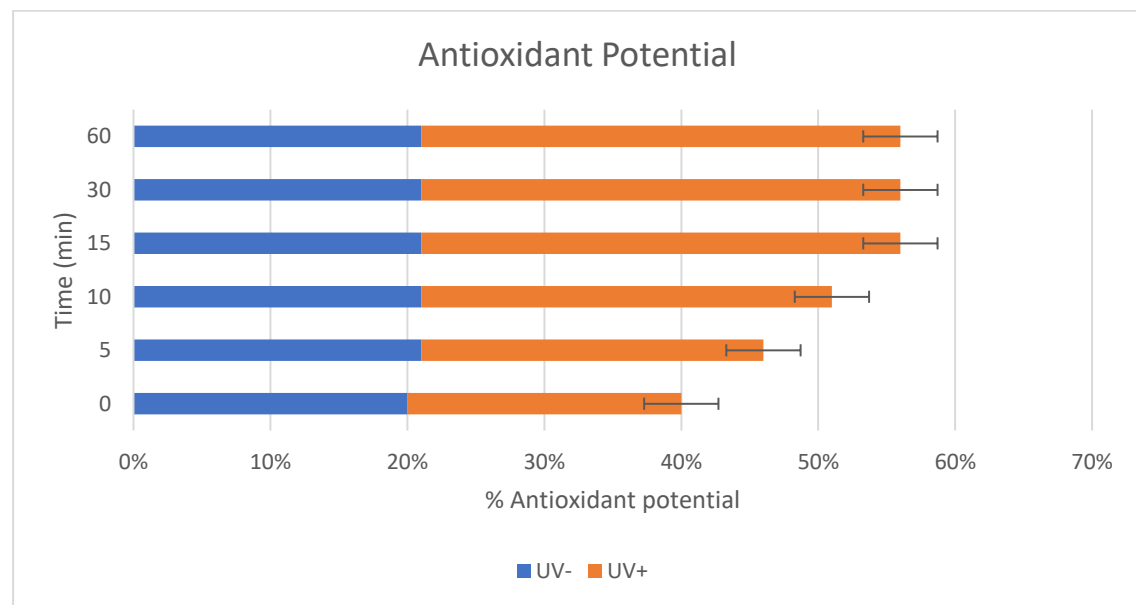


Figure 4.4.14.1. Antioxidant potential of post-harvest tea under differential UV exposure time in factory.S.D=0.05

Fig 4.4.15 shows the increase in antioxidant potential which had not increased after 15 min of exposure in the factory. During the exposure to artificial UV exposure there is the increase in antioxidant potential of about $55\% \pm 0.05$ from 15 minutes till 60minutes.

Experiment 4.4.16 : Volatile Component assay

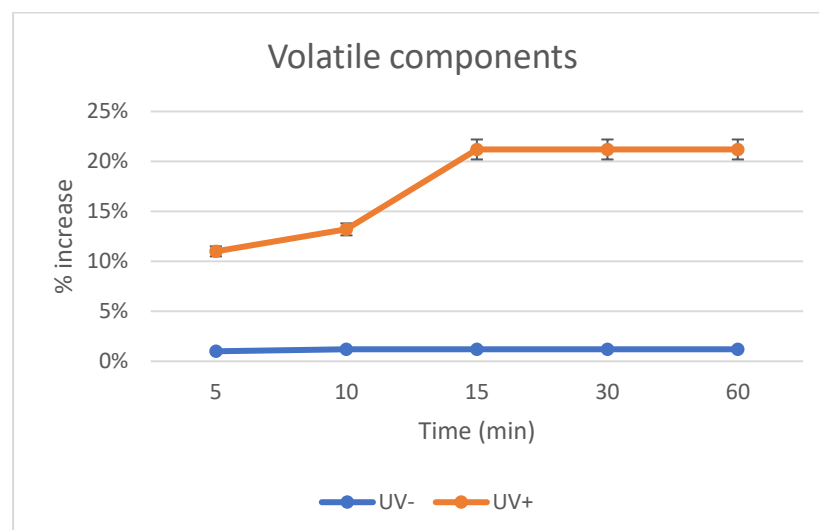


Figure 4.4.15.1: Percent increase in volatile components in UV exposed tea under UV exposure during manufacturing in the factory.S.D=0.001

Fig 4.4.70 shows that the % increase in volatile components are observed in UV treated post harvest manufactured tea during manufacturing process in factory but increase of about 20 % $\pm$ 0.001 is observed upto 115 minutes of UV radiation exposure. It is pertinent to mention here that the volatile components are the commercial values of tea.

OBJECTIVE 4: Characterisation of active metabolites present in different types of manufactured tea (White, Green, Black and Oolong) and comparison of their shelf life in different packaging under natural UV irradiance.

4.5 Biochemical Characterization of different manufactured tea:

Four different types of manufactured tea (White, Green, Oolong and Black tea) was taken for biochemical characterization by different instrumentation techniques and further assessed for therapeutic and commercial values

Experiment 4.5.1. Spectrophotometric Scan of manufactured tea

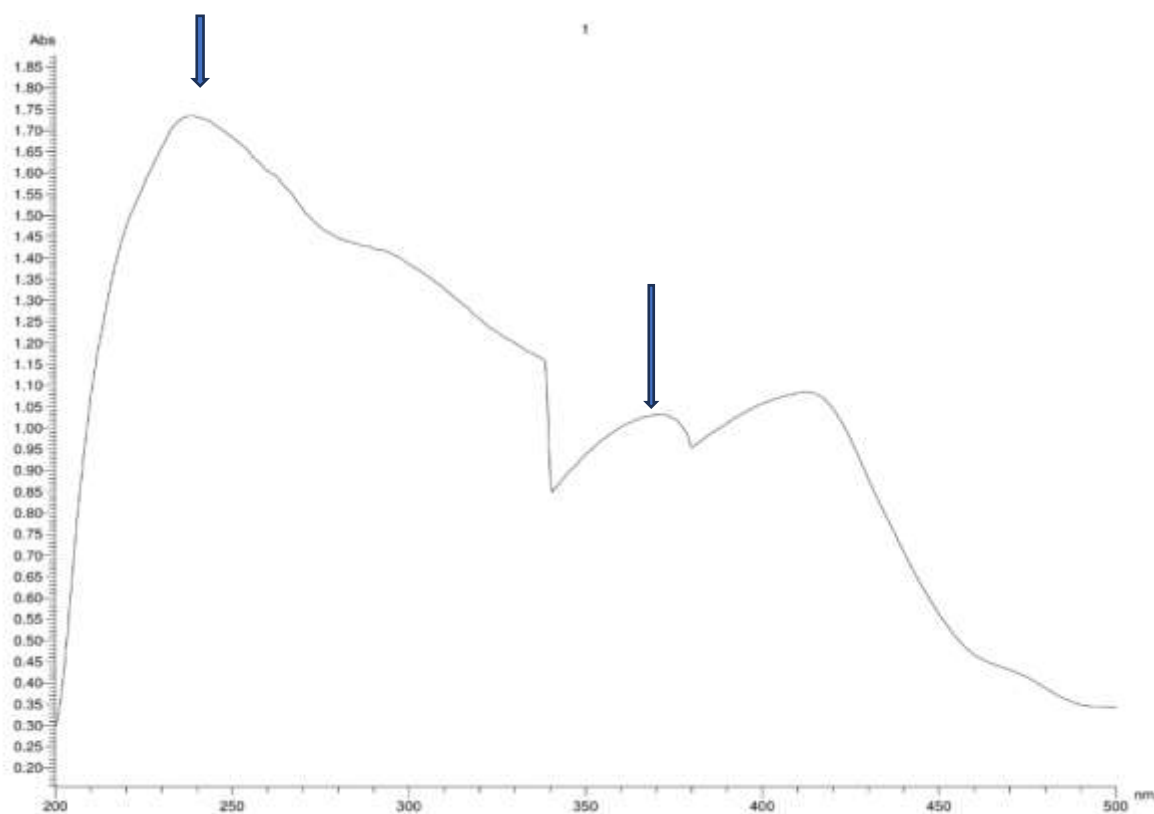


Figure 4.5.1.1a : Spectrophotometric Scan of Green tea

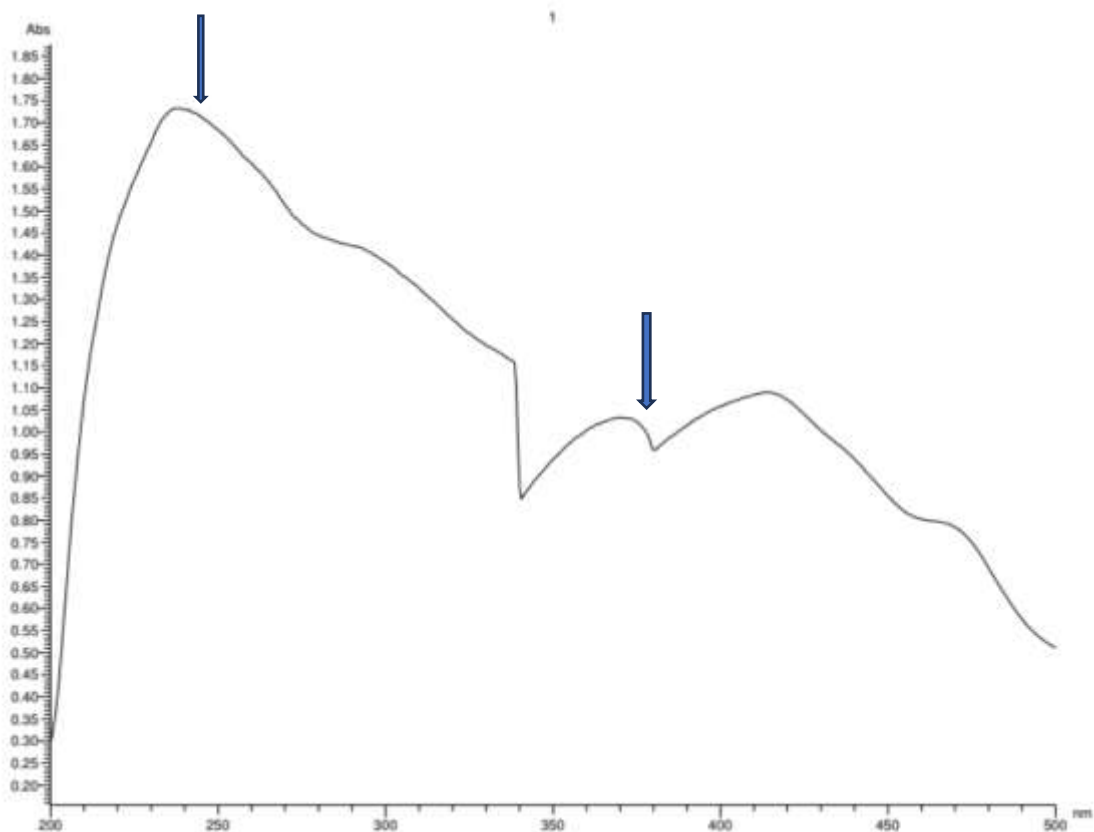


Figure 4.5.1.1b : Spectrophotometric Scan of Black tea

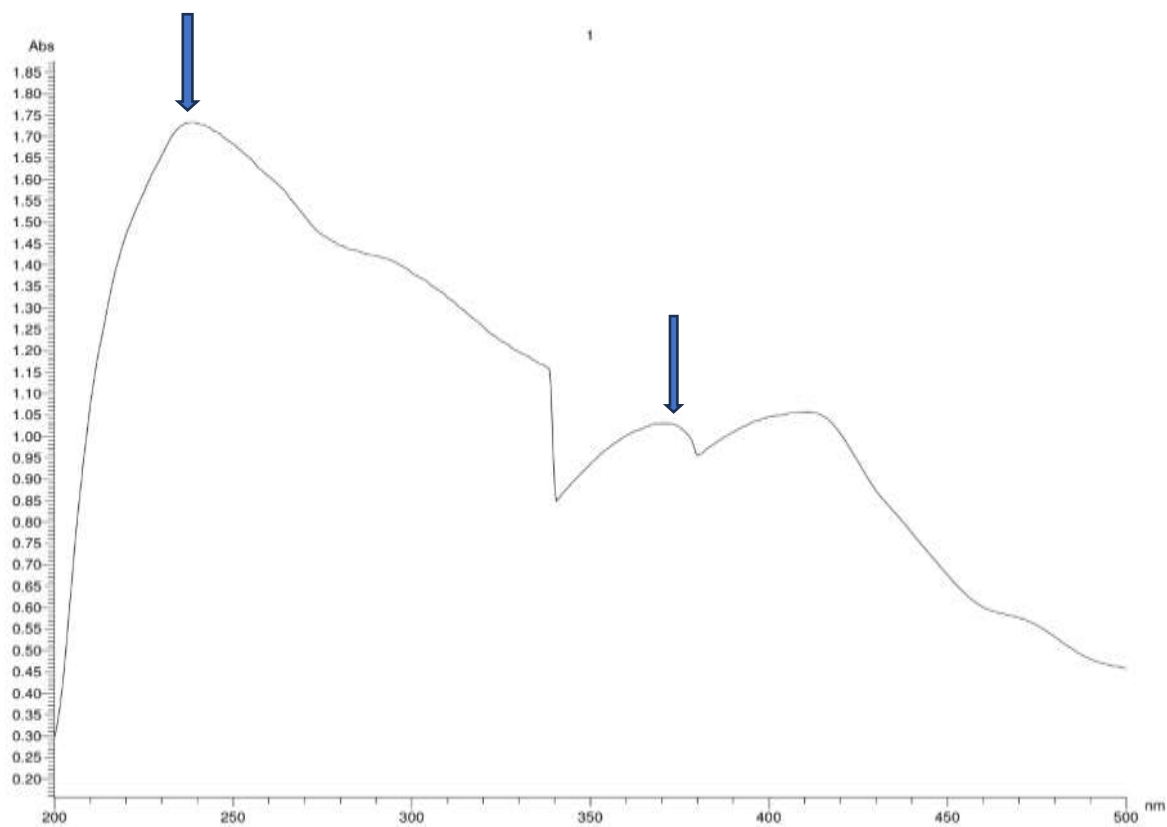


Figure 4.5.1.1c : Spectrophotometric Scan of Oolong tea

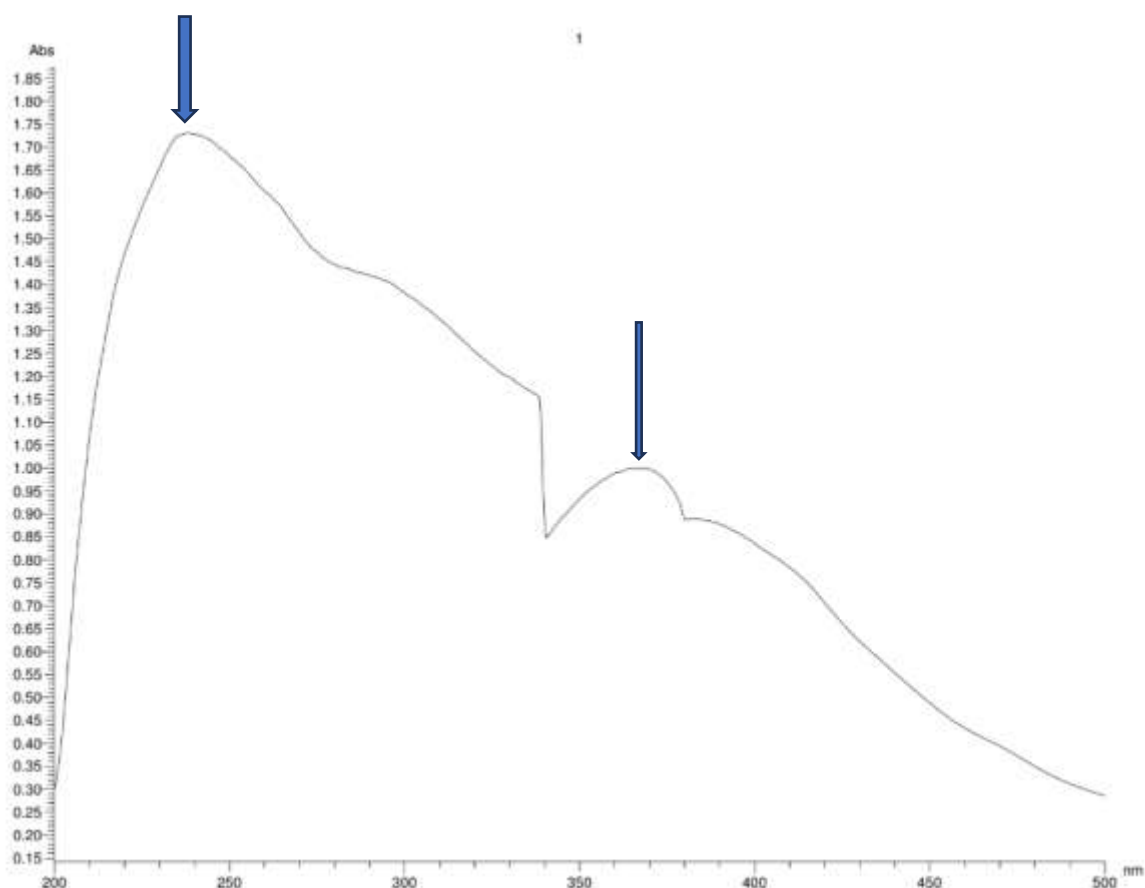


Figure 4.5.1.1d : Spectrophotometric Scan of White Tea

The absorbance of Quercetin at 250 nm, 360nm, and 425 nm are 1.71,1.0,1.12 respectively. The absorbance of White tea at 250 and 360nm are 1.71 and 0.91 respectively. The absorbance of Black tea at 250,310,460,415,470 are 1.71,1.4,1.0,1.06,0.70 respectively, 1.00 and 0.5. The absorbance of green tea at 250 nm, 340 nm, 360 nm, 410nm, and 300nm are 1.72,1.15,0.96,1.05,1.17, respectively. The absorbance of fresh tea at 300nm and 360nm are the absorbance of Oolong tea at 250nm,330nm,360nm, 410nm, 450nm are 1.71,1.11,0.94,0.97,0.54 respectively.

Quercetin (standard)	Sl.no.	0.D	Wavelength (nm)
	I	1.71	250nm
	II	1.0	360nm
	III	1.12	425nm

White Tea			
	I	1.71	250
	II	0.91	360
Black tea			
	I	1.71	250nm
	II	1.4	310nm
	III	1.0	360nm
	IV	1.06	415nm
	V	0.70	470nm
Oolong Tea			
	I	1.71	250nm
	II	1.11	330nm
	III	0.94	360nm
	IV	0.97	410nm
	V	0.54	450nm
Green Tea			
	I	1.72	250nm
	II	1.15	340nm
	III	0.96	360nm
	IV	1.05	410nm
	V	1.17	300nm

Table 4.5.24: Analysis of Spectrophotometric Scan.

Fig 4.5.1.1a,b,cand d and Table 4.5.24 shows specific band at 250 nm of quercetin which is present in all types of tea varieties and that is almost the same to 1.71-1.72; another band at

360 nm which is also present in all tea types . The concentration of Quercetin is 1mg/ml, which is almost similar in different types of tea.

Atomssa et al., (2015) studied the biochemical characterization of catechins in green tea by UV spectrophotometric scan.

Experiment 4.5.2.Spectrofluorimetric Scan of manufactured tea

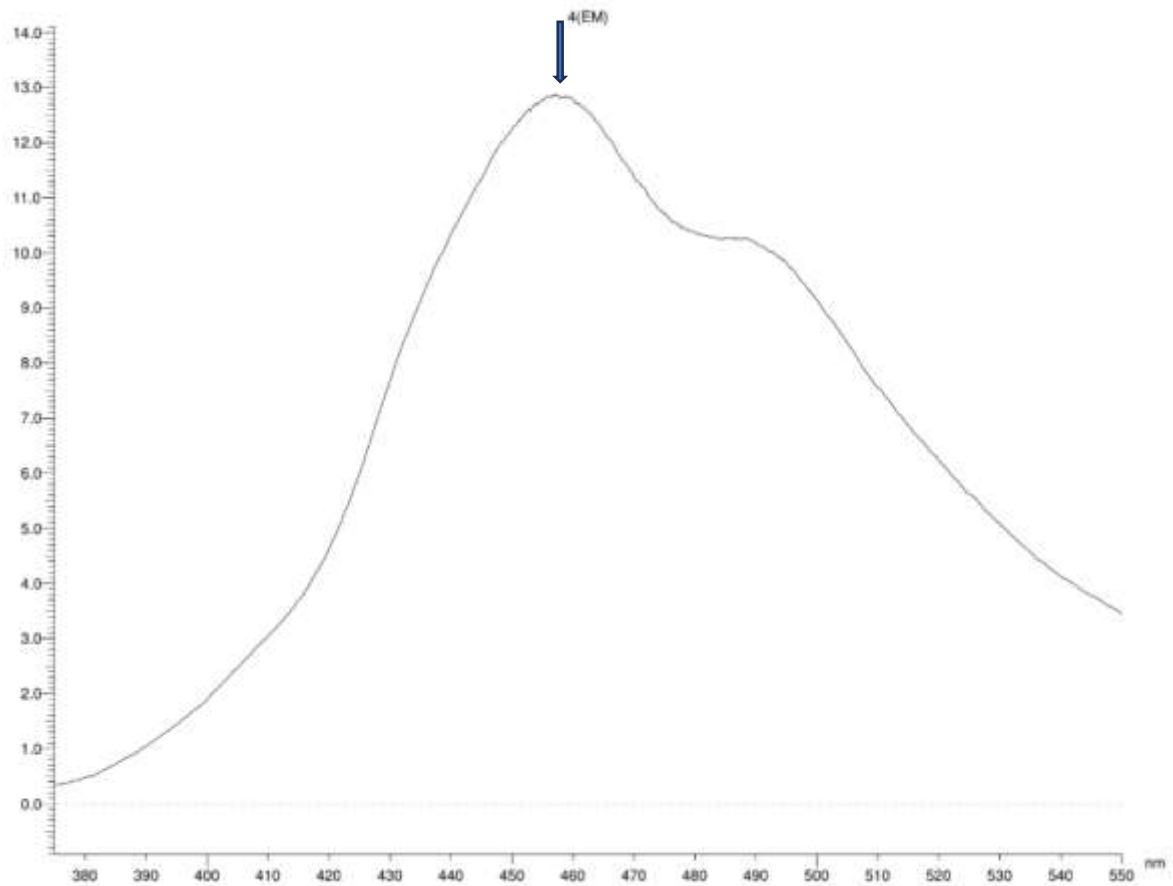


Figure 4.5.2.1a: Spectrofluorimetric Scan of Black tea

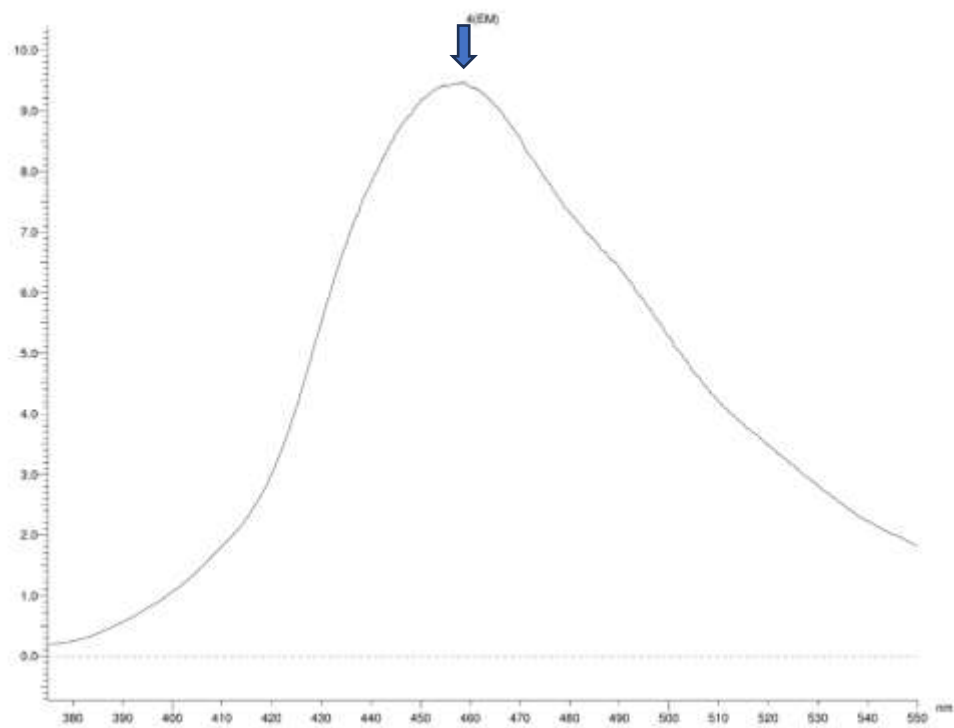


Figure 4.5.2.1b : Spectrofluorimetric Scan of Green tea

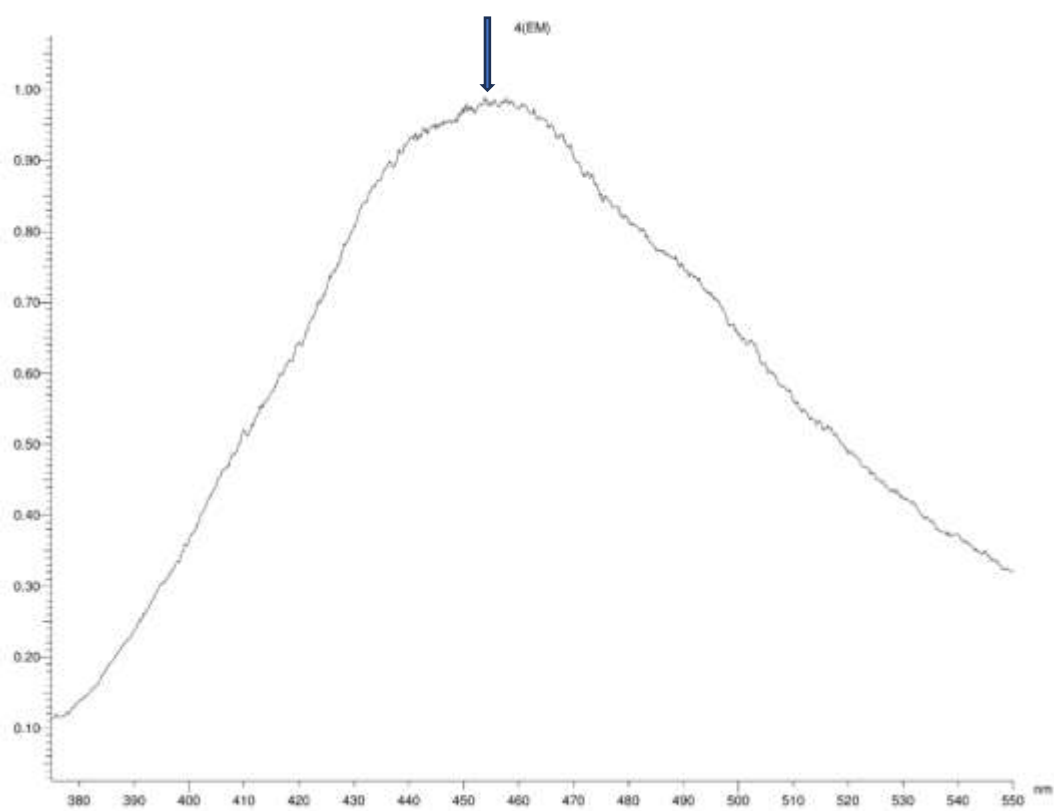


Figure 4.5.2.1c: Spectrofluorimetric Scan of Oolong tea

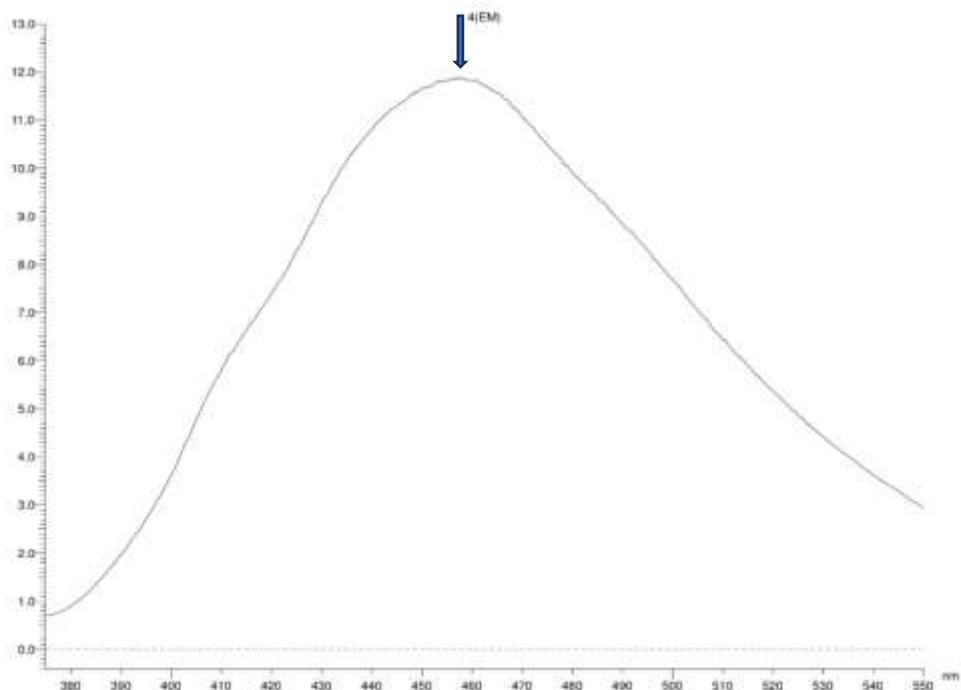


Figure 4.5.2.1d: Spectrofluorimetric Scan of White tea

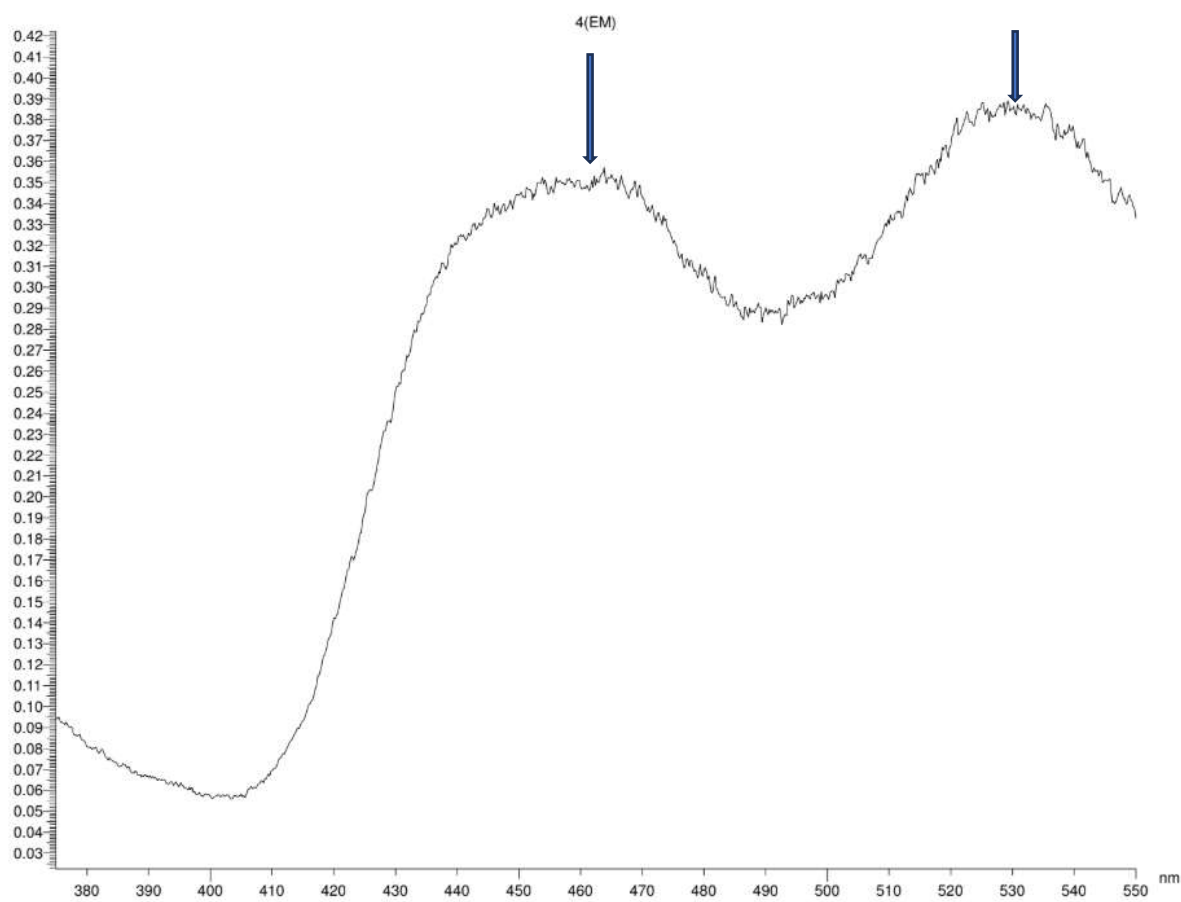


Figure 4.5.2.1e: Spectrofluorimetric Scan of Quercetin

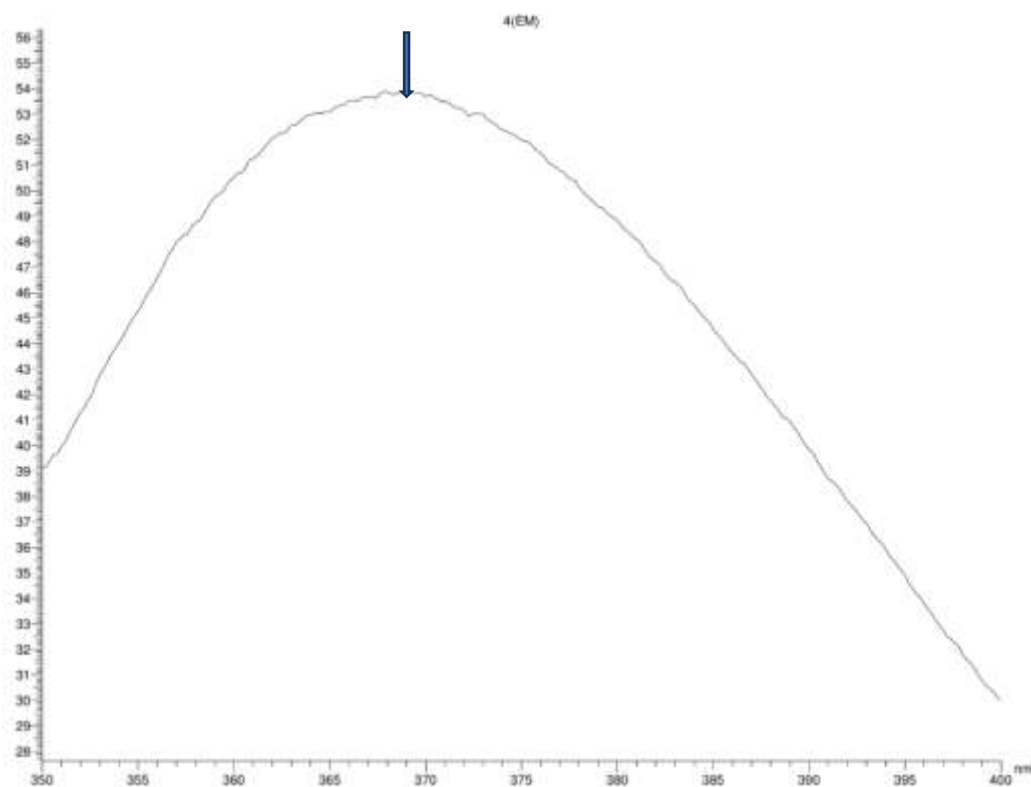


Figure 4.5.2.1f : Spectrofluorimetric Scan of EGCG

Fig 4.5.2.1 a Spectrofluorometric scan of Black Tea. The absorbance $\times 10^{-1}$ at 460 and 495nm is about 12.5 and 10 respectively.

Fig 4.5.2.1b Spectrofluorometric scan of Green Tea. The absorbance $\times 10^{-1}$ at 460 nm is about 9.

Fig 4.5.2.1c Spectrofluorometric scan of Oolong Tea. The absorbance $\times 10^{-1}$ at 452 nm is about 0.95.

Fig 4.5.2.1 d Spectrofluorometric scan of White tea. The absorbance $\times 10^{-1}$ at 455 nm is about 11.5.

Fig 4.5.2.1 e Spectrofluorometric scan of Quercetin. The absorbance $\times 10^{-1}$ at 455 and 528 nm is about 0.345 and 0.38 respectively.

Fig 4.5.2.1 f Spectrofluorometric scan of EGCG. The absorbance $\times 10^{-1}$ at 370 is about 54.

SAMPLE	WAVELENGTH (nm)	ABSORBANCE x 10 ⁻¹
Quercetin	I-455	0.345
	II-528	0.38
Epigallocatechin gallate	I-370	54.0
Oolong tea	I-452	0.95
Black tea	I-460	12.5
	II-495	10
Green tea	460	9
White tea	455	11.5

Table 4.5.25: Analysis of spectrofluorimetric scan

Fig 4.5.2.1 a,b,c,d,e,f and Table 4.5.25 show that the highest content of quercetin in white tea which is around 11.5. Spectrophotometric and spectrofluorimetric scans of Iranian teas were done by Khanchi et al., (2007).

Experiment 4.5.3. FTIR analysis of manufactured tea

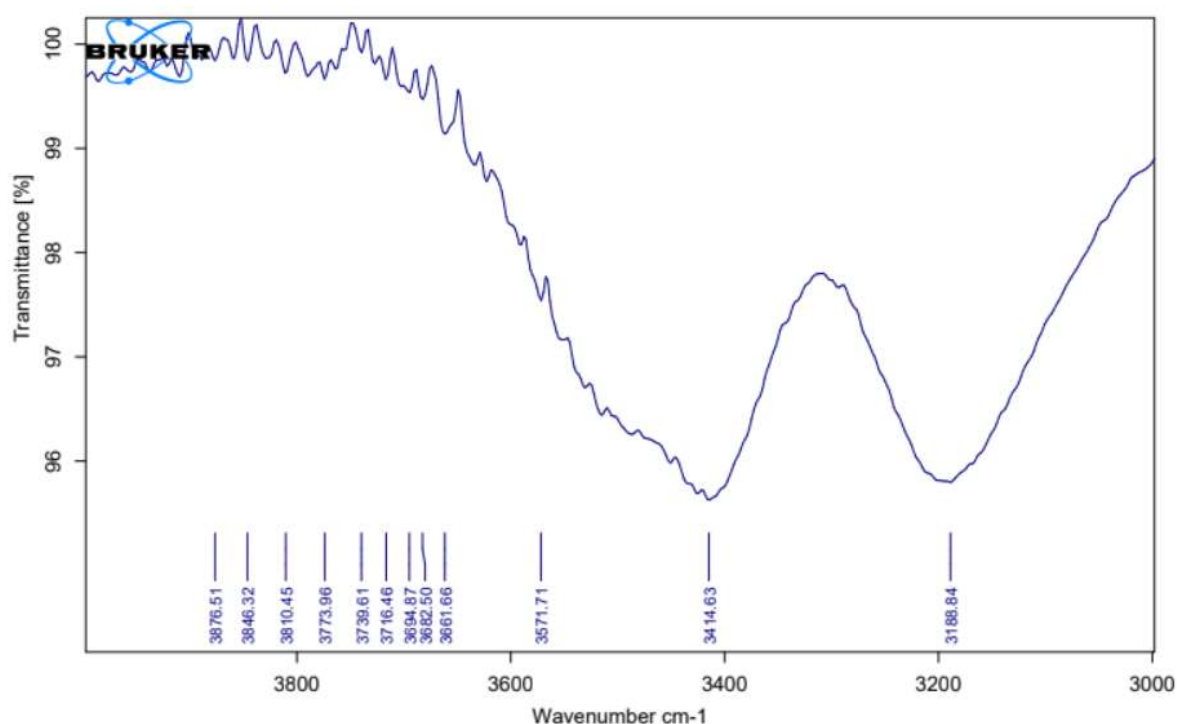


Figure 4.5.3.1a: FTIR analysis of Black tea

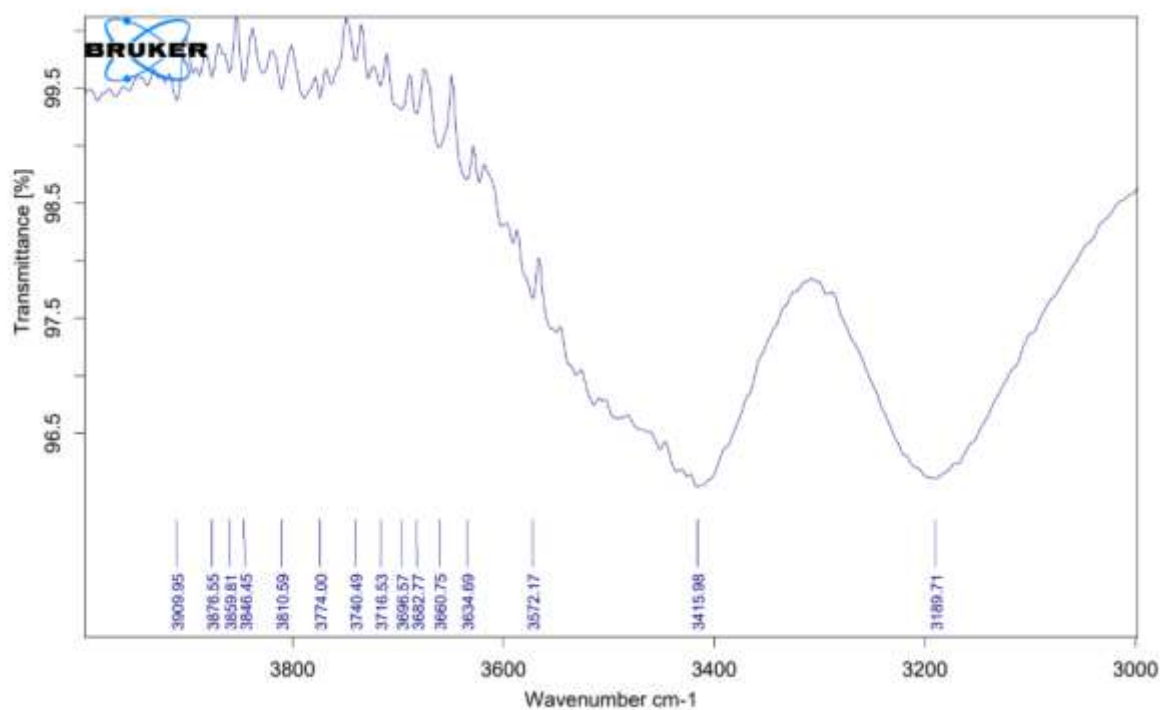


Figure 4.5.3.1b: FTIR analysis of Oolong tea

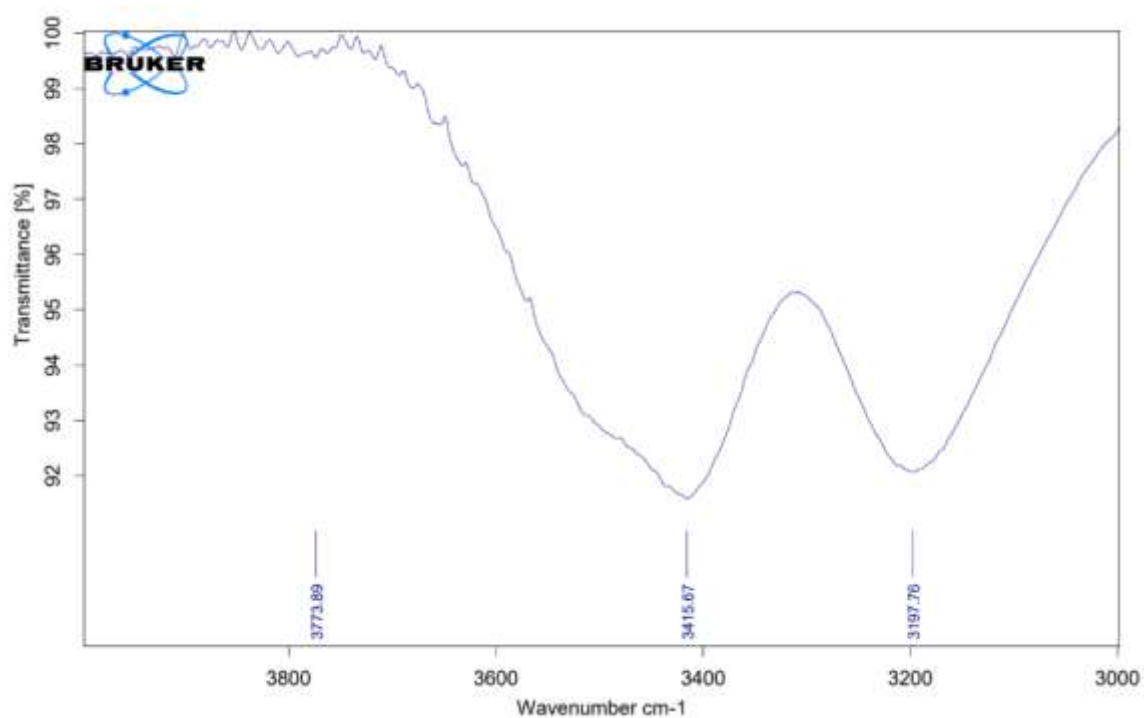


Figure 4.5.3.1c: FTIR analysis of Green tea

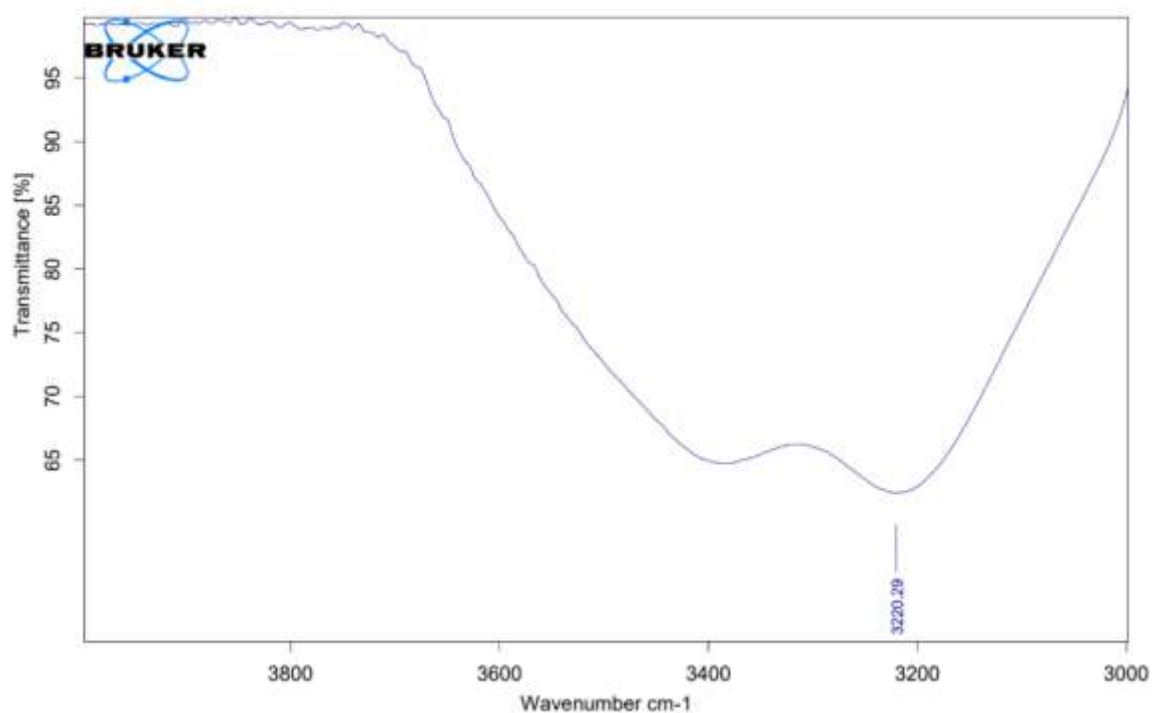


Figure 4.5.3.1.d: FTIR analysis of White tea

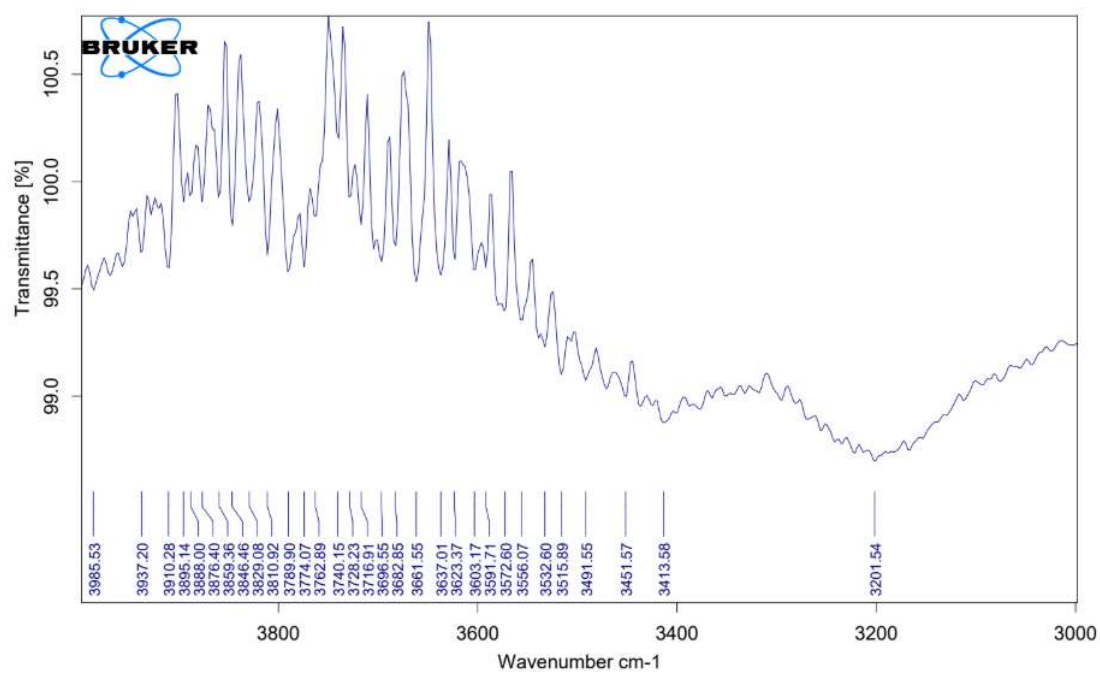


Figure 4.5.3.1e: FTIR analysis of Quercetin

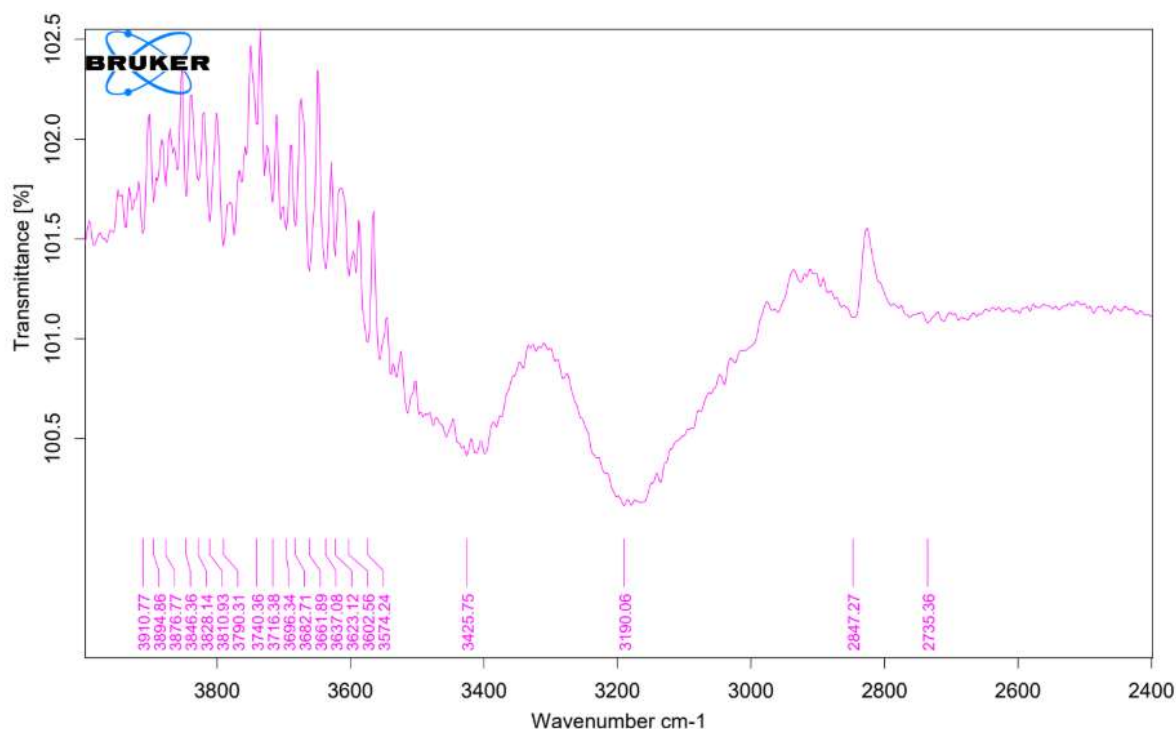


Figure 4.5.3.1f: FTIR analysis of EGCG

Figure 4.5.3.1: FTIR analysis of different tea (a Black tea, b. Oolong tea, c. Green tea, d. White tea, e Quercetin. f. Epigallocatechin gallate.)

Figure 4.5.3.1f shows the FTIR Transmittance peaks of the standards Epigallocatechin gallate. The transmittance (%) of the transmittance wavelength range 3425.75 -3109.06 is about 100.

Figure 4.5.3.1e shows the FTIR Transmittance peaks of the standards Quercetin. The transmittance (%) of the transmittance wavelength range 3600-3200 is about 99.

Tea type	Transmittance wavelength (cm ⁻¹)	Transmittance (%)
Epigallo catechin gallate	3425.75	100.4
	3190.06	100
	2847.27	101
	2735.36	101.3
Black tea	3414.63	95.5
	3186.84	96

White Tea	3415.59	95.5
	3200.41	94.5
Green Tea	3773.89	99.2
	3415.67	91.9
	3197.76	92.2
Oolong tea	3415.98	95.5
	3198.71	95.3
Quercetin	3985.53	99.5
	3937.2	99.8
	3774.07	99.5
	3463.58	98.9
	3451.57	99
	3201.94	98.5

Table 4.5.26 FTIR analysis of all the tea varieties

Fig 4.5.3.1a shows the FTIR Transmittance peaks of Black tea. The transmittance (%) of the transmittance wavelength range 3414.63- 3188.84 is about 98.

Fig 4.5.3.1b shows the FTIR Transmittance peaks of Oolong tea. The transmittance (%) of the transmittance wavelength range 3415.98- 3189.71 is about 97.

Fig 4.5.3.1c shows the FTIR Transmittance peaks of Green tea. The transmittance (%) of the transmittance wavelength range 3415.67- 3197.76 is about 95.

Fig 4.5.3.1d shows the FTIR Transmittance peaks of White tea. The transmittance (%) of the transmittance wavelength range 3400-3200 is about 99.

Fig 4.5.3.1e shows the FTIR Transmittance peaks of Quercetin. The transmittance (%) of the transmittance wavelength range 3600-3200 is about 99.

Fig 4.5.3.1f shows the FTIR Transmittance peaks of EGCG. The transmittance (%) of the transmittance wavelength range 3600-3200 is about 99.

Table 4.5.26a and Fig 4.5.3.1 show that white tea has higher transmittance within the frequency ranging from 3400-3200 cm^{-1} indicating stretching of O-H bonds, then black tea,

oolong tea, and then green tea, but the phenolic bond stretching of the epigallocatechin gallate (polyphenol catechins) . In the case of black tea (Brza et al, 2020) and green tea extracts (Senthilkumar and Thirumal,2014) similar types of transmittance patterns of FTIR spectral scan were shown. It indicates that within wavelength 3550 - 3200 cm^{-1} (indicates stretching of alcoholic/phenolic O-H group) which are lower in green tea (91.9% at 3415.67 cm^{-1}); at 3770 cm^{-1} stretching indicates specific alcohol group stretching which is there in green tea as that in quercetin, at around 3190 cm^{-1} and weak alcohol group stretching is available in all tea varieties except white tea , and is highest in black tea 96% at 3186.84 cm^{-1}

Experiment 4.5.4: LC-MS STUDY OF MANUFACTURED TEA

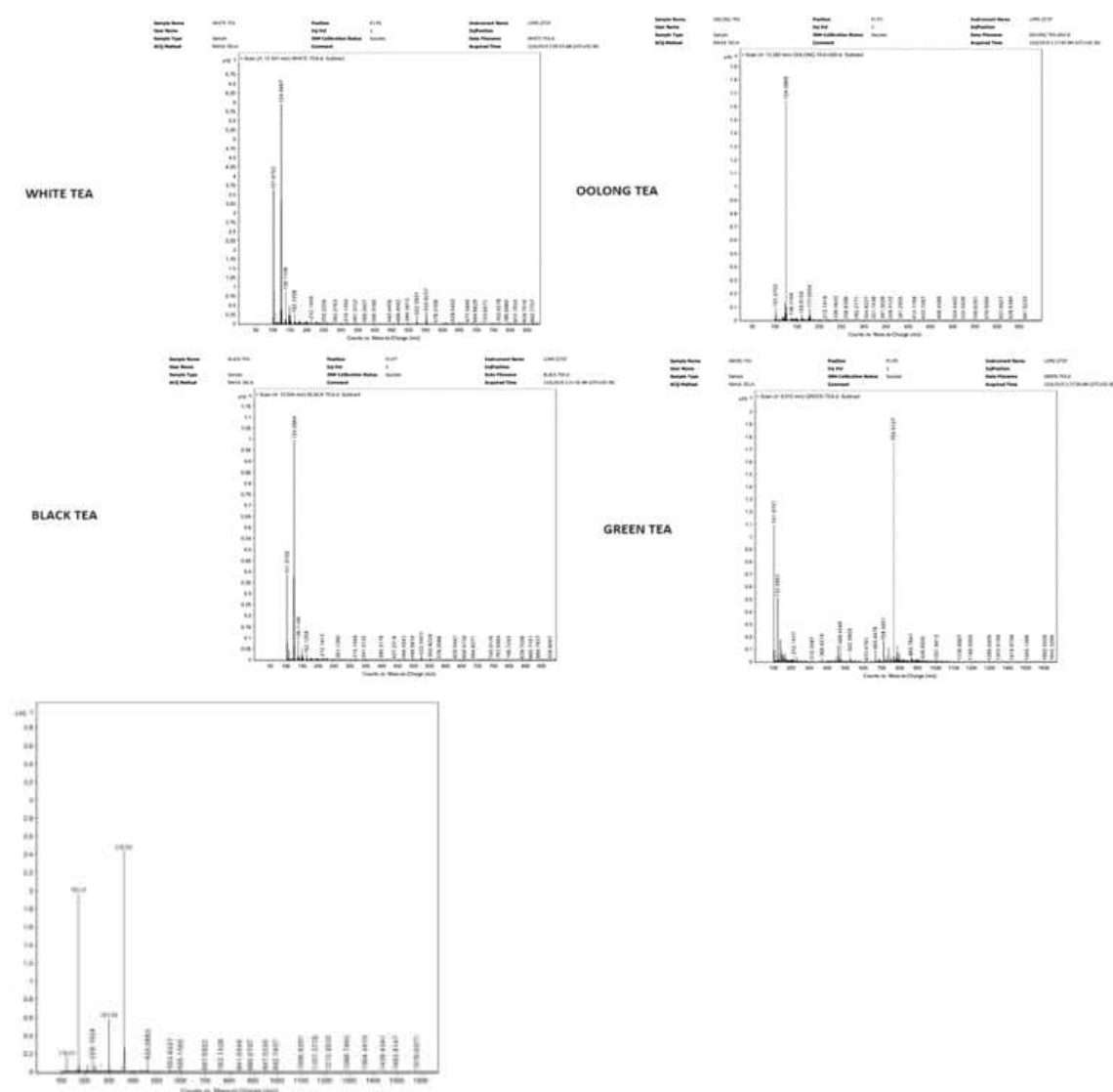


Fig 4.5.4.1a,b,c,d,e shows variation in m/z ratio of different manufactured

leaves with standard EGCG.

SL.NO.	Counts	Counts	m/z	Maximum m/z	
	Range		Ratio		
			Range		
1.	0-6.75	6.5	100-850	124.0867	
2.	0-2.2	2.1	100-900	101.0701	
3,	0-1.15	1.1	100-900	124.0864	
4.	0-3.6	3.4	100-900	195.0869	

Table 4.5.27a LC-MS data analysis for White tea.

The m/z peaks of white tea are 124.086,101.0701,124.0864,195.0869

SL.NO.	Counts	Counts	m/z	Maximum m/z	
	Range		Ratio		
			Range		
1.	0-6	1.58	50-990	104.1064	
2.	0.05-1.25	1.1	50-990	195.0866	
3.	0-8.25	7.25	50-990	124.0868	
4.	0-1.15	1	50-990	124.0864	

Table 4.5.27b LC-MS data analysis for Black tea

The m/z peaks of white tea are 104.1064,195.0866,124.0868,124.0864

SL.NO.	Counts	Counts	m/z	Maximum m/z
	Range		Ratio	
			Range	
1.	0-1.05	0.95	50-650	104.1066
2.	0-1.9	1.7	50-650	195.0870
3.	0-8	7	50-650	312.3609
4.	0-1.65	1.45	50-650	101.0702
5.	0-3	3	50-650	101.0703
6.	0-1.9	1.68	50-650	124.0865

Table 4.5.27c LC-MS data analysis for Oolong tea

The m/z peaks of Oolong tea are 104.1066,195.0870,101.0702,101.0703,124.0865

1.	0-3.8	3.4	100-1600	104.1060
2.	0-1.4	1.2	100-1600	195.0868
3.	0-9	8	100-1600	124.0866
4.	0-3.2	2.8	100-1600	124.0866
5.	0-2	1,8	100-1600	763.5127

Table 4.5.27d LC-MS data analysis for green tea

The m/z peaks of green tea are 104.1060,195.0868,124.0866,124.0866,763.5127.

LC-MS data is shown in Table 4.3.17a,b,c and Fig. 4.3.21.32a-e states that the characteristic m/z ratio of epigallocatechin gallate at around 120 which is found (124.08) in oolong tea. Han et al.,(2021) studied and identified different components in green tea by LC-MS.

Experiment 4.5.5. HPLC Study of manufactured tea

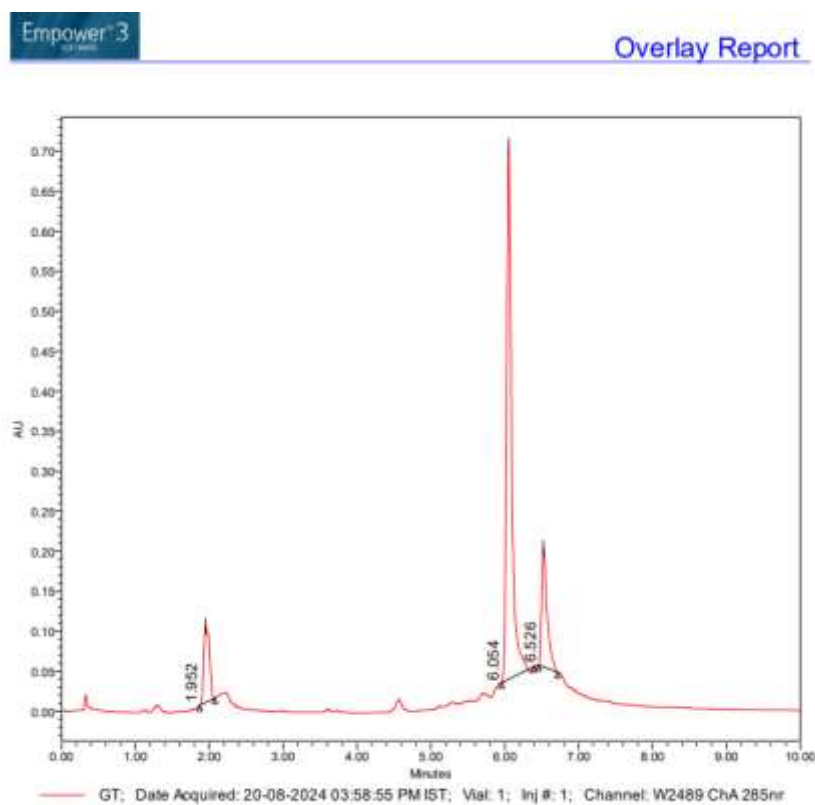


Figure 4.5.5.1a HPLC study of Green Tea

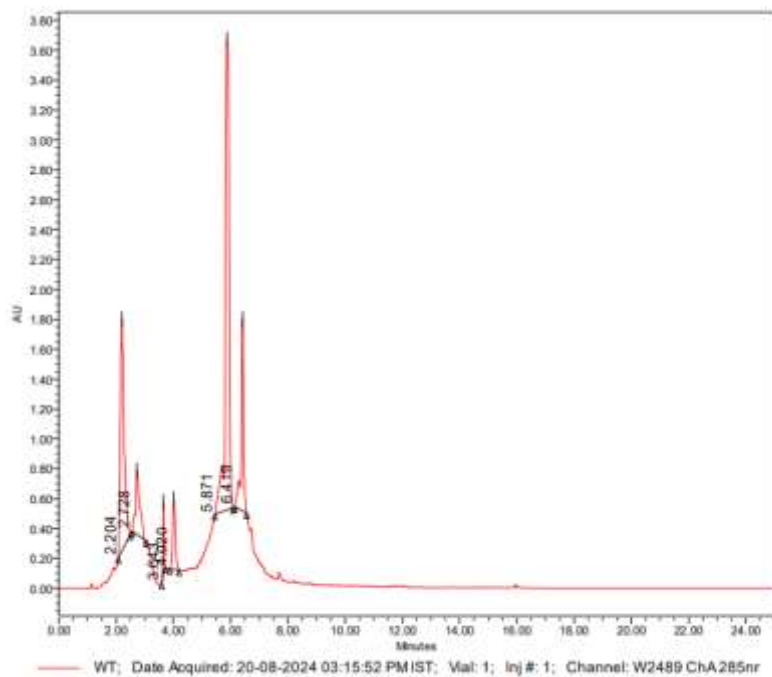


Figure 4.5.5.1b HPLC study of White tea

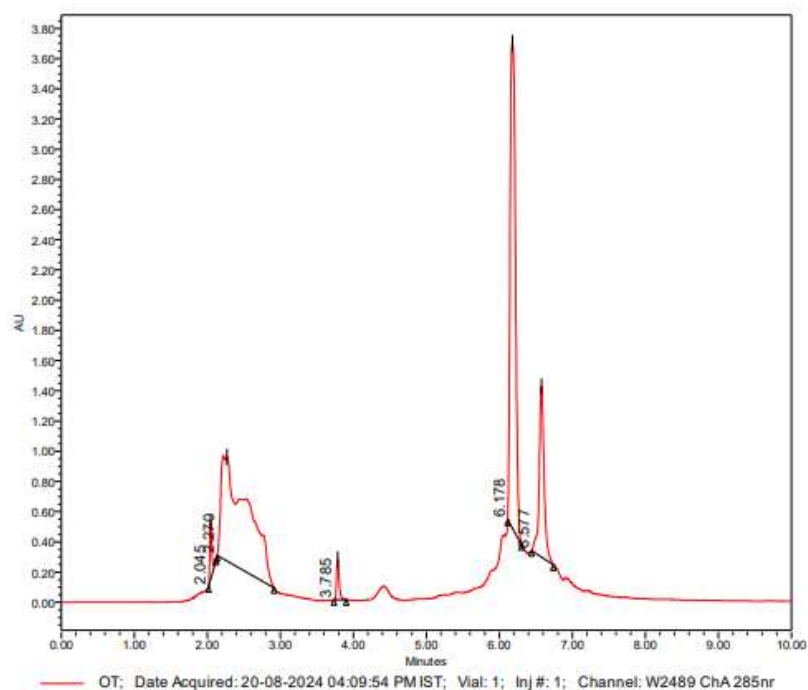


Figure 4.5.5.1 c HPLC study of Oolong Tea

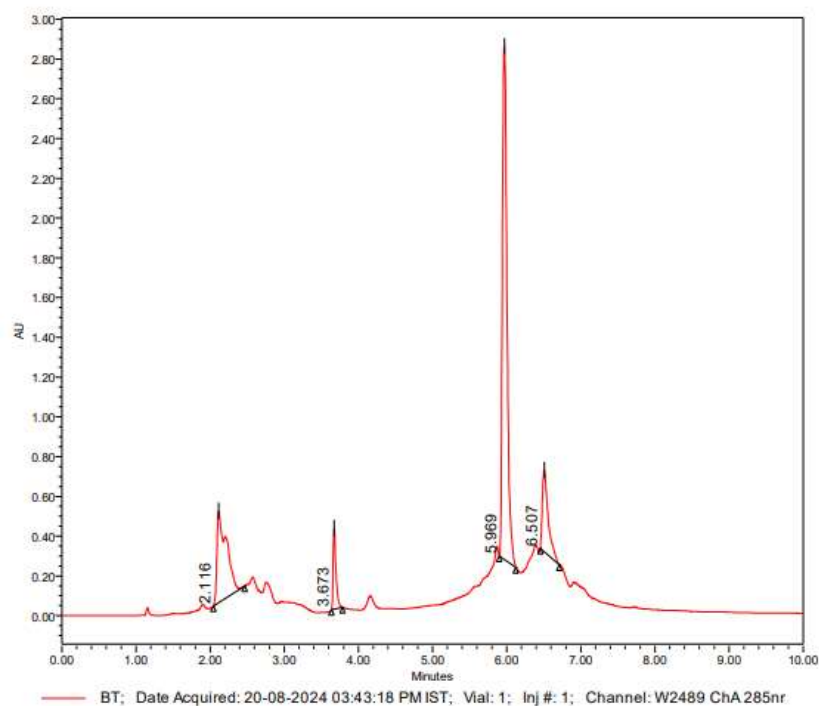


Figure 4.5.5.1 d HPLC study of Black Tea

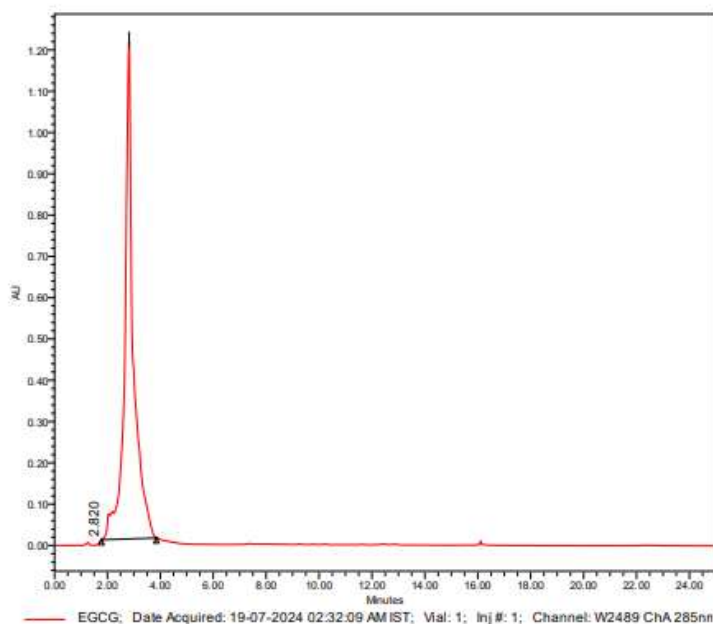


Figure 4.5.5.1 e: HPLC study of EGCG

Figure 4.5.5.1a shows the retention time of Green tea. The absorbance (AU) of the retention times of green tea for 1.952, 6.054, 6.526 minutes are 0.10, 0.05, 0.07 respectively.

Figure 4.5.5.1 b shows the retention time of White tea. The absorbance (AU) of the retention times of white tea for 2.204, 2.728, 3.643, 4.020, 5.871, 6.419 minutes are 0.20, 0.40, 0.001, 0.10, 0.50, 0.52 respectively.

Figure 4.5.5.1c shows the retention time of Oolong tea. The absorbance (AU) of the retention times of oolong tea for 2.045, 2.270, 3.785, 6.178, 6.577 minutes are 0.10, 0.25, 0.10, 0.50, 0.45 respectively.

Figure 4.5.5.1d shows the retention time of black tea. The absorbance (AU) of the retention times of black tea for 2.116, 3.673, 5.969, 6.507 minutes are 0.50, 0.40, 2.80, 0.80, respectively.

Figure 4.5.5.1e shows the retention time of EGCG. The absorbance (AU) of the retention times of EGCG for 2.820 minutes is 0.05. The concentration is 1mg/ml.

SAMPLE	RETENTION TIME (Min)	ABSORBANCE AU	CONCENTRATION mg/ml
RUTIN	7.414	0.095	1
	2.380	0.006	
	6.500	0.010	
QUERCETIN	1.290	0.001	1
EGCG	2.820	0.05	1
	1.734	0.002	
	1.907	0.008	
	1.235	0.0075	
	3.456	0.011	
GT	1.952	0.10	
	6.054	0.05	
	6.526	0.07	
WT	2.204	0.20	
	2.728	0.40	
	3.643	0.001	
	4.020	0.10	
	5.871	0.50	
	6.419	0.52	
OT	2.045	0.10	
	2.270	0.25	
	3.785	0.10	
	6.178	0.50	
	6.577	0.45	
BT	2.116	0.50	
	3.673	0.40	
	5.969	2.80	
	6.507	0.80	

Table 4.5.28a shows the retention time of Standards and different types of tea.

The figures easily show that the retention peaks of EGCG almost match those of the different varieties of manufactured teas.

Experiment 4.5.6: Polyphenol Oxidase Activity of manufactured tea.

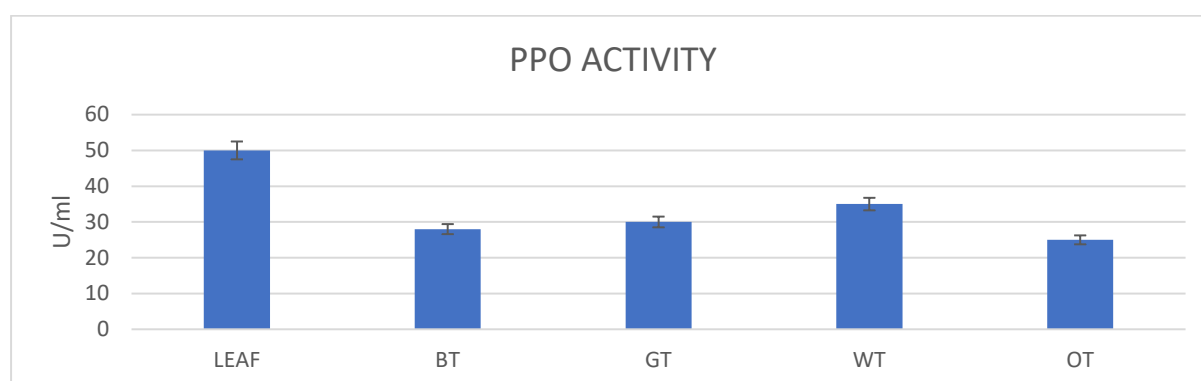


Figure 4.5.6.1: shows the variation in Polyphenol Oxidase activity in different types of manufactured tea. S.D=0.0001

Polyphenol oxidase activity is highest in case of fresh leaf. Fresh leaf shows the highest Polyphenol Oxidase activity of about 50 ± 0.0001 U/ml, followed by White tea 35 ± 0.0001 U/ml, Green Tea 30 ± 0.0001 U/ml, Black tea 28 ± 0.0001 U/ml and Oolong tea 25 ± 0.0001 U/ml. Dashtarzhaneh et al., 2024 have studied about functional roles of polyphenol oxidase enzyme in plants and fungi.

Experiment 4.5.7: Total polyphenol content of manufactured tea

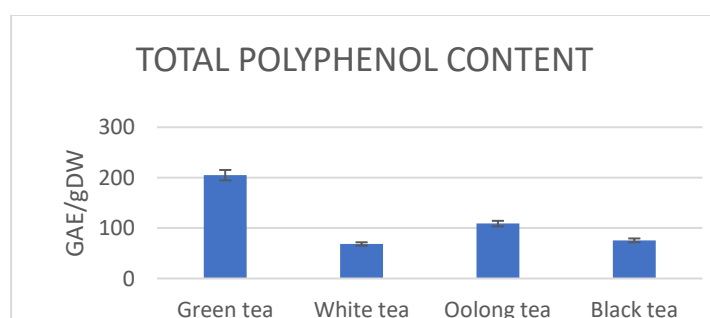


Fig 4.5.7.1: Total polyphenol content of different types of manufactured tea. S.D=0.01

Fig 4.5.7.1 shows the highest total polyphenols in green tea (205 ± 0.01 mg GAE/gDW), then Oolong tea (108.91 ± 0.01 mg GAE/gDW), black tea (75.66 ± 0.01 mg GAE/gDW) and at last white tea (68.38 ± 0.01 mg GAE/gDW). Cleverdon et al., 2018 have also shown comparative study of different levels of polyphenol contents in different types of tea, such as Rooibos tea, green tea black tea, and chamomile tea.

Experiment 4.5.8.: Total Rutin Content of the manufactured leaf

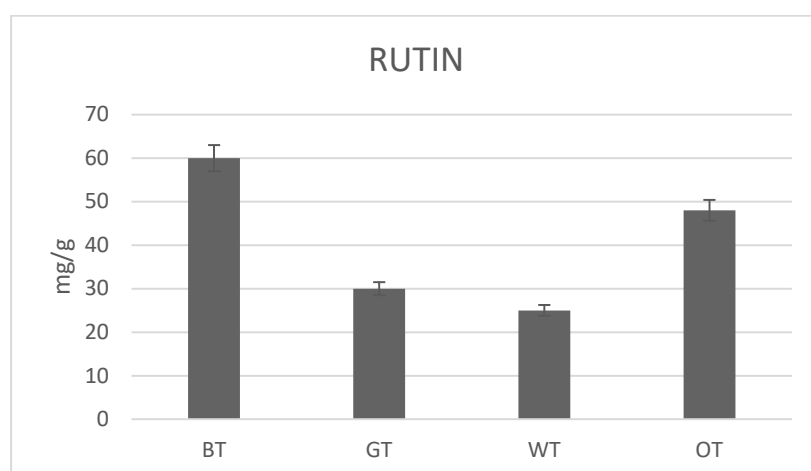


Figure 4.5.8.1 Total Rutin content of manufactured tea.S.D=0.01 Fig 4.3.25.36 shows the highest Rutin in black tea ($60 \pm 0.01 \text{ mg/g}$), then Oolong tea ($49 \pm 0.01 \text{ mg/g}$), green tea ($30 \pm 0.01 \text{ mg/g}$) and at last white tea ($24.3 \pm 0.01 \text{ mg/g}$). Atanassova et al.,2009 discussed about different levels of rutin content in different plants.

Experiment 4.5.9: Total Quercetin Content of manufactured leaf

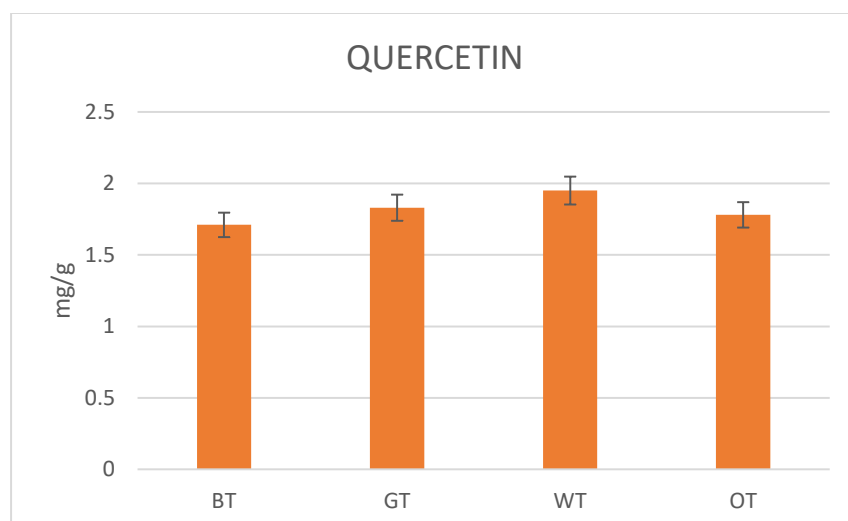


Figure 4.5.9.1:Total Quercetin content of manufactured tea.S.D=0.01

Fig 4.5.9.1 shows the highest Quercetin content in white tea ($1.9 \pm 0.01 \text{ mg/g}$), then green tea ($1.7 \pm 0.01 \text{ mg/g}$), oolong tea ($1.8 \pm 0.01 \text{ mg/g}$) and at last black tea ($1.6 \pm 0.01 \text{ mg/g}$). Aghababaei et al.,2023 have studied the health benefits of Quercetin.

Experiment 4.5.10: Total Catechin Content of manufactured leaf

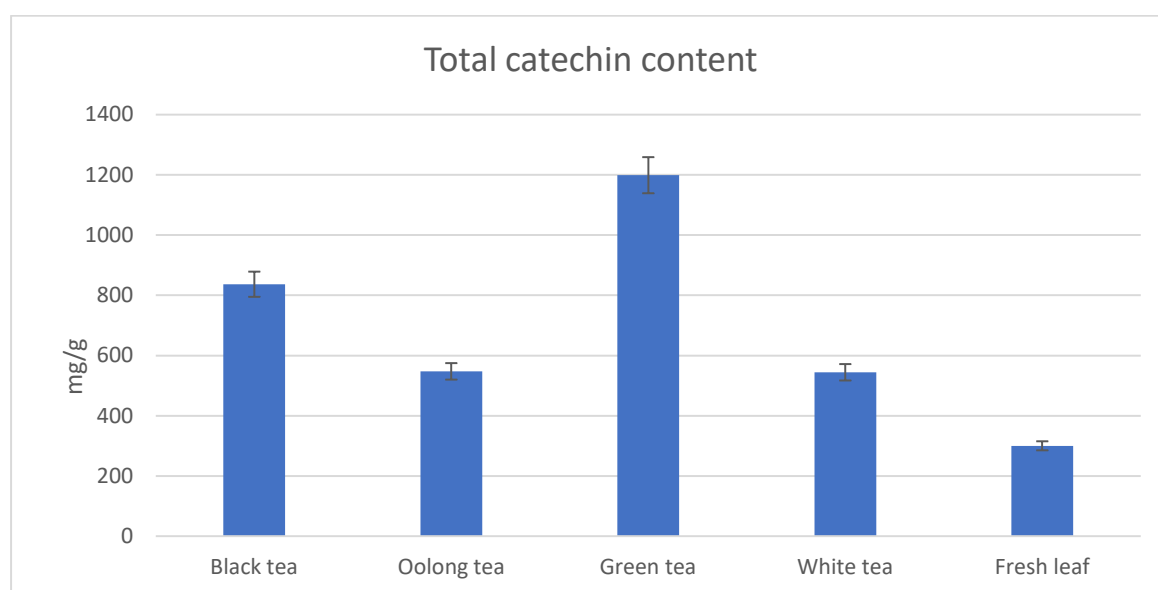


Figure 4..5.10.1: Total Catechin content of manufactured tea.S.D=0.05

Fig 4.5.10.1 shows highest catechin content in green tea ($1200 \pm 0.05\%$ mg/g) and lowest in fresh tea ($400 \pm 0.05\%$ mg/g).

Green tea is followed by black tea ($800 \pm 0.05\%$ mg/g), oolong tea ($547.3662 \pm 0.05\%$ mg/g), white tea ($544.3301 \pm 0.05\%$ mg/g) and fresh tea. Meyer et al.,2023 studied the catechin contents in commercially-available bagged, Gunpowder, and Matcha Green Teas.

Experiment 4.5.11: TLC of manufactured tea



Figure 4.5.11.1a Thin-layer Chromatography of manufactured tea with Iron Chloride-Ethanol reagent coloring agent and with standard Quercetin.

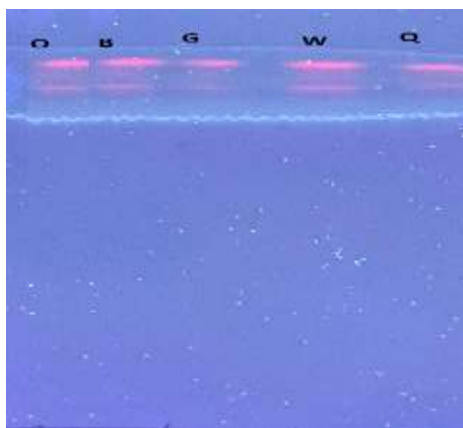


Figure 4.5.11.1b . Thin-layer Chromatography of manufactured tea with Iron Chloride-Ethanol reagent coloring agent with standard Quercetin under 254nm.

Kumar et al., (2014) have also studied the comparisons of different metabolites in various types of branded tea.

Samples	TLC Plate	R_f-I(cm)	R_f-II
Quercetin	Colour agent	0.81	
White Tea		0.81	
Green Tea		0.81	
Oolong Tea		0.81	
Black Tea		0.80	
Quercetin	UV 254	0.89	
White Tea		0.89	0.92
Green Tea		0.89	0.92
Oolong Tea		0.89	0.92
Black Tea		0.89	0.92
Quercetin	UV 366	0.44	
White Tea		0.44	
Green Tea		0.44	
Oolong Tea		0.44	
Black Tea		0.44	

Table 4.5.28b. R_f value table.

4.5.11.1 shows the R_f value of different types of tea and the standard Quercetin. The R_f factor of Quercetin, White tea, green tea and oolong tea are same that is 0.81cm. The R_f factors of

Quercetin, White tea, green tea and oolong tea are same that is 0.89cm and 0.92 at short UV wavelength whereas at long UV wavelength the R_f values are same that is 0.44.

Table 4.3. 20 a and Fig 4.5.11.1a and b show that green tea shows higher quercetin content and R_f values indicate that all the characteristic bands are present in all the tea varieties.

LANE	VOLUME%	VOLUME	HEIGHT	AREA
A Quercetin	100	94329607	56075	3036
B Black tea	24.393	23009627	55945	660
C Oolong tea	76.134	71816654	56145	2295
D Green tea	153.896	145169246	57321	3480
E White tea	29.468	27796946	58006	504

Table 4.5.29 Densitometric scan

Table 4.3.20 b shows that the catechin contents are highest in Green tea compared with the volume % of the standard epigallocatechin gallate with colorimetric spot analysis. The volume %, volume, height, and area of Quercetin are 100,94329607,56075 and 3036 The volume %, volume, height, and area of Black tea are 24.393,23009627,55945 and 660. The volume %, volume, height, and area of Oolong tea are 76.134,71816654,56145 and 2295. The volume %, volume, height, and area of green tea are 153.896,145169246,57321 and 3480. The volume %, volume, height, and area of white tea tea29.468,27796946,58006 and 504.

Experiment 4.5.12: Antioxidant assay

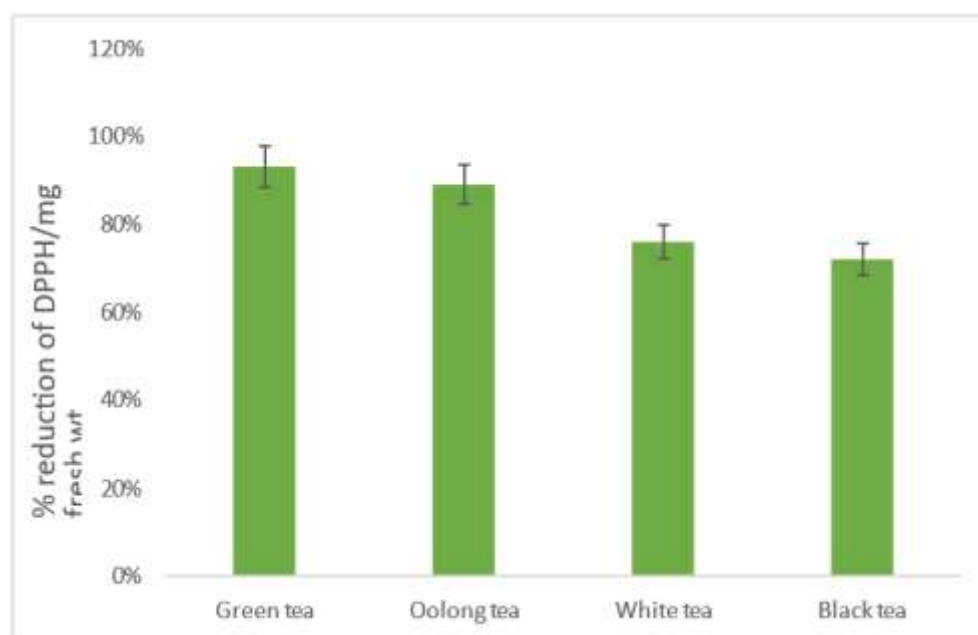


Figure 4.5.12.1: Antioxidant Potential of Different kinds of tea. S.D=0.001

The highest antioxidant potential is observed with green tea $92.5 \pm 0.001\%$ reduction of DPPH/mg fresh wt.

Experiment 4.5.13.: Antimicrobial assay

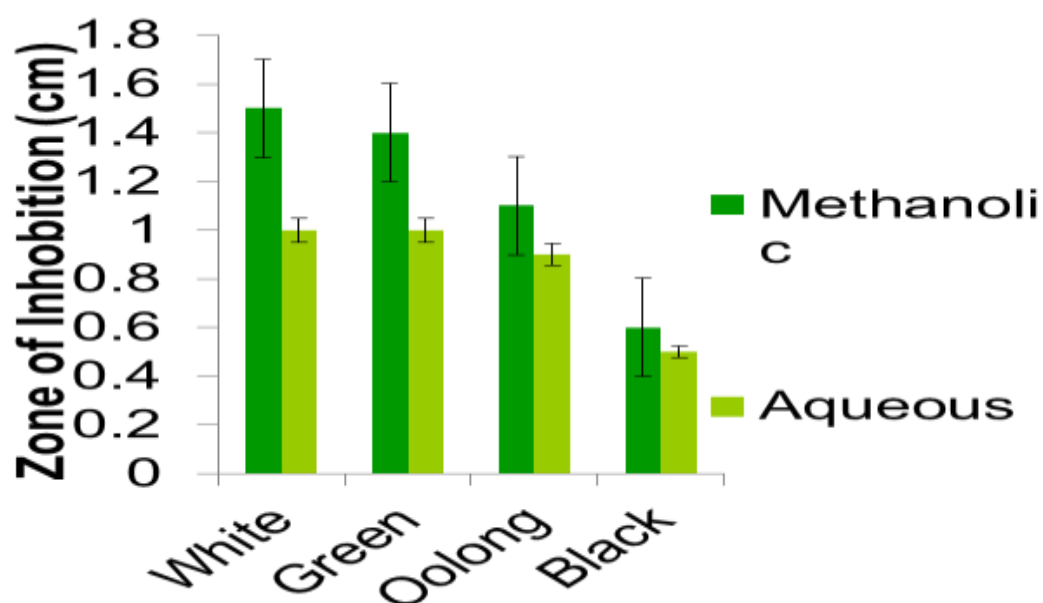


Figure 4.5.13.1a shows the antimicrobial activities of different tea extracts (Both methanolic and aqueous) against *E.coli* S.D=0.01

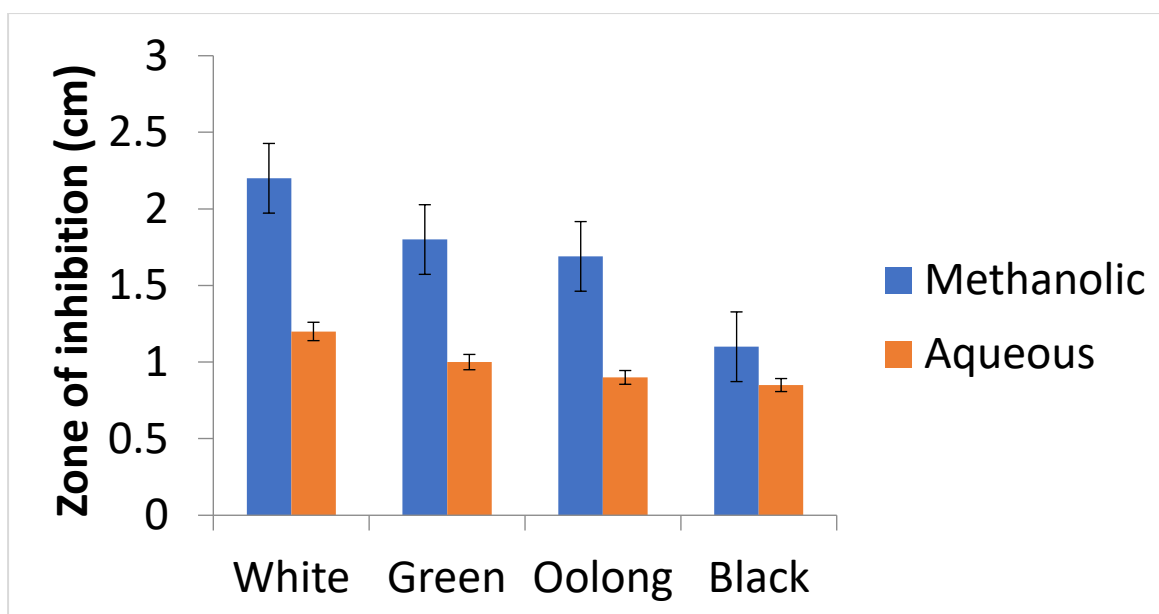


Figure 4.5.13.1b show the antimicrobial activities of different tea extracts (Both methanolic and aqueous) against *B. subtilis*. *S.D*=0.01

Methanolic extracts show a higher zone of inhibition than the aqueous extracts in various types of tea.

White tea in methanolic and aqueous extract shows 1.5 ± 0.01 cm and 1 ± 0.01 cm zone of inhibition against *E.coli*. Green tea in methanolic and aqueous extract shows 1.4 ± 0.01 cm and 1 ± 0.01 cm zone of inhibition against *E.coli*. Oolong tea in methanolic and aqueous extract shows 1.1 ± 0.01 cm and 0.9 ± 0.01 cm zone of inhibition against *E.coli*. Black tea in methanolic and aqueous extracts shows 0.6 ± 0.01 cm and 0.5 ± 0.01 cm zone of inhibition against *E.coli*.

White tea in methanolic and aqueous extract shows 2.2 ± 0.01 cm and 1.2 ± 0.01 cm zone of inhibition against *Bacillus* sp. Green tea in methanolic and aqueous extract shows 1.8 ± 0.01 cm and 1.0 ± 0.01 cm zone of inhibition against *Bacillus* sp. Oolong tea in methanolic and aqueous extracts shows 1.69 ± 0.01 cm and 0.9 ± 0.01 cm zone of inhibition against *Bacillus* sp. Black tea in methanolic and aqueous extracts shows 1.1 ± 0.01 cm and 0.85 ± 0.01 cm zone of inhibition against *Bacillus* sp.

Experiment 4.5.14. :Antifungal assay



Figure 4.5.14.1a shows the antifungal activity of different types of leaves in 1st day.S.D=0.0001

Sl.No.	Tea	Well Diameter	Control plate(mm)	Growth Inhibition(%)
1.	Oolong	7	60	90±0.0001
2.	Black	7	60	81.6±0.0001
3.	White	7	60	91.6±0.0001
4.	Green	7	60	86.6±0.0001
5.	Fresh	7	60	83.3±0.0001

Table 4.5.30.a shows that the growth inhibition

It is about 91.6±0.0001% in the case of White tea. Oolong tea shows 90±0.0001% growth inhibition, Black tea shows 81.6±0.0001% growth inhibition, Green tea shows 86.6±0.0001% growth inhibition, and fresh tea shows 83.3±0.0001% growth inhibition.

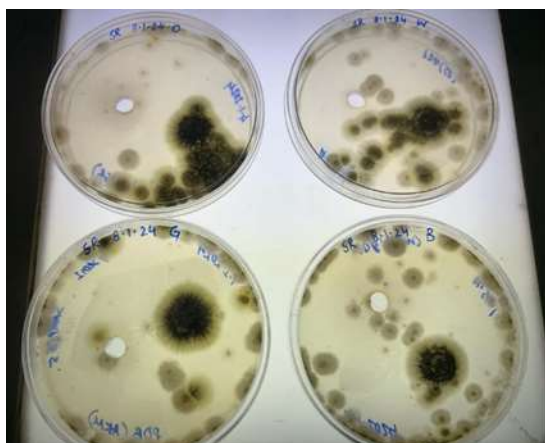


Figure 4.5.14.1 b shows the antifungal activity of different types of leaves on the 2nd day.

Sl.No.	Tea	Well Diameter	Control plate(mm)	Growth Inhibition(%)
1.	Oolong	7	80	50±0.0001
2.	Black	7	80	30±0.0001
3.	White	7	80	40±0.0001
4.	Green	7	80	50±0.0001
5.	Fresh	7	80	25±0.0001

Table 4.5.30b shows that the growth inhibition S.D=0.0001

It is about 40±0.0001% in the case of White tea, which is decreased from the 1st day. There is a decrease in growth inhibition in each of the manufactured teas. Oolong tea shows 50±0.0001% growth inhibition, Black tea shows 30±0.0001% growth inhibition, Green tea shows 50% growth inhibition, and fresh tea show 25±0.0001% growth inhibition.



Figure 4.5.14.1c shows the antifungal activity of different types of leaves on the 3rd day.S.D=0.0001

Sl.No.	Tea	Well Diameter	Control plate(mm)	Growth Inhibition(%)
1.	Oolong	7	90	5±0.0001
2.	Black	7	90	7±0.0001
3.	White	7	90	5.5±0.0001
4.	Green	7	90	5±0.0001
5.	Fresh	7	90	6±0.0001

Table 4.5.30c shows that the growth inhibition

It is about 7±0.0001% in case of White tea which is decreased from the 2nd day. There is a decrease in growth inhibition in each of the manufactured tea. Oolong tea shows 5±0.0001% growth inhibition, Black tea shows 7±0.0001% growth inhibition, White tea shows 5.5 % growth inhibition Green tea shows 5±0.0001% growth inhibition and fresh tea show 6±0.0001% growth inhibition. Shivakumar et al., 2023 documented antifungal activities of tea against *Candida albicans*.

Experiment4.5.15: Anticancer assay

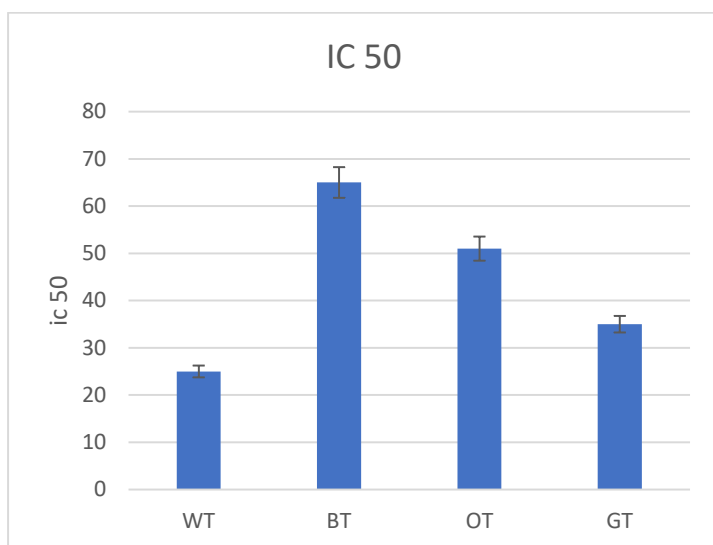


Figure 4.5.15.1 shows IC₅₀ of different types of tea.S.D=0.001

White tea shows least IC₅₀ than other tea. The low IC₅₀ indicates the high potential of the drug.IC₅₀ of Black tea, White tea, Oolong tea and Green tea are 65 ± 0.001 , 25 ± 0.001 , 51 ± 0.001 and 35 ± 0.001 respectively. The IC₅₀ assay interprets a low IC₅₀ in high altitude green tea 2nd flush. Low IC₅₀ indicates high potential of the drug. In another study, IC₅₀ is the least in the case of white tea.

Experiment4.5.16 : Antidiabetic action of different tea in in vitro by lowering serum glucose

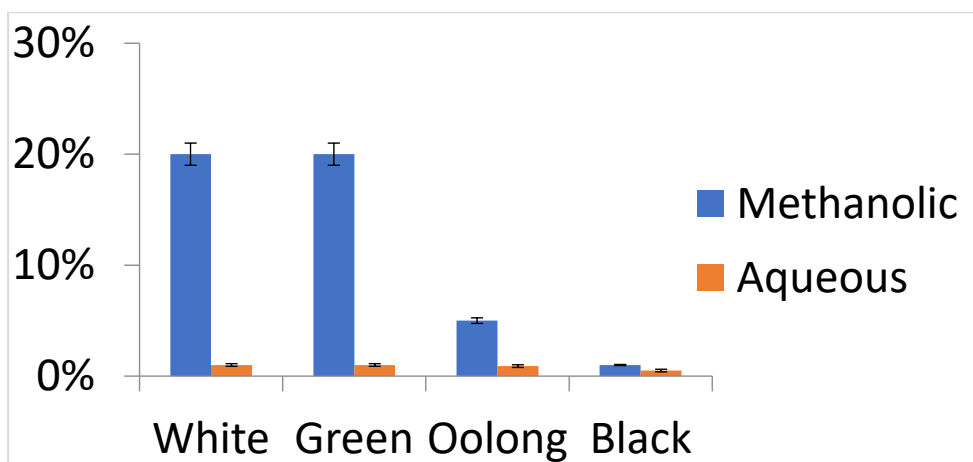


Fig 4.5.16.1shows in methanolic extracts the lowering of blood glucose is higher than the aqueous extracts.S.D=0.001

The percent inhibition of glucose in white tea in methanolic and aqueous are about 20 ± 0.001 and 1 ± 0.001 , respectively. % inhibition of glucose in green tea in methanolic and aqueous are about 20 ± 0.001 and 1 ± 0.001 , respectively. % inhibition of glucose in oolong tea in methanolic and aqueous extracts are about 5 ± 0.001 and 1 ± 0.001 , respectively. % inhibition of glucose in black tea in methanolic and aqueous are about 1 ± 0.001 and 1 ± 0.001 , respectively.

White tea shows the highest lowering of blood glucose level in methanolic solution than in the aqueous solution.

Experiment 4.5.17.: Sun protection Factor assay

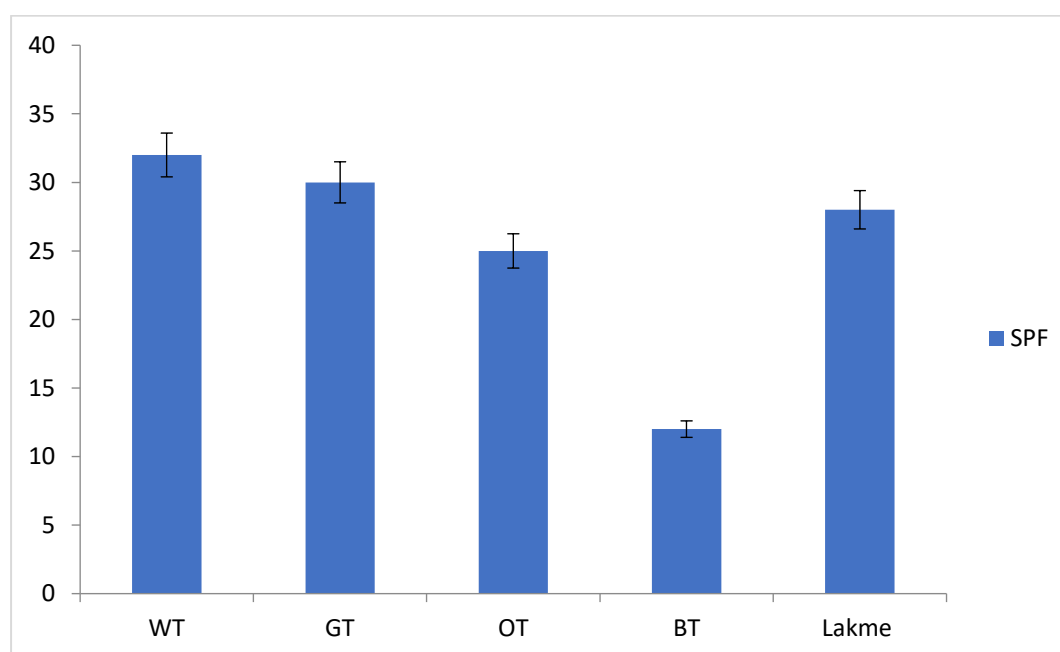


Figure 4.5.17.1 shows SPF data of different types of tea with lakme taken as standard. S.D=0.1

Fig shows White tea is showing highest SPF than other tea (32 ± 0.1).

Experiment 4.5.18: Semi-purification of the active components of different types of manufactured tea by different extraction methods and by different purification methods:

8gm of White ,Green,Oolong and Black tea before extraction was taken and the methanol added to it was 100 ml for maceration. After extraction in a rotary evaporator weight of the samples(white,Green,Oolong and Black tea) become 0.29 g,0.24g,0.24g,0.25g respectively. So the recovery percentage by rotary evaporation is 3.62%,3%, 3%, and 3.12% respectively and by Lyophilization the same starting material gives 0.18g,0.15g,0.19 g and 0.11g respectively so the recovery percentage is 2.25%,1.8%,2.37% and 1.37 % respectively.

In all cases, the recovery percentage of the rotary evaporator is higher than lyophilization.

Experiment 4.5.19 Antimicrobial Potential

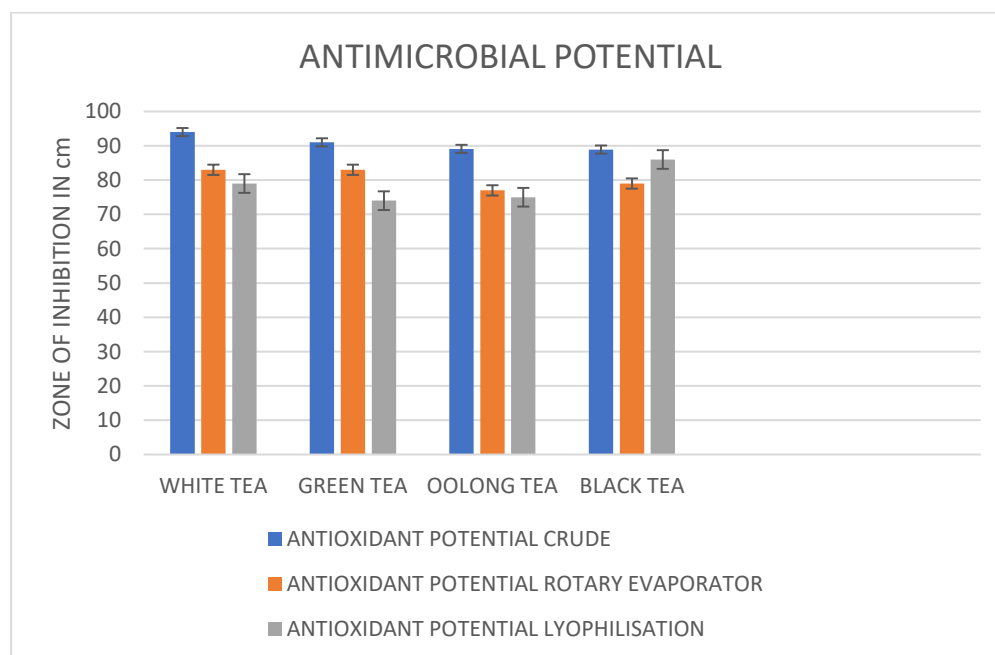


Figure 4.5.18.1 Antimicrobial potential of differentially semi purified manufactured tea.S.D=0.01

Fig shows the higher antimicrobial potential (1.2 ± 0.01 cm zone of inhibition in case of green tea) in rotary evaporation semi purified manufactured tea than the lyophilization process.

Experiment 4.5.20 Antioxidant Potential

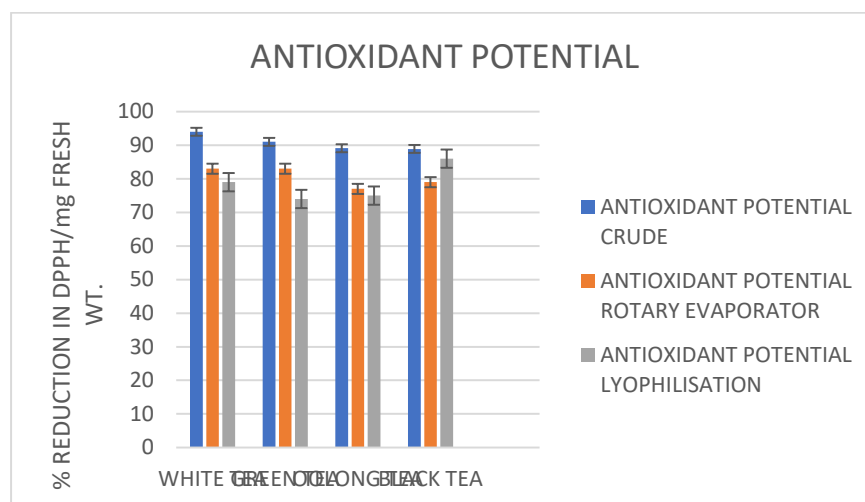


Figure 4.5.19.1 Antioxidant potential of differentially semi purified manufactured tea

.S.D=0.1

Fig shows the highest antioxidant potentials of macerated crude extracts of White tea ($94 \pm 0.1\%$ DPPH reduction/mg fresh wt.), but rotary evaporation shows higher antioxidant potential $83 \pm 0.5\%$ than lyophilisation ($79 \pm 0.5\%$ DPPH reduction/mg fresh wt) of the same tea.

Experiment 4.5.21: Measurement of ambient UV radiation for 18 months in Kolkata

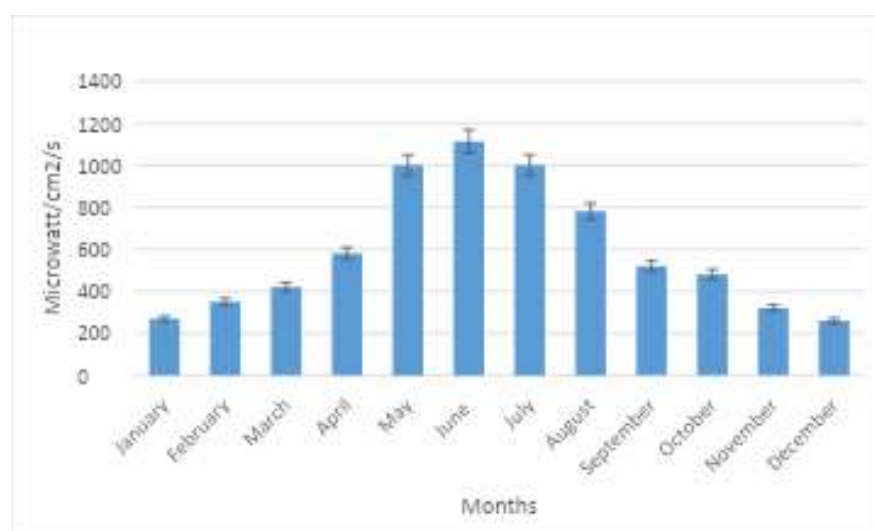


Figure 4.5.20.1 Ambient natural UV irradiance throughout the year in Kolkata: In which the different types of manufactured tea were kept.

Experiment 4.5.22: Packaging of different manufactured tea (White, Green, Oolong and Black):

The packaging were done with aluminium and silica package to assess shelf life for 18 months (best before use). All the biochemical and therapeutic parameters are measured whose methods are already mentioned.



Figure 4.5.21.1 Different tea samples packaged to assess shelf life

Experiment 4.5.23.: Moisture Content assay

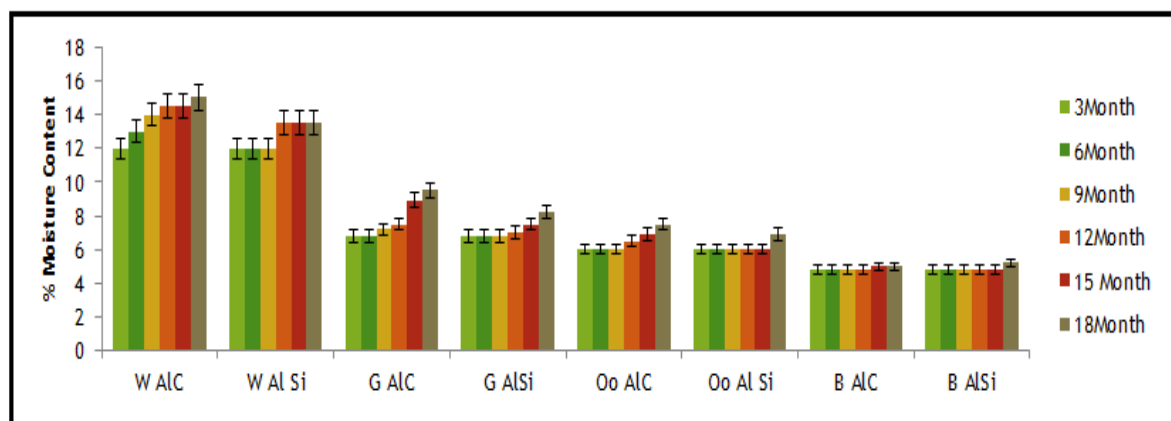


Figure 4.5.22.1 shows the percentage decrease in moisture content for different types of tea stored in Aluminium packages with Silica desiccants.

The percentage decrease in moisture content is least in Black tea with Aluminium and Silica packaging.

Experiment 4.5.24: Ash Content

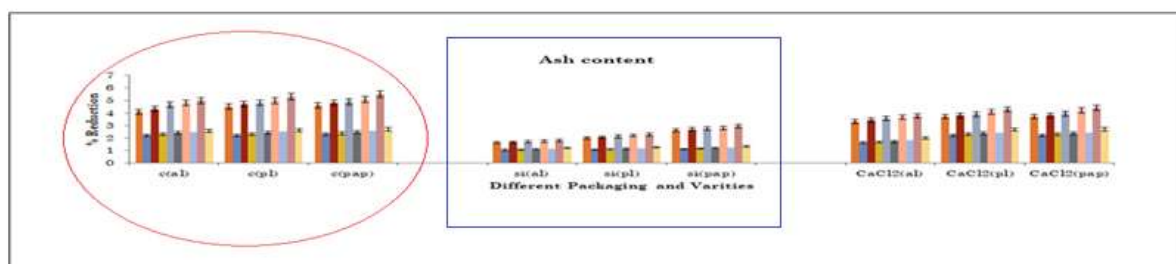


Figure 4.5.23.1 shows % decrease in ash content in different types of tea stored in different packages with different dessicants.

%decrease in ash content is least in Black tea with Aluminium and Silica packaging.

Experiment 4.5.25: % Change in amino acid and crude fibre content in different types of tea stored in different packages with different dessicants.

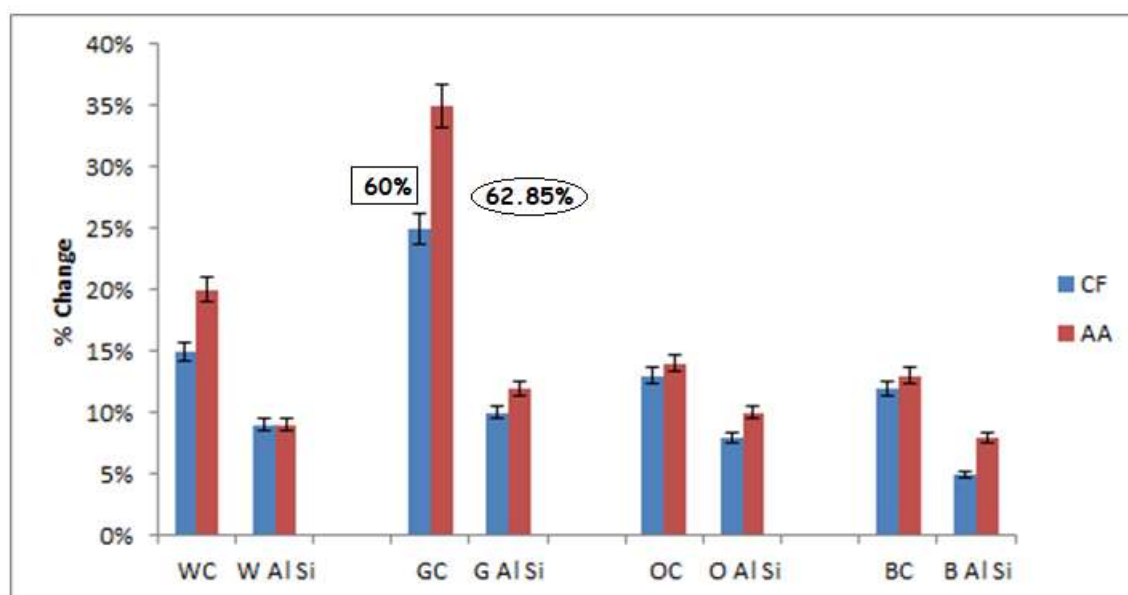


Figure 4.5.24.1 shows % change in amino acid and crude fibre content in different types of tea stored in Aluminium packages with silica dessicants.

% change in amino acid and crude fibre content is least in Black tea with Aluminium and Silica packaging.

Experiment 4.5.26: % change in catechin content in different types of tea stored in Aluminium packaging with Silica desiccants.

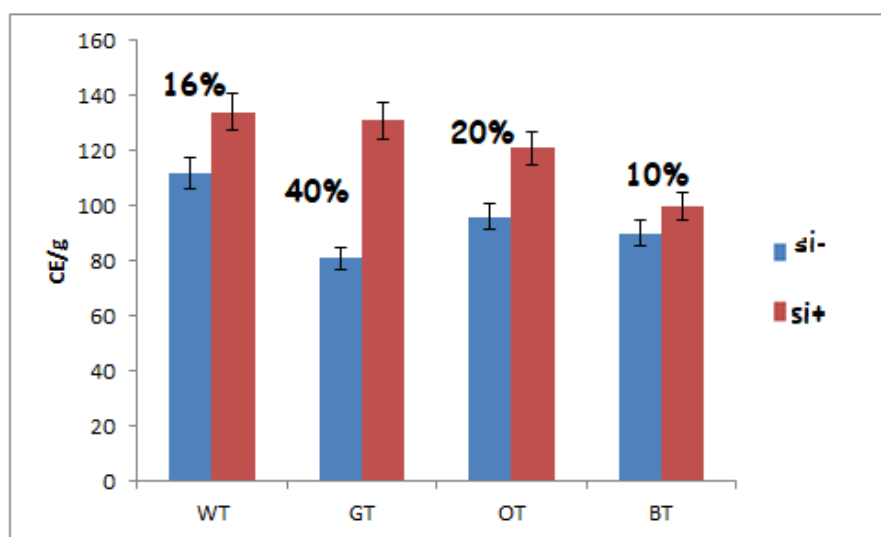


Figure 4.5.25.1 shows % reduction in catechin content in different types of tea stored in various packages with different dessicants. S.D=0.01

%change in catechin content is least in Black tea with Aluminium and Silica packaging, which is about 10%±0.01.

Experiment 4.5.27: % change in Flavonoid content in different types of tea stored in different packages with different dessicants.

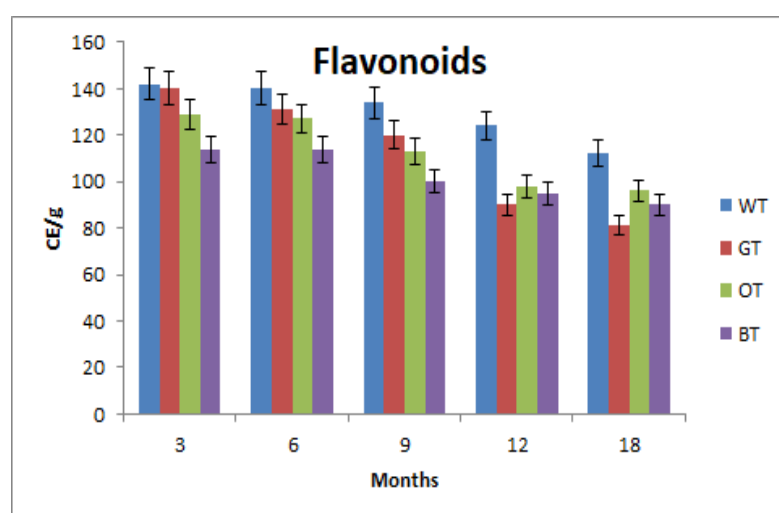


Figure 4.5.26.1 shows % reduction in flavonoid content in different types of tea stored in different packages with different dessicants.

%change in flavonoid content is least in Black tea with Aluminium and Silica packaging.

Experiment 4.5.28:% change in polyphenol content in different types of tea stored in different packages with different dessicants

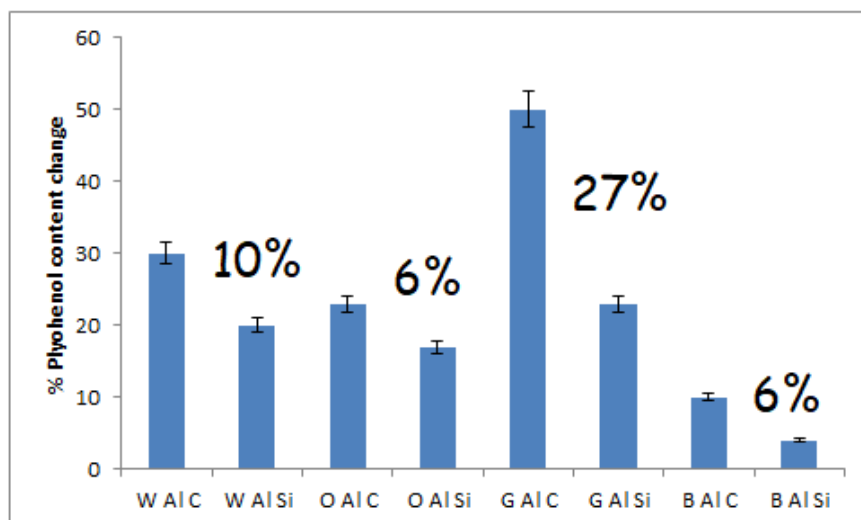


Figure 4.5.27.1 shows % reduction in polyphenol content in different types of tea stored in various packages with different dessicants.

%change in polyphenol content is least in Black tea with Aluminium and Silica packaging.

Experiment 4.5.29:% change in antioxidant potential in different types of tea stored in different packages with different dessicants

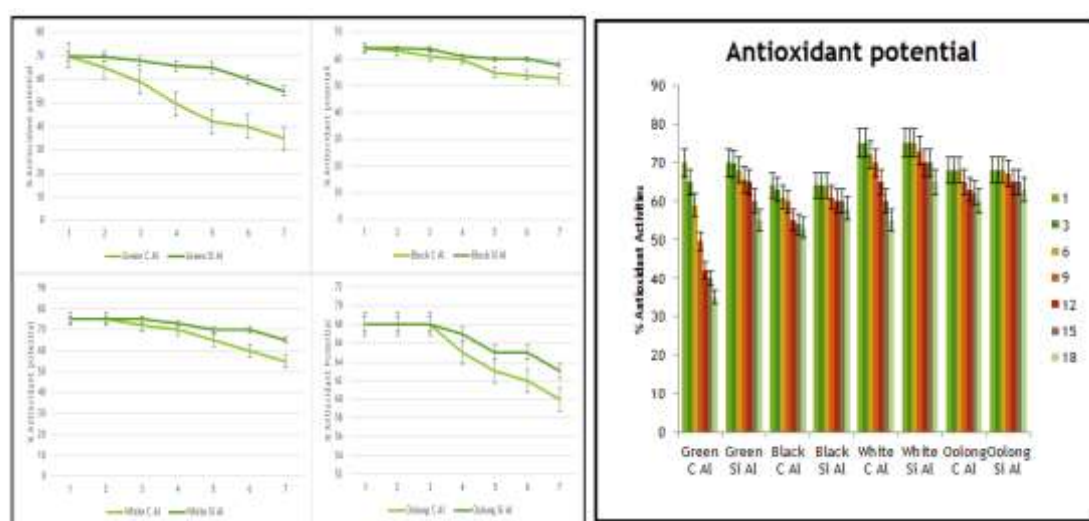


Figure 4.5 .28.1a and 4.5.29.1b shows % reduction in antioxidant potential in different types of tea stored in different packages with different dessicants.

% reduction in antioxidant potential is least in Black tea with Aluminium and Silica packaging.

BLACK TEA	14 months
GREEN TEA	12 months
WHITE TEA	9 months
OOLONG TEA	11 months

Table 4.5.31: Shelf life of different types of tea.

Aluminium and silica packaging is the best for all tea varieties for storage to increase shelf life-therapeutic/nutritional values-market values will remain almost same by combating the natural ambient UV radiation in Kolkata.

OBJECTIVE 5

4.6. Characterization of therapeutic values (antioxidant and antimicrobial). herbal and medicinal tea

Experiment 4.6: Therapeutic values (antioxidant and antimicrobial). herbal and medicinal tea .

Experiment 4.6.1: Antimicrobial Activity

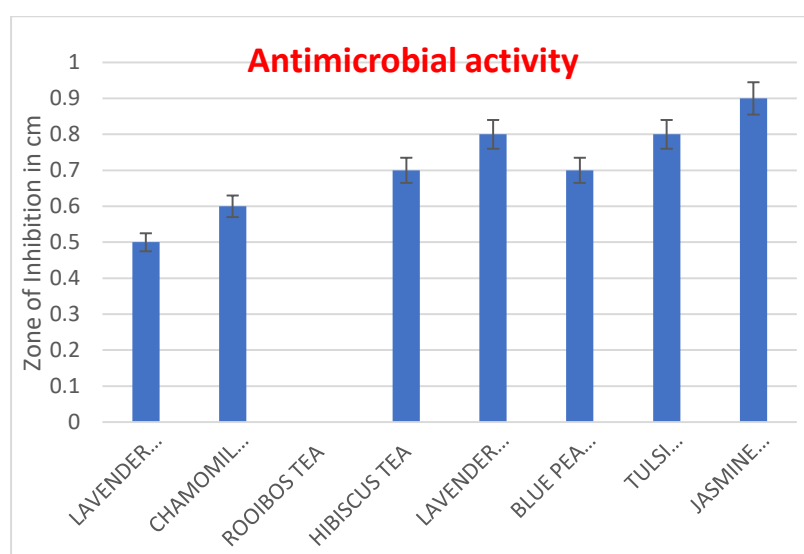


Figure 4.6.1.1 shows antimicrobial potential of herbal tea.S.D=0.001

Jasmine Hibiscus Green tea is showing highest zone of inhibition i.e. 0.9 cm against *E.coli*. The zone of inhibition of Lavender tea ,Chamomile tea ,Rooibos tea, Hibiscus tea, lavender chamomile white tea ,blue pea chamomile tea , Tulsi green tea and jasmine hibiscus green tea are $0.5\pm0.001\text{cm}$, $0.6\pm0.001\text{cm}$, $0.7\pm0.001\text{cm}$, $0.8\pm0.001\text{cm}$, $0.7\pm0.001\text{cm}$, $0.8\pm0.001\text{cm}$ and $0.9\pm0.001\text{cm}$ respectively.

Experiment 4.6.2 Antioxidant Potential

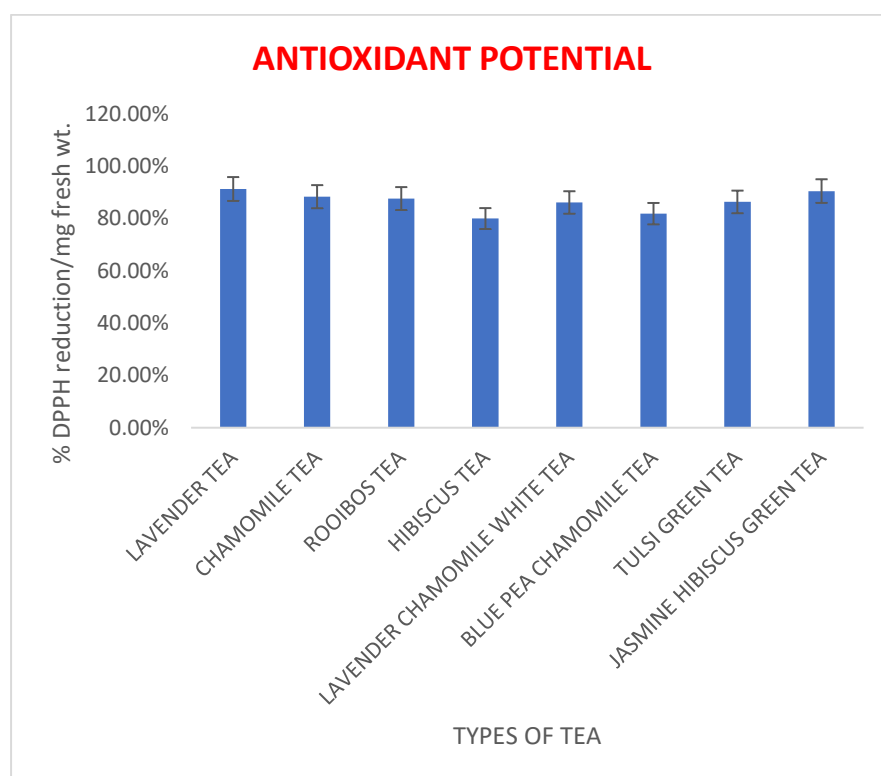


Figure 4.6.2.1 shows the antioxidant potential of herbal tea.S.D=0.1

Jasmine Hibiscus Green tea is showing the highest antioxidant potential. The antioxidant potential of Lavender tea, Chamomile tea, Rooibos tea, Hibiscus tea, lavender chamomile white tea, blue tea chamomile tea, Tulsi green tea and jasmine hibiscus green tea are $91.24\pm0.1\%$, $88.30\pm0.1\%$, $87.59\pm0.1\%$, $79.94\pm0.1\%$, $86.08\pm0.1\%$, $81.81\pm0.1\%$, $86.30\pm0.1\%$, $90.44\pm0.1\%$ respectively.

Fig 4.6.85 and 86 states that antimicrobial potential and antioxidant potential corroborate with each other.

Experiment 4.6.3: E-sensor-Based Analysis

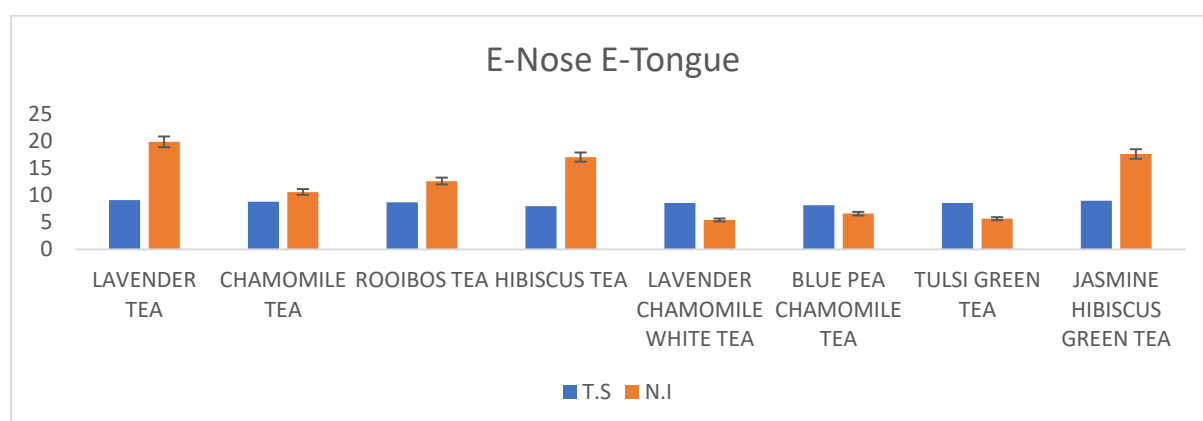


Fig 4.6.3.1 shows the Nor aroma index and taster's score of different types of herbal tea.

Jasmine Hibiscus Green tea is showing the highest Nor-aroma index and taster's score.

Experiment 4.6.2.1: Antioxidant potential of herbal teas and normal tea

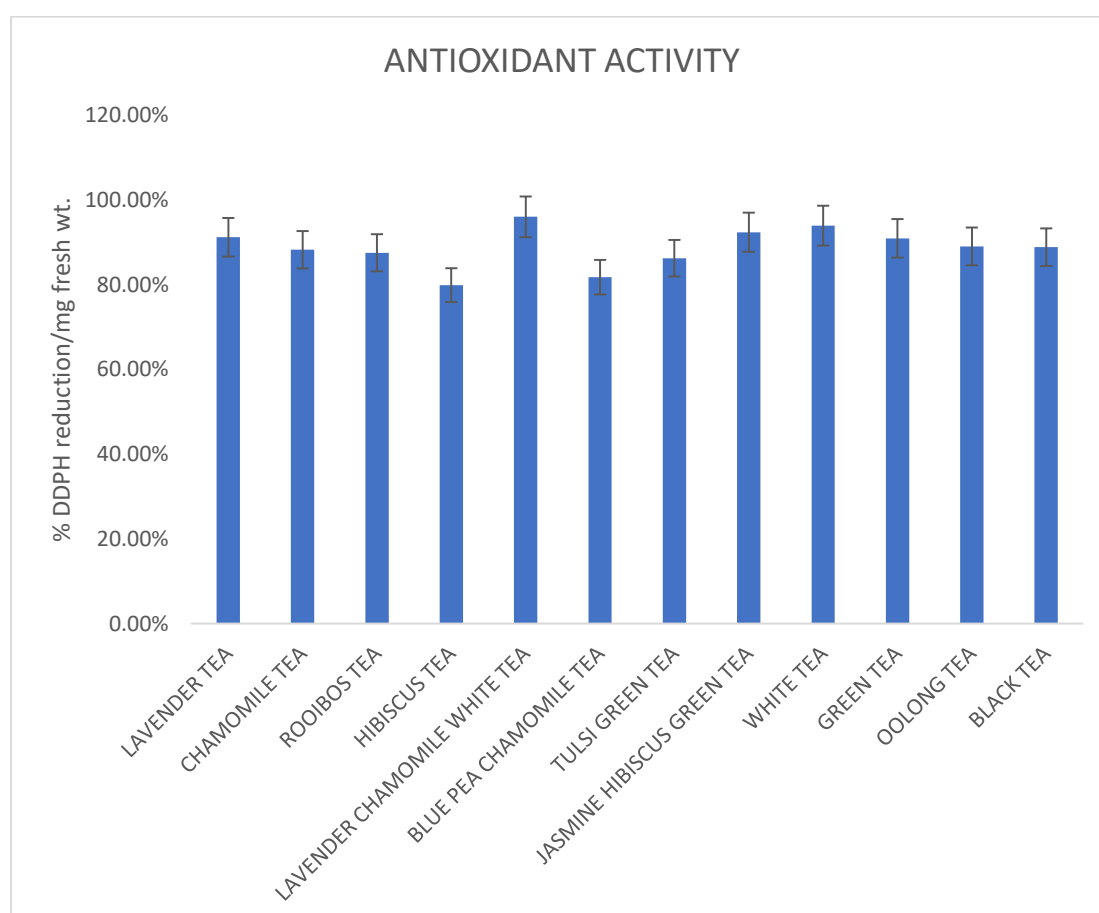


Figure 4.6.2.1.1 shows antioxidant potential of herbal teas and normal tea i.e. black tea, oolong tea, white tea and green tea. S.D=0.1

Antioxidant potential is highest for Lavender Chamomile white tea $96.08 \pm 1\%$, which is higher than white tea $94.0 \pm 1\%$. The antioxidant potential of Lavender tea, Chamomile tea, Rooibos tea, Hibiscus tea, Lavender chamomile white tea, Blue pea chamomile tea, Tulsi green tea, Jasmine hibiscus green tea, white tea, green tea, oolong tea and black tea are $91.24 \pm 1\%$, $88.30 \pm 1\%$, $87.59 \pm 1\%$, $79.94 \pm 1\%$, $96.08 \pm 1\%$, $81.81 \pm 1\%$, $86.30 \pm 1\%$, $92.44 \pm 1\%$, $94 \pm 1\%$, $91 \pm 1\%$, $89.10 \pm 1\%$, $88.90 \pm 1\%$ respectively.

Experiment 4.6.1.1: Antimicrobial activity of herbal teas and normal tea

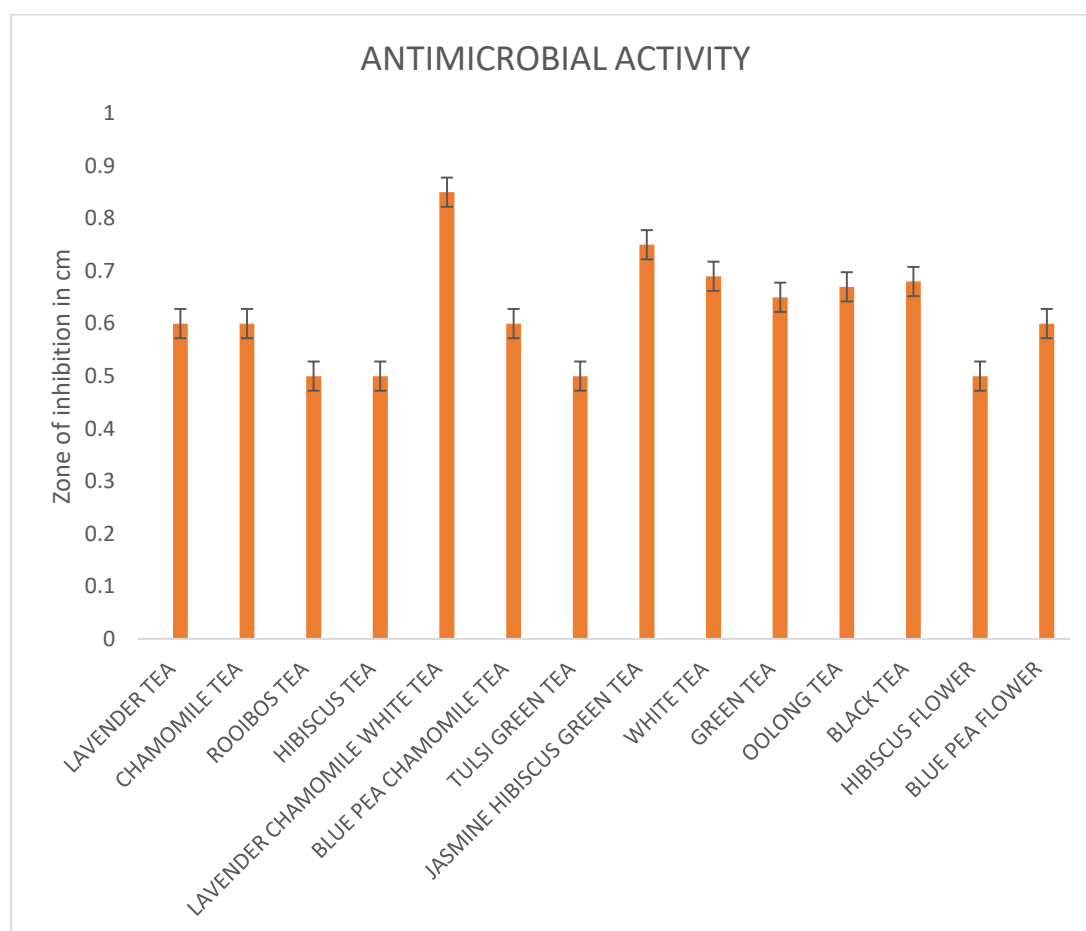


Figure 4.6.1.1.1 shows antimicrobial potential of herbal teas and normal tea i.e. black tea, oolong tea, white tea and green tea. S.D=0.01

Jasmine Hibiscus green tea is showing highest antimicrobial activities (zone of inhibition against *Streptococcus* sp. which is 0.9 ± 0.01 cm higher than normal green tea that is 0.8 ± 0.01 cm). Against *E.coli* Lavender chamomile white tea which is 0.85 ± 0.01 cm which is higher

than normal white tea which is 0.69 ± 0.01 cm. The antimicrobial potential of Lavender tea, Chamomile tea, Rooibos tea, Hibiscus tea, Lavender chamomile white tea, Blue pea chamomile tea, Tulsi green tea, Jasmine hibiscus green tea, white tea, green tea, oolong tea, black tea, hibiscus flower and blue pea flower are 0.6 ± 0.01 cm, 0.6 ± 0.01 cm, 0.5 ± 0.01 cm, 0.5 ± 0.01 cm, 0.85 ± 0.01 cm, 0.6 ± 0.01 cm, 0.5 ± 0.01 cm, 0.75 ± 0.01 cm, 0.69 ± 0.01 cm, 0.65 ± 0.01 cm, 0.67 ± 0.01 cm, 0.68 ± 0.01 cm, 0.5 ± 0.01 cm, 0.6 ± 0.01 cm respectively.

Experiment 4.6.3.1: E-sensor-Based Analysis

TEA SAMPLES	N.I
LAVENDER TEA	19.96 ± 0.02
CHAMOMILE TEA	10.66 ± 0.02
ROOIBOS TEA	12.68 ± 0.02
HIBISCUS TEA	17.13 ± 0.02
LAVENDER CHAMOMILE WHITE TEA	5.46 ± 0.02
BLUE PEA CHAMOMILE TEA	6.63 ± 0.02
TULSI GREEN TEA	5.7 ± 0.02
JASMINE HIBISCUS GREEN TEA	17.7 ± 0.02
WHITE TEA	16.5 ± 0.02
GREEN TEA	14.3 ± 0.02
OOLONG TEA	13.9 ± 0.02
BLACK TEA	12 ± 0.02

Figure 4.6.3.1a shows the Nor -aroma index of different types of herbal tea and normal tea. S.D=0.02

Jasmine Hibiscus Green tea has more nor -aroma index i.e. 17.7 than the normal green tea which is 14.3. The nor-aroma index of Lavender tea, chamomile tea, Rooibos tea, Hibiscus tea, Lavender chamomile tea, tulsi green tea, Jasmine hibiscus green tea, white tea, green tea, oolong tea and black tea are 19.96 ± 0.02 , 10.66 ± 0.02 , 12.68 ± 0.02 , 17.13 ± 0.02 , 5.46 ± 0.02 , 6.63 ± 0.02 , 5.7 ± 0.02 , 17.7 ± 0.02 , 16.5 ± 0.02 , 14.3 ± 0.02 , 13.9 ± 0.02 , 12 ± 0.02 respectively.

TEA TYPES	T.I
LAVENDER TEA	1.93±0.02
CHAMOMILE TEA	2±0.02
ROOIBOS TEA	2.16±0.02
HIBISCUS TEA	2.2±0.02
LAVENDER CHAMOMILE WHITE TEA	1.46±0.02
BLUE PEA CHAMOMILE TEA	1.73±0.02
TULSI GREEN TEA	1.6±0.02
JASMINE HIBISCUS GREEN TEA	1.76±0.02
WHITE TEA	1.39±0.02
GREEN TEA	1.29±0.02
OOLONG TEA	1.25±0.02
BLACK TEA	1.19±0.02

Figure 4.6.3.1.1b shows the Taster's score of different types of herbal tea and normal tea.S.D=0.02

Jasmine Hibiscus Green tea has more taster's score i.e. 1.76 than the normal green tea which is 1.29. Again Lavender Chamomile white tea is having more taster's score than the normal white tea. The taster's score of Lavender tea, chamomile tea, Rooibos tea, Hibiscus tea, Lavender chamomile tea, tulsi green tea, Jasmine hibiscus green tea, white tea, green tea, oolong tea and black tea are 1.93±0.02, 2±0.02, 2.16±0.02, 2.2±0.02, 1.46±0.02, 1.73±0.02, 1.6±0.02, 1.76±0.02, 1.39±0.02, 1.29±0.02, 1.25±0.02, 1.19±0.02 respectively.

Experiment 4.6.4 Statistical Analysis

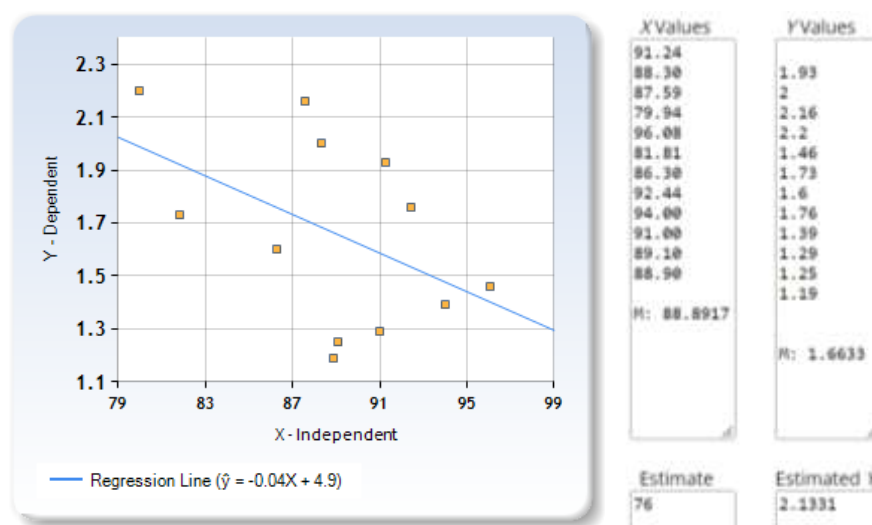


Figure 4.6.4.1a and 4.6.4.1 b shows REGRESSION ANALYSIS- E tongue-Antioxidant data of the herbal teas.

Antioxidant potential of Tulsi lemongrass tea was determined with experiment is 76%, from regression analysis the presumed Taster's index would be 2.21, practically it was observed 2.2, so this could be established as therapeutic index-S.D is 0.004

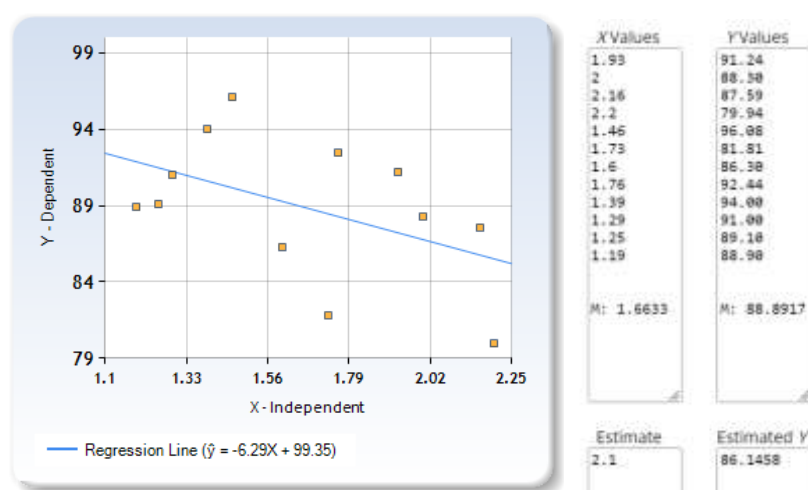


Figure 4.6.4.2a and 4.6.4.2b shows REGRESSION ANALYSIS- E tongue-Antioxidant data of the herbal teas.

Similarly the E tongue data was first derived for Tulsi ginger tea, that was 2.1 which corresponds to 86.14% antioxidant activity, which is further experimentally validated (86%); S.D is 0.07

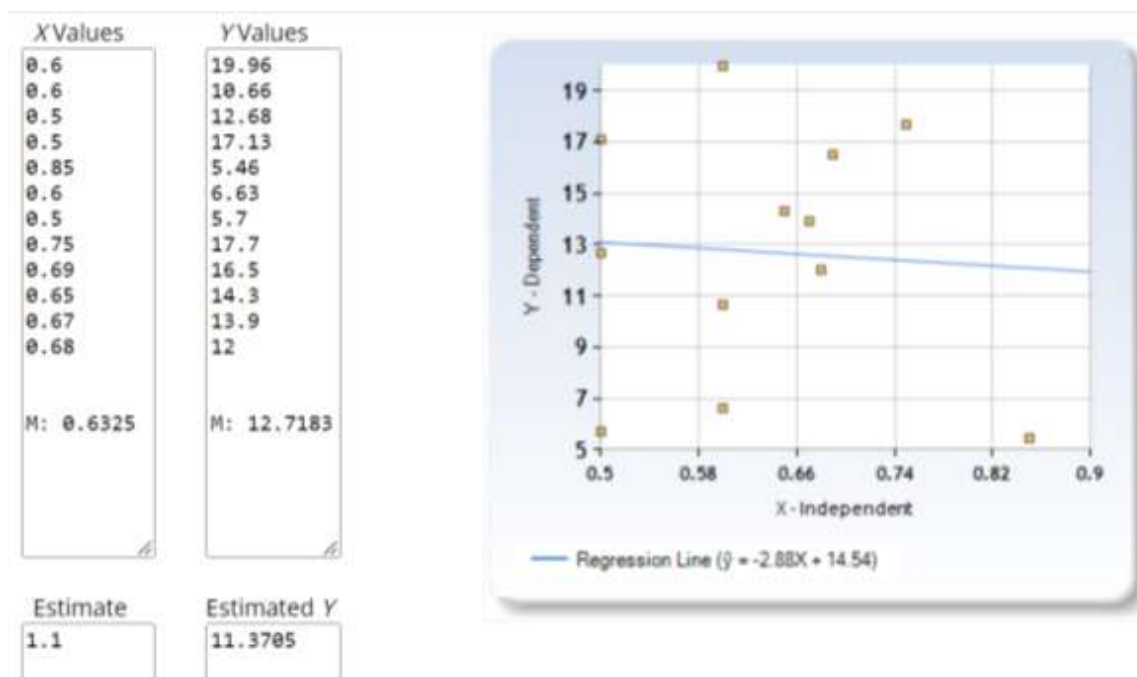


Figure 4.6.4.3 a and b shows REGRESSION ANALYSIS-E-nose-Antimicrobial data of herbal tea.

Estimated E-nose value 11 for antimicrobial zone of inhibition against *E.coli* 1.1 cm. Observed value-11.37 cm. S.D is 0.185

Experiment 4.6.5 Therapeutic potential of herbal tea and flowers

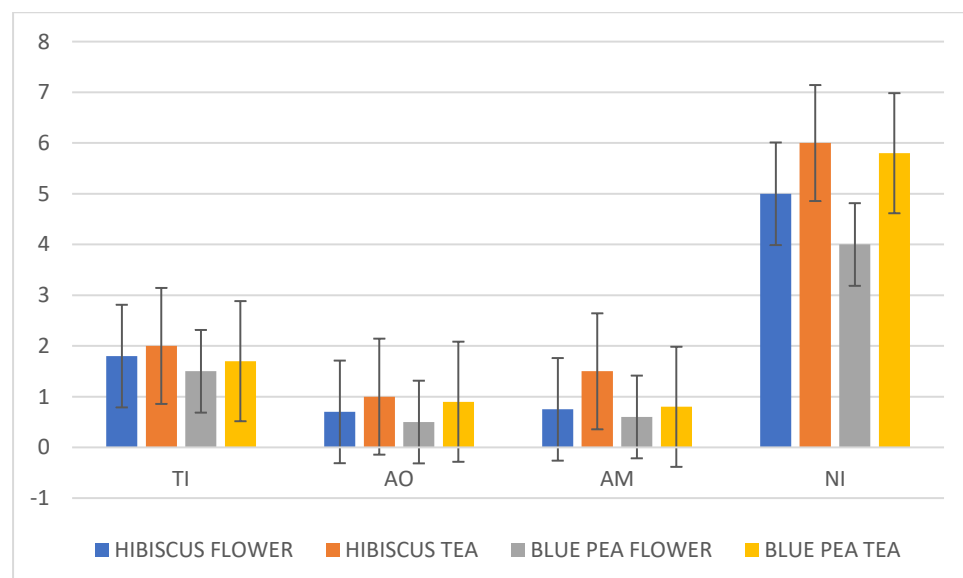


Fig 4.6.5.1 shows E-tongue value (T.I); Antioxidant potential; A.O Antimicrobial activities A.M are equivalent but E-nose value and is higher in Herbal teas than flowers.

Experiment 4.6.7 Anticancer assay

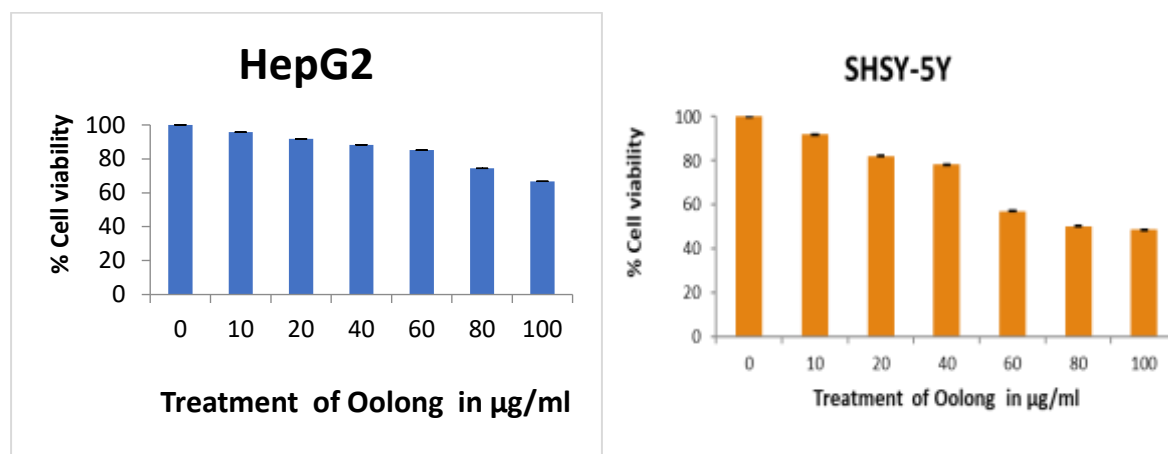


Figure 4.6.7.1a and b shows anticancer data-e-tongue/e-nose data-correlation. IC₅₀-HepG2= 100ug/ml; SHSY-5Y=60 ug/ml E-TONGUE=1.25. 60% reduction in IC₅₀ corresponds to Taster's index 1.25. Further correlation could be developed

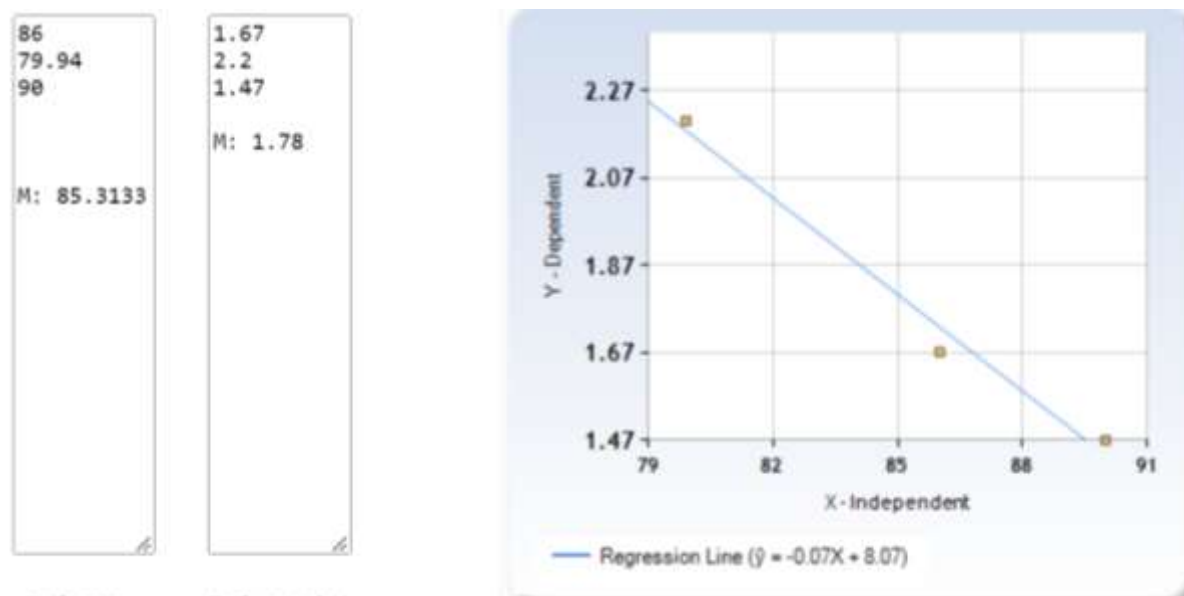


Figure 4.6.4.4 a and b Regression analysis of antioxidant and E-Tongue data.

Keeping Blue Pea Tea T.I unknown could be presumed from Regression analysis that comes as 1.7 .Experimentally it comes as 1.73; S.D is 0.01

Chapter 5

DISCUSSION

DISCUSSION

Objective 1: Documentation of agro-climatic and physiological parameters of tea from the seven valleys of Darjeeling tea gardens under natural UV irradiance.

5.1 Assessment of environmental parameters show the variation among seven valleys and their corresponding altitude and different flushes (harvest season) with respect to natural UV radiation: Horizontal (seven valleys), Vertical (altitudinal) and Seasonal (temporal) variation of natural UV radiation.

The values of UV radiation within a narrow range of clearness index values increase almost exponentially with the cosine of the solar zenith angle. Bhattacharya and Sen Mandi, 2010 clearly stated that the variation of UV radiation at high altitudes is due to different solar zenith angles. Each valley has the same solar zenith angle at any altitude so that this solar zenith angle can be taken as a unique marker parameter for geographical and environmental correlation.(Figure:4.1.1)

Ghosh and Raha, 2014, first documented the variation of UV radiation at different places in Darjeeling at different times of the day and annually.

The environmental parameters such as cloud cover, rainfall, condition of soil, cultivation management practices humidity, sunshine, and total columnar ozone concentration are all almost the same in the seven valleys of Darjeeling.(Table 4.1)

The clear evidence along with the statistical analysis shows the sharp variation among seven valleys which differs according to the solar zenith angel and there is also variation of natural UV irradiance at higher altitude.(Table 4.1.1) Figure 4.1.2 corroborates the difference in natural UV irradiance with the solar zenith angles of the seven valleys of Darjeeling (Figure 4.1.1), stating that Kurseong South is receiving a maximum dose of $5000\mu\pm0.25\text{Watt/cm}^2/\text{s}$.

The natural UV irradiance corroborates with the solar zenith angle and the highest dose of

natural UV radiation receiving valley is Kurseong South. There is significant variation in solar zenith angle in the seven valleys of Darjeeling which is highest in the case of Kurseong South tea garden. No variation is observed in each valley with different altitudes.(Figure 4.1.1)

Annual UV radiation is highest during the summer solstice in June during the 2nd flush of tea harvest in the Kurseong South tea garden. Even if the vertical variation is seen that is variation in altitudes, then it could be interpreted that during the second flush Castleton at a higher altitude among the tea gardens in Kurseong South receives more UV.(Figure 4.1.3)

Statistical analysis shows (Table 4.1.1) the significance of variation of solar zenith angles among seven valleys of Darjeeling with respect to differential natural UV irradiance.

Figure 4.1.3 shows the average annual variation of natural UV irradiance in the Kurseong South valley indicating the highest UV irradiance during the summer solstice during 2nd flush of tea harvest. Here it is pertinent to mention that the 1st flush harvest time is from the end of March to May. Annual variability and the distribution of the variation of UV radiation annually (using Temis data) throughout India, including the Darjeeling district, was mentioned by Bhattacharya et al, 2014. It is evident from Figure 4.1.4 that Castleton Garden of the 4500 ft msl altitude is receiving the highest UV irradiance during the 2nd flush season. It is pertinent to mention here that the 2nd flush harvest time is May-July is the time for summer solstice in 23.5°N and Darjeeling is situated at 27.0416 N, receiving highest and optimum dose of natural UV radiation causing to show biochemical effects on living system including plants (as well as high altitude grown tea which are exposed to differential natural dose of UV radiation).

The socio-economic dimension of the Darjeeling tea garden at different altitudes, including the variation of climatic parameters like UV radiation, was discussed by Acharya and Jairu (2020). Gateva et al., (2021) showed the effect of UV Radiation and other abiotic stress

factors on the DNA of different wild plant species grown in three successive seasons in Alpine and Subalpine regions and established that UV radiation shows the most prominent effects among them.

Different doses of natural UV radiation affect the horizontal variation that is the variation in the seven valleys of Darjeeling. It also influences the vertical variation which is the difference of altitude (vertical variation) in each garden and seasonal variation which is also called temporal variation.(Figure 4.1.2, Figure 4.1.4)

Wang et al., 2025 had describes the plant responses to environmental gradient at different niches with variable natural UV doses.

5.2 Assessment of Physiological parameters corroborates with the natural UV irradiance: Variation among seven valleys, at different altitude, at different season:

Figure 4.2.1.2b and 4.2.1.2c showed that the highest epidermal UV absorbance in terms of Dualex unit A345 was found in the case of Kurseong South Valley at the highest altitude 4500 ft msl, Castleton Garden during the summer solstice 2nd flush harvest.

Murai et al., (2009) have also studied that in the plant *Plantago asiatica*, higher altitudes show higher UV-absorbing compounds than lower altitudes.

Figure 4.2.1.3 b. Variation in Photosynthetic abilities of different flushes of different altitudes of Kurseong South Garden. Castleton in Kurseong South has the highest photosynthetic abilities in the second flush ($500\text{umol/m}^2/\text{sec}\pm 0.01$) compared to the first flush ($400\text{umol/m}^2/\text{sec}\pm 0.01$). The same is observed in the case of Upper Jungpana ($350\text{umol/m}^2/\text{sec}\pm 0.01$ in the 1st flush and $450\text{umol/m}^2/\text{sec}\pm 0.01$ in the 2nd flush), which is at a lower altitude than Castleton. Bresson et al., (2009) stated that altitudinal variations are responsible for photosynthetic abilities in plants.

Figure 4.2.1.4 b: Variation of chlorophyll content of different altitudes of Kurseong south at different flushes. Castleton has higher chlorophyll content in the second flush ($6\pm 0.025\text{mg/g}$)

than in the first flush ($5 \pm 0.025 \text{ mg/g}$). Upper Jungpana at a lower altitude than Castleton shows the same trend, i.e. it has higher chlorophyll content in the second flush ($4 \pm 0.25 \text{ mg/g}$) than in the first flush ($3 \pm 0.025 \text{ mg/g}$). Figs. 4.2.1.4a and 4.2.1.4b show that the variation of chlorophyll content is more prominent in the high altitudes of Kurseong South gardens during the second flush. Covington et al., (1975) suggested that altitude plays an important role in chlorophyll content in plants.

Epidermal UV absorbing compounds in tea plants are highest in the case of Kurseong South tea valley, especially in the highest altitude tea garden of the valley that is Castleton during the second flush. (Figure 4.2.1.2c)

Similarly the photosynthetic abilities, total chlorophyll content are more prominent in the higher altitudes of Kurseong South that is the Castleton tea garden during the second flush(temporal variation).(Figure 4.2.1.3b and 4.2.1.4b). All the physiological parameters are statistically correlated and validated with natural UV radiation and variation with respect to different altitude at different flushes.

Objective 2: Biochemical characterization and identification of therapeutic (antioxidant, antimicrobial, anticancer) and commercial potential (aroma, color) of different types of tea under natural UV irradiance.

5.3 Assessment of oxidative stress: Reactive Oxygen species and stable free radical analysis of different altitudinal and different seasonal tea varieties among seven valleys of Darjeeling show variation

Figure 4.3.1.1a and Figure 4.3.1.1b show that the higher free radicals or Reactive oxygen species are present in low-altitude-grown tea and higher in 1st flushes, which indicates the absence of some protection factor which are responsible for raising the reactive oxygen species content in terms of Malondialdehyde concentration, which is the marker of oxidative stress.

Dey et al., (2012) studied the lipid peroxidation inhibition efficacy of branded tea (*Camellia sinensis*) which are available in India. According to his study inhibition of lipid peroxidation is correlated with high polyphenol content in teas, that is higher the polyphenol content, the lower the lipid peroxidation.

Figs 4.3.2.1a and 2b show that the reactive oxygen species are least in the Kurseong South tea garden because of presence of protection factors during the 2nd flush and Castleton of Kurseong South in 2nd flush shows very low amount of ROS in terms of Malondialdehyde concentration which is the marker of oxidative stress. EPR analysis shows that there is a flavonoid-based centre having quinone or semiquinone rings that trap ROS by making them stable.

The stable free radicals are being higher at Kurseong South among all the valleys indicating better protection (peak amplitude 62 ± 0.01 cm/gm dry wt.) against oxidative stress. Polovka et al., 2003 showed the antioxidant properties of different types of tea by EPR analysis indicating that some flavonoid-based center (having quinone or semiquinone rings) can trap the reactive oxygen species by making them stable.

5.4 Biochemical Characterization of active components in different tea causing higher COMMERCIAL & THERAPEUTIC POTENTIAL under natural UV radiation

5.4.1 Antioxidant status of different tea against oxidative stress generated by natural UV irradiance:

5.4.1.1 Enzymatic antioxidant: No variation in SOD and Catalase activities among different valleys, different altitudes at different seasons

The antioxidant capacity of the cell can fight against oxidative stress generated by natural UV irradiance at different altitude at different season among seven valleys of Darjeeling.

Figure 1.4 shows the antioxidants are of two types -enzymatic and non-enzymatic antioxidants. Under natural UV radiation, there is no such variation in enzymatic antioxidants irrespective of their altitude and seasonal flushes.

There is no such variation in enzymatic antioxidants like Superoxide Dismutase (SOD--- Figure 4.3.3.1a and Table 4.3.2). Figure 4.3.3.1a shows all the gardens have almost the same content of Catalase, which is $0.52\text{U}/\pm 0.01\text{mg}$. So the enzymatic antioxidant is not the responsible candidate to protect against oxidative stress created by natural UV irradiance.

5.4.1.2 Non Enzymatic antioxidant: A huge variation in non-enzymatic antioxidants like polyphenols specially catechins, flavonoids (rutin, quercetin, epigallocatechin gallate) among seven valleys at different altitude at different seasons: characterized by different biophysical technique:

Figure 4.3.22.1b shows higher total antioxidant potential as therapeutic qualities of tea which indicates higher content in high altitude grown second flush Kurseong valley tea. To find the causative active component or metabolite causing variation the biochemical characterization have been done with different bio-instrumentation techniques and assays. Which are corroborating the higher antioxidant potential. Further with semi purification the active component was isolated and assessed for therapeutic values. Nhu-Trang et al., 2023 had demonstrated the correlation of the antioxidant capacity with the polyphenols, flavonoid content of Vietnam wild tea.

These figures 4.3.4.1b and 4.3.4.1c show that the remarkable presence of active metabolite Quercetin (flavonoid), specifically in the higher content in the high altitude of Kurseong South detected by UV spectrophotometric scan. Atomssa et al., 2015 studied the biochemical characterization of catechins in green tea by UV spectrophotometric scan. Spectrophotometric scan of high altitude and low altitude tea reveals that the concentrations

of the flavonoids such as Epigallocatechin gallate, Quercetin, and Rutin are more in the case of high-altitude 2nd flush than in low altitude in both flushes.

Figure 4.3.5.1. a, b, c and Table 4.3.2 show that the remarkable presence of active metabolite by comparing with standard Epigallocatechin Gallate, specifically higher content in high altitude of Kurseong South determined by Spectrofluorimetric scan. Sarkar et al., 2014 have demonstrated the characterization of tea polyphenols with spectrofluorimetric and other spectroscopic techniques.

In the FTIR study it is determined that the transmittance peaks of the Epigallocatechin gallate and Quercetin match with that of the high altitude indicating their presence. There is the presence of phenolic stretching and bending at different wavenumbers and the respective concentrations of Rutin are more in high altitude Kurseong South tea garden during the 2nd flush than the low altitude 1st flush.(Figs 4.3.6.1a-j). Wongsu et al., 2022 have made polyphenolic profiles of 25 herbal teas. Etseki et al., 2022 have demonstrated the FTIR fingerprinting according to quality metabolites of different tea of different geographic origin. He had demonstrated the signature transmittance peaks for polyphenols (same as our result- broad peaks around 3200-3400 cm⁻¹ for aromatic OH group bending and shorter peaks around 1580-1615, 1450-1510 cm⁻¹ for aromatic C=C bending)

Table 4.3.12a shows that the high altitude (HA-Castleton) tea clones of Kurseong South is showing higher amount of Rutin (almost equivalent retention time-7.414 and 7.548min) content during second flush by comparing with the standard for HPLC analysis.

In the HPLC study the retention peak of Rutin matches with that of the 2nd flush high altitude and the concentration of Rutin is also more in the 2nd flush high altitude.(Figures 4.3.7.1a-f)

Table 4.3.13 indicates the m/z ratio peak of LC MS chromatogram of high altitude 2nd Flush contains more Rutin than the low altitude of Kurseong South valley indicating altitudinal and seasonal variation of Kurseong South valley of Darjeeling. Figures 4.3.8.1 a-e shows LCMS

study reveals that m/z peak of Rutin corroborates with the high altitude and low altitude and the concentration of Rutin is more in case of high-altitude 2nd flush than in low altitude.

Yashin et al.,2015 have examined different chemical compositions in tea with respect to HPLC and LCMS methods of different varieties of tea.

In TLC the retention factor of the standards Epigallocatechin gallate, Rutin matches with that of the 2nd flush high altitude and low altitude. But the concentration is more in the high-altitude 2nd flush than the 1st flush.(Fig. 4.3.9.1 a and b). Ligor et al.,2008 have studied the flavonoid contents of tea and Rooibos extracts with respect to TLC methodology.

Castleton(CA) at a higher altitude than Giddhapahar(G) and Upper Jungpana(UJ) shows high theaflavin content in the 2nd flush by spectrophotometric assay.(table 4.3.11) All gardens belong to Kurseong South valley. Castleton(CA) at a higher altitude than Upper Jungpana(UJ) shows more color and hue content in the 2nd flush.(table 4.3.12) Castleton 2nd flush shows higher rutin flavonoid glycoside) and quercetin content than the low altitude Upper Jungpana.(Figure 4.3.10.1,4.3.11.1a and b)

Fig4.3.13.1a,b, c show the total polyphenol flavonoid and catechin content in high altitude Castleton 1st and 2nd flush whereas in low altitude Upper Jungpana 1st and 2nd flush, Kurseong South gardens. In each case, the high-altitude 2nd flush contains more flavonoid, polyphenol, and catechin content than the 1st flush and low altitude. Deka et al.,2020 also stated that seasonal flushes greatly impact the catechin content in tea.

Figure 4.3.12.1a, 4.3.13.1a, and 4.3.14.1a show the content of catechin, total polyphenol, and flavonoid, respectively in the seven valleys of Darjeeling. In each case, Kurseong South has the highest content of flavonoid catechin and polyphenol content. Nibir et al.,2017 examined different levels of polyphenol and their antioxidant activities in various types of Bangladesh teas.

Statistical data also interprets that there is significant positive correlation between the annual UV radiation and total flavonoid and polyphenol content than other agroclimatic parameters.

Fig. 4.3.13.1b ,4.3.14.1b and 4.3.12.1b shows that the total polyphenol, total flavonoid and total catechin content in high altitude 2nd flush is more than low altitude in other flushes.

Li et al., 2024 have shown the upregulation of tea flavonoids with natural UV radiation. Bhattacharya and SenMandi, 2011 had shown the differential values of flavonoids correlating with different doses of natural UV radiation of Darjeeling and Assam.

The biochemical characterization of such quality parameters could be tested with different biophysical techniques. HPLC, TLC help to detect soluble non volatile active metabolites (Fang et al., 2023), FTIR analysis, Spectrophotometric scan, Spectrofluorimetric scan can assess the tea active metabolites were described by Ray et al., 2024. That could further help to screen, isolate and purify the novel upregulated active marker metabolite responsible for therapeutic and commercial quality

5.4.2 Commercial Qualities: Correlated biochemical parameters are higher in high altitude grown Kurseong South 2nd flush manufactured tea

Polyphenol oxidase is the enzyme needed for the fermentation (oxidation) of the tea polyphenols like catechin to tannins like theaflavin, thearubigins which are quality indices for the liquor colour and taste of black manufactured tea. The polyphenol oxidase activity are higher in high altitude grown 2nd flush tea.(Figure 4.5.6.1)

Theaflavin percent is more in high altitude than too in 2nd flush. Thus, 2nd flush high altitude shows more β -glucosidase activity. Same is in the case of linalool content.(Fig 4.3.19.1a and table) Colour, briskness and hue content are also high in higher altitude 2nd flush than in 1st flush low altitude. Zhang et al., 2018 had shown higher polyphenol oxidase and theaflavin contents in high elevated grown New Zealand tea.

The commercial qualities include the UV upregulated flavonoid glycosides (Fig like Rutin which is being higher in high altitude grown 2nd flush Kurseong tea and same trend is followed for the Quercetin and aroma causing β -D Glucosidase enzyme which release Linalool. The linalool content is higher in high altitude grown 2nd flush Kurseong South garden (Castleton) tea which can be correlated with Gas chromatography data. Bhattacharya and Sen Mandi, 2011 have shown that increased flavonoid glycoside, β -D Glucosidase and linalool content correlated higher natural UV dose in Darjeeling tea with same tea clone of Assam.

In Gas chromatography analysis peak area and peak amplitude of volatile components in the 2nd flush high altitude are more than the 1st flush low altitude.(Figure 4.3.20.1a,b,c). Hasan et al., 2023 had shown the tea polyphenol profiling with gas chromatography responsible to show antioxidant activities.

The E-nose (Nor-aroma index) and E-tongue (Taster's scores) data of fresh leaves are also high in Kurseong South high-altitude garden Castleton during 2nd flush.(Figure 4.3.21.1a,b,c). The E-nose and E- tongue technique also help to develop the score or index to designate the commercial quality of organically grown tea (Zhi et al., 2017)

5.4.3 Therapeutic qualities: Variation among seven valleys, different altitude and season-Higher content observed in Kurseong south high altitude second flush tea-Development of Therapeutic Index

The antioxidant potential is higher in high altitude 2nd flush than in low altitude 1st and 2nd flush tea moreover the horizontal variation also the antioxidant potential is highest in case of Kurseong South.(Fig 4.3.22.1a,b,c).Thus statistically it can be correlated that annual UV radiation has a positive impact on the antioxidant potential.(table 4.3.15)

Moreover correlating the antioxidant potential of different altitudinal and seasonal tea varieties with Nor aroma indices of E-nose helped to develop the therapeutic indices which further could help to determine antimicrobial potential of unknown tea.(table 4.3.19)

Statistical analysis shows there is a negative correlation between UV radiation and IC50 values of cell viability assay (anticancer assays). (Figure 4.5.15.1) indicating that high altitudinal second flush green tea is therapeutically more potent.

High altitude tea leaves show higher inhibition of amylase and β -D glucosidase enzymes than the low altitude tea leaves. Inhibiting the activities of digestive enzymes is inhibiting available sugar formation in blood casing to show antidiabetic function(Figure 4.3.26.1a).Tamil et al.,2010 have studied about α -amylase inhibitory activity of an Indian medicinal plant, *Phyllanthus amarus*

Lowering of the blood glucose level of serum (*in vitro*) is much less in high altitude 2nd flush than in low altitude first flush and second flush. Shehadeh et al., (2021) have summarised that plant secondary metabolites like tea polyphenols can lower blood glucose levels in vitro by making adducts.(Figure 4.3.27.1)

Anti inflammatory action (by inhibing Liposoygenase activity) is higher in high altitude grown Kursoeng South garden in second flush (Castleton 2nd flush manufactured green tea, and highest in fresh leaves of 2nd flush)(Figure 4.3.28.1a,b)

Sun protection factor is highest in Kurseong South valley second flush tea. With respect to altitudinal and seasonal variation, the SPF data is more in the case of Castleton 2nd flush (Figure 4.3.29.1a,b) tea of Kurseong South valley garden. In high altitude the bioavailability of EGCG is more in plasma in the high altitude.(Figure 4.3.30.1)

Yan et al., 2020 had demonstrated the antioxidant potentials of different tea varieties due to the presence of tea polyphenols. Taylor et al., 2005 had shown the antimicrobial properties of tea catechins. Anticancer activities of tea polyphenols especially catechins along with

underlined metabolism of actions had been investigated by Cheng et al., 2020. The antidiabetic action of tea polyphenols by restoring insulin sensitivity and glucose homeostasis was demonstrated by Wei et al., 2024. It was reported by Corkovic et al., 2022 that polyphenols can show antidiabetic action by inhibiting α -amylase and β -D glucosidase activity.(Figure 4.3.26.1a) Aryaeian et al., 2017 had shown that the polyphenols have binding capacity with the sugars to make it unavailable for the cellular uptake causing to show antidiabetic action. Tea polyphenols can act as SPF (photoprotecting agent) by inhibiting the UV-induced skin damage caused by oxidative stress (Sharma et al., 2018).

5.4.4 Semi purification of active metabolite from high altitude second flush tea

The semipurified active metabolites are showing better antioxidant potential by rotary evaporation and the higher recovery was observed for high altitude grown tea.(Figure 4.3.32.1c) But in case of antimicrobial potential the lyophilisation shows better method of purification.(Figure 4.3.32.1d) Dai et al., 2022 had shown the bio activities of purified polyphenols by lyophilization from different tea.

Objective 3: Determination of therapeutic (antioxidant, antimicrobial potential) and commercial value (aroma) of tea under artificial UV radiation.

5.5 Change in Physiological and Biochemical parameters (commercial and therapeutic potentials) of tea leaf under artificial UV doses in laboratory

5.5.1 Physiological characterization of tea under different doses of artificial UV irradiance with one year old potted plant: Variation with respect to different artificial UV doses

Table shows that there is an increase in UV-absorbing compounds, photosynthetic ability and chlorophyll content with increased doses of artificial UV radiation. Figure 4.4.6.1 shows % increase in UV absorbing compounds are highest with UV5 (2100 $\mu\text{W}/\text{cm}^2/\text{s}$) artificial UV treatment but no change is observed upto 4 months.

5.5.2 Biochemical characterization of active metabolites upregulated by artificial UV radiation in laboratory with one year old potted plant:

The dose of artificial UV radiation is directly proportional to the polyphenol content of the 1-year-old potted tea plant when it is subjected to different doses of artificial UV radiation. The percent increase in polyphenol content shows that with 2100 $\mu\text{W cm}^2/\text{s}$ artificial UV exposure, there is the highest percentage increase in polyphenol content, which remains maximum for up to 4 months ($70\% \pm 0.001$). (Figure 4.4.3.1) The highest flavonoid content ($21 \pm 0.02 \text{ mg/g}$) was observed for 2100 $\mu\text{W}/\text{cm}^2/\text{sec}$ dose. The HPLC shows upregulation of Rutin compound (retention time 7.01 minute) was observed with UV treated plant.

5.5.3 Therapeutic values upregulated by artificial UV radiation in laboratory with one year old potted plant: Upregulated Rutin (flavonoid glycoside) eluent with gold nano particle shows elevated therapeutic value

Antioxidant and antimicrobial activities are also increased with increased doses of artificial UV radiation. (Figure 4.4.4.1 and 4.4.5.1). Antioxidant potential of tea under artificial UV exposure shows that the highest potential is found with UV5 that is 2100 $\mu\text{W cm}^2/\text{s}$ that is $85\% \pm 0.001$ but after 3 months no change is visible. Highest zone of inhibition is found in case of 2100 $\mu\text{W cm}^2/\text{s}$ that is about $1.6 \pm 0.01 \text{ cm}$ during the 3rd month and after that there is no change till 6th month.

Statistical analysis shows that there is a positive correlation between the treatment of the artificial UV dose with the increased biochemical and therapeutic parameters.

When artificial UV is exposed to the tea leaves post-harvest in the factory, there is also a significant rise in antimicrobial potential, antioxidant potential, and amount of the volatile components.

The upregulated sample in HPLC eluent was isolated at 7.01 minute retention time and mixed with gold nano particle, shows higher antimicrobial potential or greater therapeutic value.

Rabiee et al.,2022, have documented antimicrobial activities of gold and silver nanoparticles. Gold nanoparticle with the purified fraction of tea leaves shows more antioxidant and antimicrobial activities than the gold nanoparticle or tea leaves separately. (Figure 4.4.7.1a,1b, 4.4.8.1 and 4.4.9.1)

5.5.4 Commercial value upregulated by artificial UV radiation in laboratory with one year old potted plant

There is upregulation of Linalool percentage with highest UV exposure upto 3 months after that no change was observed.(Figure 4.4.11.1)

Thus, it can be said that artificial UV positively up regulates the therapeutic and commercial potential of the tea which could be correlated with upregulated polyphenols and flavonoids.

Variable physiological parameters like photosynthetic ability and biochemical parameters like flavonoid contents were observed by Zhang et al., 2023 with differential artificial doses of UV radiation ($15\mu\text{mol.m}^{-2}\text{s}^{-1}$, $160\mu\text{mol.m}^{-2}\text{s}^{-1}$, $240\mu\text{mol.m}^{-2}\text{s}^{-1}$) over laboratory-grown potted tea plants.

5.6 Change in Biochemical parameters (commercial potential-volatile aroma component) of tea leaf under artificial UV doses in laboratory (in UV chamber)

Fig 4.4.65 shows that the higher linalool content (responsible for volatile aroma components) in post-harvest UV-exposed tea but saturated level is observed after 240 min of exposure of UV radiation in UV chamber of laboratory. Linalool content is an index or aroma which had been increased in post harvest leaf with artificial UV treatment which calls for better industrial application with higher commercial valued tea. Yulianto et al., 2022 had shown UV-photo fermentation of tea leaves of post harvest tea to increase aroma quality.(Figure 4.4.15.1)

5.7 Change in therapeutic and commercial values of tea during post harvest manufacturing in factory with artificial UV exposure

Figure 4.4.13.1a,1b and 4.4.14.1 show that the upto 30 min of exposure the antimicrobial potential is increased but after that no change is observed that means the 30 minutes exposure of UV exposure during post harvest manufacture of tea in factory, the therapeutic potential like antimicrobial potential is increased. During the exposure to artificial UV radiation there is the increase in antimicrobial potential of about 1.2 ± 0.01 cm from 5 minutes and is constant till 60 minutes. But there is no increase in antimicrobial potential in the absence of UV radiation.

Figure 4.4.14.1 shows the increase in antioxidant potential which had not increased after 15 min of exposure in the factory. During the exposure to artificial UV exposure there is the increase in antioxidant potential of about $55\% \pm 0.05$ from 15 minutes till 60 minutes.

Figure 4.4.15.1 shows that the % increase in volatile components are observed in UV treated post harvest manufactured tea during manufacturing process in factory but increase of about $20\% \pm 0.001$ is observed upto 15 minutes of UV radiation exposure. It is pertinent to mention here that the volatile components are the commercial values of tea.

Thus artificial UV radiation in post harvest manufacturing process of tea could increase its therapeutic and commercial qualities. Wang et al., 2025 had shown the enhanced aroma of Oolong tea with artificial UV radiation during manufacturing process

Objective 4: Comparison of shelf life of different types of manufactured tea (White, Green, Black, and Oolong) in different packaging under ambient UV radiation.

5.8 Biochemical (commercial and therapeutic potential) characterization of four differentially manufactured tea (White, Green, Oolong and Black).

Fig 4.5.1.1a,b,cand d and Table 4.3.3 shows specific band at 250 nm of quercetin which is present in all types of tea varieties and that is almost the same to 1.71-1.72; another band at

360 nm which is also present in all tea types . The concentration of Quercetin is 1mg/ml, which is almost similar in different types of tea.

Atomssa et al., (2015) studied the biochemical characterization of catechins in green tea by UV spectrophotometric scan.

The spectrophotometric scan and FTIR studies reveal the presence of characteristic peaks of standards Quercetin and EGCG in varied amounts in various types of manufactured leaves. (Figure 4.5.3.1a-f).The m/z ratio in LCMS study shows the presence EGCG in varied amount in different types of manufactured leaves. (figure 4.5.4.1a-e)

Total polyphenol content is highest in green tea whereas total Rutin content is highest in black tea.(Figure 4.5.7.1,4.5.8.1) Total Quercetin content (figure 4.5.9.1)is highest in white tea and in green tea, the catechin and total polyphenol content((Figure 4.5.10.1.) are highest and thus the antioxidant potential is also highest in green tea.(Figure 4.5.12.1)

TLC also shows that the catechin contents are higher in green tea.(fig 4.5.11.1a,b). Alexander et al., 2015 had shown the variation in chemical composition of different manufactured tea with different chromatographic techniques.

The characterisation of active metabolites in different manufactured teas, such as Oolong and Black tea, using various bio-instrumentation techniques was demonstrated by Aloo et al. (2024).Antioxidant potential is highest for Green tea. Antimicrobial activities of different tea extracts against *E.coli* and *B. subtilis* depict that White tea in methanolic extract has the highest antimicrobial potential among all other types of tea in methanol and aqueous extracts. Shil et al. ,2024 have compared the antibacterial activities of tea with other plants.(Figure4.5.13.1a,b)

It is about 7% decrease in the fungal growth in antifungal assay in case of White tea which is decreased from the 2nd day. There is a decrease in growth inhibition in each of the manufactured tea. Oolong tea shows 5% growth inhibition, Black tea shows 7% growth

inhibition, White tea shows 5.5 % growth inhibition Green tea shows 5% growth inhibition and fresh tea show 6% growth inhibition.(Figure 4.5.14.1a,b,c) Shivakumar et al.,2023 documented antifungal activities of tea against *Candida albicans*.

White tea shows least IC₅₀ than other tea for anticancer potential. The low IC₅₀ indicates the high potential of the drug. IC₅₀ of Black tea, White tea, Oolong tea and Green tea are 65 ± 0.001 , 25 ± 0.001 , 51 ± 0.001 and 35 ± 0.001 respectively.(Figure 4.5.15.1) Karakas et al., 2024 had shown the anticancer action against breast cancer with white tea.

White tea shows highest lowering of blood glucose level in methanolic solution than in the aqueous solution as well as highest SPF value.(Figure 4.5.16.1,4.5.17.1) Camouse et al., 2009 had shown the topical applications of green and white tea extracts can act as photoprotecting agents

5.9 changes in Physico chemical, biochemical and therapeutic parameters of differentially manufactured tea under normal ambient UV exposure in aluminium with silica packaging

When different types of manufactured tea are stored for 18 months in different packaging it is found that the percentage decrease in moisture content and ash content are least in black tea with Aluminium and Silica packaging.(Figure. 4.5.22.1,4.5.23.1)

Percent change in amino acid and crude fiber content is also found to be least in black tea with Aluminium and Silica packaging.(Fig 4.5.24.1)

The same observation is noticed with the percentage reduction in polyphenol, flavonoid content, and antioxidant potential.(Figure 4.5.25.1,4.5.26.1,4.5.28.1a,b)

With Aluminium and Silica packaging the best before-use tag can be applied to black tea for 14 months, green tea for 12 months, white tea for 9 months, and oolong tea for 11 months.

Thus it can be said that the Aluminium and Silica packaging is best suited for the increase in the shelf life of manufactured tea under ambient UV radiation in Kolkata. Saha et al., 2017 had shown the best packaging methods of four different types of tea in different packaging

(aluminium, Silica desiccants) under normal ambient UV radiation to retain best biochemical, commercial and therapeutic qualities.

Objective 5: Characterization of therapeutic values (determination of therapeutic index) of herbal and medicinal tea.

5.10 Biochemical Characterization (commercial and therapeutic potentials) of different herbal and medicinal tea

Jasmine Hibiscus Green tea is showing the highest antioxidant potential.(Figure 4.6.2.1,4.6.2.1.1) The antioxidant potential of Lavender tea, Chamomile tea, Rooibos tea, Hibiscus tea, lavender chamomile white tea, blue tea chamomile tea, Jasmine Hibiscus green tea is showing highest antimicrobial activities (zone of inhibition against *Streptococcus* sp. which is 0.9 ± 0.01 cm higher than normal green tea that is 0.8 ± 0.01 cm.(Figure 4.6.1.1)

5.11: Development of therapeutic index for medicinal and herbal tea:

Jasmine Hibiscus Green tea is showing the highest Nor-aroma index and taster's score.(Figure 4.6.3.1.1a,1b).With the E-nose, E-tongue, antimicrobial, antioxidant, and anticancer data of the medicinal tea, the therapeutic index can be developed by applying regression analysis. Thus, the therapeutic potentials of unknown samples can be derived from the indices derived from E-nose (volatile)and E-tongue (non-volatile) data. Shyam Choudhury et al., 2022 had shown the development of therapeutic indices of different types of herbal tea with E-sensor based analysis. The quality of honey was tested by this type of data correlation with E-sensor based analysis was demonstrated by Veloso et al., 2018

Chapter 6

*SUMMARY
CONCLUSION
FUTURE
PROSPECT*

SUMMARY

Summary

Effects of NATURAL UV radiation: (Objective 1 and 2)

- ✓ Assessment of environmental parameters show the variation among seven valleys and their corresponding altitude and different flushes (harvest season) with respect to natural UV radiation: Horizontal (seven valleys), Vertical (altitudinal) and Seasonal (temporal) variation of natural UV radiation.
- ✓ Assessment of Physiological parameters corroborates with the natural UV irradiance: Variation among seven valleys, at different altitude, at different seasons

Biochemical Characterization of active components in different tea causing higher

COMMERCIAL & THERAPEUTIC POTENTIAL under natural UV radiation

- ✓ Assessment of oxidative stress: Reactive Oxygen species and stable free radical analysis of different altitudinal and different seasonal tea varieties among seven valleys of Darjeeling show variation.
- ✓ Antioxidant status of different tea against oxidative stress

generated by natural UV irradiance:

- Enzymatic antioxidant: No variation in SOD and Catalase activities among different valleys, different altitudes at different seasons.
- Non Enzymatic antioxidant: A huge variation in non-enzymatic antioxidants like polyphenol especially catechins, flavonoids (rutin, quercetin, epigallocatechin gallate) among seven valleys at different altitude at different seasons: characterized by different biophysical techniques
- Commercial Qualities: Correlated biochemical parameters are higher in high altitude grown Kurseong South 2nd flush manufactured tea.

Therapeutic qualities: Variation among seven valleys, different altitude and season-
Higher content observed in Kurseong south high altitude second flush tea-Development
of Therapeutic Index

- Semi purification of active metabolite from high altitude second flush tea

Effects of ARTIFICIAL UV radiation (Objective 3)

- ❖ Change in Physiological and Biochemical parameters (commercial and therapeutic potentials) of tea leaf under artificial UV doses in laboratory .One year old potted plant.
- ❖ Physiological characterization of tea under different doses of artificial UV irradiance with one year old potted plant: Variation with respect to different artificial UV doses.
- ❖ Biochemical characterization of active metabolites upregulated by artificial UV radiation in laboratory with one year old potted plant.
- ❖ Therapeutic values upregulated by artificial UV radiation in laboratory with one year old potted plant: Upregulated Rutin (flavonoid glycoside) eluent with gold nano particle shows elevated therapeutic value
- ❖ Commercial value upregulated by artificial UV radiation in laboratory with one year old potted plant

Post harvest tea leaf

- ❖ Change in Biochemical parameters (commercial potential-volatile aroma component) of tea leaf under artificial UV doses in laboratory (in UV chamber).
- ❖ Change in therapeutic and commercial values of tea during post harvest manufacturing in factory with artificial UV exposure

AMBIENT UV radiation and shelf life of different tea (White, Green, Oolong and Black tea)
(Objective 4)

- Biochemical (commercial and therapeutic potential) characterization of different manufactured tea (White, Black, Oolong and Green tea).

- Changes in Physico chemical, biochemical and therapeutic parameters of differentially manufactured tea under normal ambient UV exposure in aluminium with silica packaging-highest shelf life for Black tea

Characterization of therapeutic values (determination of therapeutic index) of herbal and medicinal tea (Objective 5)

- ☐ Biochemical Characterization (commercial and therapeutic potentials) of different herbal and medicinal tea
- ☐ Development of therapeutic index for medicinal and herbal tea:

SUMMARY OF THE WORK DONE:

Objective 1: Documentation of agro-climatic and physiological parameters of tea from the seven valleys of Darjeeling tea gardens under natural UV irradiance.

1. Assessment of environmental parameters shows the variation among seven valleys and their corresponding altitude and different flushes (harvest season) with respect to natural UV radiation: Horizontal (seven valleys), Vertical (altitudinal) and Seasonal (temporal) variation of natural UV radiation.
2. Measurement and documentation of agro-climatic parameters of the tea fields (seven valleys of Darjeeling and their corresponding altitudes-horizontal and vertical variations), including natural UV radiation.
3. A variation in the solar zenith angle of the seven valleys of Darjeeling was detected, which corroborates the differential data of natural UV irradiance. It selects the Kurseong South valley as the one receiving the highest UV radiation during June of the year.
4. Variation of annual UV irradiation is also observed at different altitudes of Kurseong South.

5. Assessment of Physiological parameters corroborates with the natural UV irradiance:
Variation among seven valleys, at different altitude, at different seasons
6. There is a significant positive correlation among physiological parameters like UV absorbing compounds, photosynthetic ability, with the natural UV radiance of the seven valleys of Darjeeling tea.
7. Studying the agro-climatic factors and comparing the parameters among the different tea gardens responsible for the overall growth of tea and its quality is important in the determination of the best-suited agro-climatic parameters for the improvement of the aroma of the tea leaves under natural UV radiation.

Objective 2: Biochemical characterization and identification of therapeutic (antioxidant, antimicrobial, anticancer) and commercial potential (aroma, color) of different types of tea under natural UV irradiance.

Biochemical Characterization of active components in different tea causing higher

COMMERCIAL and THERAPEUTIC POTENTIAL under natural UV radiation

8. Assessment of oxidative stress: Reactive Oxygen species and stable free radical analysis of different altitudinal and different seasonal tea varieties among seven valleys of Darjeeling show variation.
9. The biochemical characterisation shows a lesser extent of lipid peroxidation or Reactive oxygen species in high altitude grown tea, especially for Kurseong South valleys which corroborates the data of stable free radicals found by EPR analysis.
10. There is no significant variation in the enzymatic antioxidants like SOD(Superoxide Dismutase) observed in seven valleys of Darjeeling which is also applicable for the high altitude grown tea.

11. Non-enzymatic antioxidant: A huge variation in non-enzymatic antioxidants like polyphenols, especially catechins, flavonoids (rutin, quercetin, epigallocatechin gallate) among the seven valleys at different altitudes at different seasons: characterized by different biophysical techniques.
12. In the search for the non-enzymatic parameters responsible for showing the variation of the therapeutic value, total antioxidant potentials were determined, which were higher in high-altitude-grown tea and showed variation among the seven valleys of Darjeeling.
13. Bioinstrumentation techniques like FTIR analysis, HPLC, UV-Vis Spectrophotometric analysis, Spectrofluorimetric analysis, and Thin Layer chromatographic analysis state the higher active components are found in high-altitude-grown tea leaf samples of the seven valleys of Darjeeling tea, especially in Kurseong South valley.
14. With regression analysis, the natural UV fluence could be correlated with the higher flavonoid content.
15. Statistical analysis shows the positive correlation among the natural UV radiance and the therapeutic potentials like antioxidant, antimicrobial anticancer value of the seven valleys of Darjeeling tea, including altitudinal and seasonal variation
16. Commercial Qualities: Correlated biochemical parameters are higher in high altitude grown Kurseong South 2nd flush manufactured tea.
17. The polyphenol oxidase activity is found to be higher in high-altitude grown 2nd flush tea.
18. Theaflavin percent is higher in high altitude than in the 2nd flush. Thus, the 2nd flush high altitude shows more β -glucosidase activity. The same is in the case of linalool content

19. The active components were the polyphenolic group of compounds, especially flavonoids like Rutin, Quercetin, etc. are found to be higher in high altitude 2nd flush tea leaves.
20. Colour, briskness and hue content are also high in higher altitude 2nd flush than in 1st flush low altitude.
21. Volatile components were characterised with gas chromatography and non-volatile components were characterised by LC-MS analysis.
22. . Therapeutic qualities: Variation among seven valleys, different altitude and season- Higher content observed in Kurseong south high altitude second flush tea- which could help in the development of Therapeutic Index.
23. The variation in active components of the flavonoid group like flavonoid glycosides, are not only higher in altitudinal variation but also significantly higher in 2nd flush fresh leaf .
24. The antioxidant potential is higher in high altitude 2nd flush than in low altitude 1st and 2nd flush tea moreover the horizontal variation also the antioxidant potential is highest in case of Kurseong South.(Fig 4.3.40).Thus statistically it can be correlated that annual UV radiation has a positive impact on the antioxidant potential.
25. High altitude tea leaves show higher inhibition of amylase and β -D glucosidase enzymes than the low altitude tea leaves. Inhibiting the activities of digestive enzymes is inhibiting available sugar formation in blood causing to show antidiabetic function.
26. Anti inflammatory action (by inhibiting Lipooxygenase activity) is higher in high altitude grown Kurseong South garden in second flush (Castleton 2nd flush manufactured green tea, and highest in fresh leaves of 2nd flush)

27. Sun protection factor is highest in Kurseong South valley second flush tea. With respect to altitudinal and seasonal variation, the SPF data is more in the case of Castleton 2nd flush.
28. The antimicrobial properties are also higher in high-altitude 2nd Flush tea of Kurseong South tea garden Castleton.
29. Semi-purification of active metabolite from high altitude second flush tea. Semipurified active metabolites are showing better antioxidant potential by rotary evaporation, and the higher recovery was observed for high altitude-grown tea. But in the case of antimicrobial potential the lyophilization shows a better method of purification.
30. Comparing the biochemical and medicinal properties of fresh tea can be a stepping stone for drug designing purposes, out of tea leaf extracts.

Objective 3: Determination of therapeutic (antioxidant, antimicrobial potential) and commercial value (aroma) of tea under artificial UV radiation.

Change in Physiological and Biochemical parameters (commercial and therapeutic potentials) of tea leaf under artificial UV doses in laboratory

One year old potted plant:

31. Physiological characterization of tea under different doses of artificial UV irradiance with a one-year-old potted plant: Variation is observed with respect to different artificial UV doses
32. Biochemical characterization of active metabolites upregulated by artificial UV radiation in laboratory with one year old potted plant
33. Therapeutic values upregulated by artificial UV radiation in laboratory with one year old potted plant: Upregulated Rutin (flavonoid glycoside) eluent with gold nano particle shows elevated therapeutic value

34. Commercial value upregulated by artificial UV radiation in laboratory with one year old potted plant.

35. There is significant variation of the volatile and non-volatile UV absorbing polyphenolic compounds characterised by HPLC and Spectrophotometric assay under different artificial UV lamps with different doses of UV radiance.

Post harvest tea leaf:

36. Change in Biochemical parameters (commercial potential-volatile aroma component) of tea leaf under artificial UV doses in laboratory (in UV chamber) was observed.

37. Change in therapeutic and commercial values of tea during post-harvest manufacturing in a factory with artificial UV exposure.

38. The different artificial doses of UV irradiance to post-harvest tea leaves during manufacturing also show upregulation of polyphenolic compounds, which are responsible for higher therapeutic values.

39. Variation of artificial UV radiation in the laboratory can also upregulate the active metabolite, which still has the potency to show enhanced therapeutic potential, which could be correlated with the dose of UV radiation.

40. This could suggest the fact that the upregulation of UV-regulated secondary value-adding compounds can also be achieved by exposing the plants to artificial UV radiation in proper doses at non-conventional sites.

41. Isolation and characterization of UV-upregulated active metabolite (Rutin) by natural and artificial UV radiation (in the laboratory).

42. Comparing the active metabolites in different types of tea can give an idea about the significant changes in their therapeutic potential

43. Production of nano-medicine (with gold nanoparticles) and validation of therapeutic values (antioxidant and antimicrobial Potential) with the isolated upregulated active metabolite.
44. Isolation of the UV-upregulated active metabolite can be taken to the production of nano-medicine and the correlation of therapeutic qualities like antioxidant and antimicrobial potential with the E-sensor-based score to develop the therapeutic index.
45. The therapeutic values also change during storage, so proper storage conditions, including the material in which they are stored, are essential in retaining the medicinal properties of the leaves.

Objective 4: Comparison of shelf life of different types of manufactured tea (White, Green, Black, and Oolong) in different packaging under ambient UV radiation.

46. In the biochemical characterization of different types of manufactured tea by different bioinstrumentations, it is found that the concentration of standard Quercetin is almost same in different types of manufactured tea.
47. The spectrophotometric scan and FTIR studies reveal the presence of characteristic peaks of standards Quercetin and EGCG in varied amounts in various types of manufactured leaves. The m/z ratio in LCMS study shows the presence EGCG in varied amount in different types of manufactured leaves.
48. In the manufactured tea the contents of the active metabolites responsible for therapeutic potential change with each manufacturing step.
49. Antimicrobial activities of different tea extracts against *E.coli* and *B. subtilis* depict that White tea in methanolic extract has the highest antimicrobial potential among all other types of tea in methanol and aqueous extracts.

50. There is a decrease in the growth of fungus when grown with the different types of manufactured tea, which means some inhibitory properties (antifungal properties) are present in different types of manufactured tea.
51. White tea shows least IC₅₀ than other tea for anticancer potential. The low IC₅₀ indicates the high potential of the drug.
52. White tea shows highest lowering of blood glucose level in methanolic solution than in the aqueous solution as well as highest SPF value.
53. Semi-purification of active metabolite from different types of manufactured tea. Semipurified active metabolites are showing better antioxidant potential, and antimicrobial potential by rotary evaporation, and since the higher recovery was observed with the rotary evaporator method thus it can be said that rotary evaporation is a better method of purification than lyophilisation.
54. The retention of biochemical and therapeutic parameters is found to be highest with Aluminium and Silica packaging under ambient UV radiation for all types of tea (White, Green, Oolong, and black tea).
55. Percent change in amino acid and crude fiber content is also found to be least in black tea with Aluminium and Silica packaging.
56. The same observation is noticed with the percentage reduction in polyphenol, flavonoid content, and antioxidant potential.
57. With Aluminium and Silica packaging the best before-use tag can be applied to black tea for 14 months, green tea for 12 months, white tea for 9 months, and oolong tea for 11 months. Thus it can be said that the Aluminium and Silica packaging is best suited for the increase in the shelf life of manufactured tea under ambient UV radiation in Kolkata.

Objective 5: Characterization of therapeutic values (determination of therapeutic index) of herbal and medicinal tea.

58. Like true teas, herbal teas, viz. hibiscus tea, blue pea tea, chamomile tea, rooibos tea, etc, also have medicinal properties
59. The therapeutic and commercial potentials of medicinal tea are found to be greater than those of normal tea.
60. Studies have shown that initial therapeutic values are highest in white tea but are destroyed during storage under ambient UV radiation.
61. Correlating therapeutic potential with E-sensor-based analysis, E-nose (volatile components and E-tongue (non-volatile components) helps to develop therapeutic indices for all types of teas (herbal and medicinal).
62. The therapeutic indices would also help to validate the therapeutic potentials of unknown samples of medicinal and herbal teas based upon the indices derived from E-nose (volatile) and E-tongue (non-volatile) data.

CONCLUSION

Objective 1:

Natural UV radiation varies seasonally, at different altitudes and in different valleys of Darjeeling. It is correlated with physiological and agro-climatic parameter change—temporal/horizontal/vertical.

Objective 2:

There is a correlation of natural UV radiation with biochemical active components like flavonoids, polyphenols, catechins, and tannin contents in leaf and manufactured tea which vary significantly in different valleys and variation is also notable in different altitudes and seasons.

Objective 3:

There is a correlation with artificial UV dose with the physiological, biochemical-commercial, and therapeutic parameters of tea. The time of exposure in the lab and post-harvest tea during manufacturing regulates the commercial and therapeutic potential of tea.

Objective 4:

The shelf life of the manufactured tea under natural ambient UV radiation is best protected before use in different time spans. Aluminium and Silica packaging is the best for all tea varieties for storage to increase the therapeutic/nutritional values. Market values will remain almost the same by combating the natural ambient UV radiation in Kolkata. Black tea is the best one to combat the natural oxidative stress created by UV radiation.

Objective 5:

So, the correlation of antioxidant, antimicrobial and anticancer data with E-nose and E-tongue helps to derive the therapeutic indices of medicinal and herbal tea. Moreover, it would help to validate the therapeutic potentials of unknown samples of medicinal and herbal teas based upon the indices derived from E-nose (volatile) and E-tongue (non-volatile) data.

These data help to find the volatile and non-volatile component nature of the herbal/medicinal tea responsible for contribution towards antioxidant and antimicrobial properties. From the pair of known data of flower E-nose/E-tongue the antioxidant and antimicrobial data could be presumed, so after processing as a form of herbal/medicinal teas the changes in biochemical components could be presumed from therapeutic indices.

So based on these above summaries it is pertinent to mention that the natural and artificial UV radiation could enhance and upregulate the therapeutic and commercial potential in different tea and the packaging strategies could protect all those potentials during their shelf life.

FUTURE PROSPECTS

- The UV radiation could help to grow the quality tea at the non-conventional site with a proper dose along with other parameters.
- Documentation of data could provide the database to cater for the best agronomic and commercial quality of tea to repute Indian tea.
- The upregulated semipurified secondary metabolites could call for drug designing, therapeutic uses and commercialization.
- The UV upregulated tea could increase the Indian economy further.

ABBREVIATIONS USED

ANOVA	Analysis of variance
Approx	Approximately
ATP	Adenosine Tri-Phosphate
AV2	Ambari Vegetative (2) (name of tea clone)
CAT	Catalase
CE	Catechin equivalence
Cm	Centimeter
°C	Centigrade
CTC	Crush-Tear-Curl
β	Beta
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2'-diphenyl-1-picryl hydrazyl
DU	Dobson unit
E	East
E-Nose	Electronic-Nose
EPR	Electron paramagnetic resonance
E-Tongue	Electronic -Tongue
FC	Folin-Ciocalteau
Fig	Figure
Ft	Feet
FTIR	Fourier Transform Infrared Spectroscopy
FWI	Fresh weight increase

Gm	Gram
GI	Germination index
GR	Glutathione reductase
H	hour
HPTLC	High Performance Thin Layer Chromatography
IC50	Half-maximal Inhibitory concentration
Kg	Kilogram
kW	Kilowatt
LOX	Lipoxygenase
LC-MS	Liquid chromatography Mass Spectroscopy
µg	microgram
µl	micro Liter
µW	microwatt
M	Molar
m	meter
MDA	Malondialdehyde
MHA	Muller Hilton Agar
min	minute
mg	milligram
ml	millilitre
mm	millimetre
mM	millimolar
mol	molar
msl	Mean Sea level
MTT assay	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay

N	North
N	Nitrogen
NADH	Reduced form of nicotinamide adenine dinucleotide
NADPH	Reduced form of nicotinamide adenine dinucleotide phosphate
NBT	Nitro Blue Tetrazolium
nm	nanometre
OD	Optical density
PCR	Polymerised Chain Reaction
PNPG	Para-Nitrophenyl β -d-Glucopyranoside
PNP	Para nitrophenol
RPM	Revolutions per minute
ROS	Reactive oxygen species
RT- PCR	Reverse Transcription -Polymerase Chain Reaction.
Sec	Second
S	South
(S)	Substrate concentration
SD	Standard deviation
SPF	Sun Protection Factor
SOD	Superoxide dismutase
SRSWOR	Simple random sampling without replacement
TCA	Trichloroacetic acid
TE	Tris-EDTA
TLC	Thin layer chromatography
TRA	Tea Research Association
U	Unit

UV	Ultraviolet
μW	microwatt
W	Watt
W	West
wt	Weight
w/v	Weight by volume
v/v	volume by volume
WHO	World Health Organisation

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1. Hridi Halder, Reetish Raj Sahoo, Shuvrangshu Guha, Sagnik Bhattacharjee, Dyutika Banerjee, Sejuti Ray, Arpita Pareshchandra Mondal, Jaydip Ghosh, Sudeshna Shyam Choudhury "Identification and Characterization of the Antimicrobial and Active Components of Tea (*Camellia Sinensis*)". IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS), 15(1), (2020): pp. 51-58
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4. Sudeshna Shyam Choudhury, Arun Jana, Sejuti Ray, Renu Kumari Prasad, Anushree Pratihari. Development of therapeutic index of different types of herbal and medicinal teas, Journal of Medicinal Plants Studies 2022;10(4):106-112
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Seminars Attended: Paper Presentation

Participated in a poster presentation

1. National Seminar on Applications of Statistics in Natural Sciences, held in St. Xavier's College Kolkata on December 17th and 18th, 2019
2. International Symposium on Environment & Climate Crises, held in St. Xavier's College Kolkata on 20th December, 2019
3. International Conference on Ecotoxicology and Environmental Science held in Amity University, Jharkhand on the 21st and 22nd of October 2024.
4. One Day International Seminar, Frontiers in Biological Science Chapter VI held in St. Xavier's College Kolkata on 14th February 2025.

Participated in oral presentation

1. National Seminar on Nutrition Month "Poshan Maah Celebration" held in Maharani Kasiswari College on 7th September 2023.

Laurels achieved:

1. Won 2nd prize in poster presentation at the seminar: International Symposium on Environment & Climate Crises, held in St. Xavier's College Kolkata on 20th December 2019).

Comparison of Antioxidant and Antimicrobial Potential of Tea Samples from Seven Valleys of Darjeeling

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Abstract

Darjeeling, Queen of Hills, located at the foothills of the Himalaya is famous for its muscatel flavored universally acclaimed tea. Tea plantations in Darjeeling are restricted to Darjeeling and Kurseong sub-divisions. Tea is grown at an altitude ranging from 1968 ft msl-6500 ft msl. Fresh tea leaves (two leaves and a bud) were collected from seven different valleys of Darjeeling viz. Darjeeling East, Darjeeling West, Kurseong South, Kurseong North, Mirik, Upper Fagu/Rungbong and Teesta Valley. The microclimatic factors like soil, mist, fog, cloud, humidity, evaporation rate, wind speed rainfall etc. set up in the different valleys are responsible for the development of the inbuilt antioxidant properties and antimicrobial potential. The oxidative stress generated through UV radiation and other abiotic stresses induce oxidative stress in tea plants which in turn is responsible to evoke inherent antioxidant potential by inducing the synthesis of polyphenols, such as like flavonoids which are responsible for the antioxidant capacity and hence, their respective antimicrobial potentiality. In this paper the comparison of the qualities of the teas from the seven valleys of Darjeeling reflect that not only the higher altitude but an optimum altitudinal level along with the microclimatic condition is responsible to develop the elite therapeutic quality which is best for Kurseong South valley especially in the Castleton grown tea clones.

Keywords: Darjeeling tea, seven valleys, antioxidant potential, antimicrobial capacity

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INTRODUCTION

Darjeeling (27.0410° N, 88.2663° E) is a small district in the extreme north of the state of West Bengal in the Himalayan foothills of India. Darjeeling tea (*Camellia sinensis*) occupies an eminent position in world for its high quality based on its aroma, brightness and taste—all attributable to its geographical origin and impossible to replicate anywhere else or in the laboratory. The tea estates are located in three subdivisions of the district—Sadar, Kurseong and Kalimpong—under the provincial administration of the West Bengal State Government. Tea plantations in Darjeeling are restricted to Darjeeling Sadar and Kurseong sub-divisions. Tea is grown at an altitude ranging from 1968 ft–6500 ft. The seven Valleys of Darjeeling includes Darjeeling East, Darjeeling West, Kurseong

South, Kurseong North, Mirik, Upper Fagu/Rungbong and Teesta valley. Cold, misty weather, rainfall, sloppy hilly region, soil and microclimate lead to the development of terroir concept of Darjeeling tea and are responsible for the muscatel flavor and its therapeutic value. The terroir includes genotype or cultivar of the tea sample, environmental or agro-climatic factors (like microclimate including slope, soil and seasons called the different flushes) and most importantly the interactions between the genes and the environmental factors.

The critical environmental factors include UV radiation, soil, mist, fog, cloud, humidity, evaporation rate, wind speed and rainfall. In combination, they create a microclimate that is not only responsible for its aroma or flavor [1]

Biochemical characterization of different tea

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ABSTRACT:

Tea is a kind of medicinal beverage. According to different types of manufacturing processes different types of teas are available like Green, Black, Oolong and White tea. The types of teas are differentiated according to the processing parameters (oxidation/fermentation) which can change its active metabolite content. To characterize the secondary active metabolic therapeutic values/components biochemical characterization of differentially manufactured teas are needed. Which could be done with different bioinstrumentation techniques. Our study includes various analytical techniques, like FTIR analysis, LC-MS analysis, thin-layer chromatography (TLC), Spectrophotometric and Spectrofluorimetric assays and scan to characterize the differences among these different kind of manufactured tea varieties. In some cases the active metabolite content may differ from the fresh leaves also. Mainly total polyphenol and flavonoids are the main secondary active metabolites which vary in different types of tea and could be correlated with main therapeutic action like antioxidant potential.

INTRODUCTION:-

Tea (*Camellia sinensis*) is universally accepted beverage. The characteristics of the tea can vary depending on its region of origin. According to manufacturing processes mainly four different types of teas are there-white tea, green tea, oolong tea and black tea which are made from the leaves of the same species *Camellia sinensis* (Wang et al., 2022). The plant variety, the weather conditions, tea type and soil contribute to the final taste of the tea. The significant differences of tea type develop in the processing (varying levels of oxidation/fermentation) of the leaves. White and green teas are heated early on and not allowed to oxidise, oolong tea is part oxidised and black tea is oxidised (Salman et al., 2022). After white tea leaves are harvested, the leaves are withered and then dried immediately using natural sunlight, heat vents, or drying chambers. This helps prevents oxidation, giving the tea a light flavor and color and preserving some of the prized benefits of tea, such as antioxidants. For green tea, young leaves of the plant are harvested, withered, steamed, or pan-fried and then dried (Maulana et al., 2020). Oolong teas are bruised, briefly oxidised, pan fried and Black teas are rolled, fully oxidised, fired and stored. In all the cases the main secondary active metabolites like total polyphenol contents (catechins), flavonoids are changed. In White and green tea catechins like epigallocatechin gallate is prevalent but due to oxidation by polyphenol oxidase (PPO) the catechins are turned to tannins like theaflavin (TF) and thearubigins (TR) which are prevalent mostly in oolong and black tea (Musial et al., 2020).

Quercetin is also an important secondary metabolite and bioactive flavonoid which varies in different types of manufactured tea (Peng et al., 2015). All these secondary metabolites are responsible for antioxidant, antimicrobial and anticancer activities (therapeutic values) of all the tea. Biochemically different types of teas are also characterized by Marzuki et al., 2017 by spectrophotometric analysis; LC-MS and TLC by Ligor et al., 2008; FTIR analysis by Kokalj et al., 2014.

Our aim of work is to characterize the secondary active metabolic therapeutic values/components (biochemical characterization) of differentially manufactured teas with different bioinstrumentation techniques. Our study includes various analytical techniques,