PREPARATION OF TULSI (*Ocimum Sanctum*) LEAF INFUSED GREEN TEA (*Camelia sinensis)* AND ITS PHYTOCHEMICAL, ANTIOXIDANT AND SENSORY ANALYSIS

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Approval Letter

The ***dissertation Preparation of Tulsi (Ocimum sanctum) Leaf Infused Green Tea (Camelia sinensis) and its Phytochemical, Antioxidant and Sensory Analysis*** presented by **Binuta Pandey** has been accepted as the partial fulfillment of the requirements for the **B. Tech degree in food Technology.**

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Abstract

The aim of the study was to prepare Tulsi leaf incorporated green tea (blend) powder and evaluate its chemical, phytochemical, antioxidant activity and sensory analysis. Tulsi leaves (*Ocimum sanctum)* and green tea leaves *(Camellia sinensis*) were mixed in different proportions naming B, C, D, E, control A (5:95, 10:90, 15:85, 20:80, 0:100,) to obtain an optimum formulation of a blended tea using Design Expert v 13.0.1.0 software. Firstly, physiochemical analysis of all samples (green tea leaves powder, Tulsi leaves powder and blended samples) was carried out. After that, phytochemicals (phenol, flavonoid, tannin, and chlorophyll) content along with DPPH radical scavenging activity were analyzed. The sensory analysis was carried out for color, aroma, taste, astringency and overall acceptance of five tea infusions using a 9-point hedonic scale rating test. Analysis of variance (ANOVA) was done and Tukey's honesty test was performed by JMP version 14 to check the significant relationship between the mean values of the samples at $p<0.05$.

From ANOVA, by the chemical and phytochemical analysis Sample E having 80% green tea and 20% Tulsi powder was obtained as most nutritious blend among four samples of mixture formulation from statistical analysis at $P<0.05$. The chemical analysis of the blend E found moisture 73.30%, protein 17.62%, fat 6.67%, fiber 9.58%, ash 6.93%, and vitamin C 22.35 mg/g and were significantly different ($P<0.05$) than that of control product green tea and other samples. The methanolic extract of blend E had TPC 67.12±0.015 mg GAE/g, TFC 47.77±0.01 mg QE/g, TTC ±0.01 mg GAE/g, TCC 1.206±0.005 mg/g, and DPPH 76.46% inhibition and was significantly different ($P <0.05$) from the control and other blended samples except for TCC which was found similar in sample D and E. From the Sensory evaluation, product C achieved the highest mean sensory score and closely resembled the control sample which was conducted focusing on color, aroma, taste, astringency, and overall acceptability and were analyzed statistically using one-way ANOVA at a 5% level of significance

Contents

List of Tables

List of Figures

List of Color Plates

List of Abbreviation

Abbreviation	Full form
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemist
CBI	Center for promotion of imports from developing countries
DB	Dry basis
DM	Dry Matter
DW	Dry weight
DOE	Design of experiment
DPPH	2,2-Diphenyl picrylhydrazyl
DW	Dry weight
EGCG	Epigallocatechin gallate
FC	Folin-Ciocalteu
FRAP	Ferric reducing antioxidant power
GT	Green Tea
GAE	Gallic acid equivalent
GTP	Green tea powder
NTCDB	Nepal Tea and Coffee Development Board
PPO	Polyphenol oxidase
QE	Quercetin equivalent
ROS	Reactive Oxygen Species
SD	Standard deviation
TAE	Tannic acid equivalent
TCC	Total Chlorophyll Content
TTC	Total Tannin content
TFC	Total Flavonoid Content
TF	Theaflavins
TL	Tulsi leaves
TLP	Tulsi leaves powder
TPC	Total Phenolic content
TR	Thearubigins
WB	Weight basis

Part I

Introduction

1.1 General introduction

Worldwide, tea is the most consumed beverage, only surpassed by water (Wong *et al.*, 2009).The refined leaf of the *Camellia sinensis* plant is used to make tea, which is the most widely consumed beverage in the world by two thirds of people. The three types of tea are black (fermented), green (non-fermented), and oolong (semi-fermented), depending on how the leaves are collected or processed. In accordance with the various drying and fermenting methods that define their chemical makeup, these main forms of tea are produced and processed differently.

Using young tea leaves, green tea is made and then dried, graded, steamed or pan-fired, and then marketed for consumption without fermentation. The natural enzyme activities in the tea leaves must be stopped by pan firing. Before being steamed, flame-fired, or smoke-fired to create black tea, tea leaves are left to ferment for a few hours(Mukhtar and Ahmad, 2000). Tea's flavor and color are contributed by a variety of volatile and non-volatile compounds, including flavonoids, polyphenols, tannins, amino acids, and caffeine. For understanding bioactivity of tea and its physiological and pharmacological effects, it is essential to examine the chemical composition of tea and its bioactive constituents(Xu *et al.*, 2002). Green tea production requires the destruction of polyphenol oxidase, the primary enzyme in tea leaves that develops color in black tea. Various thermal processing technique such as panfrying, Steaming and roasting are carried out to inactivate polyphenol oxidase, fluidized bed drying and blanching are chosen as the ideal product (Maliepaard, 2009). Green tea's sensory qualities, such as color, bitterness, astringency, aroma, sweetness, clarity, and flavor, and its antioxidant characteristics, such as EGCG and polyphenols, are directly correlated with processing variables like steaming or roasting time, drying temperature, and drying time(Odunmbaku *et al.*, 2015).

Researchers and the general public are interested in green tea because of its many health benefits, which include its anti-oxidant, anticarcinogenic, antiangiogenic, antimutagenic, anti-hypertensive, and anti-obesity qualities (Cabrera et al., 2006).One way to increase the health benefits of green tea is to blend it with medicinal herbs that are commonly used to make herbal

teas or tisanes, expanding understanding of the connection between diet and general health and the growing popularity of herbal teas(Johnson *et al.*, 2012). Herbal teas, which have been used for thousands of years to treat and prevent illness, are made from the leaves, flowers, seeds, fruits, stems, and roots of plants other than *Camellia sinensis* (Desai *et al.*, 2007). Because they can promote relaxation, herbal teas are frequently drink for their therapeutic and energizing qualities. Herbal teas can help with digestive issues and stomach issues. They also have the ability to cleanse the body and boost immunity. Because different herbs may have different medicinal qualities, it's important to keep in mind. This allows us to customize our herbal infusions to suit our preferences for the therapeutic benefits of a cup of tea(Ravikumar, 2014). The main sources of phenolic acids and other antioxidants in today's diets are tea and herbal infusions (A. K. Atoui *et al.*, 2005b).

Tulsi is an aromatic shrub belonging to the Lamiaceae (tribe of ocimeae) basil family. It is believed to have originated in north central India and is now native to the tropical regions of the eastern world (Bast *et al.*, 2014). Tulsi (OS Linn), another name for holy basil, has been used for centuries to treat a wide range of illnesses (Dhandayuthapani *et al.*, 2015). Plants are widely used in many traditional and folk medical systems throughout Southeast Asia. Tulsi extracts in a heated mixture both inside and outside the body, cleanse, purify, and detoxify. Finely ground leaf slurry can be applied topically and is good for the skin. It is also used to treat itching, ringworm, and other skin conditions. Tea is made from its leaf extract, powdered fresh green leaves, uncooked leaves, paste, and herbal supplements (Sahoo *et al.*, 2022). Tulsi is often called "the elixir of life" because of its life-extending properties. Various plant parts are used in the Ayurvedic and Siddha Systems of Medicine for the prevention and treatment of numerous diseases and common ailments, including snake bites and scorpion stings, flatulence, migraine headaches, fatigue, skin conditions, wounds, insomnia, arthritis, digestive disorders, night blindness, diarrhea, and influenza. The leaves help to reduce anxiety and enhance memory. These include the common cold, headache, cough, flu, earache, fever, colic pain, sore throat, bronchitis, and asthma. Chewing Tulsi leaves also relieves oral infections and ulcers (B. Kumar *et al.*, 2020). Adding herbs like ginger, pepper, and Tulsi to tea enhances its taste and benefits. Herbal practitioners and healers have traditionally used herbal mixtures to enhance medicinal properties,

reduce toxicity, and improve oral taste. Folk use of mixtures is based on similar therapeutic effects as well as the assumption that the benefits add up (Carley *et al.*, 2004).

1.2 Statement of problem

Oxidative stress is characterized by an imbalance of pro-oxidants and antioxidants, favoring the former. Research indicates that oxidative stress plays a significant role in the development and progression of various diseases, including diabetes, cancer, neurodegenerative, cardiovascular, and pulmonary disorders (Toullec *et al.*, 2010). Antioxidants are crucial for preventing oxidative damage, according to epidemiological studies. Antioxidants found in fruits and vegetables can help reduce cardiovascular and neurodegenerative risks (Basak *et al.*, 2014). The harmful effects of oxidative stress on human health have become a major concern. The World Health Organization (WHO) estimates that 80% of the world's inhabitants rely on traditional medicine for their primary health care needs, and the majority of this therapy involves the use of plant extracts and active components (Krishnaiah *et al.*, 2011).

Laboratory studies have shown that Tulsi protects against toxic chemical-induced injury by increasing the body's levels of antioxidant molecules such as glutathione and enhancing the activity of antioxidant enzymes such as superoxide dismutase and catalase, which protect cellular organelles and membranes by mopping up damaging free radicals caused by a lack of oxygen(Panda and N, 2009). Plant-based antioxidants may be more effective in preventing and treating diseases due to their safety and non-toxicity compared to synthetic antioxidants (Ilaiyaraja and Khanum, 2011). There are numerous studies on green tea, but relatively few have explored the combination of green tea with other therapeutic plants or herbs. Tulsi (*Ocimum sanctum*), known for its extensive use in traditional medicine, has been recognized for its phytochemical and pharmacological properties. However, industrial-scale production and utilization of Tulsi blended with Camellia sinensis for green tea have not yet been practiced in Nepal. To address various health issues and reduce oxidative stress, it is crucial to develop functional beverages such as Tulsi-infused green tea.

1.3 Objectives

1.3.1 General objective

The general objective of this dissertation was to prepare the Tulsi leaves infused green tea and its phytochemicals, antioxidant and sensory analysis.

1.3.2 Specific objectives

1. To prepare the Tulsi leaves and green tea leaves powder.
2. To prepare an optimized sample of Tulsi leaves incorporated green tea powder using Design of experiment (DOE).
3. To study the physiochemical properties of prepared green tea leaves, Tulsi leaves and all the tea blend powder sample.
4. To prepare an extract of green tea, Tulsi and all the tea blend and quantify the phenolic content, flavonoid content, tannin content and chlorophyll of these prepared extracts.
5. To quantify the DPPH radical scavenging activity of prepared extracts.
6. To perform the sensory evaluation of infused tea and selecting the best tea blend.

1.4 Significance of the work

Herbal infusions are now commonly consumed by urban populations. People who are concerned about their health prefer nutritious and refreshing drinks that help them feel relieved and relaxed. According to a survey conducted by the Netherlands' Ministry of Foreign Affairs and the CBI (Centre for Promotion of Imports from Developing Countries), European consumers are becoming more interested in high value specialty teas with distinctive flavors. Herbal and fruit infusions, black tea, green tea, and fruit teas are all becoming significant premium goods. The main sources of phenolic acids and other antioxidants in today's diets are tea and herbal infusions(A. Atoui *et al.*, 2005a). Plant phenolics are widely recognized as potent free radical scavengers and antioxidants. Plant polyphenols use their hydrogen-donating hydroxyl groups to act as antioxidants and reducing agents(Ali and Deokule, 2008).

Tulsi green tea is such a medicinal tea which can boost up immune system and acts as antioxidant by incorporating Tulsi leaves (*Ocimum Sanctum*)(Choudhury *et al.*, 2022). Numerous

scientific research has examined the therapeutic qualities of Tulsi, including in vitro, animal, and human trials. In developing countries like Nepal, introducing tea with Tulsi (an herbal plant) can be effective as people often prefer herbal medicine over modern drugs due to their high cost and limited availability. Herbal tea can supplement poor diets with nutrients and chemicals due to its diverse composition. Combining green tea with medicinal plants, such as those found in herbal teas or tisanes, can enhance its health benefits. Herbal teas are increasingly popular as people become more aware of the impact of food on overall health(Joubert *et al.*, 2017). Numerous studies have demonstrated that compared to single active ingredients, combinations of compounds are less likely to cause disease resistance. Herbs and green tea together demonstrated a potent synergistic effect(Liu ZeHua *et al.*, 2016). By introducing blend tea, using *Ocimum sanctum* medicinal plants, and creating a balancing infusion of Camellia sinensis using *Ocimum sanctum* leaves, the dissertation may help replace the taste of tea. This will be beneficial to tea entrepreneurs who wish to develop and introduce blend green tea on a commercial scale. Furthermore, this dissertation will be helpful in advancing Nepal's and its people's socioeconomic development.

1.5 Limitation of the work

- Amino acid and alkaloid content were not determined due to unavailability of equipment and instrument.
- Pharmacological properties were not studied.

Part II

Literature review

2.1 Green tea

2.1.1 Introduction of Green tea

Tea, also known as *Camellia sinensis* (L.) is an important commercial crop that employs a considerable number of people. Green tea is among the oldest beverages in the world (Batista *et al.*, 2009). It is a popular beverage crop with medicinal, antioxidant, and antimicrobial characteristics. The tea plant has been grown throughout Asia for thousands of years. Green tea consumption dates back more than 5000 years(Yousif, 2021). Currently, more than two-thirds of the global population consumes this popular beverage(Hsu, 2005). In the seventeenth century, India transported the first green tea to Japan(Mc *et al.*, 2010). Green tea has originated from China (Thasleema, 2013). Asia produces the majority of green teas, with India leading the way, followed by China, Kenya, and Sri Lanka(Lee *et al.*, 2014). The Northeast (West Bengal and Assam) produces the majority of tea, followed by the Northern area (Himachal Pradesh) and a little amount in Southern India (Tamil Nadu). Due to variations in harvest seasons, processing techniques, tea tree species, and geographic locations, green teas from various countries may have distinct flavors, aromas, and looks(Jung, 2004).

Tea is made from the terminal leaves and shoots of the *Camellia sinensis* plant. Sinensis species has two varieties: sinensis and assamica. Camellia sinensis var. sinensis is native to southeast China, Darjeeling, and Japan. *Camellia sinensis var. assamica* is indigenous to Assam, Thailand, and Sri Lanka(Macfarlane and Macfarlane, 2009). The three primary varieties of Camellia tea are Green, Oolong, and Black tea. The distinction is in the "fermentation," which during processing actually refers to oxidative and enzymatic alterations within the tea leaves(Gunathilaka and Tularam, 2016). Green tea, a non-fermented and non-oxidized tea, is known for its distinct flavor, aroma, and health benefits, including anti-oxidative, anti-cancer, anti-atherosclerotic, and anti-inflammatory properties(Can Agca *et al.*, 2020). Catechins, a kind of flavonoid known as flavanol monomers, are abundant in green tea. Flavanols, sometimes referred to as flavan-3-ols, are present in various plant-based meals and drinks. Epicatechin

(EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3gallate (EGCG) are the catechins. A tiny quantity of vitamin C, an antioxidant and necessary nutrient, is also present in green tea (Golmovahhed and Khodaparast, 2007). Green tea has been associated to the prevention of several types of cancer, including lung, colon, esophagus, mouth, stomach, small intestine, kidney, pancreas, and mammary glands(A. Kaur *et al.*, 2015). Several epidemiological research and clinical trials revealed that green tea (black and oolong teas, to a lesser extent) may reduce the risk of several chronic diseases (Tsuneki *et al.*, 2004). This positive impact has been attributed to the high concentration of polyphenols, which are powerful antioxidants. Green tea, in particular, may lower blood pressure, lowering the risk of a stroke or coronary heart disease. Some animal studies have revealed that green tea might prevent the development of coronary heart disease by lowering blood glucose levels and body weight (Tsuneki *et al.*, 2004). Polyphenols are among the most common antioxidants extracted from higher plants. Phenolics have antioxidant action due to their redox characteristics, acting as reducing agents, hydrogen donors, and metal chelators(A. Kaur *et al.*, 2015).

Taxonomical classification of *Camellia sinensis*
Kingdom: Plantae
Sub kingdom: Tracheobionta
Division: Magnoliopsida
Order: Theales
Family: Theaceae
Genus: *Camellia*
Species: *sinensis*

Source:(McCully, 2013)

Camellia sinensis var. assamica, also known as Assam tea, is used to produce green tea(Taylor, 2003). This tea plant grows quicker, produces larger leaves, is less resistant to cold, and has a less delicate flavor than sinensis(Safran and Segal, 1996). The procedure involves roasting or boiling fresh leaves. Steaming kills enzymes that break down color pigments in leaves and inhibits polyphenol oxidase activity, including catechins, to avoid oxidation. The technique maintains the tea's green color during rolling and drying (Chacko *et al.*, 2010). After harvesting, the

leaves are quickly dried and treated to prevent fermentation(Lee, 2009). These methods maintain natural polyphenols that have health-promoting qualities(Chacko *et al.*, 2010).

2.1.2 Green tea processing

Green tea is a non-fermented form of tea. Making Japanese green tea involves harvesting, withering, heating, rolling/shaping, and drying. Pan firing is used to produce Chinese-style green tea after the withering step, with similar remaining stages for both types. White tea takes at least twice as long to wither as black tea (4-5 hours). Japanese and Chinese green teas use distinct drying procedures. The former is often steam heated, whereas the latter is dry heated to deactivate enzymes (oxidases)(Kosińska and Andlauer, 2014). Japanese green tea is made by steaming freshly collected, unfermented leaves to prevent fermentation. This results in a dry, stable product that can be withered indoors for a short duration to eliminate moisture(Yang *et al.*, 2009). Tea leaf processing affects flavor by promoting the synthesis of volatile chemicals, reducing bitterness, deactivating enzymes, reducing moisture content, and changing the look, color, and flavor of the leaf, resulting in distinct tea types(Ahmed *et al.*, 2013). Tea leaves are treated quickly after harvest to preserve flavor and prevent biochemical breakdown and spoiling.

Flowchart of green tea

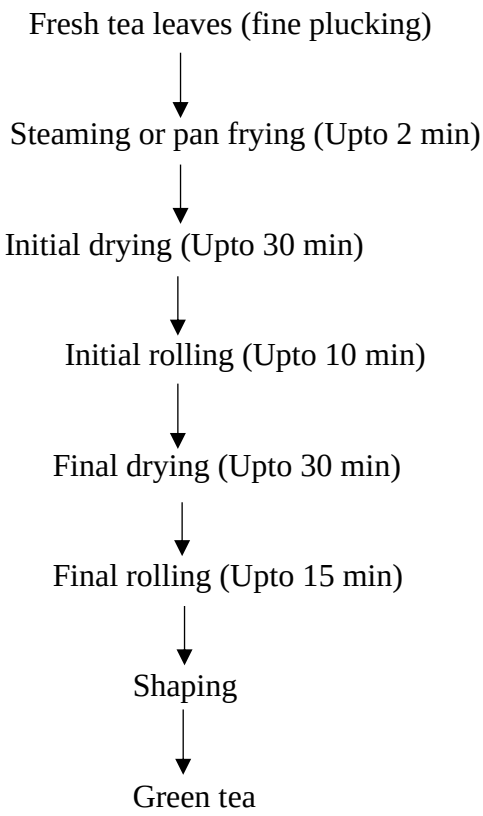


Fig 2.1 Flowchart of processing of green tea

Source : (Vishal Singh *et al.*, 2014)

2.1.2.1 Plucking

Most green tea varieties are made from young, fragile leaves. The number of leaf units gathered varies according to the variety of green tea. Many green tea varieties have a terminal bud, inter-nodes, and one to three leaves immediately beneath the bud. For several other green tea varieties, the collected unit is a single young leaf. Older leaves are typically not used to brew high-quality green teas because they are tough and have an astringent flavor. Tea leaves can be collected by hand or by machine. Many high-quality green teas are hand-plucked. Farmers can manually pluck up to 30 kg of fresh tea leaves per day. Hand plucking is the most labor-intensive and expensive step in tea production. Leaves are harvested from the same plant every four days to two weeks. Tea plants are harvested over three distinct seasons. The spring season begins in late February or early March and lasts until April. Spring tea is described as full-bodied, aromatic,

slightly bitter, and astringent. The pre-monsoon or summer season lasts from mid-May to mid-June or the end of June, whereas the monsoon harvest begins in early to mid-June and lasts until August. Monsoon season tea is typically not picked due to its low quality, characterized by a weak flavor and scent. Autumn tea, harvested from September to October, is full-bodied and less harsh than monsoon or spring teas (Ahmed, 2011).

2.1.2.2 Withering

When a tea leaf is taken from the tea plant, it undergoes a little degree of enzymatic oxidation and begins to wilt. This is normally done under sunlight (Ahmed and Stepp, 2012)or in a cool space with lots of airflow to drain the leaf of moisture(Almajano *et al.*, 2008). To get the leaves ready for more rolling, withering tries to take away moisture and make them softer. During the withering process, leaves may lose more than 25% of their weight in water. Additionally, it causes partial oxidation and the production of scent because moisture loss breaks down the cell walls. Withering causes metabolic changes such as protein conversion to amino acids, reduction of lipids and fatty acids, loss of carotenoids and chlorophylls, increase in caffeine content, and changes in sugars, organic acids, PPO activity, and volatile components(Tomlins and Mashingaidze, 1997). Green tea usually dries out for eight to ten hours(R. S. S. Kumar *et al.*, 2013)

2.1.2.3 Fixing (Pan frying/steaming)

Fixing is the process of exposing fresh tea leaves to heat for about 10-15 minutes. Fixing deactivates enzymes in leaf shoots, preventing oxidation and fermentation while maintaining a green hue. Tea shoots contain enzymes that drive the plant's growth through biochemical pathways. Tea plant enzymes include polyphenol oxidase, catalase, peroxidase, and ascorbic acid oxidase(Xu *et al.*, 2002). These enzymes are highly active when tea leaves are harvested and must be inhibited by applying high heat during the fixing process. variable enzymes have variable amounts of activity based on leaf location, plucking method, seasons, and cultivars(Obanda *et al.*, 1992). Green tea can be fixed by steaming or pan-frying. Using the pan-frying method, leaves are directly placed on a dry pan that is heated to a high temperature. It originated in the Song Dynasty in China(Xu *et al.*, 2002). This is the primary method for preparing green tea. Placing leaves over perforated steamers that release hot water-heated steam blasts is known as

steaming. In Japan, this is the most common technique for repairing. Compared to pan fixing, steaming typically maintains more color, polyphenolic content, and antioxidant bioactivity. High-quality tea requires fast, even, and high-temperature fixing, which is a property of green tea processing. About 180 degrees Celsius is the ideal temperature for pan-frying, and about 100 degrees Celsius is the ideal temperature for steaming. Leaves may turn reddish at low fixing temperatures. Overly hot temperatures cause the leaves to become dry and scorched, which causes them to turn brown and yellow and give off a smoky flavor. Moreover, leaf proteins are hydrolyzed by high temperatures. Tea leaves that have been over-dried are not ideal for rolling, which leads to fragmented pieces.

2.1.2.4 First drying

A wooden or metal drum can be used to dry leaves by swiveling them for roughly 30 minutes in 55°C heated air. During this process, the leaves lose around 50% of their remaining moisture content. Proper drying is crucial for preserving tea quality and reducing energy inputs, as physical and chemical changes occur during the process. Controlling the drying temperature prevents loss of quality and burnt taste(Xie *et al.*, 2006). Drying methods include hot-air blast oven, vacuum drying with a vacuum pump, and microwave drying with or without vacuum(Lou, 2002).

2.1.2.5 First rolling

During this procedure, leaves are rolled in a machine for 10 minutes with varying pressures. The leaves go through a cleaner to remove impurities before being crushed by a rotor vane machine. The crushed particles are then passed through a curl-turn-cut machine to make them finer. Finally, a roll breaker is used to break the twisted balls that cause the slow fermentation process. Optimal rolling is essential to avoid uneven particle crushing and loss of chemicals, as well as inappropriate chemical-enzyme mixing(Zobia Naheed *et al.*, 2007). To dry leaves faster, first break them up with a roll and then roll them out. Drying is typically done multiple times to improve product quality, remove moisture, and enhance flavor. Green tea was prepared by heating the leaves in perforated drums for a few minutes to fixate them. Green teas are needle-shaped and have dark green dried leaves. The infused leaves and scent contain more vitamin C than black and Oolong teas due to oxidation during fermentation(Zobia Naheed *et al.*, 2007).

2.1.2.6 Final drying, rolling and polishing

After a final rolling step of 15 minutes between two rotating metal plates of a rolling machine, the leaves are further dried with hot air for 30 minutes. Finally, they are polished with a polisher by pressing against a hot plate. This results in flat, bright leaves.

2.1.3 Factor affecting green tea quality

Quality matters more than yield when it comes to partially fermented tea. Environments: The quality is mostly influenced by two factors: soils and climate. The quality of tea can be influenced by high elevations and cultural methods (such as weeding, tillage, irrigation, plant protection, and harvesting). The quality of green tea is influenced by a number of elements, including tea plant cultivars, tea forms, fertilization and plucking techniques, and processing technologies like as withering, shaking, panning, rolling, and drying (Chiu, 1989). For a given type of green tea, the height of the tea plant, quantity of leaves, and length of harvest are all influenced by the weather.

China is often thought to require heavier fixing for tender leaves, lighter fixing for adult leaves, and higher fixing temperatures initially, then lower later. In order to deactivate the enzymes, a high temperature and extended fixing time are necessary because tender leaves have higher enzyme activities than mature leaves. Fixing tender leaves more deeply increases the rate of protein hydrolysis (just as fixing times increase the amount of amino acids) (Cheng, 2006). A sufficient amount of sunlight and appropriate shading are required to produce healthy green tea. In order to raise the amino acid content of green tea and improve its quality, nitrogen should be added. Green tea has been found to be superior to other tea varieties that contain the antioxidant (−)-epicatechin gallate (Cheng, 2006).

2.1.4 Harmful effect of green tea

Green tea has favorable impacts on human health, but greater dosages may have unknown harmful effects. Three major reasons contribute to the harmful consequences of excessive tea drinking (black or green): (1) the caffeine concentration, (2) the presence of aluminum, and (3) the effects of tea polyphenols on iron absorption. Patients with cardiac issues or significant cardiovascular difficulties should avoid green tea. Caffeine consumption should be limited to one or

two cups per day for pregnant and lactating women due to the potential for an increase in heart rhythm. To avoid the diuretic effects of caffeine, it's vital to limit consumption of green tea while taking certain medications(Baskar, 2014). Research indicates that tea plants can acquire significant amounts of aluminum. Controlling aluminum intake is crucial for people with renal failure, as it can build in the body and lead to neurological problems (Costa *et al.*, 2002). Green tea catechins may bind to iron and reduce its bioavailability in the diet(Hamdaoui *et al.*, 2003).

Furthermore, the effects of green tea catechins may differ between individuals.
Green tea extract contains EGCG, a cytotoxic compound that can cause acute cytotoxicity in liver cells, a key metabolic organ (Schmidt *et al.*, 2005). study indicated that drinking more green tea may lead to oxidative DNA damage in the pancreas and liver of hamsters(Takabayashi *et al.*, 2004). Green tea extract at a high dose (5% of diet for 13 weeks) causes thyroid enlargement (goiter) in normal rats (Sakamoto *et al.*, 2001). The high-level therapy altered thyroid hormone concentrations in plasma. Drinking large amounts of green tea is unlikely to create deleterious consequences in humans.

2.1.5 Chemical constituents of green tea

Tea leaf composition varies depending on environment, season, horticultural practices, plant type, and age. Green tea's chemical composition is comparable to that of the leaves (Mukhtar and Ahmad, 2000). This chemical composition is complex consisting of proteins (15-20% dry weight) whose enzymes constitute an important fraction; amino acids (1-4% dry weight) such as teanine or 5-N-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, lysine; carbohydrates (5-7% dry weight) such as cellulose, pectins, glucose, fructose, sucrose; lipids as linoleic and a-linolenic acids, sterols like stigmasterol; vitamins (B, C, and E); xanthine bases (like caffeine and theophylline); pigments (like chlorophyll and carotenoids); volatile compounds (like alcohols, esters, lactones, and hydrocarbons); minerals and trace elements (5% dry weight) like Ca, Mg, Cr, Mn; Fe, Cu, Zn, Mo, Se, Na, P, Co, Sr, Ni, K, F, and Al (Cabrera *et al.*, 2006).

Green tea leaves are rich in polyphenols, particularly flavonoids, making it a valuable source of these compounds (Cabrera *et al.*, 2006). Green tea's most abundant catechin is epigallocatechin-3-gallate (EGCG), accounting for 50-80% of its total composition. It is also thought to be

the most bioactive component in green tea. Other minor catechins are epicatechin-3-gallate (ECG), epigallocatechin (EGC), epicatechin, and catechins. Caffeine, theanine, theaflavins, theorubigins, quercetin, and other phenolics including gallic acid and chlorogenic acid make up the remaining solids in green tea(Rains *et al.*, 2011).

Table 2.1 Proximate composition of green tea

Proximate	Percentage (dry matters)
Moisture	4.8
Protein	18
Crude fat	2.4
Crude fiber	15.3
Ash	5.6
Nitrogen free extractives	53.6

Source: (Rubab *et al.*, 2020)

2.2 Tulsi

2.2.1 Introduction

Tulsi, an aromatic shrub of the Lamiaceae (tribeocimeae) family of basil, is believed to have originated in north central India and is currently a native of the tropical regions of eastern and eastern Europe(Bast *et al.*, 2014). Tulsi is referred to as "The Incomparable One," "Mother Medicine of Nature," "The Queen of Herbs," and is highly esteemed in Ayurveda for its potent medical and spiritual qualities. It is also called as the "elixir of life (N. Singh *et al.*, 2002). Tulsi is traditionally consumed in a variety of forms, including herbal tea, dried powder, fresh leaf, and combined with honey or ghee. For millennia, dried Tulsi leaves have been sprinkled with stored grains to repel insects (Khan *et al.*, 2010). Tulsi may act as a COX-2 inhibitor, similar to current medicines, because to its high eugenol content(Gaber *et al.*, 2015). The plant is planted all over India, from the Andaman and Nicobar Islands to the Himalayas, where it is grown up to 1800 meters above sea level (Tewari *et al.*, 2013). In addition, Malaysia, Australia, West Africa, and a few Arab nations are rich in it. This herbaceous, highly branching annual plant grows all over India and is revered by Hindus. The plant is often planted in gardens and in close proximity to

temples. It spreads through seeds. Today, Tulsi is grown commercially for its oil, which is highly volatile. Tulsi can be used to treat epilepsy, asthma, dyspnea, hiccups, cough, skin and haematological illnesses, parasite infections, neuralgia, headache, wounds, and inflammation(Ayurvedic Pharmacopoeia Committee %J Government of India and Family Welfare. New Delhi, 2001)and oral conditions (Hebbar *et al.*, 2004). A drop of the leaf juice has been used to treat earaches (Dadysett, 1899).Although the tea infusion has been used to treat hepatic and stomach conditions(Chopra *et al.*, 1992). There are three generally described forms of Tulsi. Two botanically and phytochemically different cultivars of *Ocimum tenuiflorum* (also known as *Ocimum sanctum L.*) are known as Rama or Sri Tulsi (green leaves) and Krishna or Shyama Tulsi (purplish leaves) (Kothari *et al.*, 2005), whereas the third variety of Tulsi , *Ocimum gratissimum*, is also referred to as Vana or wild or woodland Tulsi (dark green leaves)(Orwa *et al.*, 2009). The former has higher therapeutic value and is often used for worship. Other species widely found in India include O. *canum*, O. *basilicum*, O. *kilimandscharicum*, O. *ammericanum*, O. *camphora*, and O. *micranthum* (Bhargava and Singh, 1981).

Its therapeutic powers have contributed significantly to science since ancient times and are still used in modern study(Das and Vasudevan, 2006). It has various medicinal effects, including aromatic, stomachic, carminative, demulcent, diaphoretic, diuretic, expectorant, alexiteric, vermifuge, and febrifuge (Gupta *et al.*, 2002). Tulsi plants are a valuable source of medicine and pharmaceuticals, with several secondary metabolites and essential oils derived from them (Vinod Singh *et al.*, 2010). Tulsi has proven to be quite beneficial in numerous animal models. *Ocimum sanctum* is a plant with numerous medicinal properties, including analgesic, anti-ulcer, anti-arthritic, immunomodulatory, anti-asthmatic, antifertility, anticancer, anticonvulsant, antidiabetic, antihyperlipidemic, anti-inflammatory, antioxidant, antistress, memory enhancer, and neuroprotective properties(Kadian *et al.*, 2012)

Taxonomical classification of Tulsi

Kingdom:	Plantae
Sub kingdom:	Tracheobionta
Super division:	Spermatophyta
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Asteridae
Order:	Lamiales
Family:	Lamiaceae
Genus:	Ocimum
Species:	O. sanctum

Source:(Shival *et al.*, 2020)

2.2.2 Morphology of Tulsi

Tulsi is a fragrant plant that grows up to 30-60cm tall when fully mature(Thakur *et al.*, 2021).The plant known as *Ocimum sanctum* has roots that are externally blackish-brown, inside pale violet; thin, wiry, branching; hairy, soft in nature. The stem is erect, herbaceous, woody, and branching. It has a hairy, sub-quadrangular structure, and is internally cream colored with an exterior purplish-brown to black color. The bark of the stem is fibrous, and the xylem is short. The stem has a delicately scented scent. The leaves are pubescent on both sides and measure 2.5–5 cm in length and 1.6–3.2 cm in width. The petiole is hairy, slender, and is 1.5–3 cm in length. It has a distinct taste and a fragrant scent. The leaves grow up to 5 cm long(Thakur *et al.*, 2021). The Ocimum sanctum flower is small and has close whorls. It has a purplish or crimson color. The bracts are about 3 mm long and broad, and the pedicels are slender and pubescent. The calyx is ovoid or campanulate, with a bilipped length of 3–4 mm. The upper lip is broadly obovate or suborbicular, with a short apiculate, while the lower lip is 170 mm longer than the upper, with four mucronate teeth—the two lateral being short and the central ones being the largest. corolla, pubescent, about 4 mm long, and fragrant in fragrance; strong flavor. Flowering

began after 136 days and lasted up to 195 days, with seeds maturing after 259 days (Thakur *et al.*, 2021).

Fruit consists of four nutlets, each containing one seed and enclosed in an enlarged, veined, membrane calyx. Nutlets are sub-globose or broadly elliptic, slightly compressed, almost smooth, and have a pungent taste and aromatic aroma. They are also pale brown or reddish with a small black marking at the point where they attach to the thalamus. Tulsi seeds are oval to spherical, brown, and mucilaginous when soaked in water. They are also 0.1 cm long, have a little notch at the base, taste strongly, and have no smell (Shival *et al.*, 2020).

2.2.3 Proximate constituents of Tulsi leaves

The presence of carbohydrates, protein, fiber, fat, and moisture was found in the proximate analysis, which aids in determining the medicinal plant's nutritional worth(Nile and Khobragade, 2009).

Table 2.3 Proximate composition of Tulsi leaves

Proximate composition	% dry matters
Crude Fat	6.25
Crude Fiber	16.72
Protein	20.64
Moisture	12
Carbohydrate	39.58
Total ash	4.82

Source: (Vidhani *et al.*, 2016)

2.2.4 Chemical constituents and pharmacological activity of Tulsi

Ocimum sanctum L. has a very complex chemical makeup that includes a variety of nutrients and other substances that are biologically active. Different growing, harvesting, processing, and storage conditions have a substantial impact on the quantity of many of these constituents, which are still not fully understood (Pattanayak *et al.*, 2010). The chemical constituents and pharmacological activities of *Ocimum sanctum* are summarized in the table below 2.5

Table 2.4 Major and sub group of natural product present in different parts of *Ocimum sanctum.L*

Plant part	Active component	Compound	Pharmacological activities
Leaf	Flavonoids, Alkanoids, Saponins, Tannins, Phenols, Anthocynins, Terpenoids, Steroils	Eugenol,Eugenal,Urosolicacid,Carvacol,Linalool, Caryophyllene,Limatrol,Caryophyllene, Methyl carvicol, Anthocyans	Anti-stress, Anti-chronic, Anti-hypolipidemic, Anti-oxidant, Anthelmintic,Anti-malarialactivity(againstplasmodiumvivex),Anti-fungal(against ring worm and also skindiseases),Anti-fertilityactivity,Anti-cancer(carsinigenic),Anti-viral- activity
Steam	Phenols,Saponins, Flavonoids,Triterpenoids, Tannins.	Rosmarinicacid,Apigenin, Cirsimaritin,Isothymusin, Isothymonin.	Genitourinary system disorders
Seed	Fattyacids, Sitosterol.	Sugar(xyloseand polysaccarides)	Reduced blood and urinary uric acid level in albino rabbits.
Whole plant	Flavonoids, Alkanoids, Saponins, Tannins, Phenols, Anthocynins, Triterpenoids, Tannins	Eugenol,Rosmarinicacid, Linalool,Ursolic acid,Flavonoids(e.g Apigenin, Luteolin)	Control diabetes mellitus, anti-dot for dog bite, scorpion bite and insects bite
Roots	Triterpenoids	Beta-sitosterol, Stigmasterol	Anti-inflammatory,Antimicrobial, Adaptogenic

Source (Siva *et al.*, 2016)

2.2.5 Medicinal application of Tulsi

The roots, leaves, and seeds of tulsi possesses significant medicinal properties. It is utilized internally as well as externally. Tulsi leaves are excellent for reducing or treating practically all fevers. Holy basil kills the microorganisms that cause dental cavities, plaque, tartar, and foul breath, while also protecting the teeth. It also contains astringent effects, which cause the gums to tighten, preventing the teeth from falling. However, Tulsi contains substances such as mercury, which has strong germicidal qualities but can be detrimental to the teeth if in close contact for an extended period of time. Tulsi, a powerful detoxifier, an excellent analgesic, and a mild diuretic, is also very useful in the easy dissolving of kidney stones and discharging them painlessly through the urinary tract. Tulsi contains essential oils that have antibiotic, disinfecting, antibacterial, and antifungal properties. Applying a smooth paste of Tulsi leaves to skin rashes, acne, pimples, wounds, and other conditions is particularly effective. External application to the skin eliminates excess oil from the skin's surface. Consuming Tulsi leaves on a regular basis can protect eyes against free radical damage, including cataracts, macular degeneration, glaucoma, visual abnormalities, and ophthalmia. This is due to the high antioxidant concentration of essential oils, vitamins A, and C. Tulsi leaves' preventive effects are extremely beneficial for treating bug bites and stings. Tulsi has specified actions on the respiratory system. It’s hot and sharp properties effectively dissolve mucus. It provides remarkable results in cough owing to Kapha, allergic bronchitis, asthma, and eosinophilia. The components found in the essential oils of Tulsi, such as vitamin C, camphene, eugenol, and cineole, not only provide relief from pulmonary infections but also assist relieve lung congestion. It is a good blood purifier and is helpful in heart disease. Vitamin C and other antioxidants such as eugenol protect the heart from the harmful effects of free radicals. Tulsi contains phytochemicals like eugenol that may prevent the growth of oral cancer and other malignancies(Choudhary, 2020).

2.2.6 Immunomodulatory activities of Tulsi

Tulsi improves immune response by boosting both cellular and humoral immunity. Higher antibody titre in response to typhoid antigen challenge indicated an enhanced humoral immune response, while higher lymphocyte count indicated an enhanced cellular immunity(Savitri Godhwani *et al.*, 1988). It has anti-inflammatory properties similar to aspirin, but without any

negative effects. It relieves pain and inflammation associated with arthritis. Studies on rats with Freund's adjuvant-induced arthritis, formaldehyde-induced arthritis, and turpentine oil-induced joint edema found that oil of Tulsi dramatically reduced symptoms of arthritis and edema(Vinod Singh *et al.*, 2010). Fixed oil of Ocimum sanctum was discovered to have considerable anti-inflammatory action against carrageenan- and other mediator-induced paw edema in rats. *Ocimum sanctum* may be a beneficial anti-inflammatory drug since it inhibits both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism.

2.2.7 Safety evaluation of Tulsi

Tulsi has been the focus of several scientific investigations, with over a hundred articles on its pharmacology and wide variety of medicinal applications in only the previous decade. Numerous in vitro and animal studies attest to Tulsi leaf having potent pharmacological actions that include adaptogenic, metabolic, immunomodulatory, anticancer, anti-inflammatory, antioxidant, hepatoprotective, radioprotective, antimicrobial, and antidiabetic effects, which have been extensively reviewed before(Tabassum *et al.*, 2010). Tulsi has been demonstrated in experiments to reduce both acute and chronic inflammation in rats. Paw edema caused by carrageenan, granuloma caused by croton oil, and exudates at a dosage of 500 mg/kg, bw/day were used in this test. According to certain research, the oils extracted from fresh O. sanctum leaves and seeds exhibit anti-inflammatory properties that are induced in experimental animals by carrageenan, histamine, serotonin, and prostaglandin E2. Prior to the injection of phlogistic drugs, these experimental rats were given essential oil (200 mg/kg, bw) and fixed oil (0.1 ml/kg, bw). The results were compared with the standard medication flurbiprofen. When compared to the saline-treated control, it was seen that Tulsi extracts may considerably lessen the edema. It had less of an impact than the typical medication, though(S. Singh and Agrawal, 2008).

For thousands of years, Tulsi has been utilized as a medicinal herb with no known negative side effects. Numerous scientific investigations have been carried out to assess the plant's harmful effects. Bhargava and Singh(Bhargava and Singh, 1981) tested the toxicity of an ethanolic extract of Tulsi on adult mice to determine the lethal dose. Ocimum sanctum's approximate LD50 was determined to be 4505±80 mg/kg body weight (bw) when administered orally and 3241±71 mg/kg bw when administered intraperitoneally (ip). Mice received ip injections of

Tulsi leaf aqueous and alcoholic extracts in graduated doses (3500–6300 mg/kg, bw), and mortality was tracked for 72 hours. When administered at doses up to 5 g/kg, bw ,the aqueous extract did not cause any acute toxic symptoms (100% survival), and the alcoholic extract was well tolerated (80% survival) up to a dose of 4 g/kg, bw. The acute LD50 values (30) for aqueous and alcoholic extracts were 6200 mg/kg bw and 4600 mg/kg bw, respectively(Devi and Ganasoundari, 1995). The fixed oil was well tolerated up to 30 ml/kg, but a dose of 55 ml/kg resulted in 100% mortality. The LD50 for oil was 42.5 ml/kg. In a 14-day trial of rats given 3 ml/kg/day of O. sanctum fixed oil intraperitoneally, no adverse effects on subacute toxicity were seen(Madhuri, 2008).

2.3 Phytochemicals in plants

Phytochemicals are naturally occurring compounds found in plants that offer health advantages. Metabolites are classified as primary (carbohydrates, proteins, and lipids) or secondary (polyphenols, steroids, alkaloids) depending on their role in plant metabolism. Secondary metabolites are a class of chemical compounds with reduced molecular weight that arise in the plant kingdom and have distinct bioactivity (Kossel, 1891) They play no direct function in plant growth or development, but have evolved as a defense against biotic and abiotic stressors. Bioactive substances are supplementary nutritious components found in small amounts in food(Kris-Etherton *et al.*, 2002) and depending on dosage have positive benefits on human health. Polyphenols, flavonoids, terpenoids, alkaloids, and plant sterols are examples of phytochemicals that vary in nature based on their chemical structure and function and that significantly contribute to the bioactivity displayed by plants

2.3.1 Classification of phytochemical

2.3.1.1 Phenols/ Polyphenols

Polyphenols are fragrant secondary plant metabolites found across the plant world. They have an aromatic benzene ring with hydroxyl substituents produced from the shikimate pathway or phenyl propanoid metabolism(Bravo, 1998). Nowadays, over 8000 polyphenolic structures have been shown to have a variety of biological activities due to their ability to inhibit or stop the production of reactive nitrogen species (RNS) and reactive oxygen species (ROS)(Croft, 1998).

They are effective in preventing a number of oxidative stress-related illnesses, including cancer, heart disease, and neurological conditions(Manach *et al.*, 2005). Their concentrations in plants are also strongly related to the degree of stress factors such as UV radiation, light intensity, low temperature, drought, and nutritional deficiencies (Bernhoft *et al.*, 2010).

The primary and most important function of phenol is its antioxidant activity. They act as free radical scavengers, which are produced in response to excessive UV exposure. Similarly, they have been shown to have a variety of biological impacts. Phenolic acids play several biological activities, including increased bile synthesis, lower cholesterol and lipid levels, and antibacterial activity against Staphylococcus aureus (Ghasemzadeh *et al.*, 2010).

Table 2.5 Major classes of phenolic compounds in plant

S.N	No of carbon atom	Basic skeleton	Class
1	6	C_6	Simple phenols Benzoquinones
2	7	$C_{6-}C_1$	Phenolic acids
3	8	$C_{6-}C_2$	Acetophenones Tyrosine derivatives
4	9	$C_{6-}C_3$	Hydroxycinnamic acid, Coumarin
5	10	$C_{6-}C_4$	Naphthoquinones
6	13	$C_{6-}C_{1-}C_6$	Xanthones
7	14	$C_{6-}C_{2-}C_6$	Stilbenes
8	15	$C_{6-}C_{3-}C_6$	Flavonoids
9	18	$(C_{6-}C_3)2$	Lignans
10	30	$(C_{6-}C_{3-}C_6)2$	Bioflavonoids
11	N	$(C_{6-}C_{3-}C_6)n$	Condensed tannins

Source:(Saxena *et al.*, 2013)

2.3.1.2 Flavonoids

Flavonoids are a type of polyphenolic chemical that is commonly found in fruits and vegetables. They are organic substances classified as secondary metabolites, which means they are not directly involved in the growth or development of plants. Flavonoids are practically ubiquitous in plants and are known as the pigments responsible for the colors of leaves, particularly in autumn.

They are abundant in seeds, citrus fruits, olive oil, tea, and red wine. They are low molecular weight compounds made up of a three-ring structure with different replacements(Kesarkar *et al.*, 2009).It exist in both free form and as glycosides. These chemicals appear as yellow and white plant pigments**.** More than 4000 flavonoids have been discovered so far, 500 of which are present in their free state. Flavonoids have anti-inflammatory, anti-allergic, antioxidant, antithrombotic, and vaso protective activities. They also help protect plants from microbes and insects(Watson, 2000). The ability of flavonoids to act as antioxidants is dependent on their chemical structure. Flavonoids' antioxidant and free radical scavenging properties rely on their chemical structure, including the location of hydroxyl groups(Heim *et al.*, 2002).

2.3.1.3 Tannin

Tannins are highly prevalent polyphenols in the plant kingdom(Bernhoft *et al.*, 2010). Tannins differ from other plant polyphenols due to their ability to attach to proteins, basic chemicals, pigments, big molecules, metallic ions, and exhibit antioxidant capabilities (Okuda and Ito, 2011). Tannins are present in a wide variety of plants and environments around the world. Lower plants, such as algae, fungi, and mosses, have minimal tannin content. Tannins are found in various plants, including tea leaves, coffee beans, and herbs (Savolainen, 1992). Tannins are divided into hydrolysable or condensed. Hydrolysable tannins produce gallic and ellagic acids, referred to as gallo tannins or egallitannins (Bressani *et al.*, 1983). When heated, they generate pyrogallic acid(Savolainen, 1992). Hydrolysable tannins include theaflavins (tea) , daidezein, genistein, and glycitein (Doughari *et al.*, 2009). Research indicates that some phenols have a strong correlation with condensed tannin, whereas others have a poor correlation with hydrolysable tannin (Baldwin *et al.*, 1987).

Tannin-rich plant extracts offer anti-inflammatory, antibacterial, antioxidant, and hemostatic benefits (Anesini *et al.*, 2008). In the dye industry, tannins are used to make caustics and inks for cationic dyes (such as iron gallate ink). Tannins are used in the food industry to clear fruit juices, wine, and beer. Commercial uses for tannins include colorants for textiles, coagulants for rubber, and antioxidants in wine, beer, and fruit juice(Gyamfi and Aniya, 2002). Tannins have recently drawn more scientific interest, particularly in light of the growth in deadly illnesses like cancer and AIDS. The hunt for novel lead molecules for the launch of novel drugs has grown in

significance as the biological effects of plant extracts containing tannins have been extensively studied (Saxena *et al.*, 2013).

2.3.1.4 Chlorophyll

All plants naturally contain the pigment chlorophyll, especially in their leaves (Humphrey, 2004).Levels in certain species might range from 1000 to 2000 parts per million by wet weight. Because of their relationship with bioactive yellow/orange carotenoid pigments and their fundamental role in photosynthesis, blue-green pigments are frequently disregarded. However, they might contribute to the prevention of chronic diseases(Caballero *et al.*, 2015).

Dietary chlorophyll mostly consists of lipophilic derivatives such chlorophylls a and b, found in fresh fruits and vegetables, as well as metal-free pheophytins and pyro-pheophytins. Commercial-grade sodium copper chlorophyllin (SCC) and other water soluble chlorophyll derivatives, such as chlorophyllides and pheophorbides, expand the range of dietary chlorophylls (Caballero *et al.*, 2015). Green tea contains chlorophyll, a pigment found naturally in tea leaves and other green plants. Chlorophyll pigments are green pigments found in photosynthetic cells. According to (Ošťádalová *et al.*, 2015)they are a key component of photosynthesis because they absorb light. The word "chlorophyll" was first used exclusively to describe the green pigments that higher plants employ for photosynthesis; later, it was expanded to encompass all photosynthetic porphyrin pigments (Ošťádalová *et al.*, 2015). The amount of chlorophyll in tea leaves and non-fermented teas impacts the final color of the green tea infusion, making it a crucial pigment. Black tea's dark color results from the fermentation process, which changes chlorophylls into pheophorbides and pheophytins (Harbowy *et al.*, 1997). (Wei *et al.*, 2011)claimed that the environmental and plant-growing circumstances affect the amount of chlorophyll in tea plants, with higher levels being associated with lower temperatures and higher relative humidity.

2.3.1.5 Caffeine

Caffeine (1, 3,7-trimethylxanthine) is a naturally occurring alkaloid found in a variety of plants, including tea leaves, cocoa beans, coffee beans, and cola nuts. It is a widely used mental stimulant around the world. Toxicity studies have shown that healthy persons can consume up to 400 mg of caffeine per day. Caffeine levels in beverages like Coke are typically 26 mg per 250ml. A study by the Radiological Society of North America in 2005 has found that consuming

caffeine in moderation can boost memory and reaction times. Excessive use may result in anxiety, irritability, insomnia, sensory disturbances, diuresis, gastrointestinal issues, increased breathing, and hepatic and renal system dysfunctions(Heckman *et al.*, 2010).

Caffeine is an important ingredient in tea beverages, responsible for its distinct and attractive flavor. Additionally, it acts as a strong antioxidant, boosting the antioxidant capacity of beverage(Koláčková *et al.*, 2020). Its concentration may be affected by the time of harvest and the age of the leaves; the older the leaves, the lower the caffeine content. The amount of caffeine in tea varies according on the variety, weather conditions, and brewing procedure(Koláčková *et al.*, 2020). Caffeine's advantages are derived from its antioxidant ability, which neutralizes reactive oxygen species while enhancing antioxidant enzyme activity and total glutathione. Caffeine in regular doses may limit chronic oxidative stress, lowering the incidence of free radical-mediated diseases (Stefanello *et al.*, 2019).

2.3.1.6 Tea polyphenols

Tea leaves, wine, fruits, and vegetables all contain high levels of polyphenols. Tea contains around 4000 bioactive compounds, with polyphenols accounting for one-third of those. Other components contained in tea include alkaloids (such as caffeine, theophylline, and theobromine), amino acids, carbohydrates, chlorophyll, volatile organic compounds (which contribute to tea odor), fluoride, aluminum, minerals, and trace elements(Koch *et al.*, 2019).Tea polyphenols include catechins, flavanols, flavanones, phenolic acids, glycosides, and plant pigments (aglycons). They're soluble in water, ethanol, methanol, and acetone. Tea polyphenols derived from green tea leaves operate as a natural antioxidant, scavenging active oxygen radicals. Tea polyphenols outperform butylated hydroxyanisole, butylated hydroxytoluene, and dl-αtocopherol in terms of antioxidant activity. Furthermore, tea polyphenols are less toxic(Pan *et al.*, 2003).

Tea leaves include three polyphenol groups: catechins, theaflavins, and thearubigenes. Green tea leaves contain flavonoids and phenolic acids, making up up to 30% of the fresh leaf dry weight, compared to only 10% in black tea. Green and black tea contain comparable amounts of flavonoids but have distinct chemical structures. Tannins are also produced through polyphenol oxidation. Tannins are divided into two types: hydrolysable (water-soluble) tannins (such as

phenolic acids and gallic acid esterified polyols) and non-hydrolyzable tannins (condensed polymers of flavonoid particles)(Gramza *et al.*, 2005).

2.3.2 Extraction technique of phytochemicals

Extraction is the process of employing certain solvents in conventional extraction techniques to separate a plant's medicinally active parts from its inactive or inert constituents (Azwanida, 2015).

It is an essential first step in the research of medicinal plants and has a big influence on the final result. Chemicals with equal polarity in solid plant material are dissolved by extraction solvents. In order to extract physiologically active compounds from plant material, the type of solvent used is essential for the best outcomes. The following solvents are frequently used to extract active ingredients: ether, acetone, water, methanol, ethanol, and chloroform(Stéphane *et al.*, 2021). The choice of appropriate extraction techniques is also essential for both qualitative and quantitative investigations of bioactive chemicals from plant sources. Plant materials can be extracted using a variety of extraction techniques

Numerous techniques, such as maceration, infusion, decoction, percolation, digestion, Soxhlet extraction, superficial extraction, ultrasound assisted extraction, and microwave extraction, were used to extract medicinal plants. Furthermore, thin-layer chromatography, high-performance liquid chromatography, paper chromatography, and gas chromatography were used to separate and purify the secondary metabolites(Ingle *et al.*, 2017). Non-conventional technologies are environmentally friendly, utilize fewer synthetic and organic chemicals, minimize operational time, and improve extract production and quality during the past 50 years. Promising non-conventional approaches include supercritical fluid extraction, ultrasound-assisted extraction, enzyme-assisted extraction, and microwave-assisted extraction. Conventional extraction methods, like Soxhlet, are still used as a benchmark to evaluate the performance of new approaches(Azmir *et al.*, 2013). The majority of these procedures rely on the extractive properties of various solvents, as well as the use of heat and/or mixing. Plant extraction solvents often include methanol, acetone, chloroform, petroleum ether, and hexane. In some studies, liquid nitrogen has also been employed as an extraction method (Karuna *et al.*, 2000).

2.3.3 Factor affecting phytochemical content

2.3.3.1 Cultivar Effect

Plant phytochemicals are largely impacted by genetic composition. Research suggests that differences in phytochemical compounds across cultivars are larger than across plants grown under different conditions(Tiwari and Cummins, 2013). The phenolic profile of horticultural crops like potato, faba bean, tomato, garlic, globe artichoke, and cardoon is significantly influenced by genetic elements(Colla *et al.*, 2013).

2.3.3.2 Extraction method and solvent used

Plant activity and phytochemical concentration are regulated by extract preparation processes and solvent type. Methanol extracts produced substantial phytochemical concentrations. Samples from the Highland and Semi-arid zones had more antioxidant activity than those from the Tropical zone, which had the lowest. Different agro-climatic conditions affect the phytochemicals, total phenolic content (TPC), and antioxidant potential of the alovera plant(S. Kumar *et al.*, 2017). According to the study by (Vidic *et al.*, 2014) there is a notable variation in the phytochemical contents as a result of the different extraction techniques employed, with soxhlet extraction outperforming ultrasound extraction of the sample. Different solvents, varying maturation levels, and climatic conditions could all be the cause of the data variances (Rababah *et al.*, 2010).

2.3.3.3 Environmental conditions

The quantity of phytochemicals may be affected by environmental elements such exposure to sunlight, temperature fluctuations, and regional climate conditions (Rababah *et al.*, 2010). Numerous factors, such as soil type, temperature, light, water content, and mineral composition, are said to affect the total phytochemical content of plants (Rajbhar *et al.*, 2015). The best possible fertilization is necessary to provide adequate levels of phytochemicals. High doses of fertilizer containing nitrogen, phosphorus, and potassium may increase vegetative growth and yield while reducing phytochemical levels (Tiwari and Cummins, 2013). Seasonal changes can drastically affect the phytochemical makeup of plants when exposed to different temperatures (Usano-Alemany *et al.*, 2014).

2.3.3.4 Growth condition

The stages of plant growth have an impact on the quantity of phytochemicals found. The research indicates that whereas leaves have modest phenolic and flavonoid contents during the early growth stage, total alkaloids gradually increase during the growth and development stages. Aloevera active components and antioxidant capacity fluctuate depending on its growth stage (Hu *et al.*, 2003) The phytochemicals are impacted by variations in plant species or even maturation stages (Hu *et al.*, 2003)

2.3.3.5 Post harvest storage conditions

The quantity and quality of phytochemicals are significantly influenced by storage temperature, gas composition, and chemical application. The breakdown of phytochemicals may be postponed at lower temperatures. When compared to fresh form, high temperatures significantly affect the phenolic, flavonoid, tannin, and antioxidant activity. This effect is not present at lower temperatures. According to certain studies, the drying process and length of time spent in hot air can affect the concentration of these compounds (Li *et al.*, 2012).

2.3.3.6 Other factors

Factors affecting phenolic compound extraction include chemical composition, raw material, storage conditions, and time. Important considerations include extraction and verification techniques, standards, and interference (Alara *et al.*, 2021).

2.3.4 Importance of phytochemicals

The mechanisms by which phytochemicals function can be diverse. They may disrupt specific metabolic processes, stop the growth of microorganisms, or change the signaling networks that regulate gene expression. Both chemotherapeutic and chemo preventative medicines can be used with phytochemicals; chemoprevention refers to the use of compounds to stop, reverse, or delay the development of cancer. Because chemo-preventive phytochemicals may share some molecular pathways with cancer treatment, they can therefore be employed in cancer treatment(Manson, 2003).

Plant extracts and essential oils can suppress bacterial development by disrupting the phospholipid bilayer of the cell membrane, resulting in increased permeability and cellular component loss. They can also affect enzymes involved in energy production and structural component creation, as well as degrade genetic material. Research suggests that the mechanism of action involves rupture of the cytoplasmic membrane, disruption of the proton motive force, electron flow, active transport, and coagulation of cell contents (Kotzekidou *et al.*, 2008). Plants use phytochemicals for protection and reproduction, including color, odor, phytoalexins, hormones, antifeedants, toxins, and allelochemicals. Numerous phytochemicals found in plant diets have been linked to a decreased risk of chronic noncommunicable diseases like type 2 diabetes and heart disease(Liu *et al.*, 2018). Over the past 20 years, bioactive phytochemicals have been extensively studied using in vitro and in vivo models, offering important insights into structure-function interactions that could be in charge of lowering the risk of disease

2.4 Antioxidant activity

Antioxidants are substances that, when added to food, prevent, slow down, or completely stop oxidation and food quality degradation. Antioxidants in the body reduce the likelihood of oxidative stress-induced degenerative illnesses. Antioxidants can delay or prevent oxidation caused by reactive oxygen species or ambient oxygen. They are used to stabilize petrochemicals, food, cosmetics, medicines, and polymeric materials (Pisoschi and Negulescu, 2011; Upadhyay *et al.*, 2014)

Reactive oxygen species, often known as free radicals, include singlet oxygen, superoxide anion, peroxyl, hydroxyl, and nitrite, which produce oxidative stress and cellular damage. Antioxidants are chemicals that defend cells from harmful impacts. Natural antioxidants are crucial for maintaining health and preventing chronic diseases such as atherosclerosis, myocardial and cerebral ischemia, carcinogenesis, neurological disorders, diabetes, pregnancy, rheumatoid arthritis, DNA oxidation, and aging (Doughari *et al.*, 2009). Antioxidants work by scavenging "free-oxygen radicals," producing a "relatively stable radical" in the process(G. Kaur *et al.*, 2016). The body's natural antioxidant defenses, including glutathione and catalases, can neutralize free radicals(Jayaprakash *et al.*, 2015). To compensate for this lack, extrinsic natural antioxidants such as vitamin C, flavones, beta carotene, and plant-based products should be

employed(Jayaprakash *et al.*, 2015). Tinospora cordifolia lowers lipid peroxidation and reactive free radicals in a diabetic rat model induced with alloxan. It stimulates antioxidant enzymes such as catalase and glutathione, which indicate anti-oxidant characteristics(Sivakumar and Rajan, 2010). According to certain research, Tinospora cordifolia controls enzyme levels, regulating the generation of reactive species, and maintaining a stable oxidative load by influencing lipid peroxidation and glutathione levels(Sivakumar and Rajan, 2010). The body produces reactive oxygen species (ROS) as a result of many metabolic processes. The partial reduction of oxygen results in both radical and non-radical oxygen species. Cellular processes such as signaling, development, and defense require low levels of oxygen species. Oxidative stress is caused by excessive ROS or ineffective antioxidant control (Bertout et al., 2004).Oxidative stress can damage nucleic acids, proteins, and lipids, resulting in chronic diseases like stroke, Alzheimer's, Parkinson's, atherosclerosis, diabetes, cancer, and osteoporosis(Ienco *et al.*, 2011). The human body naturally manufactures antioxidants such as glutathione and ubiquinol. The body's main antioxidants are vitamin E (αtocopherol), vitamin C (ascorbic acid), and β-carotene, but other enzymes also help scavenge free radicals. The body cannot generate certain micronutrients, hence they must be obtained through diet(DIPLOCK, 1994). Polyphenols, the majority of antioxidants derived from higher plants, exhibit biological action in the form of antibacterial, anticarcinogenic, anti-inflammatory, antiviral, antiallergic, estrogenic, and immune-stimulating properties(Graf *et al.*, 2005). As a result, using dietary antioxidant supplements has gained popularity among people in general to combat illnesses, including cancer

2.5 Synergistic effect of combining green tea and Tulsi leaves

The fundamental notion behind synergy is that it is more advantageous to use an entire plant with a variety of compounds cooperating rather than a single component to accomplish a certain goal (Malongane *et al.*, 2017).According to (Manikanta, 2023) adding Tulsi leaf powder to green tea enhanced its nutritional value and acceptance. The nutrients were more evenly distributed in the blends when compared to green tea alone. Additionally, the herbal tea brews were extremely good when compared to green tea alone. The herbal tea made from green tea and Tulsi leaves has a distinct flavor and is rich in antioxidants and therapeutic benefits in addition to its nutrient-dense qualities. Additionally, the combination possesses antibacterial qualities, such as enhanced effectiveness against foodborne illness-causing microbes. Their bioactive components,

including as tannins, catechins, and flavonoids, which inhibit microbial growth and guard against cellular damage, most likely work in concert to produce this synergistic effect(Jain *et al.*, 2011). This blend is also well-known for its ability to boost metabolic health and immune function, offering consumers who are concerned about their health a natural, functional beverage substitute(Ortiz-Islas *et al.*, 2024).

2.6 Sensory evaluation

The study of sensory science in food science focuses on how individuals utilize their senses—taste, smell, and texture—to experience and respond to various meals and beverages. It evolved to provide a methodical and scientific approach to researching how humans react to and interpret these foods and their components (Tuorila *et al.*, 2009).The area of sensory evaluation has grown significantly in the second half of the twentieth century, along with the rise of the processed food and consumer products sectors(Lawless and Heymann, 2010). Customers must evaluate a product and indicate how much they enjoy it. This helps with menu planning so that items that are popular with many customers can stay on the menu while items that are unpopular with many customers can be taken off (Wichchukit *et al.*, 2015). The sensory evaluation reduces the potentially biasing effects of brand identification and other information influences on customer perception and includes a collection of methods for precisely measuring human reactions to foods. As a result, it makes an effort to separate the sensory qualities of foods and gives managers, food scientists, and product developers crucial and practical information regarding the sensory aspects of their products (Tuorila *et al.*, 2009).

One of the most important measures for sensory analysis is consumer acceptability. It usually uses a scaling technique to measure how much a consumer likes or dislikes a product employing inexperienced customers (Tuorila *et al.*, 2009). However, there are other aspects of consumer research than acceptability level(Drake, 2007)consumer perception, sentiments, and the connection between descriptive sensory attributes, instrumental data, and consumers' thoughts about a product(Venturi *et al.*, 2016).

2.6.1 9-point hedonic scale rating

Many rating systems have been established for determining the extent of like (Lim *et al.*, 2009; Rosas-Nexticapa *et al.*, 2005) among which the 9-point hedonic scale is perhaps the most used sensory testing scale in the last 60 years in food research. The scale was first established to help US servicemen organize their menus in canteens. The scale consists of nine verbal categories ranging from 'like extremely' as '9' to 'dislike extremely' as '1' for subsequent quantitative and statistical analysis, and responses to the verbal categories are treated as responses to numerical values along a preference continuum, namely a 'numbers only' scale(DR, 1952; Peryam and Pilgrim, 1957). A typical hedonic test involves 75-150 regular product users. The test would include numerous different versions of the product and would take place at a central location or sensory testing center. Affective tests may require a higher sample size to account for the considerable heterogeneity of individual preferences, ensuring statistical power and sensitivity. This provides an opportunity to identify customer categories who prefer alternative product styles, such as colors or flavors. Additionally, it might provide insight into why people like or dislike a product

2.7 Sensory parameter of tea

Table 2.6 Sensory parameter of tea

Attributes	Description
1) Aroma	
Sweet—caramel, maple syrup	Aromatics associated with materials that also have a sweet taste, such as molasses, caramelized sugar and maple syrup
Honey-sweet typical honey	Aromatics associated with the sweet, caramelized flora and woody aromatic associated with honey.
Green—cut grass, mint	Aromatics associated with green cut grass, fresh-cut grass, mint
Cooked spinach	Aromatics associated with cooked spinach.
Dry green herbal—chai tea	Aromatics associated with “Green” flavor typical of dried grass or dried herbs.
Earthy—boiled potatoes, damp potting soil	Aromatics associated with damp soil, wet foliage, and damp potting soil
Perfume—floral, lavender	Aromatics associated with having a light fragrant aromatic characteristic of lavender

Woody cinnamon, dry dusty, bark	Aromatics associated with dry fresh-cut wood; bark, cinnamon, dust.
2. Tase flavors	
Bitter—quinine, caffeine	Flavors associated with the taste on the tongue stimulated by solutions of caffeine, quinine.
Sweet—caramel, maple syrup	Flavors associated with materials that also have a sweet taste, such as molasses, caramelized sugar, and maple syrup.
Honey—sweet typical honey	Flavors associated with the sweet, caramelized flora and woody aromatic associated with honey.
Rooibos	Flavors associated with a combination of honey, woody and herbal-floral notes with a slightly sweet taste and subtle astringency.
Green—cut grass, mint	Flavors associated with green cut grass, fresh-cut grass, mint.
Cooked spinach	Flavors associated with cooked spinach.
Dry green herbal—chai tea	Flavor associated with "Green" flavor typical of dried grass or dried herbs
Earthy—boiled potatoes, damp potting soil	Flavor associated with damp soil, wet foliage, or slightly undercooked boiled potato, damp potting soil.
Fruity—peach, mango-like	Flavor associated with a mixture of peach-mango like fruits.
Perfume—floral, lavender	Flavor associated with a light fragrant aromatic characteristic of lavender.
Woody cinnamon, dry dusty, bark	Flavor associated with dry fresh-cut wood; bark, cinnamon, dust.
Medicinal	Flavor associated with dried grass or dried herbs used in herbal medication.
3.Aftertaste	
Bitter	Aftertaste associated with the taste on the tongue stimulated by solutions of caffeine, quinine
Green—cut grass, mint	Aftertaste associated with green cut grass, fresh-cut grass, mint.
Cooked spinach	Aftertaste associated with cooked spinach.
Dry green herbal—chai tea	Aftertaste associated with "Green" flavour typical of dried grass or dried herbs
Woody—cinnamon, dry dusty, bark	Aftertaste associated with dry fresh-cut wood, bark, cinnamon, dust.

Earthy—boiled potatoes, damp potting soil	Aftertaste associated with damp soil, wet foliage, or slightly undercooked boiled potato, or damp potting soil. Mouthfeel
4)Mouthfeel	
Astringent/dry	The chemical feeling factor on the tongue or other skin surfaces of the oral cavity described as puckering/dry and associated with tannins or alum (unripe banana, strong tea, anise, allspice)

(Malongane *et al.*, 2020)

PART III

MATERIALS AND METHOD

3.1 Materials

The materials that are included in my dissertation are as follow:

3.1.1 Green tea

For this study, the leaves of Camellia sinensis were chosen as the plant material. It was collected from village of Illam, helped by local people.

3.1.2 Tulsi leaf

It was collected from the periphery of central campus of technology. Krishna Tulsi (*Ocimum tenuiflorum*) was used for analysis which has purple leaves with serrated edges and small pink flowers in summer.

3.1.3 Packaging materials

Sample was kept in a polyethylene bag during the analysis. Tea bag used during sensory evaluation was made from food grade nonwoven fabric

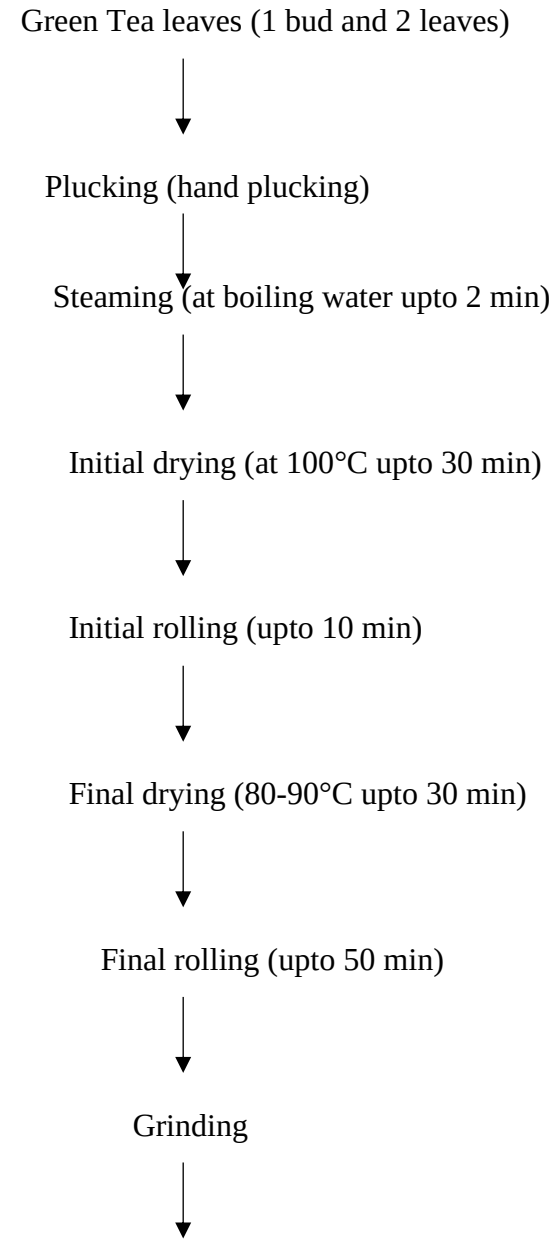
3.1.4 Equipment and Chemicals

All materials, equipment, and chemicals required were used from the laboratory of the Central campus of Technology, Dharan. All the materials, equipment, and chemicals used in the research purpose are listed in Appendix A.

3.2 Methods

3.2.1 Preparation of green tea powder

The flowchart for the preparation of green tea powder is as follows



Source:(Vishal Singh *et al.*, 2014)

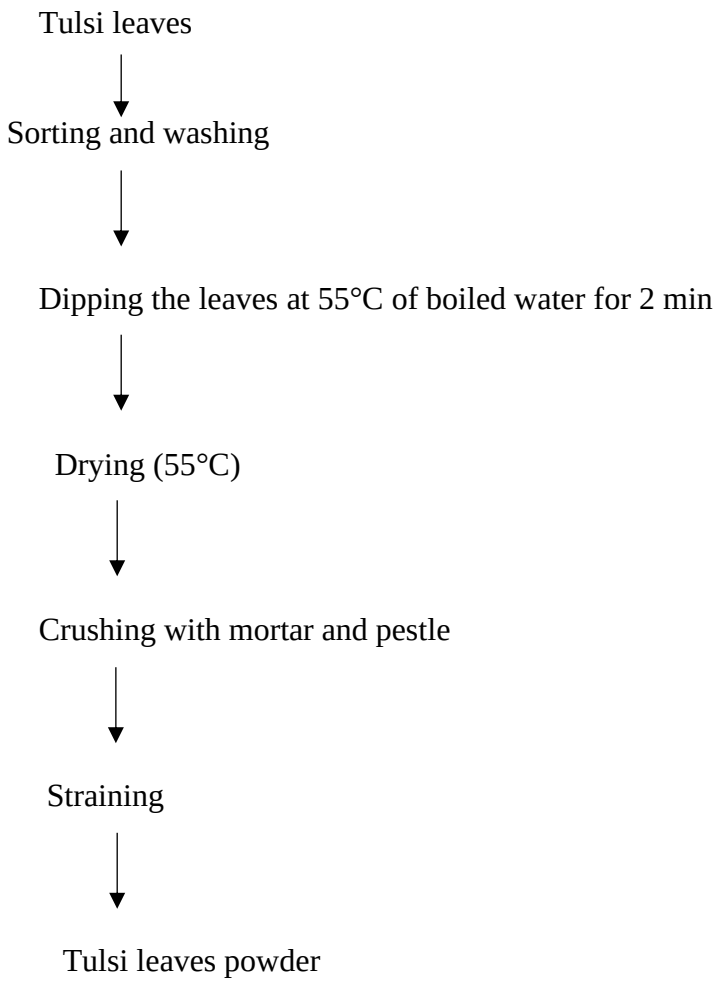
Fig 3.1 flowchart for the preparation of green tea powder

The method of making green tea powder started with the physical harvesting of two and one bud of green leaves. For up to two minutes, the freshly harvested leaves were steam-cooked in

boiling water to deactivate enzymes and maintain their green hue. To reduce the moisture content, the leaves were then first dried at 100°C for up to 30 minutes. After the leaves were partially dried, they were rolled for ten minutes or more to break up the structure and shape them, which enhanced the flavor development. The leaves were further dried at 80–90°C for 30 minutes in order to get the proper moisture content. A last roll lasting up to 50 minutes was used to fine-tune the leaf texture and improve product quality. The leaves were finely crushed and strained through 60 mesh size sieve to obtain uniform size. To ensure freshness and quality, the green tea powder was wrapped in polyethylene bags before storage and shipping.

3.2.2 Preparation of Tulsi leaves powder

The flowchart for the preparation of Tulsi leaves powder is as follows



Source: (Ameer *et al.*, 2022)

Fig 3.2 Flowchart for the preparation of Tulsi leaves powder

Tulsi leaf powder was prepared by first collecting Tulsi leaves, which were then sorted and washed with water to remove extraneous elements. The leaves were then steam blanched for 2 minutes by dipping in 55°C of warm water to deactivate enzymes and maintain nutritional value. After blanching, the leaves were dried at 55°C until they reached their required moisture level. The dried leaves were crushed to a fine powder, strained in 60 mesh size strainer and kept in a polyethene bag.

3.3 Experimental design

Design Expert v. 13.0.1.0 software was used to create various Tulsi leaf-infused green tea powder formulations. A simple mixing design was utilized to create different blend formulations. Four mix formulations were created using Tulsi and green tea extract, with yields ranging from 80% to 95% for green tea. Based on the output, five different formulations were obtained as shown in Table.

Table 3.1 Different formulation of Tulsi leaf powder and green tea powder

Product	**Formulation**
Control (A)	100% Green tea
B	5%Tulsi+95% Green tea
C	10% Tulsi +90% Green tea
D	15% Tulsi + 85% Green tea
E	20% Tulsi + 80 % Green tea
T	100% Tulsi leaves powder

3.4 Preparation of mixed tea blend

All the samples as per Design Expert v. 13.0.1.0 software was prepared on total of 3gm for analysis as shown in below Table 3.2

Table 3.2 Sample preparation for analysis

Sample code	Green tea powder (gm)	Tulsi leaves powder (gm)
A	3	0
B	2.85	0.15
C	2.7	0.3
D	2.55	0.45
E	2.4	0.6
T	0	3

3.5 Analytical procedure

3.5.1 Determination of moisture content

The moisture content of samples was measured as per method described by AOAC (2005)

3.5.2 Determination of protein content

The protein content of samples was determined by Kjeldahl method as described by AOAC (2005).

3.5.3 Determination of crude fat

The crude fat of samples was determined as per method described by AOAC (2005).

3.5.4 Determination of crude fiber

The crude fiber of samples was determined as per method described by AOAC (2005).

3.5.5 Determination of ash content

The ash content of samples was determined as per method described by AOAC (2005).

3.5.6 Determination of vitamin C content

The vitamin C content of samples was determined as per method described by AOAC (2005)

3.6 Preparation of plant extracts for phytochemical analysis

The plant extracts were prepared using solvent extraction with methanol. The organic extraction will be performed using the Soxhlet extraction method. To extract 20 g of dried plant powder, place it in a glass thimble and add 250 mL of methanol. The extraction process is continued until the solvent in the Soxhlet apparatus's siphon tube turns clear. The extract is cooked in a hot water bath at 350C until the solvent evaporates. The dried plant extract is kept refrigerated at 2-8°C for future use(Jaradat *et al.*, 2015).

3.7 Phytochemical analysis

3.7.1 Total phenolic content determination

The plant methanolic extract's total phenolic content (TPC) was assessed using a modified spectrophotometric method(Sangeetha *et al.*, 2014). For the analysis, 1 mg/ml aqueous solutions of methanolic extract are produced. Mixing 0.5 mL of plant extract solution, 2.5 mL of 10%
Folin Ciocalteu's reagent diluted in water, and 2.5 mL of 7.5% sodium carbonate (Na2CO3) aqueous solution yielded the reaction mixture. The samples are then incubated for 45 minutes in a thermostat set to 45 degrees Celsius. A spectrophotometer set at 765 nm is used to measure absorbance. For each analysis, the samples are produced in triplicate and the mean absorbance value is determined. The same procedure is repeated for the standard solution of gallic acid and the calibration line is constructed. Based on the measured 40 absorbance, the concentration of gallic acid equivalent is expressed in terms of (mg of GAE/g of extract)

3.7.2 Total flavonoid content determination

The total flavonoid content was determined using a modified aluminum chloride assay method(Shraim *et al.*, 2021).). In this method, 2 ml of the solution was pipetted into a test tube, and 0.2 ml of 5% Sodium Nitrate (NaNO2) was added, allowing it to stand for 5 minutes. Subsequently, 0.2 ml of Aluminum Chloride (AlCl3) was pipetted into the tube, and the mixture is allowed to stand for an additional 5 minutes. Next, 2 ml of 1N Sodium Hydroxide (NaOH) was added to the tube, and the volume was adjusted to 5 ml. After 15 minutes, the absorbance was measured at 510 nm against a reagent blank. The obtained results are then correlated with the

standard curve of Quercetin (20, 40, 60, 80, 100 µg/ml). The total flavonoid content was expressed as milligrams of quercetin equivalents (QE).

3.7.3 Total tannin content determination

The total tannin content was assessed using the Folin-Ciocalteu technique. In a volumetric flask (10 ml), add 0.1 ml of sample extracts, 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu reagent, and 1 ml of 35% Sodium Carbonate (Na2CO3) solution. Dilute to 10 ml with distilled water. After shaking thoroughly, the mixture was let to sit at room temperature for 30 minutes. Gallic acid reference standard solutions (20, 40, 60, 80, and 100 µg/ml) were made using the same method as previously described. The absorbance of the test and standard solutions was measured against a blank at 725 nm using a UV/visible spectrophotometer. Tannin concentration was expressed as milligrams of Gallic Acid equivalent per gram of extract t (mg GAE/g of extract) (Blainski *et al.*, 2013).

3.7.4 Chlorophyll content determination

Chlorophyll was extracted in 80% acetone and absorbance was measured using a spectrophotometer at 663nm for chlorophyll a and 645nm for chlorophyll b(Gogoi and Basumatary, 2018). The chlorophyll content was determined using the absorbance coefficients and calculated by following empirical formula

Chl a, mg/g tissue = 12.7(*A*663) - 2.69(*A*645) × V = 1000 × W

Chl b, mg/g tissue = 22.9(A645) - 4.68(A663) × V = 1000 × W.

Total chlorophyll (mg/g tissue) = Chl a + Chl b (calculated above)

Total chlorophyll (mg/g tissue) = Chl a + Chl b (calculated above)

Where A is the absorbance at a specific wavelength, V is the final volume of chlorophyll extract, and W is the fresh weight of the extracted tissue.

3.8 Determination of DPPH radical scavenging activity

To measure the DPPH radical scavenging activity, 1 milliliter of the sample extract and 4 milliliters of a 0.01% methanolic DPPH solution were placed in a test tube. The test tube was subsequently left in the dark for 30 minutes at ambient temperature (28°C). At 517 nm, the absorbance

was then determined with a UV-vis spectrophotometer. The sample was swapped out for methanol in a parallel control(Reena Patel *et al.*, 2015)

The DPPH scavenging activity was calculated using the formula

DPPH scavenging activity (%) = $\frac{(Ac-As)\ 100}{Ac}$

Where, Ac represents for absorbance of control;

As represents for absorbance of test sample

3.9 Preparation of blended tea in teabag packaging

To make blended tea, mix green tea powder with Tulsi leaves powder at the prescribed ratio as per table 3.2. The tea was thoroughly combined, resulting in an ideal mixture. The tea bags 39 were then filled with 3 grams of the blended tea. The teabag's top was tied with string, sealed, and labeled. Teabags were tightly sealed to prevent leaks

3.10 Sensory analysis of Tulsi leaves incorporated green tea

Tea samples were sensory evaluated using a 9-point hedonic scale to determine the tea's preferred rating for flavor, aroma, appearance, and general acceptability. The hedonic rating test was employed to evaluate consumers' acceptance of food goods. With both inexperienced and experienced panelists, this approach might be applied (Karki, 2022).

For the sensory evaluation, blended tea was brewed with 3 g using the same water volume of 150 ml of freshly boiled water for 5 minutes. All samples were served in 150 ml cups coded with random alphabets under warm conditions. The panel room was maintained free of food/chemical odors, unnecessary sound, and mixing of daylight. Each panelist was provided with an evaluation card (Appendix B) to record their opinions on sensory observations. Potable water was provided for rinsing between the samples, and verbal communication among the panelists was prohibited. The sensory parameters, including brew color, aroma, taste, mouthfeel, and overall acceptance, were analyzed. The grading system was based on a 9-point hedonic rating scale, where 9 represented "Like extremely," 8 stood for "Like very much," 7 indicated "Like," 6 signified "Like slightly," 5 denoted "Neither like nor dislike," 4 represented "Dislike

slightly," 3 indicated "Dislike moderately," 2 stood for "Dislike," and 1 represented "Dislike extremely."

3.11 Statistical method

The software IBM SPSS statistics version 27 was used to perform a one-way analysis of variance (ANOVA) on the triplicate data from each experimental analysis. Means were compared using fisher's unprotected LSD at 5% level of significance($P<0.05$) and MS excel for graphical representation of data.

PART IV

RESULT AND DISCUSSION

Tea (Camellia sinensis) leaves and Krishna Tulsi (*Ocimum tenuiflorum*) leaves were collected from KANYAM, Illam and periphery of central campus of technology respectively. Both Tea leaves and Tulsi leaves were processed further to prepare green tea and Tulsi leaves powder. It was then mixed in different proportions to obtain an optimum formulation of a blended tea using DOE (Sample A, B, C, D, E and T). Then physiochemical, phytochemical and sensory analysis of all samples were carried out to determine the best tea blends.

4.1 Chemical analysis

4.1.1 Chemical analysis of Tulsi leaf powder

The chemical composition of Tulsi leaf powder is shown in Table.

Table 4.1 Chemical composition of Tulsi leaf powder

Parameter	Values
Moisture content (% wb)	85.32± 0.53
Crude protein (% db)	19.20± 0.65
Crude fat (% db)	6.25± 0.08
Crude fiber (% db)	17.53± 0.45
Ash (% db)	8.06± 0.34
vitamin C (mg/100gm, db)	64.41± 0.74

*Values are the means of triplicate determination ± Standard Deviation. (All values are expressed in dry basis expect for moisture)

The moisture, protein, fat, crude fiber, ash and vitamin C of Tulsi leaf powder was found from the analysis as shown in above table. In this study, moisture content was 85.32 %. Tulsi leaves had 87% moisture content, similar to our findings(Parmar *et al.*, 2017). The crude protein value was obtained as 19.20 % and the crude protein content of 20% in Tulsi leaves powder according to (Priya and Peddha, 2023). The value of crude fat and crude fiber was found to be 6.25%and 17.53% respectively. The values of the fat composition of dried Tulsi powder, ranged between 2% to7% which is found to be in range and crude fiber of 17.55%(Priya and Peddha, 2023).

Similarly the value of ash was found to be 8.06% but it was reported to be 9% by(Raman, 1981). The vitamin C content of Tulsi powder we obtained was 64.41mg/g and it was 65 mg/g according to (Raman, 1981) . The difference in the data might be due to the variation in temperature during drying of leaves

4.1.2 Chemical analysis of Green Tea leaves and the blends

The chemical composition of green tea leaves and all the blended samples are shown in Table 4.2.

Table 4.2 Chemical composition of green tea leaves and the blends

Parameter	Green tea (A)	Sample B	Sample C	Sample D	Sample E
Moisture content (%wb)	70.07 ± 0.06^{a}	70.80 ± 0.02^{b}	71.63 ± 0.07^{c}	72.40 ± 0.08^{d}	73.30 ± 0.26^{e}
Crudeprotein (%db)	17.22 ± 0.02^{a}	17.32 ± 0.02^{b}	17.42 ± 0.02^{c}	17.50 ± 0.0^{d}	17.62 ± 0.02^{e}
Crude fat (% db)	6.80 ± 0.01^{d}	6.76 ± 0.005^{c}	6.73 ± 0.005^{bc}	6.71 ± 0.01^{b}	6.67 ± 0.02^{a}
Crude fiber (%db)	7.7 ± 0.10^{a}	8.1 ± 0.10^{b}	8.54 ± 0.08^{c}	9.06 ± 0.15^{d}	9.58 ± 0.01^{e}
Ash (%db)	6.58 ± 0.01^{a}	6.65 ± 0.01^{b}	6.74 ± 0.02^{c}	6.83 ± 0.05^{d}	6.93 ± 0.05^{e}
VitaminC (mg/100gm,dw)	11.29 ± 0.11^{a}	14.30 ± 0.09^{b}	16.68 ± 0.18^{c}	19.66 ± 0.15^{d}	22.35 ± 0.18^{e}

*Values are the means of triplicate determination ± Standard Deviation. (All values are expressed in dry basis expect for moisture). Value in the column having different superscripts are significantly different at 5% level of significant

The above table shows the proximate compositions of green tea (A), and four blended samples (B, C, D, and E), highlighting variations in moisture, protein, fat, fiber, ash and vitamin C levels. In the blended samples the concentration of Tulsi was gradually increased from B to E.

Green tea (A), Sample B, Sample C, Sample D, and Sample E exhibit a substantial increase in moisture content from 70.07% to 73.30%. The mean moisture level of each sample was statistically distinct ($p < 0.05$).sample E had the maximum moisture content of 73.30% ± 0.26,

whereas green tea (A) had the lowest at 70.07% ± 0.06.Green tea had a moisture level of 77.2%, similar to the value we studied (Jabeen *et al.*, 2019).

Protein content of green tea was found as 17.22%. The protein content of green tea was found to be 18.06 and14.32% respectively which align with our analyzed value(Ahmad *et al.*, 2014)(Rubab *et al.*, 2020). Sample B has 17.32%, Sample C has17.42 %, Sample D has 17.50 %, and Sample E has 17.62% protein content. The mean protein level of each sample was statistically distinct ($p < 0.05$) as denoted by different subscripts. This increase in protein content reflect the protein rich nature of Tulsi.

The fat content of green tea (sample A) was measured as 6.80%. Similarly sample B, C, D and E has fat content of 6.76%, 6.74%, 6.71% and 6.67% respectively. Fat content of sample decreased from B to E and there is also no statical difference between samples. However, addition of Tulsi significantly does not impact the fat content of blends, this may be due to slightly lower fat content of Tulsi than green tea.

The fiber content of green tea was measured as 7.7%. Crude fiber of green tea ranged from 4.37 to 20.8% (Aroyeun, 2012). Variations in drying temperature, processing conditions, and tea leaf variety may account for the observed variance. Sample B, C, D and E contain crude fiber of 8.1%,8.54%,9.06 %and 9.58% respectively. This constant increasing trend implies a positive relationship with Tulsi addition and fiber content, with substantial statistical differences between samples, as indicated by the superscripts. These could be due to the high fiber content of Tulsi leaves.

The ash content of green tea was measured as 6.58%. Ash content of green tea was 6.94% (Tandale *et al.*, 2023). In the blend samples B, C, D and E it was found6.65%,6.74%,6.83% and 6.93% respectively. The ash content of each sample was statistically distinct ($p < 0.05$) with different subscripts, and gradually increases when concentration of Tulsi increased in green tea and that might be due to the high ash content of Tulsi leaves.

Vitamin C of green tea was determined 11.29 mg/ 100gm. Vitamin C levels in unbrewed leaf green tea varied from <3 to 178 mg/100g (Somanchi *et al.*, 2017). In the blend samples B, C, D and E it was found 14.30±0.09, 16.68±0.18, 19.66±0.15, 22.35±0.18 mg/g respectively. The mean vitamin C level of each sample was statistically distinct ($p < 0.05$) with different

subscripts, and gradually increases when concentration of Tulsi increases in green tea and that might be due to the high vitamin C content of Tulsi leaves.

4.2 Phytochemical Analysis

All samples extract phytochemical content were analyzed using spectrophotometer. The value obtained are as follows. **Table 4.3** Phytochemical analysis of green tea and the blends

Sample	TPC (mg GAE/g dry weight)	TFC (mg QE/g dry weight)	TTC (mg GAE/g dry weight)	TCC (mg/g)
A	70.52 ± 0.01^{f}	52.166 ± 0.115^{d}	38.59 ± 0.01^{f}	0.92 ± 0.01^{ab}
T	60.33 ± 0.015^{a}	25.71 ± 0.64^{a}	11.24 ± 0.005^{a}	2.28 ± 0.015^{d}
B	70.43 ± 0.02^{e}	51.87 ± 0.017^{d}	38.50 ± 0.01^{e}	0.843 ± 0.12^{a}
C	69.22 ± 0.02^{d}	51.55 ± 0.005^{d}	37.65 ± 0.01^{d}	1.046 ± 0.005^{bc}
D	68.76 ± 0.01^{c}	49.61 ± 0.03^{c}	36.32 ± 0.01^{c}	1.123 ± 0.011^{c}
E	67.12 ± 0.015^{b}	47.77 ± 0.01^{b}	35.02 ± 0.01^{b}	1.206 ± 0.005^{c}

*Values are the means of triplicates and figures in the parenthesis are standard deviation of the triplicates. Values in the column having different superscripts are significantly different at 5% level of significant

4.2.1 Total Phenolic Content (TPC)

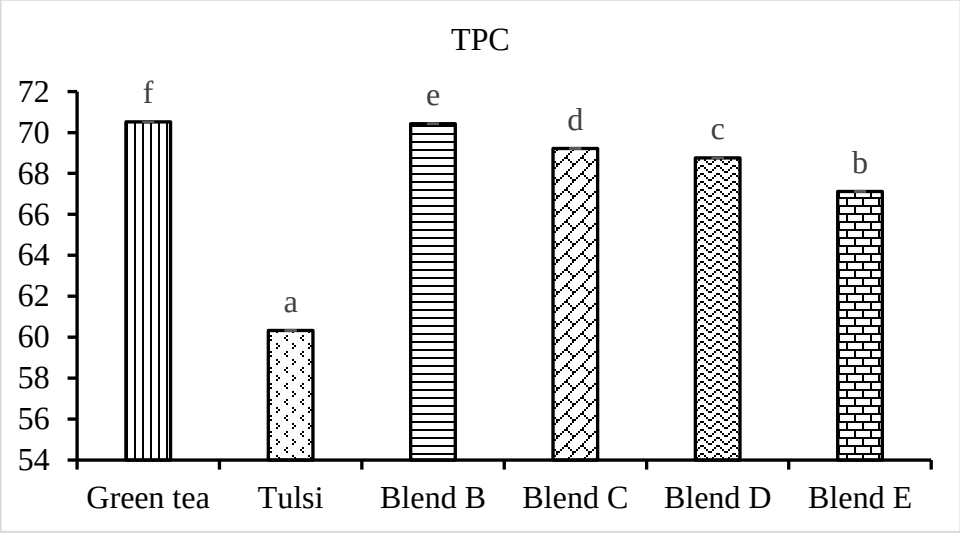


Fig 4.1 Bar diagram for TPC of different samples [Values in the figure having different alpha-bets at top are significantly different (P<0.05)]

The TPC of green tea was found to be 70.52 ± 0.01mg GAE/g dry weight which was calculated by using the calibration curve and absorbance value. The TPC of green tea's methanolic extract was 76 mg GAE/g dry weight(Anita *et al.*, 2014). Weather, cultivar effect, and varying maturation levels could all be contributing factors to the data variances.

The TPC of methanol extract of Tulsi was found to be 60.33±0.015 mg GAE/g which was calculated by using the calibration curve and absorbance values. TPC of methanol leaf extract of Tulsi was found to be 48.89±1.59 (Guleria *et al.*, 2013). TPC of methanol leaves extract of Tulsi was 180.21 ± 0.89 mg GAE/g dried extract(Pathak and Niraula, 2019). And similarly TPC of methanol extract of Tulsi was 92.37±1.81 mg GAE/g (Aluko *et al.*, 2012).Plant source variability, maturation stage, drying and storage methods, extraction time and temperature, analysis of plant material during different seasons might be the reason for this variation of data. The polyphenol content of plants and plant meals can be impacted by environmental edaphic factors such as rainfall, soil type, and sun exposure, according to(Rajbhar *et al.*, 2015).

The TPC values of blended samples B, C, D and E were 70.43±0.02, 69.22±0.02, 68.76±0.015, and 67.12±0.01 mg GAE/g dry weight, measured using the calibration curve and absorbance values. From the figure 4.1 is found that there are significance differences($P<0.005$) among samples with decreasing TPC contain. The decreased in TPC may be due to the lower TPC content of Tulsi than green tea.

4.2.2 Total Flavonoid Content (TFC)

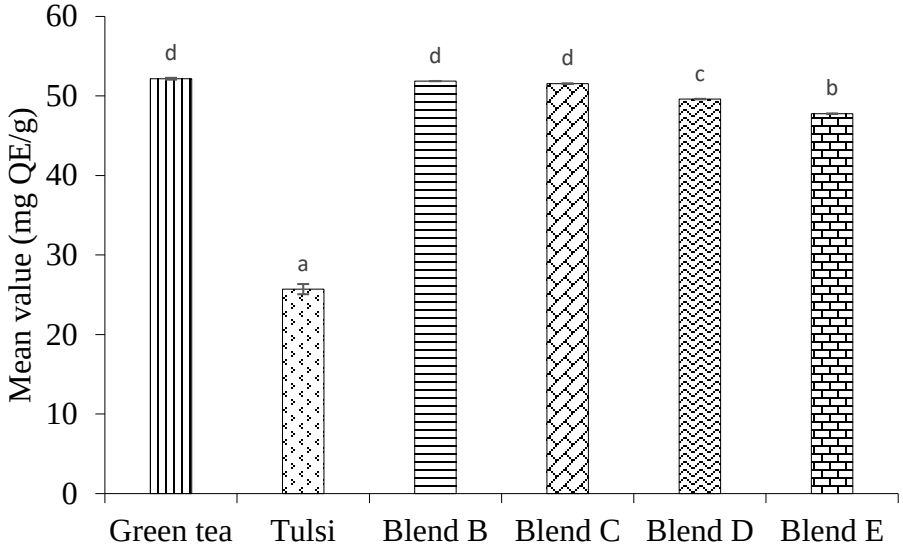


Fig 4.2 Bar diagram for TFC of different samples [Values in the figure having different alphabets at top are significantly different (P<0.05)]

Green tea extract had a TFC of 52.166 ± 0.115 mg QE/g dry weight, determined using the calibration curve and absorbance data from spectrophotometer (see Figure 2). After methanol extraction, aged Camellia sinensis green tea leaves had a flavonoid content of 70.3 ± 4.428 mg QE/g dry weight(Acharya *et al.*, 2013). Variations in data may occur due to factors like as maturity level, cultivar effect, harvesting timing, and weather.

The TFC of methanol extract of Tulsi leaves is 25.71 ± 0.64 mg QE/g which was quantified by using the calibration curve as well as the absorbance values (Figure E.1). The TFC of methanol extract of leaf of Tulsi leaves was found to be 67.70 ±1.04 mg QE/g (Ilyas, 2024). Similarly , the TFC of methanol extract of leaf of Tulsi was found to be 67.11 ± 0.43 mg QE/g (Pathak and Niraula, 2019). The differences in the value of TFC may be due to difference in maturity, growth conditions, post-harvest factors, cultivar effect, extraction methods, and season during analysis.

The TFC values of blended samples B, C, D and E were 51.87±0.017, 51.55±0.005, 49.61±0.03 and 47.77±0.01 mg QE/g dry weight, respectively, measured using the calibration curve and absorbance values. Figure 4.1 shows no significant differences (P < 0.005) among

samples A, B and C with subscripts denoted by (d). This may be due to the addition of low amount of Tulsi. While with increased addition of Tulsi leaves to green tea diminishes its flavonoid concentration. This suggests that the mixture may alter the antioxidant profile of tea, affecting its nutritional and health benefits.

4.2.3 Total Tannin Content (TTC)

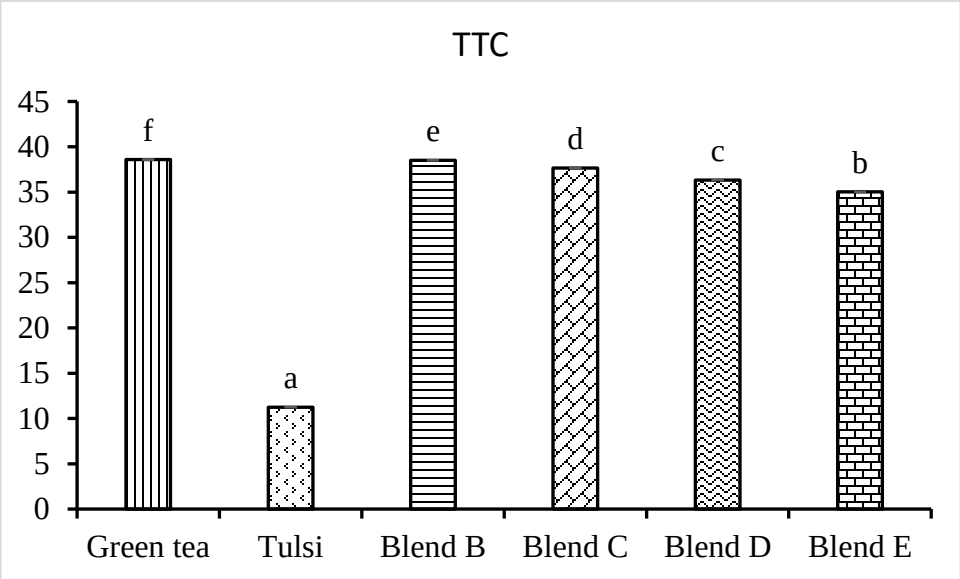


Fig 4.3 Bar diagram for TTC of different samples [Values in the figure having different alphabets at top are significantly different (P<0.05)]

Green tea had a tannin concentration of 38.59 ± 0.037 mg GAE/g, as determined by the calibration curve and absorbance values (Figure 3). Green tea contains 37 ± 2.6 mg GAE/g dry weight of tannin according to (Pathak and Niraula, 2019). A number of factors, such as the plant's genotype, climate, soil, harvest time, storage, processing, treatment, and vegetative stage, can affect the tannin content (TC).

The tannin content of Tulsi leaves was found to be 11.25 ± 0.07 mg GAE/g quantified by using the calibration curve as well as the absorbance values (Figure.3).The tannin content of hexane extract of Tulsi was 260.8 ± 11.5 mg CE/kg d w) (Basak *et al.*, 2014). Differences in the plant's growth stage, extraction technique, environmental elements like soil quality and climate, when to harvest, and processing techniques could all contribute to the value discrepancy. The Tannin content of blended samples B, C, D, and E were 38.50± 0.07, 37.65,36.32 and 35.02 mg GAE/g

dry weight, measured using the calibration curve and absorbance values. The figure shows significant differences ($P < 0.005$) among samples. Data shows that adding Tulsi leaves to green tea reduces its tannin concentration. Tulsi leaves' beneficial chemicals may interact with tannins, reducing their solubility and availability, leading to a drop in tannin levels.

4.2.4 Chlorophyll content

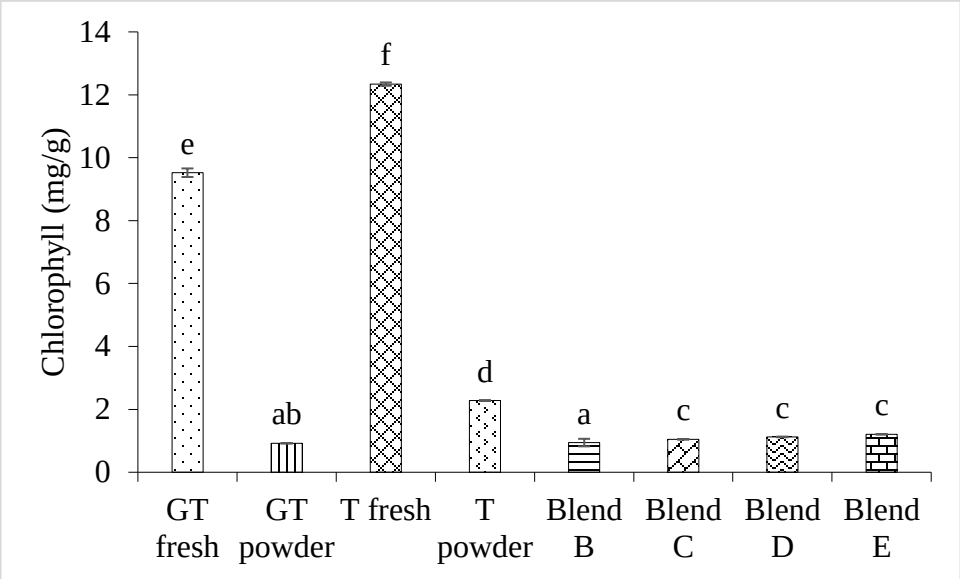


Fig 4.4 Bar diagram for Chlorophyll of different samples [Values in the figure having different alphabets at top are significantly different ($P<0.05$)]

Chlorophyll concentrations varied greatly among samples, including fresh green tea and Tulsi leaves, powdered versions, and four blends. Fresh Tulsi leaves contained the maximum chlorophyll concentration (12.34±0.052 mg/g), followed by fresh green tea leaves (9.523±0.136 mg/g).

Chlorophyll content of fresh Tulsi leaves was 10.32 ± 0.12 mg/gm which is similar to our analyzed value(Ghoshal and Research, 2013). Chlorophyll content of fresh green tea leaves similar to our analyzed value(Chen *et al.*, 2021). The process of drying had a significant effect on the chlorophyll content as chlorophyll is a sensitive pigment which can breakdown easily and the powdering process also reduced chlorophyll levels. Tulsi powder retained 2.28±0.015 mg/g and green tea 0.92±0.01 mg/g amount of Chlorophyll. Green tea powder contains 1.12 to 1.89 mg/gm chlorophyll (Ošťádalová *et al.*, 2015) .Tulsi powder contain 5.97 ± 0.02 mg/gm chlorophyll with 100% acetone (De *et al.*, 2015). By limiting exposure to elements that normally

induce chlorophyll degradation, such as heat, oxidation, and enzymatic activity, the greatest chlorophyll content can be achieved. Thermal degradation is reduced and the green hue associated with chlorophyll is maintained by reducing drying time and allowing lower drying temperatures.

Samples B, C, D, and E, showed chlorophyll contents of 0.943± 0.12, 1.046±0.005, 1.123±0.011, and 1.206 ±0.005 mg/g, respectively. Here the chlorophyll content of blend increases with increasing addition of Tulsi which is due to the more chlorophyll content of Tulsi than green tea.

4.2.5 Antioxidant activity analysis

The percentage inhibition of DPPH radical scavenging activity of green tea, Tulsi leaves and blend is shown in Figure 4.5

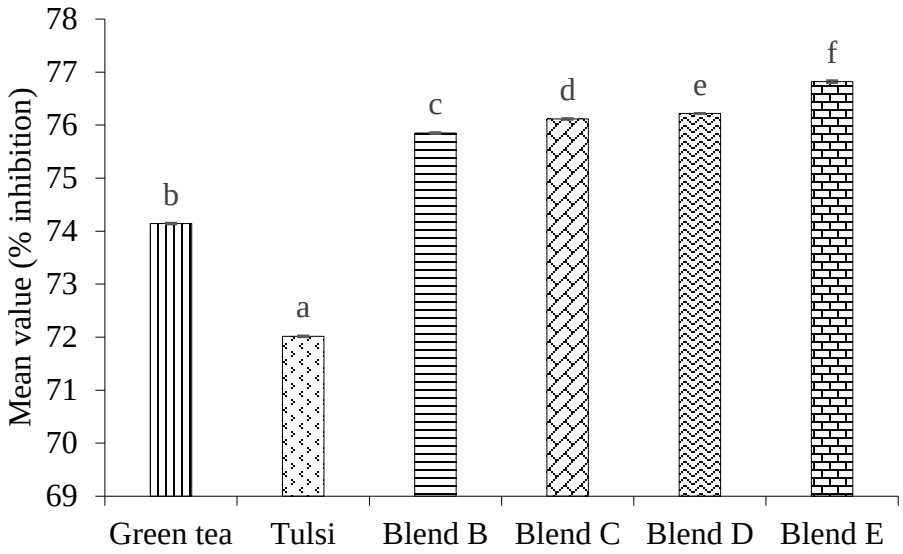


Fig 4.5 Bar diagram for Chlorophyll of different samples [Values in the figure having different alphabets at top are significantly different (P<0.05)]

The DPPH radical scavenging activity of green tea was found to be 74.173% inhibition. The methanolic extract of Camellia sinensis green tea has 94.10 percent suppression of DPPH radical scavenging activity (Namdev *et al.,* 2015). The DPPH values may vary depending on the plant sections used, the season, and the maturity of the plants at the time of study. The DPPH radical scavenging activity of Tulsi leaves was 71 % inhibition and this is near to our value which was

72.02% inhibition (Balaji *et al.*, 2011). The results of DPPH-free radical scavenging assay indicate that the Tulsi extracts can scavenge free radicals through mechanisms that donate electrons or hydrogen, and as a result, they ought to be strong enough to stop harmful free radical-mediated chain reactions from starting in vulnerable matrices, such as biological membranes (Hakkim *et al.*, 2007). The DPPH radical scavenging activity of blend B, C, D, and E was found to be 75.872%, 76.100%, 76.236%, 76.846% inhibition respectively. The higher activity observed in all blends suggests that Tulsi and other ingredients may work in concert to greatly increase the mixes' antioxidant capability. The findings demonstrate that the DPPH value can be changed by the herbal green tea formulation.

4.3 Sensory analysis

Sensory evaluation was done by 10 Panelist for the color, aroma, taste, astringency, and overall acceptance of the infusion on a 9-point hedonic scale. Statistical analysis (One-way ANOVA) was conducted ($p < 0.05$). Table 4.1 summarizes the sensory evaluation and statistical analysis results.

Table 4.4 Average mean of sensory score

Sample	Color	Aroma	Taste	Astringency	Overall Acceptance
A	7.50 ± 0.972^{b}	6.60 ± 1.174^{a}	7.0 ± 0.816^{b}	8.000 ± 0.47^{a}	7.0 ± 0.816^{b}
B	7.30 ± 0.823^{b}	6.50 ± 0.707^{a}	7.0 ± 0.667^{b}	7.700 ± 0.632^{a}	6.9 ± 0.78^{b}
C	7.70 ± 0.483^{b}	7.30 ± 0.919^{b}	8.30 ± 0.823^{c}	6.850 ± 0.669^{b}	8.2 ± 0.91^{c}
D	6.40 ± 0.699^{a}	6.30 ± 0.949^{a}	6.10 ± 0.994^{a}	5.90 ± 0.567^{c}	5.9 ± 0.87^{a}
E	6.10 ± 0.994^{a}	6.0 ± 1.633^{a}	6.30 ± 1.337^{a}	5.700 ± 0.567^{c}	5.5 ± 1.35^{a}

*Values are the means of triplicates and figures in the parenthesis are standard deviation of the triplicates. Values in the column having different superscripts are significantly different at 5% level of significant.

4.3.1 Color

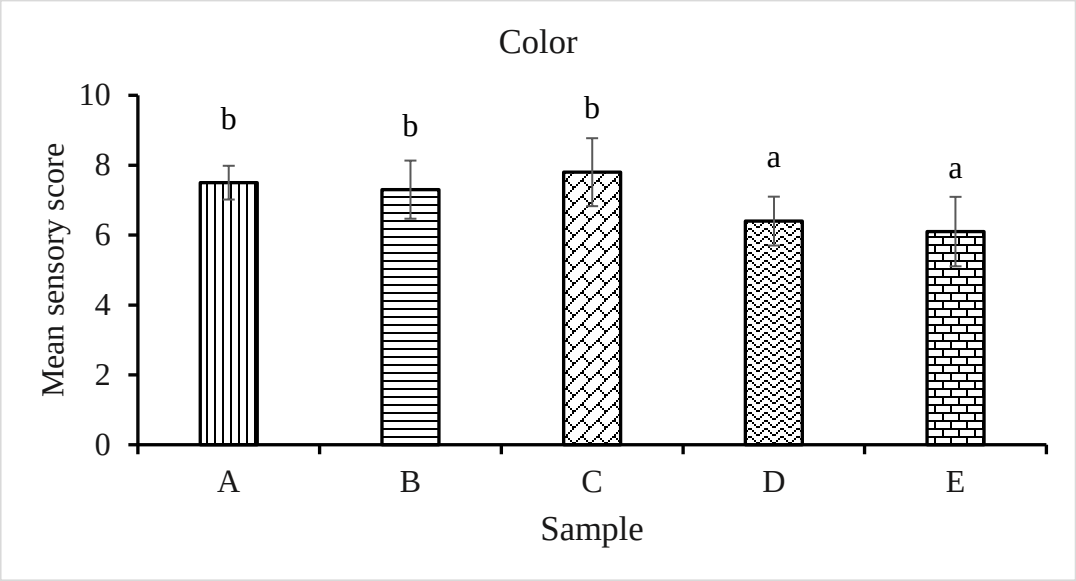


Fig 4.6 Bar diagram for Color of different samples [Values in the figure having different alphabets at top are significantly different (P<0.05)]

The mean sensory score for the color of samples A, B, C, D and E was found to be 7.50, 7.30, 7.70, 6.40 and 6.10 respectively. Samples A, B and C had high scores, indicating a higher color preference, whilst Sample D and E received the lowest value, implying that it was the least favored color. Above table shows no statistically significant difference across the samples ($p<0.05$) with Samples A, B and C classified as (b) indicating no significant difference. Samples D and E were marked with a (a)indicating no substantial differences between them but lower than those of A, B and C sample.

Statistical analysis revealed that samples A, B and C had superior color. This increased preference could be due to the light brownish green which panelists deemed more appealing. In contrast, Sample D and E had the lowest score, possibly because of more greenish color, which was less visually appealing to the panelists. Color is an important quality element, particularly in food choices, because it significantly impacts first impressions. According to(Liu *et al.*, 2018) intended infusion color is primarily yellowish green, and color is a significant quality indicator for the flavor of green tea infusions. The primary variables influencing the color shift of dried green tea are temperature and drying duration. Non-enzymatic browning reactions, which are significant occurrences during food processing, may arise from prolonged drying at high

temperatures. A variety of processes, including the Maillard reaction, caramelization, and ascorbic acid oxidation, are included in non-enzymatic browning.

4.3.2 Aroma

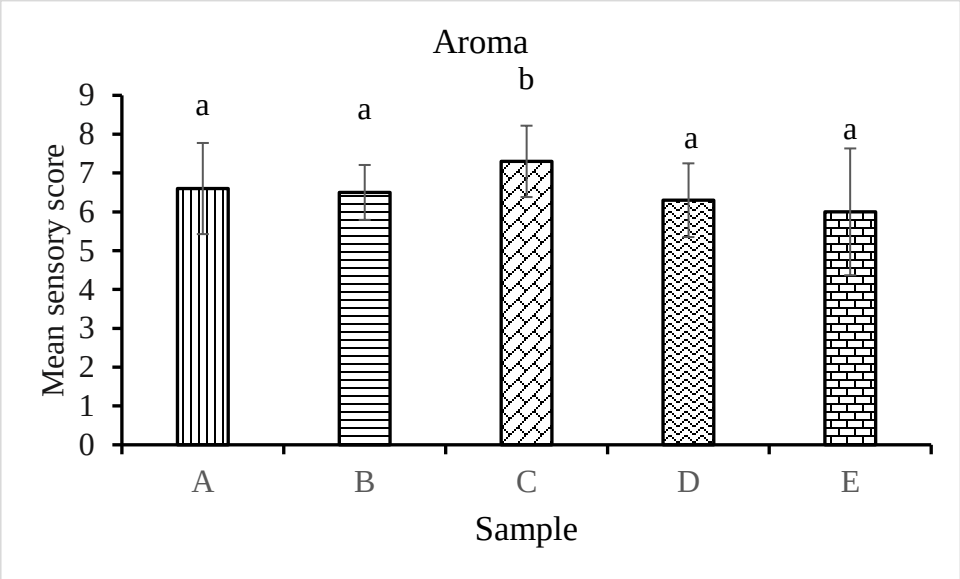


Fig 4.7 Bar diagram for aroma of different samples [Values in the figure having different alphabets at top are significantly different (P<0.05)]

The mean sensory score for the aroma of samples A, B, C, D and E was found to be 6.60,6.50, 7.30, 6.30 and 6.000 respectively. Sample C received the highest aroma score (7.30), and Sample E had the lowest (6.000). Sample C marked with a (b) exceeded other sample in terms of aroma, with a statistically significant difference ($p<0.05$). However, Samples A, B, D and E which all belong to the (a) grouping, are significantly different from Samples C. In comparison to other sample C had an acceptable aroma. This may be due to the optimal balance of Tulsi and green tea, which contributed to an acceptable aroma.

Processing and storage circumstances can affect the delicate scent profile of green tea; problems during these phases might change the aroma even if the basic chemical components stay the same(Zhao *et al.*, 2024).The high temperatures needed to process tea can result in Maillard reactions, which give the tea a burnt smell(Al-Abbasy *et al.*, 2024). Previous studies have shown that green tea smelled better than black tea. This is probably because green tea leaves are

younger and fermentation is controlled, which alters the amount of thearubigins, caffeine, and catechins(Tanaka *et al.*, 2020).

4.3.3 Taste

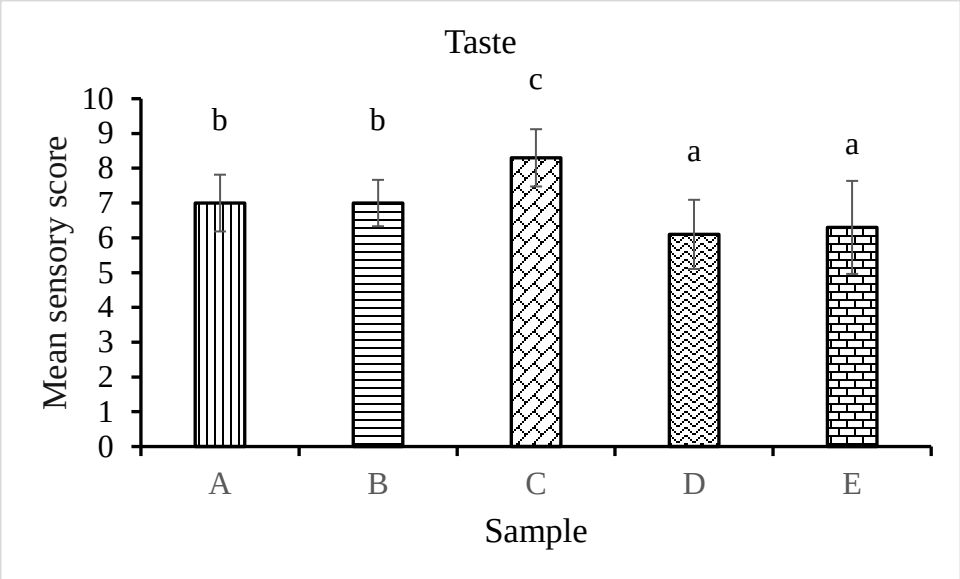


Fig 4.8 Bar diagram for taste of different samples [Values in the figure having different alphabets at top are significantly different (P<0.05)

The mean sensory score for the taste of samples A, B, C, D and E was found to be 7.0, 7.0, 8.30,6.10 and 6.30 respectively. Sample C received the highest taste score (8.30) and sample D had the lowest (6.10). Samples A and B which all belong to the (b) grouping, did not differ significantly from each other and Sample C denoted by (c) was found to be significantly differ from other sample and Sample D and E denoted by (a) had also found no significant difference from one another.

The preference for Sample C could be attributed to a balance of green tea and Tulsi leaves which provide astringent and pleasant aftertaste features moderated by the polyphenol-amino acid ratio, which influences bitterness and astringency levels. Sample D lower taste score indicates a lower astringent flavor, which is most likely due to a lower polyphenol-amino acid ratio and higher Tulsi leaf concentration and Sample A and B also found to be less preferred than C due to its lower amount of Tulsi leaves and higher bitter taste of green tea. The bitterness of the samples was reduced as the concentration of Tulsi leaves increased because the bitterness of

Tulsi leaves was eliminated during roasting. This finding is consistent with prior research on tea processing, where procedures such as microwave drying minimize astringency but hot airdrying increases bitterness(Kidist Teshome *et al.*, 2013).

When evaluating commercial tea's sensory qualities, caffeine plays a crucial role in the formation of taste(Adnan *et al.*, 2013).

4.3.4 Astringency

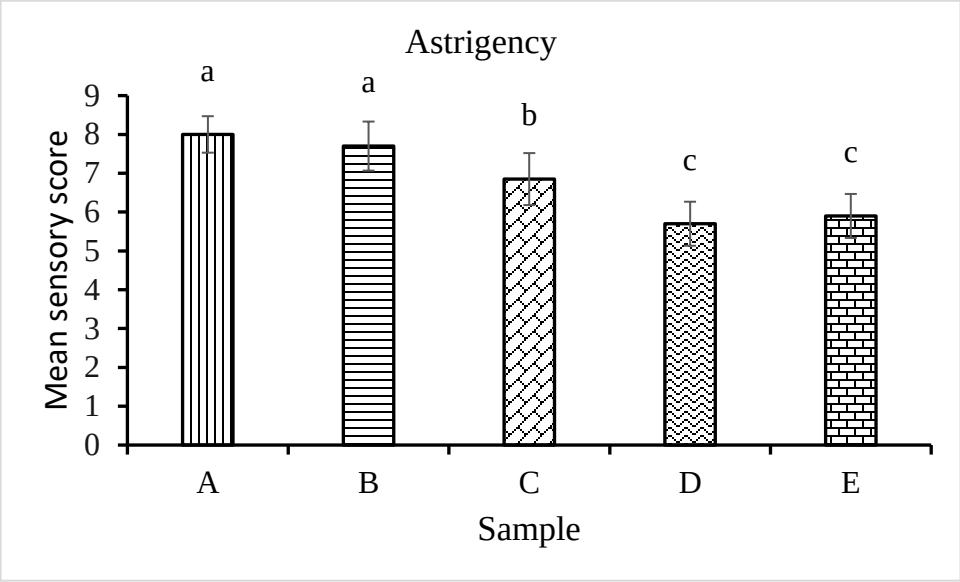


Fig 4.9 Bar diagram for Astringency of different samples. Values in the figure having different alphabets at top are significantly different ($p<0.05$)

The mean sensory score for the astringency of samples A, B, C, D and E was found to be 8.0, 7.7, 6.8,5.9 and 5.7 respectively. Sample A received the highest mean score for astringency, whereas sample E had the lowest mean score. Table shows sample A and B had symbol (a) with no significant differences in astringency while sample C denoted by symbol (b) are significantly different ($p<0.05$). Sample D and E denoted by (c) was also found to have no significant difference between them.

Sample A and B was superior based on the astringency of infusion from statistical analysis. Since sample A was control sample of green tea and it was bitter so it has rich astringency profile and sample B had little Tulsi on it which provide the balance on astringency so the sample A

and B are more acceptable to panelists. As the Tulsi increases in other sample which may alter the astringency profile of green tea. According to(Liu *et al.*, 2018) the intensity of bitterness and astringency increased with longer infusion time, higher water temperature, smaller particle size, and lower water/tea ratio. Some high-quality teas are thought to be bitter in the tongue but sweet in the throat. High-quality teas prioritize fragrant, sweet, and delicate qualities above bitter and astringent ones.

4.3.5 Overall Acceptance

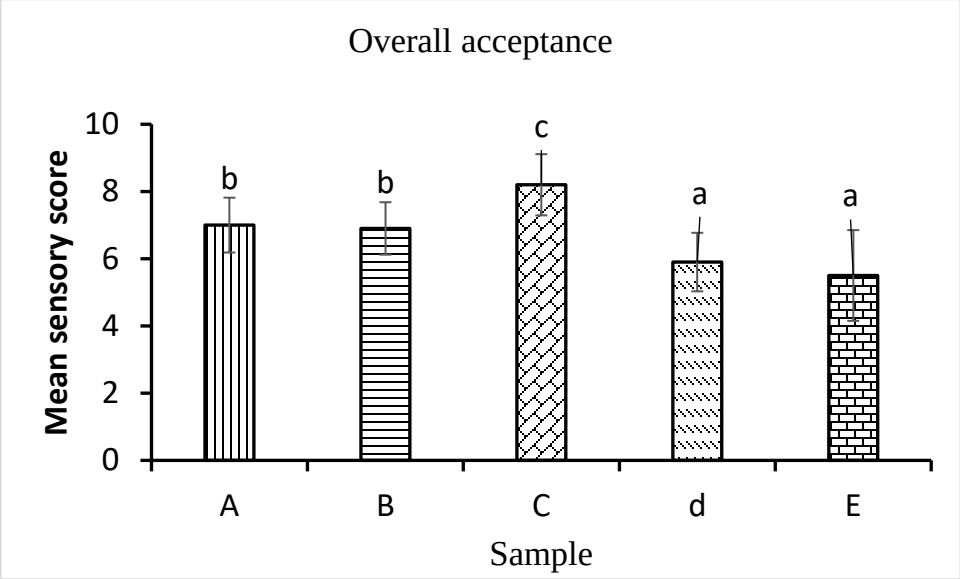


Fig 4.10 Bar diagram for overall acceptance of different samples. Values in the figure having different alphabets at top are significantly different ($p<0.05$)

The mean sensory score for the overall acceptance of samples A, B, C, D and E was found to be 7.0, 6.9, 8.2,5.9 and 5.5 respectively. Sample C received the Highest overall acceptance score, whereas Sample E had the lowest mean score. Above Table shows that the general acceptance of the brew differed significantly ($p<0.05$). Samples A & B had symbol(b) indicates no significant difference and sample D and E denoted by (a) also found to have no significant difference in acceptability. Sample A and B, Dand E and C with a symbol (c) differ from the other and found to be more acceptable one. This could be attributed to the formulation's more appealing color, aroma, taste, and astringency.

(Liu *et al.*, 2018) discovered that samples of green tea infusion had overall acceptance scores ranging from 3.81 to 6.38. To assess overall acceptability, green tea infusions were scored on flavor and color (apart from aroma). Samples of black and green tea received acceptability ratings ranging from 4 to 8. A number of factors, such as caffeine, amino acids, catechins, thearubigins, and theaflavins, are used to assess the quality of commercial tea. High concentrations of chemical and volatile components in tea samples improve their sensory attributes, including their general acceptability(Shafi *et al.*, 2022).

PART V

Conclusion and recommendation

5.1 Conclusions

On the basis of proximate analysis, phytochemical analysis, DPPH radical scavenging activity and sensory characteristics of green tea (*Camellia sinensis*), Tulsi (Camellia sinensis) and Tulsi leaves incorporated green tea (blend) powder were studied. As a result of the research, the following results were drawn**:**

1. Proximate analysis showed that incorporating Tulsi leaves powder in green tea boosted moisture, protein, ash, fiber and vitamin C content while fat remains constant.
2. From Phytochemical analysis, Adding Tulsi to green tea increases chlorophyll levels, while lowering Phenolic, flavonoids and tannins contain.
3. The DPPH radical scavenging activity analysis shows varying antioxidant capacities in green tea, Tulsi leaves, and their blends with significant synergistic effects.
4. Sample E (highest Tulsi) showing the greatest improvements, making it the top blend for enhanced nutrition from chemical and phytochemical analysis.
5. From the sensory analysis green tea (90%) powder in combination with Tulsi (10%) leaf powder (sample C) was selected as best tea blend powder and was found highly acceptable to consume.

5.2 Recommendations

1. Green tea blended with *Ocimum sanctum* in a 90:10 ratio was found to be highly preferred by panelist, indicating its potential for large-scale production as a distinctive tea variety.
2. Effect of other herbs incorporation on phytochemical, antioxidant and sensory properties of green tea can be studied.
3. Different parts of *Ocimum sanctum* can be used for analysis.

PART VI

Summary

To prepare the blend, a simple mixture design was applied. *Camellia sinensis* tea is a globally popular beverage, second only to water. Scientific research has focused on the biological and chemical properties of teas. *Ocimum sanctum* is renowned in Ayurveda and traditional medicine for its effective medicinal properties. The plant's leaves are widely utilized and helpful, and its extract is an excellent source of antioxidants for nutraceutical applications.

A simple mixture design was used to make the blend. Five powder blend compositions were created namely: Control (100% green tea), B (95% green tea + 5% Tulsi), C (90% green tea + 10%Tulsi), D (85% green tea + 15% Tulsi), and E (80% green tea + 20% Tulsi). Tulsi powder, green tea powder, and four blend formulations were subjected to chemical analysis in order to determine their levels of moisture, fat, protein, crude fiber, ash, and vitamin C. Moisture, protein, ash, vitamin C, and fiber content all increased in the blend except the fat. Among all the samples, blend sample E was identified as the most nutritious based on the chemical analysis with moisture 73.30%, protein 17.62%, fat 6.67%, fiber 9.58%, ash 6.93%, and vitamin C 22.35 mg/g. After that, all samples were subjected to phytochemical analysis (TPC, TFC, TTC, TCC, and DPPH radical scavenging activity). The results showed that all the blend's phenolic and chlorophyll levels was increased, while its flavonoid, tannin content decreased. DPPH radical scavenging activity was also seen synergistically enhanced. From the phytochemical analysis, sample E was again identified as the most nutritious blend with TPC 72.03±0.015 mg GAE/g and TCC 2.496±0.145 mg/g and DPPH 68.746% inhibition while TFC 47.77±0.06 mg QE/g and TTC 30.500±0.060 mg GAE/g was found lower than other blends. However, sample E was found most accepted due to its higher in both chemical and phytochemical properties. All the physiochemical and phytochemical properties of sample E was found to be significantly different ($P<0.05$) from green tea and other blends.

Control A sample of green tea, along with four blend formulations, was created for tea cup testing. The sensory examination assessed color, aroma, taste, astringency, and overall acceptability. Data was evaluated using one-way ANOVA with a 5% level of significance. Product C had the highest mean sensory score and closely matched the control sample.

Reference

Acharya, P. P., Genwali, G. R. and Rajbhandari (2013). Isolation of catechin from acacia catechu willdenow estimation of total flavonoid content in camellia sinensis kuntze and camellia sinensis kuntze var. assamica collected from different geographical region and their antioxidant activities. *Scientific world* 11, no. **11** (11), 32-36.

Adnan, M., Ahmad, A., Ahmed, A., Khalid, N., Hayat, I. and Ahmed,(2013). Chemical composition and sensory evaluation of tea (Camellia sinensis) commercialized in Pakistan. *Pak. J. Bot* **45** (3), 901-907.

Ahmad, R. S., Butt, M. S., Huma, N., Sultan, M. T., Arshad, M. U., Mushtaq, Z. and Saeed, F. J. (2014). Quantitative and qualitative portrait of green tea catechins (GTC) through HPLC. *International Journal of Food Properties* **17** (7), 1626-1636.

Ahmed, S. (2011). "Biodiversity and ethnography of tea management systems in Yunnan, China". City University of New York. [1124519106].

Ahmed, S. and Stepp, J. (2012). Green Tea: The Plants, Processing, Manufacturing and Production. *Tea in health and disease prevention In*.). [9780123849373].

Ahmed, S., Stepp and prevention, d. (2013). Green tea: The plants, processing, manufacturing and production. *Tea in health and disease prevention* 19-31.

Al-Abbasy, O. Y., Younus, S. A., Rashan, A. I., Ahmad, and Biotechnology, A. (2024). Maillard reaction: formation, advantage, disadvantage and control. *Food Science and Applied Biotechnology* A review. **7** (1), 145-161.

Alara, O. R., Abdurahman, N. H. and Ukaegbu, (2021). Extraction of phenolic compounds: A review. *Current research in food science* **4**, 200-214.

Ali, A. and Deokule, S. S. (2008). Comparison of Phenolic Compounds of Some Edible Plants of Iran and India. *Pakistan Journal of Nutrition* 7,**8**. 10.3923/pjn.2008.582.585.

Almajano, M. P., Carbó, R., Jose Angel, L. and Gordon, M. (2008). Antioxidant and antimicrobial activities of tea infusions. **108**, 55-63. 10.1016/j.foodchem.2007.10.040.

Aluko, B., Oloyede, O. and Afolayan, A. J. (2012). Phytochemical and nutrient compositions of the leaves of Ocimum canum Sims. *African Journal of Biotechnology* **11** (63), 12697-12701.

Ameer, S. M., Chawla, Science and Management. (2022). Development of Herbal Milk Shake Dip Bags from Milk Powder, Tulsi Powder and Turmeric Powder. *International Journal of Research in Engineering, Science and Management* **5** (3), 42-46.

Anesini, C., Ferraro, G. E., Filip,and chemistry, f. (2008). Total polyphenol content and antioxidant capacity of commercially available tea (Camellia sinensis) in Argentina. *Journal of agricultural and food chemistry* **56** (19), 9225-9229.

Anita, P., Sivasamy, S., Kumar, P. M., Balan, I. N., Ethiraj, and pharmacy, c. (2014). In vitro antibacterial activity of Camellia sinensis extract against cariogenic microorganisms. *Journal of basic and clinical pharmacy* (2014) **6** (1), 35.

Anonymous. (1981). Announcements. *Cambridge University Press*. **15** (1), 110-112. 10.1016/0033-5894(81)90122-8.

Aroyeun, S. O. J. W. S. D. (2012). "Crude fibre, water extracts, total ash, caffeine and moisture contents as diagnostic factors in evaluating green tea quality." *WORLD SUSTAINABLE DEVELOPMENT* (2012).

Atoui, A., Mansouri, A., Boskou, G. and Kefalas, P. (2005a). Tea and herbal infusions: Their antioxidant activity and phenolic profile. *Food chemistry* **89**, 27-36. 10.1016/j.foodchem.2004.01.075.

Atoui, A. K., Mansouri, A., Boskou, G. and Kefalas, Panagiotis Kefalas (2005b). Tea and herbal infusions: their antioxidant activity and phenolic profile. *Food chemistry* **89** (1), 27-36.

Ayurvedic Pharmacopoeia Committee %J Government of India,and Family Welfare. New Delhi, (2001). The ayurvedic pharmacopoeia of India. *Journal of Pharmaceutical Education and Research* **1** (1), 144-145.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K., Mohamed, A., Sahena, F., Jahurul, M., Ghafoor, K., Norulaini, N. and Omar, A. Jahurul (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of food engineering* **117** (4), 426-436.

Azwanida, N. J. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med aromat plants* **4** (196),2167-0412.

Balaji, R., Prakash, G., Suganya, P. and Aravinthan, K. J. (2011). Antioxidant activity of methanol extract of Ocimum tenuiflorum (dried leaf and stem). *Int J Pharm Sci Rev Res* **3**, 20-27.

Baldwin, I. T., Schultz, J. C. and Ward, D. J. (1987). Patterns and sources of leaf tannin variation in yellow birch (Betula allegheniensis) and sugar maple (Acer saccharum). *Journal of Chemical Ecology* **13**, 1069-1078.

Basak, P., Mallick, P., Mazumder, S., Verma, A. S. and Toxicology. (2014). Assessment of antioxidant, anti-inflammatory, anti-cholinesterase and cytotoxic activities of tulsi (Ocimum sanctum) leaves. *Advances in Pharmacology and Toxicology* **15** (1), 19.

Baskar, S. J. (2014). Role of green tea in dental problems. *Int J Cur Res Chem Pharm Sci* **1**, 73-77.

Bast, F., Rani, P. and Meena, D. j (2014). Chloroplast DNA phylogeography of holy basil (Ocimum tenuiflorum) in Indian subcontinent. *The Scientific World Journal* **2014** (1), 847482.

Batista, G., Cunha, C., Scartezini, M., Heyde, R., Bitencourt, M. and Fabricio De Melo, S. (2009). Prospective double-blind crossover study of Camellia sinensis (green tea) in dyslipidemias. *Arquivos brasileiros de cardiologia* **93**, 128-134.

Bernhoft, A., Siem, H., Bjertness, E., Meltzer, M., Flaten, T., Holmsen, E. J. and Letters, O. (2010). Bioactive compounds in plants–benefits and risks for man and animals. *The Norwegian Academy of Science and Letters, Oslo (2010)* 13-14.

Bhargava, K. and Singh, N. (1981). Anti-stress activity of Ocimum sanctum Linn.

Blainski, A., Lopes, G. C. and De Mello, J. C. (2013). Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from Limonium brasiliense L. *Molecules* **18** (6), 6852-6865.

Bravo, L. J. N. r. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition reviews* **56** (11), 317-333.

Bressani, R., Elias, L., Wolzak, A., Hagerman, A. E. and Buttler.L.G (1983). Tannin in common beans: methods of analysis and effects on protein quality. *Journal of Food Science* **48** (3), 1000-1001.

Caballero, B., Finglas, P. and Toldrá, F. (2015). "Encyclopedia of food and health". Academic Press. [0123849535].

Cabrera, C., Artacho, R. and Giménez, R. J. (2006). Beneficial effects of green tea—a review. *Journal of the American College of Nutrition* (2006) **25** (2), 79-99.

Can Agca, A., Vural, N. and Sarer, E. (2020). Determination of volatile compounds in green tea and black tea from Turkey by using HS-SPME and GC-MS. *İstanbul Journal of Pharmacy* **50**. 10.26650/IstanbulJPharm.2019.0075.

Carley, K., Kamneva, N. and Reminga, J. (2004). Response Surface Methodology. An overview to analyze multivariate data." *Indian J. Microbiol. (2022): 241-248* **9**.

Chacko, S. M., Thambi, P. T., Kuttan, R. and Nishigaki, (2010). Beneficial effects of green tea: a literature review. *Chinese medicine* (2010) **5**, 1-9.

Chen, Y., Chen, J., Zhang, Z., Xu, Y., He, Z., Liu, C., Wang, R., Wang, X., Peng, Y. and Peng, S. (2021). Study on the anti-aging physiological characteristics and molecular mechanism of camellia oleifera.

Cheng, T. O. (2006). All teas are not created equal: the Chinese green tea and cardiovascular health. *International journal of cardiology* **108** (3), 301-308.

Chiu, W. (1989). Factors affecting the production and quality of partially fermented tea in Taiwan. *International Symposium on the Culture of Subtropical and Tropical Fruits and Crops 275*. 57-64.

Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1992). "Glossary of Indian medicinal plants". Council of Scientific & Industrial Research.

Choudhary, S. K. (2020). Ethnobotany and the Sacred Divine Plant: Tulsi. *Res. Rev. J* **5**, 242-244.

Choudhury, S. S., Ghosh, J., Jana, A. K., Roy, S., Prasad, R. K. and Pratihar, A. J. (2022). Development of therapeutic index of different types of herbal and medicinal teas. *Journal of Medicinal Plants Studies* **10** (4).

Colla, G., Rouphael, Y., Cardarelli, M., Svecova, E., Rea, E., Lucini, L. J. and Agriculture. (2013). Effects of saline stress on mineral composition, phenolic acids and flavonoids in

leaves of artichoke and cardoon genotypes grown in floating system. *Journal of the Science of Food and Agriculture* **93** (5), 1119-1127.

Comstock, M. J. Phenolic Compounds in Food and Their Effects on Health I, Copyright, 1992 Advisory Board, Foreword: Analysis, Occurrence, and Chemistry. *In*.). ACS Publications. [1947-5918].

Costa, L. M., Gouveia, S. T. and Nóbrega, J. A. (2002). Comparison of heating extraction procedures for Al, Ca, Mg, and Mn in tea samples. *Analytical sciences* **18** (3), 313-318.

Croft, K. D(1998). The chemistry and biological effects of flavonoids and phenolic acids a. *Annals of the New York Academy of Sciences* **854** (1), 435-442.

Dadysett, H. J. (1899). ON, The various domestic remedies,with their effects used by the people of india for certain diseases of ear. *The Lancet* **154** (3968), 781-782.

Das, S. K. and Vasudevan, D. (2006). Tulsi: The Indian holy power plant.

De, B., Bhandari, K., Singla, R. K., Katakam, P., Samanta, T., Kushwaha, D. K., Gundamaraju, R. and Mitra, A. J(2015). Chemometrics optimized extraction procedures, phytosynergistic blending and in vitro screening of natural enzyme inhibitors amongst leaves of Tulsi, Banyan and Jamun. *Pharmacognosy Magazine* **11** (Suppl 4), S522.

Desai, V. R., Ramkrishnan, R., Chintalwar, G. J. and Sainis, K. J. (2007). G1-4A, an immunomodulatory polysaccharide from Tinospora cordifolia, modulates macrophage responses and protects mice against lipopolysaccharide induced endotoxic shock. *International immunopharmacology* (2007) **7** (10), 1375-1386.

Devi, P. U. and Ganasoundari, A. J. (1995). Radioprotective effect of leaf extract of Indian medicinal plant Ocimum sanctum. *Indian Journal of Experimental Biology* **33** (3), 205-208.

Dhandayuthapani, S., Hasan, A. and Rathinavelu, A. (2015). Apoptosis Induction by Ocimum sanctum Extract in LNCaP Prostate Cancer Cells. *Journal of medicinal food* **18**. 10.1089/jmf.2014.0008.

DIPLOCK, A. T. (1994). Antioxidants and free radical scavengers. *In:* "New Comprehensive Biochemistry" (Vol. 28).). pp. 113-130. Elsevier. [0167-7306].

Doughari, J. H., Human, I. S., Bennade, S. and Ndakidemi (2009). Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic

resistant verocytotoxin producing bacteria. *Journal of medicinal plants Research* **3** (11), 839-848.

DR, Peryam (1952). Advanced taste-test method. *Food Eng* **7**, 58-61.

Drake, M.A(2007). Invited review: Sensory analysis of dairy foods. *Journal of dairy science* **90** (11), 4925-4937.

Gaber, A., Hassan, M., Dessoky, E.-D. and Attia, A. (2015). In vitro Antimicrobial Comparison of Taif and Egyptian Pomegranate Peels and Seeds Extracts. *J. Appl. Biol. Biotechnol* **3**, 012-017. 10.7324/JABB.2015.3203.

Ghasemzadeh, A., Jaafar, H. Z. and Rahmat, (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (Zingiber officinale Roscoe). *Molecules* **15** (6), 4324-4333.

Ghoshal, S. J. and Research, P. (2013). Population dynamics and biochemical fluctuations in relation to the infestation of tetranychus neocaledonichus andre on the leaves of Tulsi (Ocimum sanctum). **2** (3), 225-231.

Gogoi, M. and Basumatary, M. J. (2018). Estimation of the chlorophyll concentration in seven Citrus species of Kokrajhar district, BTAD, Assam, India. *Molecules* **5** (1), 83-87.

Golmovahhed, G. and Khodaparast, M. (2007). Antioxidant activity of various extracts of old tea leaves and black tea wastes (Camellia sinensis L.). *Food Chemistry* **100**, 231–236. 10.1016/j.foodchem.2005.09.046.

Graf, B. A., Milbury, P. E. and Blumberg, J. B. (2005). Flavonols, flavones, flavanones, and human health: epidemiological evidence. *Journal of medicinal food* **8** (3), 281-290.

Gramza, A., Korczak, J. and Amarowicz, R. (2005). Tea polyphenols-their antioxidant properties and biological activity-a review.

Guerreiro Pereira, C., Rodrigues, M. J., Nawrot-Hadzik, I., Matkowski, A. and Custódio, L. (2024). Seasonal and Geographic Dynamics in Bioproperties and Phytochemical Profile of Limonium algarvense Erben. *Molecules* **29**. 10.3390/molecules29020481.

Guleria, S., Tiku, A., Singh, G., Koul, A., Gupta, S., Rana, S. J. and biotechnology. (2013). In vitro antioxidant activity and phenolic contents in methanol extracts from medicinal plants. *Journal of plant biochemistry and biotechnology* **22**, 9-15.

Gunathilaka, D. and Tularam, A. (2016). The Tea Industry and a Review of Its Price Modelling in Major Tea Producing Countries. *Manag. Strategy* **7**. 10.5430/jms.v7n1p21.

Gupta, S., Prakash, J. and Srivastava, S. J.(2002). Validation of traditional claim of Tulsi, Ocimum sanctum Linn. as a medicinal plant. *Journal of Pharmacognosy and Phytochemistry* **40** (7), 765-773.

Gyamfi, M. A. and Aniya, Y. J. (2002). Antioxidant properties of Thonningianin A, isolated from the African medicinal herb, Thonningia sanguinea. *Biochemical Pharmacology* **63** (9), 1725-1737.

Hakkim, F. L., Shankar, C. G., Girija, S. J. and chemistry, f. (2007). Chemical composition and antioxidant property of holy basil (Ocimum sanctum L.) leaves, stems, and inflorescence and their in vitro callus cultures. *Journal of agricultural and food chemistry* **55** (22), 9109-9117.

Hamdaoui, M. H., Chabchoub, S., Hédhili, A. J and biology. (2003). Iron bioavailability and weight gains to iron-deficient rats fed a commonly consumed Tunisian meal 'bean seeds ragout'with or without beef and with green or black tea decoction. *Journal of trace elements in medicine and biology* **17** (3), 159-164.

Harbowy, M. E., Balentine, D. A., Davies, A. P. and Cai, Y. J.(1997). Tea chemistry. **16** (5), 415-480.

Hebbar, S., Harsha, V., Shripathi, V. and Hegde, G. J. (2004). Ethnomedicine of Dharwad district in Karnataka, India—plants used in oral health care. *Journal of ethnopharmacology* **94** (2-3), 261-266.

Heckman, M. A., Weil, J. and De Mejia, E. G (2010). Caffeine (1, 3, 7-trimethylxanthine) in foods: a comprehensive review on consumption, functionality, safety, and regulatory matters. *Journal of food science* **75** (3), R77-R87.

Heim, K. E., Tagliaferro, A. R. and Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *The Journal of nutritional biochemistry* **13** (10), 572-584.

Hsu, S. (2005). Green tea and the skin. *Journal of the American Academy of Dermatology* **52**, 1049-1059. 10.1016/j.jaad.2004.12.044.

Hu, Y., Xu, J., Hu, Q. J. and chemistry, f. (2003). Evaluation of antioxidant potential of Aloe vera (Aloe barbadensis Miller) extracts. *Journal of agricultural and food chemistry* **51** (26), 7788-7791.

Humphrey, A. J. (2004). Chlorophyll as a color and functional ingredient. *Journal of food science* **69** (5), C422-C425.

Ienco, E. C., LoGerfo, A., Carlesi, C., Orsucci, D., Ricci, G., Mancuso, M. and Siciliano, G. J. (2011). Oxidative stress treatment for clinical trials in neurodegenerative diseases. *Journal of Alzheimer's Disease* **24** (s2), 111-126.

Ilaiyaraja, N. and Khanum, F. (2011). Antioxidant Potential of Tinospora cordifolia Extracts and their Protective Effect on Oxidation of Biomolecules. *Pharmacognosy Journal* **3**, 56–62. 10.5530/pj.2011.20.11.

Ilyas, M. (2024). Proximate and Phytochemical Analysis To Assess The Therapeutic Potential Of Ocimum Sanctum. **31**, 1700-1707. 10.53555/jptcp.v31i6.6748.

Ingle, K. P., Deshmukh, A. G., Padole, D. A., Dudhare, M. S., Moharil, M. P., Khelurkar, V. C. J. and Phytochemistry. (2017). Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. *Journal of Pharmacognosy and Phytochemistry* **6** (1), 32-36.

Jabeen, S., Alam, S., Saleem, M., Ahmad, W., Bibi, R., Hamid, F. S. and Shah, H. U. (2019). Withering timings affect the total free amino acids and mineral contents of tea leaves during black tea manufacturing. *Arabian Journal of Chemistry* **12** (8), 2411-2417.

Jain, D. P., Pancholi, S. S., Patel, R. J. and research. (2011). Synergistic antioxidant activity of green tea with some herbs. *Journal of advanced pharmaceutical technology & research* **2** (3), 177-183.

Jaradat, N., Hussen, F. and Al Ali, (2015). Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of Ephedra alata Decne. **6** (6), 1771-1778.

Jayaprakash, R., Ramesh, V., Sridhar, M., Sasikala, C. J and sciences, b. (2015). Antioxidant activity of ethanolic extract of Tinospora cordifolia on N-nitrosodiethylamine (diethylnitrosamine) induced liver cancer in male Wister albino rats. *Journal of Pharmacy and bioallied sciences* **7** (Suppl 1), S40-S45.

Johnson, R., Bryant, S. and Huntley, (2012). "Green tea and green tea catechin extracts: an overview of the clinical evidence." *Maturitas* **73** (4), 280-287.

Joubert, E., De Beer, D. and Malherbe, C. J. Malherbe (2017). Herbal teas—Exploring untapped potential and strengthening commercialisation. *South African Journal of Botany* (110), 1-3.

Jung, D. J. H., Seoul, Korea. (2004). "Components and Effects of Tea."*Hongikjae, Seoul, Korea*

Kadian, R., Parle, M. J. and sciences, l. (2012). Therapeutic potential and phytopharmacology of tulsi. *International journal of pharmacy & life sciences* **3** (7).

Karki, S. (2022). Phytochemical,anti-oxidant and sensory analysis of gurjo stem incorporated green tea,Department of Food Technology Central Campus of Technology Institute of science and technology.

Karuna, S. B., Rajendrakumar, C. and Reddy, A. J. (2000)." Aldose reductase in rice (Oryza sativa L.): stress response and developmental specificity." *Plant science* **160** (1), 149-157.

Kaur, A., Kaur, M., Kaur, P., Kaur, H., Kaur, S. and Kaur, K. (2015). "Estimation and comparison of total phenolic and total anti-oxidant in green tea and black tea." *Global Journal of Bio-Science and Biotechnology* 4, no. 1 (2015): 116-120.

Kaur, G., Prabhakar, D. P., Lal, U. and Suttee, A. (2016). "Phytochemical and Biological Analysis of Tinospora cordifolia." *International journal of toxicological and pharmacological research* **8**, 297-305.

Kesarkar, S., Bhandage, A., Deshmukh, S., Shevkar, K. and Abhyankar, Mukta Abhyanka. (2009)." Flavonoids: an overview." *Journal of Pharmacy Research***2** (6), 1148-1154.

Khan, A., Ahmad, A., Akhtar, F., Yousuf, S., Xess, I., Khan, L. A. and Manzoor, N. J. R. i. m. (2010)." Ocimum sanctum essential oil and its active principles exert their antifungal activity by disrupting ergosterol biosynthesis and membrane integrity. "*Research in microbiology***161** (10), 816-823.

Kidist Teshome, K. T., Adugna Debela, A. D. and Weyessa Garedew, W. G. (2013)."Effect of drying temperature and duration on biochemical composition and quality of black tea (Camellia sinensis L.) O. Kuntze at Wush Wush, south western Ethiopia."(2013): 235-240

Koch, W., Zagórska, J., Marzec, Z. and Kukula-Koch, W. J. (2019). "Applications of tea (Camellia sinensis) and its active constituents in cosmetics." *Molecules* **24** (23), 4277.

Koláčková, T., Kolofiková, K., Sytařová, I., Snopek, L., Sumczynski, D. and Orsavová, J. J. (2020). "Matcha tea: analysis of nutritional composition, phenolics and antioxidant activity." *Plant Foods for Human Nutrition* **75**, 48-53.

Kosińska, A. and Andlauer, W. (2014). Antioxidant capacity of tea: effect of processing and storage. *In:* "Processing and impact on antioxidants in beverages".). pp. 109-120. Academic Press, 2014..

Kossel, A.j (1891). Ueber die chemische Zusammensetzung der Zelle. **278**, 181-186.

Kothari, S., Bhattacharya, A., Ramesh, S., Garg, S. and Khanuja, (2005). "Volatile constituents in oil from different plant parts of methyl eugenol-rich Ocimum tenuiflorum Lf (syn. O. sanctum L.) grown in South India." *Journal of Essential Oil Research* **17** (6), 656-658.

Kotzekidou, P., Giannakidis, P., Boulamatsis, A. J. and Technology. (2008). "Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens in vitro and on the fate of inoculated pathogens in chocolate." *LWT-Food Science and Technology* **41** (1), 119-127.

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., Griel, A. E. and Etherton, T. D. (2002). "Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer.*"The American journal of medicine* **113** (9), 71-88.

Krishnaiah, D., Sarbatly, R., Nithyanandam, R. J. F. and processing, b. (2011). "A review of the antioxidant potential of medicinal plant species." *Food and bioproducts processing* **89** (3), 217-233.

Kumar, B., Bajpai, V., Tiwari, S. and Pandey, R. (2020). "Phytochemistry of plants of genus Ocimum". CRC Press. [1003014852].

Kumar, R. S. S., Murugesan, S., Kottur, G., Gyamfi, D. J. and prevention, d. (2013)."Black tea: The plants, processing/manufacturing and production."*Tea in health and disease prevention* **5**, 41-57.

Kumar, S., Yadav, A., Yadav, M. and Yadav, J. P. (2017). "Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of Aloe vera (L.) Burm. f." *BMC research notes* **10**, 1-12.

Lawless, H. T. and Heymann, H. (2010). "Sensory evaluation of food: principles and practices". Springer Science & Business Media. [1441964886].

Lee, J. (2009). "Green tea: Flavor characteristics of a wide range of teas including brewing, processing, and storage variations and consumer acceptance of teas in three countries". Kansas State University. [110956922X].

Lee, J., Chambers, D. and Chambers, E. (2014). "A comparison of the flavor of green teas from around the world."*Journal of the Science of Food and Agriculture* **94**. 10.1002/jsfa.6413.

Li, H., Tsao, R. and Deng, Z.J (2012)." Factors affecting the antioxidant potential and health benefits of plant foods." *Canadian journal of plant science* **92** (6), 1101-1111.

Lim, J., Wood, A. and Green, (2009)."Derivation and evaluation of a labeled hedonic scale." *Chemical senses* **34** (9), 739-751.

Liu, Y., Luo, L., Liao, C., Chen, L., Wang, J. and Zeng, L. J. (2018). "Effects of brewing conditions on the phytochemical composition, sensory qualities and antioxidant activity of green tea infusion: A study using response surface methodology." *Food Chemistry* **269**, 24-34.

Liu ZeHua, L. Z., Luo ZiWen, L. Z., Jia CaiXia, J. C., Wang DongMei, W. D. and Li DengWu, L. D. (2016). "Synergistic effects of Potentilla fruticosa L. leaves combined with green tea polyphenols in a variety of oxidation systems." *Journal of food science 81, no. 5 (2016): C1091-C1101.*

Lou, L. J. F. T. (2002). "Applications of microwave technology in the tea processing."*Fujian Tea* 23-25.

Macfarlane, A. and Macfarlane, I. (2009). "The empire of tea". Abrams. [1468306014].

Madhuri, S. (2008). Studies on oestrogen induced uterine and ovarian carcinogenesis and effect of ProImmu in rats. PhD thesis, Rani Durgavati Vishwa Vidyalaya, Jabalpur, MP, India,

Maliepaard, R. (2009). "Inactivation of polyphenol oxidase in Camellia sinensis for the production of high quality instant green tea". University of Pretoria (South Africa). [9798380999540].

Malongane, F., McGaw, L. J., Debusho, L. K. and Mudau, (2020)."Sensory characteristics and volatile compounds of herbal teas and mixtures of bush tea with other selected herbal teas of South Africa."*Foods* **9** (4), 496.

Malongane, F., McGAW, L. J., Mudau, F. N. and Agriculture. (2017)."The synergistic potential of various teas, herbs and therapeutic drugs in health improvement: a review." *Journal of the Science of Food and Agriculture* **97** (14), 4679-4689.

Manach, C., Mazur, A. and Scalbert, A. J. (2005)."Polyphenols and prevention of cardiovascular diseases." *Current opinion in lipidology* **16** (1), 77-84.

Manikanta,(2023)."v SV, DS, Dr SS. Blended tulsi-drumstick herbal tea: Quality and organoleptic properties." *Pharma Innov* **12** (3), 19-23.

Manson, M. M (2003)."Cancer prevention–the potential for diet to modulate molecular signalling." *Trends in molecular medicine* **9** (1), 11-18.

Mc, S., Thekekara, P., Kuttan, R. and Nishigaki, I. (2010). "Beneficial effects of green tea: A literature review." *Chinese medicine* **5**. 10.1186/1749-8546-5-13, 1-9.

McCully, W. F. (2013). The antibacterial activity of tea infusions and their effect against the hospital pathogen clostridium difficile. Cardiff University,

Mondal, S., Mirdha, B. and Mahapatra, S. (2009). "The science behind sacredness of Tulsi (Ocimum sanctum Linn)."*Indian J Physiol Pharmacol***53**, 291-306.

Mukhtar, H. and Ahmad, N. J (2000)."Tea polyphenols: prevention of cancer and optimizing health."*The American journal of clinical nutrition* **71** (6), 1698S-1702S.

Namdev, P., Gupta, R. K.and Phytochemistry. (2015)."Herbal green tea formulation using Withania somnifera stems, Terminalia arjuna bark, Cinnamonbark and Tinospora cordifolia stems and nutritional & phytochemical analysis." J*ournal of Pharmacognosy and Phytochemistry***4** (2), 282-291.

Nile, S. and Khobragade, (2009). "Determination of nutritive value and mineral elements of some important medicinal plants from western part of India." **8**, 79-88.

Obanda, M., Owuor, P. and Njuguna, C. (1992). "The impact of clonal variation of total polyphenols content and polyphenol oxidase activity of fresh tea shoots on plain black tea quality parameters." (1992): 129-133.

Odunmbaku, L., Babajide, J., Shittu, T., Aroyeun, S. and Eromosele, C. (2015). "Effect of process variables on the chemical constituents and sensory characteristics of Nigerian green tea." (2015): 510.

Okuda, T. and Ito, H. J. M. (2011). "Tannins of constant structure in medicinal and food plants—hydrolyzable tannins and polyphenols related to tannins." *Molecules* **16** (3), 2191-2217.

Ortiz-Islas, S., Espinosa-Leal, C. A., González-Rodríguez, T. and García-Lara, S. J. F. (2024)." Enhancing the Antioxidant Activity of Tea (Camellia sinensis) Through Common Herbal Infusions."*Foods* **13** (20), 3284.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., Simons, A. Jand Version, S. G. (2009). Psidium guajava. **4**.

Ošťádalová, M., Tremlová, B., Pokorná, J. and Král, M. J.(2015)." Chlorophyll as an indicator of green tea quality." *Acta Veterinaria Brno* **83** (10), 103-109.

Pan, X., Niu, G., Liu, H. J. and Intensification, P. P. (2003). "Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves." *Chemical Engineering and Processing: Process Intensification* **42** (2), 129-133.

Panda, V. and N, S. (2009)." Evaluation of cardioprotective activity of Ginkgo biloba and Ocimum sanctum in rodents." *Alternative Medicine Review* **14**, 161-171.

Parmar, M. R., Kumpavat, M. T., Doshi, J. S. and Kapdi, S. S. (2017). "A comparative study on drying of basil leaves." *Agricultural Engineering International: CIGR* **19** (1), 169-177.

Pathak, I. and Niraula, (2019). "Assessment of total phenolic, flavonoid content and antioxidant activity of Ocimum sanctum Linn." *Journal of Nepal chemical society* **40**, 30-35.

Pattanayak, P., Behera, P., Das, D. and Panda, S. K.(2010). "Ocimum sanctum Linn. A reservoir plant for therapeutic applications: An overview." *Pharmacognosy reviews***4** (7), 95.

Peryam, D. R. and Pilgrim, F. J.(1957). "Hedonic scale method of measuring food preferences." *Food technology* (1957).

Pisoschi, A. M. and Negulescu, G. P. (2011). "Methods for total antioxidant activity determination: a review." *Biochem Anal Biochem* **1** (1), 106.

Priya, S. and Peddha, M. S (2023). "Physicochemical characterization, polyphenols and flavonoids of different extracts from leaves of four varieties of tulsi (Ocimum sp.)." *South African Journal of Botany* **159**, 381-395.

Rababah, T. M., Banat, F., Rababah, A., Ereifej, K. and Yang, (2010). "Optimization of extraction conditions of total phenolics, antioxidant activities, and anthocyanin of oregano, thyme, terebinth, and pomegranate." *Journal of Food Science* **75** (7), C626-C632.

Rains, T. M., Agarwal, S. and Maki, K. C. (2011). "Antiobesity effects of green tea catechins: a mechanistic review." *The Journal of nutritional biochemistry* **22** (1), 1-7.

Rajbhar, K., Dawda, H. and Mukundan, U. J (2015). "Polyphenols: Methods of extraction." *Sci. Revs. Chem. Commun***5** (1), 1-6.

Ravikumar, C. (2014). "Review on herbal teas." *Journal of Pharmaceutical Sciences and Research* **6**, 236-238.

Reena Patel, R. P., Yogesh Patel, Y. P., Prasant Kunjadia, P. K. and Anju Kunjadia, A. K. (2015). "DPPH free radical scavenging activity of phenolics and flavonoids in some medicinal plants of India." (2015): 773-780.

Rosas-Nexticapa, M., Angulo, O. and O'mahony, M. J. (2005). "How well does the 9-point hedonic scale predict purchase frequency?." *ournal of sensory studies* **20** (4), 313-331.

Rubab, S., Rizwani, G. H., Bahadur, S., Shah, M., Alsamadany, H., Alzahrani, Y., Shuaib, M., Hershan, A., Hobani, Y. H. and Shah (2020). "Determination of the GC–MS analysis of seed oil and assessment of pharmacokinetics of leaf extract of Camellia sinensis L." *Journal of King Saud University-Science* **32** (7), 3138-3144.

Safran, J. and Segal, Z. V. (1996). "Interpersonal process in cognitive therapy". Jason Aronson. [1568218583].

Sahoo, D. D., Tabassum, Y. and Sharma, D. J. (2022)." Multiple health benefits of Tulsi plants." *Journal of Medicinal Plants* **10** (5), 95-102.

Sakamoto, Y., Mikuriya, H., Tayama, K., Takahashi, H., Nagasawa, A., Yano, N., Yuzawa, K., Ogata, A. and Aoki, N. J. A. o. t. (2001)." Goitrogenic effects of green tea extract catechins by dietary administration in rats." *Archives of toxicology* **75**, 591-596.

Sangeetha, S., Deepa, M., Sugitha, N., Mythili, S. and Sathiavelu, A. J. I(2014). "Antioxidant activity and phytochemical analysis of Datura metel." *Int J Drug Dev Res* **6** (4), 46-53.

Savitri Godhwani, S. G., Godhwani, J. and Vyas, D. (1988). "Ocimum sanctum-a preliminary study evaluating its immunoregulatory profile in albino rats." (1988): 193-198

Savolainen, H. J. (1992). "Tannin content of tea and coffee."*Journal of Applied Toxicology* **12** (3), 191-192.

Saxena, M., Saxena, J., Nema, R., Singh, D., Gupta, A. J. and phytochemistry. (2013). "Phytochemistry of medicinal plants." *Journal of pharmacognosy and phytochemistry* **1** (6), 168-182.

Schmidt, M., Schmitz, H.-J., Baumgart, A., Guedon, D., Netsch, M., Kreuter, M.-H., Schmidlin, C., Schrenk, D. J. F. and Toxicology, C. (2005). "Toxicity of green tea extracts and their constituents in rat hepatocytes in primary culture." *Food and Chemical Toxicology* **43** (2), 307-314.

Shafi, J., Tahira, F., Saleem, H., Asif, M., Mirza, Z. and Chemistry, E. (2022)." Characterization and Analysis of Polyphenols in Green and Black Tea Brands Available in Commercial Market of Pakistan." *Pakistan Journal of Analytical & Environmental Chemistry* **23** (1), 50-60.

Shival, A., Bornare, A., Shinde, A. and Musmade, D. J.(2020). General introduction, classification, morphology, phytoconstituents, traditional & medicinal uses, pharmacological activities of tulsi (Ocimum Sanctum). **9** (9), 701-713.

Shraim, A. M., Ahmed, T. A., Rahman, M. M. and Hijji, Y. M. (2021). "Determination of total flavonoid content by aluminum chloride assay: A critical evaluation." *Lwt* **150**, 111932.

Singh, N., Hoette, Y. and Miller, D. R. (2002). "Tulsi: The mother medicine of nature". International Institute of Herbal Medicine. [8188007005].

Singh, S. and Agrawal, S. (2008)." Anti-Asthmatic and Anti-Inflammatory Activity of Ocimum sanctum."*InternationalJournalofpharmacognosy***29**,306-310. 10.3109/13880209109082904.

Singh, V., Amdekar, S. and Verma, O. (2010). "Ocimum sanctum (tulsi): Bio-pharmacological activities."*Webmed Central Pharmacol* **1** (10), 1-7.

Singh, V., Verma, D. K. and Singh, (2014). "Processing technology and health benefits of green tea." *Popular Kheti* **2** (1), 23-30.

Siva, M., Shanmugam, K., Shanmugam, B., Venkata, S. G., Ravi, S., Sathyavelu, R. and Mallikarjuna, K. J.(2016). "Ocimum sanctum: a review on the pharmacological properties."*Int. J. Basic Clin. Pharmacol* **5** (3), 558-565.

Sivakumar, V. and Rajan, M. D(2010). "Antioxidant effect of Tinospora cordifolia extract in alloxan-induced diabetic rats." *Indian journal of pharmaceutical sciences* **72** (6), 795.

Somanchi, M., Phillips, K., Haile, E. and Pehrsson, P. J.(2017). "Vitamin C content in dried and brewed green tea from the US retail market."*The FASEB Journal* **31**, 956.958-956.958.

Stefanello, N., Spanevello, R. M., Passamonti, S., Porciúncula, L., Bonan, C. D., Olabiyi, A. A., da Rocha, J. B. T., Assmann, C. E., Morsch, V. M., Schetinger, and Toxicology, C. (2019). "Coffee, caffeine, chlorogenic acid, and the purinergic system." *Food and Chemical Toxicology* **123**, 298-313.

Stéphane, F. F. Y., Jules, B. K. J., Batiha, G. E.-S., Ali, I. and Bruno, L. N. (2021). "Extraction of bioactive compounds from medicinal plants and herbs." *Natural medicinal plants* 1-39.

Tabassum, I., Siddiqui, Z. and Rizvi, S. (2010). "Effects of Ocimum sanctum and Camellia sinensis on stress-induced anxiety and depression in male albino Rattus norvegicus." *Indian Journal of Pharmacology* **42**, 283-288. 10.4103/0253-7613.70108.

Takabayashi, F., Tahara, S., Kaneko, T. and Harada, N. J. B. (2004). "Effect of green tea catechins on oxidative DNA damage of hamster pancreas and liver induced by N-nitrosobis (2-oxopropyl) amine and/or oxidized soybean oil." *Biofactors* **21** (1-4), 335-337.

Tanaka, T., Matsuo, Y. J. C. and Bulletin, P. (2020)." Production mechanisms of black tea polyphenols." *Chemical and Pharmaceutical Bulletin* **68** (12), 1131-1142.

Tandale, A. T., Nayak, B., Xavier, K., Devangre, A., Gore, S. B. and Balange, A. K (2023). "Phenolic Content and Characterization of Tea Shrub Bottom Leaves." *Agricultural Science Digest* **43** (4), 478-481.

Taylor, S. (2003). "TEA| Types, Production, and Trade."(2003): 5737-5743.

Tewari, D., Kumar, P. and Sharma, P. (2013). "Pharmacognostical Evaluation of Elaeocarpus sphaericus (Rudraksha) Leaves."*International Journal of Pharmacognosy and Phytochemical Research* **5**, 147-150.

Thakur, S., Choudhary, S., Walia, B., Chaudhary, G. J. and Review. (2021). "Tulsi-a review based upon its ayurvedic and modern therapeutic uses."*International Journal of Research and Review* **8** (5), 263-272.

Thasleema, S. (2013). "Green tea as an antioxidant- A short review." *Journal of Pharmaceutical Sciences and Research* **5**, 171-173.

Tiwari, U. and Cummins, E. J(2013). "Factors influencing levels of phytochemicals in selected fruit and vegetables during pre-and post-harvest food processing operations."*Food research international* **50** (2), 497-506.

Tomlins, K. and Mashingaidze, A. J. (1997)." Influence of withering, including leaf handling, on the manufacturing and quality of black teas—a review." *Food chemistry* **60** (4), 573-580.

Toullec, A., Gerald, D., Despouy, G., Bourachot, B., Cardon, M., Lefort, S., Richardson, M., Rigaill, G., Parrini, M., Lucchesi, C., Bellanger, D., Stern, M.-H., Dubois, T., Sastre-Garau, X., delattre, O., Vincent-Salomon, A. and Mechta-Grigoriou, F. (2010). "Oxidative stress promotes myofibroblast differentiation and tumour spreading." *EMBO molecular medicine* **2**, 211-230. 10.1002/emmm.201000073.

Tsuneki, H., Ishizuka, M., Terasawa, M., wu, J., Sasaoka, T. and Kimura, I. (2004). "Effect of Green tea on blood glucose levels and serum proteomic patterns in diabetic (db/db) mice and on glucose metabolism in healthy humans." *BMC pharmacology* **4**, 18. 10.1186/1471-2210-4-18.

Tuorila, H., Monteleone, E. J and Technology. (2009). "Sensory food science in the changing society: Opportunities, needs, and challenges."*Trends in Food Science & Technology* **20** (2), 54-62.

Upadhyay, N., Ganie, S. A., Agnihotri, R. K., Sharma, R. J and Phytochemistry. (2014)." Free radical scavenging activity of Tinospora cordifolia (Willd.) Miers." *Journal of Pharmacognosy and Phytochemistry* **3** (2), 63-69.

Usano-Alemany, J., Palá-Paúl, J., Rodríguez, M. S.-C. and Herraiz-Peñalver, D. J(2014). "Chemical description and essential oil yield variability of different accessions of Salvia lavandulifolia." *Natural Product Communications* **9** (2), 1934578X1400900236.

Venturi, Francesca, Chiara Sanmartin, Isabella Taglieri, A. Nari, and Gianpaolo Andrich. "Effect of the baking process on artisanal sourdough bread-making: A technological and sensory evaluation." *Agrochimica: International Journal of Plant Chemistry, Soil Science and Plant Nutrition of the University of Pisa: **60**, 3, 2016* (2016): 222-234.

Vidhani, S. I., Vyas, V. G., Parmar, H. J., Bhalani, V. M., Hassan, M. M., Gaber, A., Golakiya, B. A. J. and Technology. (2016). "Evaluation of some chemical composition, minerals fatty acid profiles, antioxidant and antimicrobial activities of Tulsi (Ocimum sanctum) from India." *American Journal of Food Science and Technology* **4** (2), 52-57.

Vidic, D., Tarić, E., Alagić, J., Maksimović, M. J., Bosnia, T. o. and Herzegovina. (2014). "Determination of total phenolic content and antioxidant activity of ethanol extracts from Aloe spp." *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina* **42**, 5-10.

Watson, R. R. (2000). "Vegetables, fruits, and herbs in health promotion". CRC press. [0429118236].

Wei, K., Wang, L., Zhou, J., He, W., Zeng, J., Jiang, Y. and Cheng, H. J(2011). "Catechin contents in tea (Camellia sinensis) as affected by cultivar and environment and their relation to chlorophyll contents." *Food chemistry* **125** (1), 44-48.

Wichchukit, S., O'Mahony,and Agriculture. (2015). "The 9-point hedonic scale and hedonic ranking in food science: some reappraisals and alternatives." J*ournal of the Science of Food and Agriculture* **95** (11), 2167-2178.

Wong, C.-C., Cheng, K.-W., Chao, J., Peng, X., Zheng, Z., Wu, J., Chen, F. and Wang, M. (2009). "Analytical methods for bioactive compounds in teas". CRC Press: Boca Raton, FL.

Xie, Y.-b., Song, L.-n. and Yang,(2006). Application of heat pump drying technology and its developmental trend. *Food Chemistry* **4**, 12-16.

Xu, N., Chen, Z. J. T. B. and potential, t. (2002). Green tea, black tea and semi-fermented tea. 35-57.

Yang, Z., Kinoshita, T., Tanida, A., Sayama, H., Morita, A. and Watanabe, N. J. (2009). "Analysis of coumarin and its glycosidically bound precursor in Japanese green tea having sweet-herbaceous odour." *Food Chemistry* **114** (1), 289-294.

Yousif, I. (2021). "In vivo, Antibacterial Effect of Green and Black Tea Extracts on Infected Liver and Kidney of Rats." *Tikrit Journal for Agricultural Sciences* **19**. 10.25130/tjas.19.4.9.

Zhao, X., Yu, P., Zhong, N., Huang, H. and Zheng, H. J. B. (2024)." Impact of Storage Temperature on Green Tea Quality: Insights from Sensory Analysis and Chemical Composition." *Beverages* **10** (2), 35.

Zobia Naheed, Z. N., Barech, A., Sajid, M., Khan, N. and Rafaqat Hussain, R. H. (2007)." Effect of rolling, fermentation and drying on the quality of black tea."(2007): 577-580.

APPENDICES

Appendix A

Materials, Equipment and Chemicals

1. **Materials required**

   Beakers, Conical flasks, Volumetric Flask, Measuring cylinder, Glass Slides, Coverslips, Petri plates, Inoculating loop, Spatula, Wire gauge, Test tubes, Screwcap tubes, Pipettes, Glass rods, Filter paper, Funnel, etc.

2. **Equipment required**

   Micropipette, Pipette, Microscope, Grinder, Incubator, Refrigerator, Digital Balance, Hot Plate, Cabinet Dryer, Water Bath Shaker, Soxhlet Apparatus, Rotatory Evaporator, Autoclave, Spectrophotometer, Hot air oven.

3. **Chemicals required**

   Methanol, Ethanol, Gallic acid, DPPH, Quercetin, Aluminum chloride, Ascorbic acid, Ferric chloride, Sodium nitrate, Sodium hydroxide, Gram's iodine, Crystal violet, Safranin, DMSO, Conc.H2SO4, Conc. HCl, etc.

Appendix B

ANOVA result for analysis of different Chemical parameters of sample

B.1 One-way ANOVA for Moisture

Source	DF	SS	MS	F	Sig
Sample	4	19.478	4.870	267.754	<0.001
Error	10	0.18	0.018		
C. Total	14	19.66			

B.2 One-way ANOVA for protein

Source	DF	SS	MS	F	Sig
Sample	4	0.287	0.072	219.69	<0.001
Error	10	0.003	0.01		
C. Total	14	0.29			

B.3 One-way ANOVA for Fat

Source	DF	SS	MS	F	Sig
Sample	4	0.04	0.010	62.33	<0.001
Error	10	0.002	0.013		
C. Total	14	0.041			

B.4 One-way ANOVA for fiber

Source	DF	SS	MS	F	Sig
Sample	4	6.753	1.688	167.82	<0.001
Error	10	0.101	0.01		
C. Total	14	6.854			

B.5 One-way ANOVA for Ash

Source	DF	SS	MS	F	Sig
Sample	4	0.917	0.229	168.561	<0.001
Error	10	0.014	0.001		
C. Total	14	0.931			

B.6 One-way ANOVA for Vitamin C

Source	DF	SS	MS	F	Sig
Sample	4	294.633	73.65	3269.82	<0.001
Error	10	0.225	0.02		
C. Total	14	294.85			

Appendix C

Gallic acid standard curve

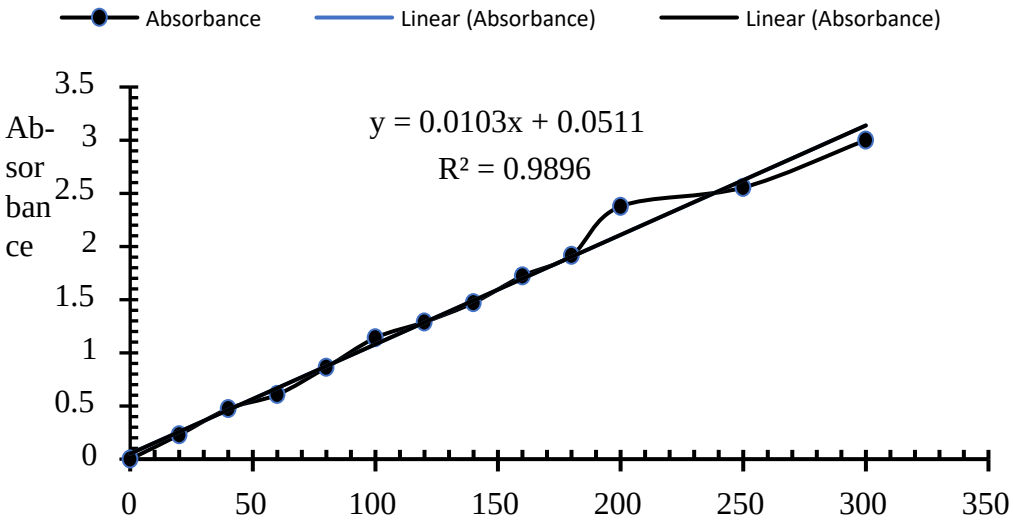


Figure E.1 Gallic acid standard curve of phenol

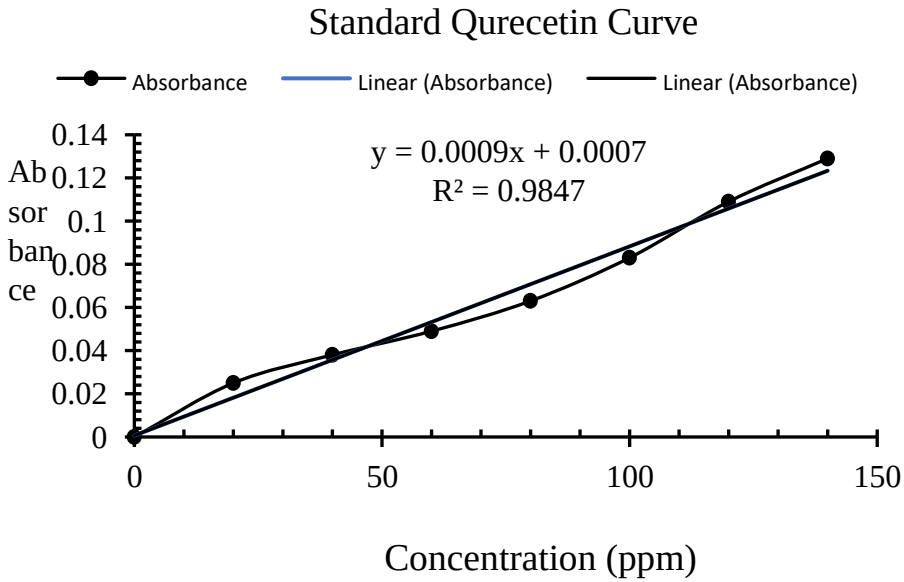


Figure E.2 Standard Qurectin curve of flavonoid

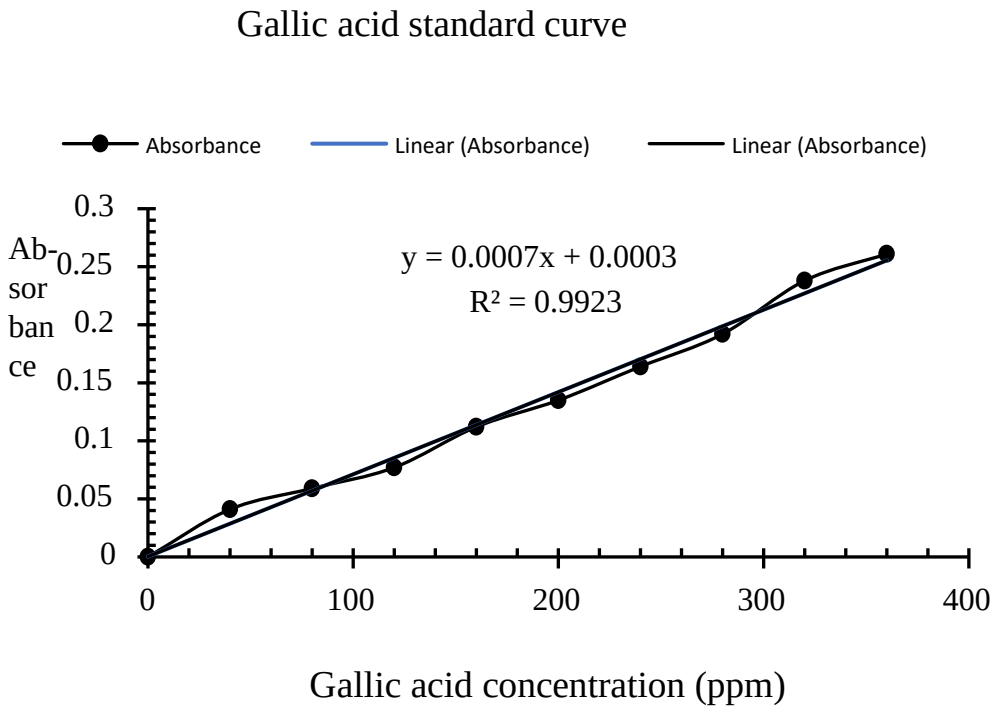


Figure E.3 Gallic acid standard curve of tannin

Appendix D

D 1 One-way ANOVA for DPPH inhibition

Source	DF	SS	MS	F	Sig
Sample	5	48.996	9.799	35967.67	<0.001
Error	12	0.003	0.01		
C. Total	17	48.99			

Connecting Letters Report

level						mean value
T	a					72.016
A		b				74.143
B			c			75.85
C				d		76.116
D					e	76.22
E						f 76.86

Levels not connected by same letter are significantly different.

Appendix E

Sensory Analysis Score Card

Name of the panelist: …… Date: ……

Name of the product: Tulsi leaf incorporated green tea

Dear panelist, you are provided with 5 samples of Tulsi leaves incorporated green tea on each proportion with variation on Tulsi leaf powder content with control sample. Please, test the following samples of tea and check how much you prefer for each of the samples. Give the point for your degree of preference for each sample as shown below.

Judge the characteristics on the 1-9 scale as below:

Like extremely – 9 Like slightly – 6 Dislike moderately – 3

Like very much – 8 Neither like nor dislike – 5 Dislike very much – 2

Like moderately – 7 Dislike slightly – 4 Dislike extremely – 1

SN	Samples	Color	Aroma	Taste	Astrigency	Overall Acceptance
1	A					
2	B					
3	C					
4	D					
5	E					

Any comments : ……..…… Signature: ……

Appendix F

: Sensory analysis output

F 1 One-way ANOVA for color

Source	DF	SS	MS	F	Sig
Sample	4	62.0	15.500	23.250	<0.001
Error	45	30.0	0.667		
C. Total	49	92.0			

Connecting Letters Report

level			mean value
E	a		7.50
D	a		7.30
B		b	7.70
C		b	6.40
A		b	6.10

Levels not connected by same letter are significantly different.

F 2 One-way ANOVA for Aroma

Source	DF	SS	MS	F	Sig
Sample	4	32.520	8.130	6.407	<0.001
Error	45	57.100	1.269		
C. Total	49	89.620			

Connecting Letters Report

level			mean value
E	a		6.0
D	a		6.30
B	a		6.50
A	a		6.60
C		b	8.30

Levels not connected by same letter are significantly different

F 3 One-way ANOVA for Taste

Source	DF	SS	MS	F	Sig
Sample	4	50.52	12.630	13.828	<0.001
Error	45	41.100	0.913		
C. Total	49	91.620			

Connecting Letters Report

level			Mean
E	a		6.30
D	a		6.10
A		b	7.0
B		b	7.0
C			8.30

Levels not connected by same letter are significantly different

F 4 One-way ANOVA for Astringency

Source	DF	SS	MS	F	Sig
Sample	4	41.080	10.270	11.355	<0.001
Error	45	40.700	0.904		
C. Total	49	81.780			

Connecting Letters Report

level			Mean
E		c	5.70
D		c	5.90
A	a		8.0
B	a		7.7
C		b	6.85

Levels not connected by same letter are significantly different

F 5 One-way ANOVA for Overall Acceptance

Source	DF	SS	MS	F	Sig
Sample	4	44.280	11.070	11.694	<0.001
Error	45	42.600	0.947		
C. Total	49	86.880			

Connecting Letters Report

Level				Mean
E	a			5.5
D	a			5.9
A		b		7.0
B		b		6.9
C			c	8.2

Levels not connected by same letter are significantly different

COLOR PLATES

P1 Dried powder of green tea and Tulsi leaves

P2 Fresh Tulsi leaves and green tea leaves for drying

P3 Determination of protein by Kjeldal method

P4 Phytochemical work

P5 Tea bag containing tea samples

P6 Using of spectrophotometer

P7 Sample prepared for sensory

P8 Filtration of extract

THANK YOU