PHYTOCHEMICALS, ANTI-OXIDANT AND SENSORY ANALYSIS OF GURJO (*Tinospora sinensis*) STEM INCORPORATED GREEN TEA (*Camellia sinensis*)

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Phytochemicals, Anti-oxidant and Sensory Analysis of Gurjo (*Tinospora sinensis)* Stem Incorporated Green Tea (*Camellia sinensis*)

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Approval Letter

The ***dissertation*** entitled ***Phytochemicals, Anti-oxidant and Sensory Analysis of Gurjo (Tinospora sinensis) Stem Incorporated Green Tea (Camellia sinensis)*** presented by **Sunita Karki** has been accepted as the partial fulfillment of the requirements for the **B. Tech degree in Food Technology.**

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(Sunita Karki)

Abstract

The present work was carried out to prepare gurjo stem incorporated green tea (blend) powder and evaluate its sensory, phytochemical analysis and antioxidant activity. Gurjo stem (*Tinospora sinensis*) and green tea leaves (*Camellia sinensis*) were mixed in different proportions naming Sample A, B, C, D and Control (35:65, 17.5:82.5, 26.25:73.75, 8.75:91.25, 0:100) to obtain an optimum formulation of a blended tea using Design Expert v 13.0.1.0 software. Green tea, gurjo stem and the selected tea blend was subjected to organic solvent of methanolic extraction. Major phytochemicals (phenol, flavonoid, and tannin) content along with DPPH radical scavenging activity were determined for all the prepared extracts. The sensory analysis was carried out for color, aroma, taste, mouthfeel 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 $p<0.05$. R version 4.02 was used for multivariate analyses using correlation and principal component analysis (PCA).

From ANOVA and PCA result, Sample D having 91.25% green tea and 8.75% gurjo powder was obtained as best blend among four samples of mixture formulation. The Sample D was found to be superior than others samples on the basis of color, aroma, taste, mouthfeel and overall acceptance of infusion from statistical analysis at $P<0.05$. The moisture, protein, fat, crude fiber and ash content in blend were found to be 5.267%, 21.534%, 2.709%, 14.214% and 5.946% and were significantly different ($P<0.05$) than that of control product green tea. As there was no significance difference in nitrogen free extractive (NFE) of blend and green tea, the NFE in blend was found to be 55.590%. The methanolic extract of blend had TPC 69.353 mg GAE/g, TFC 49.781 mg QE/g, TC 28.167mg GAE/g dry weight, DPPH radical scavenging activity 69.143% inhibition and was significantly different ($P<0.05$) than that of control product green tea. The principal component analysis (PCA) showed that the closet proximity of blend extract with TPC and DPPH had strong correlation. Likewise, TC & DPPH and TC & TFC were found to be very strongly correlated with each other (99.7%) followed by TPC & DPPH and TPC & TC (99.4%) respectively. This finding indicated that TPC, TFC, TC and % DPPH of blend was found to be significantly different from green tea.

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List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemist
AYUSH	Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy
CE	Catechin equivalents
DB	Dry basis
DOE	Design of experiment
DPPH	2,2-Diphenyl picrylhydrazyl
DW	Dry weight
EGCG	Epigallocatechin gallate
FC	Folin-Ciocalteu
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
NTCDB	Nepal Tea and Coffee Development Board
PCA	Principal component analysis
PPO	Polyphenol oxidase
QE	Quercetin equivalent
SD	Standard deviation
TAE	Tannic acid equivalent

Abbreviation	Full form
TC	Tannin content
TFC	Total flavonoid content
TF	Theaflavins
TPC	Total Phenol content
TR	Thearubigins
WB	Weight basis
WHO	World Health Organization

Part I

Introduction

1.1 General introduction

Tea (*Camellia sinensis*) is the most widely consumed beverage on the world, and it can be prepared in a variety of ways. It is a member of the *Camellia* genus and the Theaceae family. There are two types of tea: Chinese variation *Camellia sinensis* var. *sinensis* and Assamese variety *Camellia sinensis* var. *assamica* (Kaundun and Matsumoto, 2002; Parmar *et al.*, 2012).

Tea is grown in approximately 30 countries throughout the world, in both tropical and sub-tropical climates, and it is thought to have originated in Southeast Asia. When consumed without milk or sugar, it is a natural, pleasant, cool, thirst-quenching beverage that is almost calorie-free (Adnan *et al.*, 2013). As the world's population lives longer, evidence suggests that making dietary and lifestyle adjustments at any age might enhance vascular, metabolic, and cognitive health, lowering the burden of non - communicable disease (Calder *et al.*, 2018). According to more clinical research, individuals must prioritize plant materials such as fruit, vegetables, grains, nuts, and oils while lowering their intake of red and processed meat, as well as sugary drinks, to obtain a balanced diet (Schulze *et al.*, 2018).

Green tea has generated a lot of interest among researchers and the general public because of its multiple health benefits, including antioxidant, anticarcinogenic, antiangiogenic, antimutagenic, antihypertensive, and anti-obesity qualities (Cabrera *et al.*, 2006). Green tea drinking, on the other hand, has been linked to mild to moderate effects on four major global killers: cancer, type 2 diabetes, stroke, and atherosclerosis-related cardiovascular events, according to meta-analysis reports (Johnson *et al.*, 2012). One method for increasing the health-promoting effects of green tea is to blend it with medicinal plants commonly used in herbal teas or tisanes. A growing understanding of the connection between nutrition and one's general wellbeing and the popularity of most herbal teas (Joubert *et al.*, 2017). Herbal teas are prepared from leaves, flowers, seeds, fruits, stems, and roots of plants other than *Camellia sinensis*, and have been used for health care and illness prevention for thousands of years (Deetae *et al.*, 2012). They are simple, effective, cheap, caffeine-free, and drug-free methods to receive the taste and health benefits of herbs (Killedar and Pawar, 2017).

Tinospora sinensis (Gurjo) is a widely accepted herbal medicine employed solely as therapeutic drugs in South Asia, with a plethora of pharmacological processes. This plant is also known as Guduchi or Amrita. Based on the findings obtained from Ayurveda and ethnobotanical studies, *Tinospora sinensis* possesses a vast array of pharmacological applications (Singh *et al.*, 2003). Several extracts of *Tinospora sinensis* like aqueous, alcohol, methanol, chloroform, ethanol, acetone, etc. are chiefly used in pharmaceutical, pre-clinical and clinical trial. Alkaloids, glycosides, steroids, phenolics, flavonoids, saponins, aliphatic chemicals, and polysaccharides are among the active ingredients in this herb. Antioxidant activity is provided by each of these substances (Onkar *et al.*, 2012). *Tinospora sinensis* stem is effectively used in health care management, mostly in general fatigue, dyspepsia, fever, urinary disease, constipation, burning pain, diarrhea, blood accumulation, and jaundice treatments. It is also recognized that *Tinospora cordifolia* has immunomodulatory characteristics (Modi *et al.*, 2021).

1.2 Statement of the problem

Excess of free radicals in the body leads to oxidative stress. Oxidative stress is the result of an imbalance in pro-oxidant/antioxidant homeostasis that leads to the generation of toxic reactive oxygen species (ROS), such as hydrogen peroxide, organic hydro peroxides, nitric oxide, superoxide and hydroxyl radicals etc. (Nimse and Pal, 2015). There is growing evidence that oxidative damage to tissue and cellular components plays a significant role in a variety of human diseases and aging processes, either as a primary or secondary cause. Many of the recent landmarks in scientific research have shown that in human beings, oxidative stress has been implicated in the progression of major health problems by inactivating the metabolic enzymes and damaging important cellular components, oxidizing the nucleic acids, leading to cardiovascular diseases, eye disorders, joint disorders, neurological diseases (Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis), atherosclerosis, lung and kidney disorders, liver and pancreatic diseases, cancer, ageing, disease of the reproductive system including the male and female infertility etc. (Rahman *et al.*, 2012).

There are many experiments about green tea, but relatively very few studies have only combined green tea with other therapeutic plants or herbs. *Tinospora sinensis* (gurjo) have been used for preparation of juice and evaluate its phytochemicals and pharmacological

properties but industrial scale production and utilization of *Tinospora sinens*is blending with *Camellia sinensis* for blend green tea has not been practiced yet in Nepal. To treat numerous diseases and reduce oxidative stress on human health, functional beverages like gurjo incorporated green tea must be developed.

1.3 Objectives

1.3.1 General objective

The general objective of this dissertation was to prepare the gurjo stem powder incorporated green tea powder (tea blend powder) and its phytochemicals, anti-oxidant and sensory analysis.

1.3.2 Specific objectives

- To prepare the gurjo (*Tinospora sinensis*) stem incorporated green tea powder in different proportion using Design of Experiment (DOE).
- To perform the sensory evaluation of infused liquid tea based on 9-point hedonic scale rating and principal component analysis (PCA) and thus selecting the best tea blend.
- To study the physiochemical properties of green tea, gurjo stem and the best tea blend powder.
- To prepare an extract of green tea, gurjo, best tea blend and quantify the phenolic content, flavonoid content and tannin content of these prepared extracts.
- To quantify the DPPH radical scavenging activity of prepared extracts.
- To carry out PCA and evaluate the correlation between phenolic content, flavonoid content, tannin content and DPPH radical scavenging activity.

1.4 Significance of the study

Antioxidant-rich plants are the focus of intense interest since recent reports have expressed safety concerns over the use of synthetic antioxidants (Taghvaei and Jafari, 2015). Plant phenolics in general are known to be powerful free radical scavengers and antioxidants. Plant polyphenols act as reducing agents and antioxidants by the hydrogen-donating property of their hydroxyl groups (Aberoumand and Deokule, 2008).

As a source of many physiologically active compounds with protective activity, *Tinospora sinensis* stem is very potential to be used as an ingredient for functional food development (Khan *et al.*, 2020). *Tinospora* species have a wide range of actions, including antidiabetic, antioxidation, anticancer, anti-inflammation, antibacterial, antiosteoporosis, and immune stimulation effects, according to modern pharmacological research and clinical practices. *Tinospora* genus is often used in clinics to maintain and support the immune system, prevent upper respiratory infections, cure diabetes, as adjuvant therapy in cancer, and to protect the liver (Yang *et al.*, 2004).

In developing countries like Nepal, introduction of tea with gurjo (an herbal plant) can be very effective as people here mostly rely on herbal medicine rather than modern drugs due to their higher cost and limited access. These 'herbal tea' contains a wide range of substances and could serve an important role in supplying nutrients and chemicals to compensate for poor diets. Combining green tea with medicinal plants typically used in herbal teas or tisanes is one strategy for enhancing the health-promoting properties of green tea. Most herbal teas are popular due to rising health awareness regarding the importance of food with one's general well-being (Joubert *et al.*, 2017).

However, there has yet to be a systematic review of these investigations. As a result, extra and thorough research on the plant could provide useful and essential facts that could aid in the promotion of the plant's value as well as serve other researchers and academia in future research (Sengupta *et al.*, 2011). The dissertation might help to replace the taste of the tea by introducing blend tea, utilize the medicinal plants (*Tinospora sinensis*) and prepare a balancing infusion of *Camellia sinensis* using *Tinospora sinensis* stem, which will be helpful to tea entrepreneurs to develop and launch blend green tea in a commercial level. Moreover, this dissertation will prove to be beneficial in contributing to the socioeconomic development of Nepal and the people.

1.5 Limitations of the study

- Antiglycation activity and Alkaloid content were not determined.
- Pharmacological properties were not studied.

1.6 Delimitations of the study

- A single variety of *Camellia sinensis* and *Tinospora sinensis* were used.

- Only methanolic extraction technique was used to prepare the extract.

Part II

Literature review

2.1 Green Tea

2.1.1 Introduction of green tea

Green tea (*Camellia sinensis*) is one of the most popular beverages worldwide and the main type of tea manufactured in China (Fu *et al.*, 2020). As a typical non-fermented and non-oxidized tea, green tea is famous not only for its elegant flavor, characteristic taste, and unique aroma but also for its health-promoting effects, such as anti-oxidative, anti-cancer, anti-atherosclerotic, and anti-inflammatory effects (Tan *et al.*, 2019). Currently, more than two thirds of the world population consumes this popular beverage (Hsu, 2005).

Many antioxidants isolated from higher plants are polyphenols. These polyphenols have been reported to exhibit biological activity as antibacterial, anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic, and immune-stimulating effects. Phenolics possess antioxidant activity due to their redox properties which allow them to act as reducing agents, hydrogen donors, and metal chelating potential. Comparing with the other natural antioxidant such as vitamins C, F and S-carotene, tea polyphenols are reported to possess stronger antioxidant activity in vitro lipoprotein oxidation model (Shrestha *et al.*, 2010).

Black and green teas are treated differently during the manufacturing process. To avoid fermentation and create a dry, dependable beverage, green tea is brewed by steaming freshly collected leaves. As a result of the steaming procedure, which destroys the enzymes responsible for breaking down the color pigments in the leaves, the tea is able to maintain its green color while being rolled and dried. These techniques maintain natural polyphenols and their health-promoting properties (Hursel and Viechtbauer, 2009).

2.1.2 History of green tea

During the 17th century, India exported the first green tea to Japan (Chacko and Thambi, 2010). Green tea originates from China (Thasleema and Aafrin, 2013). The green tea craze, which originated in China, has spread worldwide and is now the second most popular

beverage after water. Two primary varieties of *Camellia sinensis* are *Camellia sinensis* var. *sinensis* and *Camellia sinensis* var. *assamica* which is shown in Table 2.1. The sinensis plant strain is originated from China, tracing back to 2737 B.C. The assamica plant strain primarily is inhabitant to the Assam region in Northern India. According to legend, tea was discovered accidentally by either a man named Shien Non-Shei or the Emperor Shen Nung. Either way, green tea soon became popular among wealthy Chinese nobles (Senanayake, 2013). An annual production of 2.5 million tons of tea leaves is made throughout the world, with green tea making up 20% of the total. Green tea is mostly consumed in Asia, some parts of North Africa, the United States, and Europe (Anon., 2014).

Table 2.1 Taxonomic classification of *Camellia sinensis*

Taxonomic classification	
Kingdom:	Plantae
Sub-kingdom:	Tracheobionta
Division:	Magnoliopsida
Order:	Thales
Family:	Theaceae
Genus:	*Camellia* (L.)
Species:	*Camellia sinensis* (L.) Kuntze
Main varieties:	*Camellia sinensis* var. *assamica*
	Camellia sinensis var. *sinensis*

Source: McCully (2013)

2.1.3 Proximate composition of green tea

Due to the chemical composition of green tea, which is one of the most widely consumed drinks, it is recognized to provide many health advantages. More than 700 different chemical compounds can be found in green tea leaves. Proteins, carbohydrates, vitamins, minerals, lipids, theanine, caffeine, simple polyphenols, flavanols, organic acids, sugars, alkaloids, pigments, and volatile chemicals are all present in it (Xu *et al.*, 2021). Nitrogen-free extract (NFE) are consisting of carbohydrates, sugars, starches, and a major portion of materials

classed as hemicellulose in feeds. When crude protein, fat, water, ash, and fiber are added and the sum is subtracted from 100, the difference is NFE (Santana *et al.*, 2015; Sood, 2015). The proximate composition of green tea in terms of dry weight basis (Ahmad *et al.*, 2014; Rubab *et al.*, 2020) is shown in Table 2.2.

Table 2.2 Proximate composition of green tea

Proximate composition	Percentage (%)
Moisture (wb)	4.88 ± 0.09
Protein (db)	18.06 ± 1.06
Crude fat (db)	2.49 ± 0.13
Crude fiber (db)	15.35 ± 1.05
Ash (db)	5.60 ± 0.21
Nitrogen Free Extractives/NFE (db)	53.68 ± 1.75

Source: Ahmad *et al.* (2014); Rubab *et al.* (2020)

The higher moisture content in green tea samples may be due to exclusion of fermentation process during processing of green tea as compared to black tea because during this process much of the polyphenols are destroyed that retain moisture content (Ahmad *et al.*, 2014). Yao *et al.* (2006) also observed 70% of commercial tea samples having moisture content of 6.6% or less and 30% sample containing more moisture percentage up to 8% which can have negative effect on shelf life of the product, so for the better quality of the product moisture percentage should be controlled between 2.5-6.5%. The highest amount of protein and fat contents in green tea may be due to no fermentation of green tea during processing (Rehman *et al.*, 2002). Higher fiber content of tea may be by using the stems like impurities during its processing while low fiber content in tea samples due to use of younger leaves of tea plant. Moreover, the process of curling, tearing, and crushing also destroyed the structure of tea leaf and thus fiber content might be effected (Bashir *et al.*, 2019). The ash content are good sources of minerals and higher amount of crude fiber helps to prevent constipation (Mohammed and Sulaiman, 2009).

2.1.4 Methods of processing of green tea

Green tea is a variety of tea that hasn't been fermented. The stages of making Japanese green tea include harvesting, withering, steaming, rolling/shaping, and drying. In a similar way, pan firing is used to produce Chinese style green tea following the withering stage, with the remaining stages being the same for both types. White tea requires at least twice as long to wither as black tea (4-5 h). Both Japanese and Chinese green teas have different drying methods. The former is usually steam heated whereas the latter is dry heated to deactivate enzymes (oxidases) (Kosinska and Andlauer, 2014). Japanese green tea is produced by steaming young, unfermented leaves as soon as they are harvested in order to prevent fermentation. This produces a dry, stable product that might be withered indoors for a brief period of time to eliminate moisture (Yang *et al.*, 2009).

2.1.5 Flowchart of green tea

The flowchart of green tea is shown in Figure 2.2 as

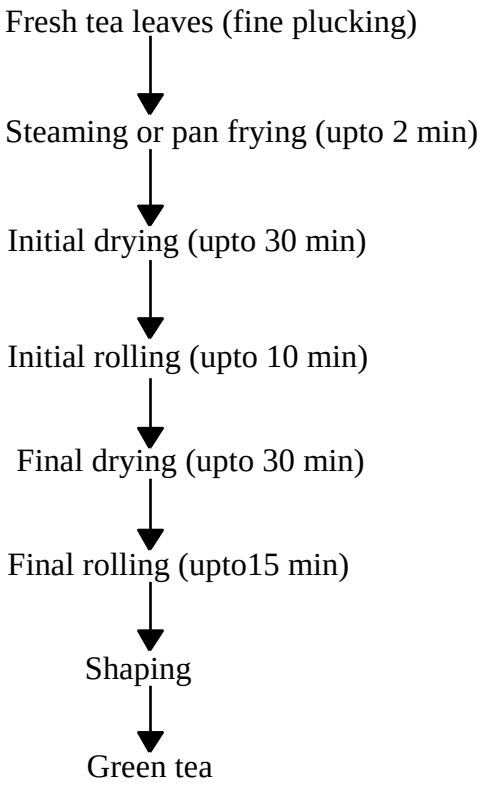

Fig. 2.1 Green tea manufacturing process

Source: Singh *et al.* (2014)

For understanding the above technology, stepwise processing has been below:

2.1.5.1 Plucking

To produce high-quality tea, the buds and shoots with two or three leaves must be cut off and processed. Tender and uniform tea leaves are typically favored for green teas with the finest grade (such Longjing, Biluochun, Gyokura, etc.). Immature shoots and coarse leaves are not acceptable since the standard flush is based on the leaf standard which is listed in Table 2.3. Selecting coarse leaves lowers the quality of tea that has been produced. After being plucked, the shoots are typically left to dry for 1-3 h on the ground or on bamboo trays in order to produce a grassy scent and achieve the ideal degree of moisture for the best possible green tea quality (Singh *et al.*, 2014).

Table 2.3 Percentage of standard flush for standard leaf plucking

Percentage of standard flush	Leaf standard
More than 75%	Fine plucking
60-75%	Medium plucking
Less than 60	Coarse plucking

Source: Singh *et al.* (2014)

2.1.5.2 Fixing (Pan-frying or Steaming)

In order to prevent fermentation and maintain the leaves' green color, fixing is a technique that lowers or stops the enzyme activities in green leaves in order to prevent fermentation and maintain the leaves green color. While steam-fixing is often done at 100°C, pan-fixing is usually performed at a temperature more than 180°C. The leaves are extracted once more after being passed through a rotating drum with hot steam added for up to 2 min. In this stage, the amount of steam utilized is important since too much steam might ruin the leaves while too little steam can cause fermentation to begin and affects the color of tea (Adnan *et al.*, 2013).

2.1.5.3 First drying

For drying, leaves can be swiveled in a wooden or metal drum for around 30 min in warm air at 55°C. During this process, the leaves lose around half of their moisture content. Because the physical and chemical changes that take place throughout the drying process affect the final tea's quality, good drying process control is necessary to maintain quality while consuming less energy. In order to avoid burnt taste and quality loss, the drying temperature is controlled (Xie *et al.*, 2006). Long time drying at high temperature could result in non-enzymatic browning reactions, which are important phenomena that occur during food processing (Lin *et al.*, 2010; Yan *et al.*, 2018).

2.1.5.4 Rolling

In this stage of processing, leaves are rolled in a rolling machine for around 10 min at various pressures. The leaves are cleaned to remove impurities prior to being fed into the rolling machine. Crushed particles are then fed through a rotor vane machine for additional crushing, a curl-turn-cut machine to modify the crushed particles, and finally a roll breaker to dislodge the twisted balls that are responsible for the slow fermentation process. Optimal rolling is necessary because insufficient rolling produces uneven crush particles and excessive rolling causes chemical loss and inappropriate chemical-enzyme combination (Naheed *et al.*, 2007). Roll breaking is used to release compressed or compacted leaves, which is then followed by rolling to hasten the drying process. To enhance flavor, remove moisture, and improve the final product, drying is typically performed many times (Naheed *et al.*, 2007) .

2.1.5.5 Final drying, rolling and polishing

The leaves are then subjected to hot air for up to 30 min to further dry them, followed by 15 min of rolling between two rotating metal plates in a rolling machine, and finally polishing with the help of a polisher by forcing the leaves up against a hot plate. This makes the leaves smoother and bright. When it comes to aesthetics, this phase is crucial (Singh *et al.*, 2014).

2.1.6 Factors affecting green tea quality

Quality is more important than quantity when it comes to partially fermented tea. Environmental factors: Soils and climate are two significant determinants of quality. High elevations and cultural traditions may affect the quality of tea (such as tillage, weeding,

fertility control, irrigation, plant protection, and harvesting). The quality of green tea is affected by a number of elements, including tea plant cultivars, tea shapes, fertilization and harvesting techniques, as well as processing techniques (withering, shaking, panning, rolling, and drying) (Chiu, 1990).

Tea quality, a major factor of its market value, depends on metabolite content and is primarily influenced by the amounts and contents of both non-volatile and volatile components (Rawat *et al.*, 2007; Tudu *et al.*, 2009). Non-volatile components are generally responsible for taste, while volatile components provide aroma (Ma *et al.*, 2018). The taste of tea is attributed to a variety of non-volatile ingredients, such as caffeine (for briskness), catechins (for bitterness), and amino acids (for freshness) (Chen *et al.*, 2014; Xu *et al.*, 2018). These substances determine the soup color and taste of tea, and any changes in their concentrations reflect tea quality (Zhang *et al.*, 2017). The taste and aroma of tea are determined by the maturity of the fresh tea shoots used as a raw material for processing, in addition to the manufacturing process and geographic differences (Han *et al.*, 2016; Wen *et al.*, 2019).

To enhance the quality of green tea, nitrogen should be utilized to increase the amino acid content of the beverage. The amino acids in tea have a significant role in color production which may be oxidized by catechins resulting in tea liquor color (Ying *et al.*, 2005). For other high quality teas, bitter and astringent have negative connotations while aromatic, sweet and delicate are desired characteristics (Ahmed and Stepp, 2012).

2.1.7 Health benefits of green tea

Animal studies have shown that green tea catechins offer some defense against degenerative diseases. In studies using hepatoma-treated rats, green tea has been shown to have antiproliferative and hypolipidemic characteristics. It has also been shown to prevent hepatotoxicity and act as a post-initiation mammary cancer preventative agent. Green tea catechins could also act as antitumorigenic agents (Roomi *et al.*, 2007) and as immune modulators in immunodys function caused by transplanted tumors or by carcinogen treatment (Vanessa and Gary, 2004). Moreover, green tea, its extract, and its isolated constituents were also found to be effective in preventing oxidative stress (Babu *et al.*, 2006) and neurological problems (Unno *et al.*, 2007). Drinking green tea has also been related to

reducing the risk of developing cancers of the kidney, pancreas, esophagus, mouth, stomach, small intestine, lung, and mammary glands, among other cancers (Koo and Cho, 2004).

Numerous epidemiological research and clinical trials have demonstrated that drinking green tea can reduce the risk of a number of chronic diseases, with black and oolong teas having a weaker impact (Zaveri, 2006). High concentrations of polyphenols, powerful antioxidants, are likely to be the cause of this advantageous result. Particularly green tea may lower blood pressure, reducing the risk of heart attack and stroke. By reducing blood glucose levels and body weight, green tea has been proven in animal studies to protect against the development of coronary heart disease (Tsuneki *et al.*, 2004).

Green tea extracts and green tea polyphenols (GTPs) had a beneficial effect on the proliferation and activity of bone cells (Park *et al.*, 2003) . Hepatic stellate cell proliferation is linked to the evolution of liver fibrosis in chronic liver disorders, and EGCG has a potential inhibitory effect on these cells' proliferation (Dorchies *et al.*, 2003; Sakata *et al.*, 2004). Green tea boosts immune system function by protecting it from oxidants and radicals. GTPs have been shown to protect against Parkinson's, Alzheimer's, and other neurodegenerative disorders in recent studies (Pan *et al.*, 2003; Weinreb *et al.*, 2004).

2.1.8 Harmful side effects of green tea

The effects of green tea and its constituents may be advantageous up to a point, but excessive levels may have unknown adverse effects. Green tea provides a lot of health advantages. Additionally, not everyone may experience green tea catechins effects in the same way. The principal metabolic organ of the body, the liver, is the target of acute cytotoxicity caused by green tea extract's EGCG (Schmidt et al., 2005). According to a different study, consuming more green tea damages the oxidative DNA in hamsters pancreas and liver (Takabayashi *et al.*, 2004) .

In pancreatic beta-cells in vivo, Yun *et al.* (2006) discovered that EGCG functions as a pro-oxidant rather than an antioxidant. As a result, a high green tea intake may be harmful to diabetic animals' ability to regulate hyperglycemia. Green tea extract caused thyroid enlargement (goiter) in normal rats when given at a high dose (5 % of diet for 13 wk.) (Sakamoto *et al.*, 2001; Satoh *et al.*, 2002). As a result of this high-level therapy, thyroid

hormone plasma concentrations were changed. However, it is unlikely that even a very high dietary intake of green tea will have negative consequences on humans.

Harmful effects of tea overconsumption (black or green) are due to three main factors: its caffeine content, the presence of aluminum, and the effects of tea polyphenols on iron bioavailability. Green tea should not be consumed by anyone who has heart disease or major cardiovascular issues. Women who are pregnant or nursing shouldn't consume more than one or two cups of caffeine each day because it can alter their heart rhythm. Avoiding green tea while taking certain drugs is especially important due to the diuretic effects of caffeine (Bruneton, 2001).

Numerous studies have demonstrated that tea plants are able to store large amounts of aluminum. This is an important factor to take into account for people with renal failure since aluminum can build up in the body and cause neurological diseases. Therefore, it is important to restrict the consumption of foods high in this metal (Costa *et al.*, 2002). Similarly, green tea catechins may have a preference for iron, and green tea infusions can considerably lower the bioavailability of iron from food (Hamdaoui *et al.*, 2003).

2.2 Gurjo (*Tinospora sinensis*)

Since the beginning of time, plants have been important to people as a source of food and medicine. The popularity of medicinal plants is rising at the moment due to the various phytochemicals that are useful for therapeutic purposes (Rani *et al.*, 2015). *Tinospora sinensis* (Lour.) Merr. belongs to the Menispermaceae family and is known in India by different names such as giloy, guduchi, and amrita. It is well known in Ayurveda and traditional medicine for its admirable therapeutic efficiency (Sankhala *et al.*, 2012). Because of its capacity to provide freshness, energy, and long life, it is known as the "Nectar of Immotality" (Upadhyaya *et al.*, 2011). *Tinospora sinensis,* which grows on neem trees (*Azadirachta indica*), has a high medicinal potential and is known as ‘Neem giloy' because of its synergistic impact (Mittal *et al.*, 2014).

The stem is a more widely used and beneficial component of the plant than the leaves (Sarala *et al.*, 2012) and its extract has been shown to be a good source of antioxidant for nutraceutical purposes, providing protection against cardiovascular disease, premature aging, and cancer (Ilaiyaraja and Khanum, 2011). Traditionally, *Tinospora sinensis* (Lour.)

Merr. leaf and stem juice has been used to cure chronic rheumatism, ulcerated sores, and piles by disinfecting the fresh stem and leaves (Rajgopal *et al.*, 2013). The Newar community in Kathmandu and far west Nepal are seen to use the juice, powder, or liquid of *Tinospora sinensis* (Lour.) Merr. for diabetes and gastritis (Balami, 2004) .

In China, the plant is used to relieve wind dampness, stimulate blood circulation, relax joint and muscle, relieve pain, and reduce swelling (Xu *et al.*, 2010). In India's North Central Western Ghats, the stem decoction of *Tinospora sinensis* (Lour.) Merr. is used to cure bone fractures and strengthen bones (Upadhya *et al.*, 2012). *Tinospora sinensis* (Lour.) Merr. has been reported to be used as a substitute for *Tinospora cordifolia* (Willd.) Miers (Khare, 2007). The common names and taxonomic classification of gurjo according to Sharma *et al.* (2010) is shown in Table 2.4 and Table 2.5.

Table 2.4 Botanical names of gurjo

Botanical names	
Sanskrit:	Guduchi, amrita
Bengali:	Giloe, gulancha
Hindi:	Giloya
Gujarati:	Gado, galo
Telugu:	Duyutige, teppatige
English:	Heartleaf moonseed
Malayalam:	Amruthu, Chittamruthu
Tamil:	Shindilakodi

Source: Sharma *et al.* (2010); Biswasroy *et al.* (2020)

Table 2.5 Taxonomic classification of gurjo

Taxonomic classification	
Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Ranunculales
Family:	Menispermaceae
Genus:	*Tinospora*
Main species:	*Tinospora cordifolia*
	Tinospora sinensis

Source: Sharma *et al.* (2010); Meshram *et al.* (2013)

Some of the medicinally important species are *Tinospora cordifolia* , *Tinospora sinensis*, *Tinospora crispa*, *Tinospora cordifolia*, *Tinospora malabarica*, *Tinospora tomentosa*, *Tinospora uliginosa*, etc. (Neeraja and Margaret, 2013).

2.2.1 Plant morphology

Tinospora cordifolia is a large, deciduous, extensively spreading and climbing shrub with several elongated twining branches. Different parts of exhibits different types of morphology which are described below.

2.2.1.1 Root

Roots are aerial, threadlike, long filiform, threadlike, squarish, and extend downward from mature branches or cut parts of stems, sometimes reaching the earth by continuously lengthening (Spandana *et al.*, 2013).The dried aerial roots have a light grey-brown or creamy white color, are odorless, and have a bitter flavor. Starch can be found throughout the aerial root's parenchyma.

2.2.1.2 Stem

The stem of this plant is succulent, with long, filiform, fleshy, and climbing tendencies. The branches give rise to aerial roots. The dried stem is slender, cylindrical, and somewhat twisted. The outer bark is thin and papery, and the color ranges from brown to greyish. Transverse sectioning of the stem reveals a wheel-like structure. The stem powder has a characteristic aroma and bitter flavor and is creamish brown to dark brown in color. The stem is used to treat dyspepsia, fever, and urinary tract infections. The starch extracted from the stem, known as "Guduchi-satva," is extremely nutritious and digestive, and is used to treat a variety of conditions (Bishayi *et al.*, 2002).

2.2.1.3 Leaves

The leaves of this plant are membranous, simple, alternate, with long petiole approximately 15 cm which is round, pulvinate, heart shaped, twisted partially and half way round (Nasreen *et al.*, 2010). In the beginning, the leaves are a bright green color, but as they age, they turn yellowish green to yellow. The leaves are harsh and have an unremarkable odor. The lamina is ovate-cordate, measuring 10- 20 cm long and 8-15 cm wide. Protein, calcium, and phosphorus are abundant in leaves (Sharma *et al.*, 2013).

2.2.1.4 Flowers

The flowers are tiny and unisexual, and the color is a greenish yellow. Male flowers are grouped together, while female flowers are found alone. There are six sepals in each of two series of three. The outer sepals are smaller than the inner ones. Petals are six in number, smaller than sepals, free-floating, and membranous. Summer is when flowers bloom (March to June) (Sinha *et al.*, 2004).

2.2.1.5 Fruit

Fruits are fleshy, single-seeded, and occur in clusters of one to three. These are drupelets with a subterminal scar on a thick stem. The fruit is ovoid in shape, with a smooth texture and a scarlet or orange red color (Nasreen *et al.*, 2010).

2.2.1.6 Seed

Seeds are white, bean shaped and curved. Embryo also turned in to curve shape automatically (Singh *et al.*, 2003).

2.2.2 Chemical constituents and biological activity of gurjo

The chemical constituents and biological activities of *Tinospora cordifolia* are summarized in Table 2.6.

Table 2.6 Major and sub groups of natural products present in different parts of *Tinospora cordifolia* and their biological activities.

Active component	Compound	Plant part	Biological activity
Alkaloids	Berberine, Choline, Palmatine, Tembetarine	Stem, Root	Anti-viral infections, Anti-cancer, Anti-diabetes
Diterpenoid lactones	Furanolactone, Tinosporin, Isocolumbin	Whole plants	Anti-inflammatory, Anti-hypertensive, Anti-microbial
Terpenoids	Epoxy-clerodane diterpene	Stem	Anti-inflammatory
Glycosides	Cordifolioside A, Syrigin, Tinocordiside	Stem	Immunomodulation, Anti-cancer
Steroids	β–sitosterol, Makisterone A	Shoots	Anti-inflammatory
Sesquiterpenoid	Tinocordifolin	Stem	Antiseptic

Source: Upadhya *et al.* (2010); Sundarraj *et al.* (2012); Biswasroy *et al.* (2020)

2.2.3 Proximate composition of gurjo

The proximate study (Nile and Khobragade, 2009; Rahal *et al.*, 2014; Tyagi *et al.*, 2020; Modi *et al.*, 2021) revealed the presence of carbohydrate, protein, fiber, fat, and moisture which helps to determine the nutritive value of the medicinal plant gurjo. Nitrogen free extractives (NFE) vary from basic sugar to more complex compounds, like starch and fiber. The findings derived from the proximate assessment of the oven dried stem sample's nutritional values are summarized in Table 2.7.

Table 2.7 Proximate composition of dried *Tinospora cordifolia* stem

Proximate Composition	% In dried sample
Moisture content	10.01
Total ash	7.05
Crude fat	1.77
Crude fiber	26.99
Protein content	8.06
Carbohydrate	46.11

Source: Modi *et al.* (2021)

2.2.4 Ethnobotanicals and traditional uses of gurjo

Those species have been used for therapeutic purposes for a long time. According to research, they are employed as an adjunctive therapy in cancer and liver protection, as well as a treatment for oral, skin, respiratory, and urinary tract infections, ulcerations, and diabetes (Zhang *et al.*, 2010).

Ayurveda states that *Tinospora sinensis* has an unpleasant flavor that is bitter, pungent, and astringent. Even at the molecular level, the bitter flavor is considered to enhance metabolic activity. It has been shown to be effective in treating gastrointestinal conditions such dyspepsia, flatulence, gastritis, jaundice, diarrhea, splenomegaly, and hemorrhoids. It is currently more likely to be a research topic and plays a part in the treatment of metabolic illnesses like diabetes and kidney disorders. It is prescribed for intermittent fevers, infective conditions, urinary disorders, skin diseases, and eye diseases. It is frequently used as an ingredient to treat gout and rheumatoid arthritis in combination with other herbs. Fractures are treated with the whole plant that has been properly ground. Additionally, *Tinospora sinensis* is regarded as a general tonic and has a positive impact on health equipped with a variety of nutritive ingredients (Panchabhai *et al.*, 2008). The numerous and significant

ethnomedicinal characteristics that even this genus contains could serve as a foundation for further study into the phytochemical and pharmacological characteristics of this species.

2.2.5 Immunomodulatory activity of gurjo

Few studies have focused on the immunomodulatory abilities and mechanisms of *Tinospora sinensis. Tinospora sinensis* stem contains an ingredient known as arabinogalactan polysaccharide (G1-4A), which controls cytokines and nitric oxide excretion to protect mouse macrophages from endotoxic shock brought on by lipopolysaccharide (Desai *et al.*, 2007). Different polysaccharides, such as arabinose, glucose, and fructose, may be involved in *Tinospora sinensis* immunomodulatory action (Sharma *et al.*, 2012) and induces a nonspecific immune response (Alexander and Kirubakaran, 2010); however, the mechanism is poorly elucidated. Additionally, supplementing mice with *Tinospora sinensis* results in splenomegaly, an increase in macrophages, T cells, and B cells, as well as an increase in the production of antiapoptotic genes in immune cells (Raghu *et al.*, 2009).

Many active compounds, including N-methyl-2-pyrrolidone, N-formylannonain, 11-hydroxymustakone, Cordifolioside A, Tinocordiside, Syringin, and Magnoflorine, are found in *Tinospora sinensis* (Sharma *et al.*, 2012), usually show practical immunomodulatory and cytotoxic effects. It was reported that such active components could boost macrophage phagocytic properties by causing free radicals to be produced in human neutrophils (More and Pai, 2012).

2.2.6 Safety evaluation of gurjo

Tinospora herbs have a long history of use in medical contexts, which confirms to their effectiveness as medicines. When used in tolerance, there have been no negative effects reported for this genus. For children and newborns, *Tinospora sinensis* is utilized as a growth booster. *Tinospora sinensis* has no negative effects in an acute toxicity investigation with a dose of 3 g/kg, and the experimental rats did not die (Agrawal *et al.*, 2002). More and Pai (2012) suggested approaches and interventions from AYUSH to address COVID-19 health challenges. The prophylactic care and as add on to standard care for *Tinospora cordifolia* aqueous extract with a 500 mg bid for 15 days or 1 month (as directed by Ayurveda physician with warm water. The herb has been reported to be safe even in high doses for long term, clinical studies have also shown it to be safe in long term use.

When administered to rats in doses of 0.1 g/kg for a period of 12 wk., *Tinospora sinensis* has no adverse effects on liver and renal function markers. Leukocytosis and neutrophilia increased in rats, but in healthy persons it had no effect (Panchabhai *et al.*, 2008). *Tinospora sinensis* treatment has no clastogenic or DNA-damaging effects on bone marrow erythrocytes and peripheral blood cells (Chandrasekaran *et al.*, 2009). And no neurological impairment or marked central nervous system depressant activities were shown (Karkal and Bairy, 2007).

Cytotoxicity was evaluated in terms of LC_{50} (lethality concentration). The result showed that the extract of *Tinospora cordifolia* was found to be toxic with an LC_{50} value of 232.64µg/ml. The bioactive component present in the plants could be the result of its pharmacological effects that support the traditional use of plants (Shrestha and Lamichhane, 2021). The literature survey on hepatic effect by *Tinospora cordifolia* (TCF) on 6 male Wister rats with alcohol intoxication of duration on treatment 200 mg extract (water and sediment) /kg brew/day for 15 days shows significant improvements in the total protein content, the liver function tests and the lipid profile tests except for high-density lipoprotein cholesterol. Also, significantly lower levels of lipid peroxidation and glutathione (GSH). The liver histology showed improvements despite the collection of many lymphocytes within the hepatic parenchyma and the prominent Kuepfer cells (Chavan *et al.*, 2017). Also, the literature survey on hepatic effect by *Tinospora crispa* (TCP) on 1 man of duration on treatment 10 pellets/day for 4 weeks shows centrilobular necrosis with inflammatory cell infiltration compatible with a toxic etiology and complete recovery after discontinuation (Langrand *et al.*, 2014).

2.2.7 Medicinal applications of Gurjo

Tinospora sinensis is widely used in traditional ayurvedic medicine in India due to its biological activities, which include anti-inflammatory, immunomodulatory, anti-oxidant, anti-diabetic, anti-periodic, anti-spasmodic, anti-neoplastic, anti-stress, anti-leprotic, anti-malarial, hepato-protective, anti-allergic, and anti-arthritic activity, as well as various fevers, asthma, diabetes, dyspepsia, jaundice, urinary difficulties, skin illnesses, and chronic diarrhea and dysentery are all treated using *Tinospora sinensis* (Sharma *et al.*, 2012).

- The root and stem of *Tinospora cordifolia* an antidote to snake bite and scorpion sting (Zhao *et al.*, 1991).

- The stem is bitter, stomachic, diuretic, stimulates bile secretions, allays thirst, enriches the blood and cures jaundice (Nayampalli *et al.*, 1988).

- The juice of plant stem is useful in diabetes, dyspepsia, vaginal and urethral discharges (Singla *et al.*, 2010).

- The bark of this plant acts as Anti-allergic, Anti-spasmodic, Anti-pyretic, Anti-leprotic (Asthana *et al.*, 2001).

- The powder of root and stem is used along with milk for treatment of cancer (Bhatt and Sabnis, 1987).

- The whole plant of *Tinospora cordifolia* used in scabies in swine, diarrhea, Urinary diseases, syphilis, skin diseases, bronchitis, to promote longevity, increase body's resistance and Stimulate the immune system (Kapur *et al.*, 2008).

- The dry stem crude extract of this plant which was poly saccharide in nature shows a poly clonal B-cell mitogen activity and Active components of stem extract enhanced the humoral response in mice (Kalikar *et al.*, 2008).

- Giloy *(Tinospora cordifolia*) juice which is a mixture of Giloy herb and tulasi leaves is used against monkey malaria (Vashist *et al.*, 2011).

- The stem aqueous extract of *Tinospora cordifolia* shows anti-inflammatory effect in both acute an sub-acute models of inflammation (Jana *et al.*, 1999).

- It is used in treatment of jaundice because it reduces body heat (Sangeetha *et al.*, 2013).

- The stem of this plant regulates the blood sugar level due to the presence of alkaloids (Patel and Mishra, 2011).

2.3 Phytochemicals

Phytochemicals are non-nutritive, bioactive chemical substances that are present in plants and function as disease defenses (Kumar *et al.*, 2018). Studies suggest that up to 10,000 distinct phytochemicals may have the power to influence conditions like cancer, stroke, and metabolic syndrome. The main components of the plant are a mixture of fatty acids,

polysaccharides, alkaloids, glycosides, steroids, sesquiterpenoids, aliphatic compounds, and sesquiterpenes (Singh *et al.*, 2003). Phytochemical constituents are the biochemical compounds used as the precursors for the development of drugs (Abidemi, 2013) which is shown in Table 2.8.

Table 2.8 Bioactive phytochemicals in medicinal plants

Classification	Main Group of Elements	Biological Function
Non-starch polysaccharides.	Cellulose, hemicellulose, gums, mucilages, pectins, lignins	Water holding capacity, delay in nutrient absorption, binding toxins and bile acids.
Antibacterial & Antifungal	Terpenoids, alkaloids	Inhibitors of microorganisms, reduce the risk of fungal infection.
Antioxidants	Polyphenolic compounds, flavonoids, carotenoids, tocopherols, ascorbic acid	Oxygen free radical quenching, inhibition of lipid peroxidation
Anticancer	Carotenoids, polyphenols, curcumine, flavonoids	Inhibitors of tumor, inhibited development of lung cancer, anti-metastatic activity
Detoxifying Agents	Reductive acids, tocopherols, phenols, indoles, aromatic isothiocyanates, coumarins, flavones, carotenoids, retinoids, cyanates, phytosterols	Inhibitors of procarcinogen activation, inducers of drug binding of carcinogens, inhibitors of tumourogenesis
Other	Other Alkaloids, terpenoids, volatile flavor compounds, biogenic amines	Neuropharmacological agents, anti-oxidants, cancer chemoprevention

Source: Saxena *et al.* (2013)

2.3.1 Classification of phytochemicals

2.3.1.1 Phenolic

The largest and most prevalent class of phytochemicals in the plant kingdom are phenolic phytochemicals. Phenolics are a type of chemical compounds with a hydroxyl group (-OH) that is directly linked to an aromatic hydrocarbon group (Altiok, 2010). Based on the numbers of carbon atoms present in its structure, phenolic are categorized as Table 2.9.

Table 2.9 Major classes of phenolic compounds in plants

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, Coumarins
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: Altiok (2010)

2.3.1.1.1 Biological activity of phenolic acids

Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators (Wang *et al.*, 1996; Cao *et al.*, 1997). In food plants,

phenolic acids can combine with sterols, alcohols, glucosides, and hydroxyfatty acids to create glycosides or esters. Numerous biological effects of phenolic acids have been discovered. The biological functions of phenolic acids include increasing the synthesis of bile, lowering blood cholesterol and lipid levels, and acting as an antibiotic against bacteria like *Staphylococcus aureus* (Silva *et al.*, 2007). Phenolic acid has antiulcer, anti-inflammatory, antioxidant, cytotoxic and anticancer, antispasmodic, and antidepressant properties, to mention a few (Ghasemzadeh *et al.*, 2010). Numerous studies have revealed a strong correlation between phenolic acids and free radical scavenging activity (Sultana *et al.*, 2007; Moein *et al.*, 2008; Dudonne *et al.*, 2009; Kim, 2012).

2.3.1.2 Flavonoids

In the kingdom of plants, flavonoids are abundant secondary metabolites. They can exist in both free form (as aglycones) and as glycosides, and they vary in the kind, number, and locations of their substituents as well as the degree to which they are saturated. The amount and placement of hydroxyl groups in the various groups of flavonoids molecules, in particular, have a significant impact on their ability to act as antioxidants (Maisuthisakul *et al.*, 2007; Moein *et al.*, 2008; Gonzalez *et al.*, 2013; Bhaigyabati *et al.*, 2014). DPPH radical scavenging activity is a property of distinct flavonoid compounds that might have varying affinities (Hirano *et al.*, 2001; Firuzi *et al.*, 2004; Meda *et al.*, 2005). The most common classes are the flavones, flavanols, flavanones, catechins, isoflavones and anthocyanidins, which accounts for around 80% of flavonoids (Pinheiro and Pedro, 2012).

2.3.1.2.1 Biological activity of flavonoids

A wide class of substances known as flavonoids protects biological systems against the negative effects of oxidative processes on macromolecules like DNA, proteins, carbohydrates, and lipids (Atmani *et al.*, 2009; Praveen *et al.*, 2012). Although practically every category of flavonoids has the ability to act as powerful antioxidants, flavonoids are also known to have antibacterial, cytotoxic, anti-inflammatory, and anticancer activities. As compared to vitamins C, E, and carotene, flavonoids like luteolin and catechins are more powerful antioxidants. There have been numerous claims made about flavonoids anti-inflammatory, enzyme-inhibiting, antibacterial, oestrogenic, anti-allergic, antioxidant, vascular, and cytotoxic anticancer properties (Tapas *et al.*, 2008; Kumar *et al.*, 2018).

2.3.1.3 Tannin

Plant polyphenols called tannins are astringent and bitter; they either attach to and precipitate proteins or shrink them. Tannins range in molecular weight from 500 to more than 3000. Worldwide, tannins can be found in many types of plants and habitats. Algae, fungus, and mosses are lower plants that do not have a lot of tannin. Numerous studies revealed that tea leaves, coffee beans, herbs, and other plants all contain tannins (Savolainen, 1992; Namdev and Gupta, 2015). Tannins are divided into hydrolysable tannins and condensed tannins. Hydrolysable tannins, upon hydrolysis, produce Gallic acid and ellagic acid and depending on the type of acid produced, the hydrolysable tannins are called Gallo tannins or egallitannins (Bressani *et al.*, 1983). On heating, they form pyrogallic acid (Savolainen, 1992; Bizuayehu *et al.*, 2016). Common examples of hydrolysable tannins include theaflavins (from tea), daidezein, genistein and glycitein (Doughari *et al.*, 2009). Studies found that some phenols are positively correlated with condensed tannin whereas some are poorly correlated with hydrolysable tannin (Baldwin *et al.*, 1987).

2.3.1.3.1 Biological activity of tannin

Tannin-containing plant extracts are used as anti-inflammatory, antiseptic, antioxidant, and hemostatic pharmaceuticals (Dolara *et al.*, 2005; Anesini *et al.*, 2008). Tannins are also utilized to make inks and as caustics for cationic dyes (tannin dyes) in the dyestuff industry (iron gallate ink). In the food industry, tannins are used to clarify wine, beer, and fruit juices. Other commercial applications for tannins include textile dyes, coagulants in the manufacture of rubber, and antioxidants in the manufacturing of fruit juice, beer, and wine (Gyamfi and Aniya, 2002). In contrast, the phenolic compounds known as tannins have powerful antioxidant effects, have been found to be anti-mutagenic and anti-carcinogenic, and are used to treat cancer patients (Ramakrishnan *et al.*, 2006). Recently, tannins have attracted scientific attention, particularly in light of the increase in fatal diseases like AIDS and various cancers. Since that the biological effects of plant extracts containing tannin have been extensively studied, the search for new lead molecules for the introduction of unique pharmaceuticals has acquired growing importance (Saxena *et al.*, 2013; Namdev and Gupta, 2015).

2.3.2 Factors affecting phytochemical content

2.3.2.1 Cultivar effect

Genetic composition is the main determining factor which can directly influence the phytochemicals of vegetables, since it has been shown that the differences in phytochemical compounds between cultivars are greater than those between plants of the same cultivar grown under different conditions (Hu, 2012). The strong effect of genetic materials on the phenolic profile has been demonstrated in several horticultural crops such as potato, faba bean, tomato, garlic, globe artichoke and cardoon (Rouphael *et al.*, 2016).

2.3.2.2 Extraction method and solvent used

Plant activities and phytochemical concentration are influenced by extract production techniques and solvent type. Methanol extracts produced high levels of phytochemical concentration. Samples from the Highland and Semi-arid zones had more antioxidant activity than samples from the Tropical zone, which had the lowest. The phytochemicals, total phenolic content (TPC), and antioxidant capacity of the Alovera plant are impacted by various agro-climatic situations (Kumar *et al.*, 2017). The study done by Vidic *et al.* (2014) shows that there is significant difference in the phytochemical contents due to the difference in the method of extraction used where soxhlet extraction was better than ultrasound extraction of the sample. The variations in data may be due to different solvents, different maturity level and weather conditions (Rababah *et al.*, 2010; Izreen and Fadzelly, 2013; Waleed *et al.*, 2014; Dhanani *et al.*, 2017). The phytochemicals values were affected by the extraction methods used during analysis (Praveen *et al.*, 2012).

2.3.2.3 Environmental conditions

The amount of phytochemicals may be influenced by environmental factors such as sunlight exposure, temperature change, and local climatic circumstances (Oh *et al.*, 2008). The overall phytochemical content of plants is reportedly influenced by a number of elements, including mineral composition, soil type, temperature, light, and water content (Rajbhar *et al.*, 2015). To ensure favorable levels of phytochemicals, optimal fertilization is essential. Nitrogen, phosphorus, and potassium fertilizer applied at high rates may improve vegetative growth and production while lowering phytochemical levels (Tiwari and Cummins, 2013; Santana *et al.*, 2015). Seasonal fluctuations can significantly alter the phytochemical

composition of plants when they are exposed to varying temperatures (Usano-Alemany *et al.*, 2014).

2.3.2.4 Growth conditions

Plant growth stages affect the amount of phytochemicals present. According to the research, the total alkaloids gradually increase during the growth and development stages, while leaves have low phenolic and flavonoid contents during the early growth stage. Diverse developmental phases of Aloe vera have different active ingredients and varying levels of antioxidant capability (Hu *et al.*, 2003). The difference in maturity stage or even due to difference in species of plant affect the phytochemicals (Sivakumar and Rajan, 2010).

2.3.2.5 Post- harvest factors

Due to fungal decay, physiological issues, pests, mechanical damage, over ripeness, and insufficient temperature and relative humidity during storage or transport, phytochemicals are very susceptible to deterioration during postharvest, which may cause significant losses in quality components, including phytochemicals (Yahia, 2018).

2.3.2.6 Post- harvest storage conditions

The quantity and quality of phytochemicals are significantly influenced by the storage temperature, the composition of the atmosphere gas, and the application of chemicals. A lower temperature can delay phytochemical degradation. High temperature also significantly alters the total phenolic, flavonoid, tannin, and antioxidant activity when compared to fresh form, in contrast to lower temperature. However, depending on the drying techniques employed and the length of time exposed to hot air, their concentration may change (Li *et al.*, 2012).

2.3.2.7 Others

The appropriate extraction of phenolic compounds depends on a number of variables, including their chemical make-up, the raw material, storage conditions, and time. It also relies on the techniques used for extraction and quantification, the standards selected, and the existence of interference (Dimcheva and Karsheva, 2018).

2.3.3 Importance of phytochemicals

Phytochemicals may operate through a variety of different methods. They might prevent the growth of microbes, obstruct certain metabolic functions, or alter the signaling pathways that control gene expression. Phytochemicals can be employed as either chemotherapeutic or chemo preventative agents, with chemoprevention denoting the use of substances to inhibit, reverse, or delay carcinogenesis. As a result, chemo-preventive phytochemicals can be used in cancer treatment since they may share some molecular pathways with cancer treatment (Manson, 2003).

Plant extracts and essential oils may act against bacterial strains in a variety of ways, including by interfering with the phospholipid bilayer that helps make up the cell membrane, increasing permeability and the loss of cellular components as a result, harming the enzymes responsible for cellular energy production and the synthesis of structural components, and destroying or inactivating genetic material. In general, the disruption of the cytoplasmic membrane, disruption of the proton motive force, electron flow, active transport, and coagulation of cell contents are thought to be the mechanism of action (Kotzekidou *et al.*, 2008). Some specific modes of actions are antimicrobial activity, antioxidants, anti-carcinogenesis, anti-ulcer, anti-diabetic, anti-inflammatory (Doughari *et al.*, 2009).

2.4 Anti-oxidant and its activity

Antioxidants are compounds that, when added to food, prevent, slow down, or stop oxidation and the degradation of food quality. Antioxidants in the body lower the risk of degenerative diseases brought on by oxidative stress. Antioxidants are substances that have the ability to prevent or delay oxidation processes that are influenced by reactive oxygen species or ambient oxygen. They are used to stabilize petrochemicals, food items, cosmetics, and pharmaceuticals as well as polymeric materials (Pisoschi and Negulescu, 2011; Upadhyay *et al.*, 2014).

Reactive oxygen species, often known as free radicals, such as singlet oxygen, superoxide anion, peroxyl, hydroxyl, and nitrite, cause oxidative stress and cellular damage. Antioxidants are substances that protect cells from these effects. Natural antioxidants are essential for maintaining good health and protecting against chronic and degenerative diseases like atherosclerosis, myocardial and cerebral ischemia, carcinogenesis, neurological

disorders, diabetes pregnancy, rheumatoid arthritis, DNA oxidation, and aging (Doughari *et al.*, 2009).

Antioxidants function by scavenging "free-oxygen radicals," creating a "relatively stable radical" in the process (Bhawya and Anilakumar, 2010). Natural antioxidant defenses produced by the body, such as glutathione or catalases, can neutralize free radicals that are produced within the body (Jayaprakash *et al.*, 2015). As a result, this deficiency needs to be made up for by using exogenous natural antioxidants such vitamin C, flavones, beta-carotene, and natural plant products (Sivakumar and Rajan, 2010; Upadhyay *et al.*, 2011). In a diabetic rat model (alloxan-induced diabetes), *Tinospora cordifolia* significantly lowers the control of the lipid peroxidation process, resulting in a reduction in the level of reactive free radical species. It also upregulates antioxidant enzymes like catalase and glutathione, indicating its anti-oxidant effects (Sivakumar and Rajan, 2010). According to a study, *Tinospora cordifolia* alters the levels of several enzymes, which in turn regulates the production of these reactive species and keeps the oxidative load constant by controlling the lipid peroxidation process and glutathione level (Jayaprakash *et al.*, 2015).

Many different compounds that can scavenge free radicals are found in plants, including phenols, flavonoids, vitamins, and terpenoids, which are very active antioxidants. Ascorbic acid, vitamin E, carotenoids, flavanols, and phenolics, which have the ability to scavenge free radicals in the human body, are found in many plants, citrus fruits, and leafy vegetables. Phytochemicals with significant antioxidant activities have been found to be essential for linked to lower of various diseases (Omojate *et al.*, 2014).

2.4.1 Effect of extraction on anti-oxidant activity

The biological activity of plant extracts differed significantly depending on the extraction method used, highlighting the importance of choosing the right extraction process. The solvents with different polarities had significant effects on antioxidant activity. In the work done by Dhanani *et al.* (2017), the antioxidant activity of *Withania somnifera* varied with both the solvent (ethanol, ethanol: water and water) used as well as extraction techniques (conventional, UASE and MASE).

2.4.2 Effect of solvent on anti-oxidant activity

The solvent employed for extraction may have an impact on the phytochemical concentration in the extract, which may then have an impact on their antioxidant activity. Improved phenolic compound extraction can increase the antioxidant activity of the extracts because phenolic compounds and solvent have similar polarities. Sometimes there can be synergies between the phenolic antioxidants found in extracts, increasing antioxidant activity. However, the antioxidant activity of the extracts cannot be credited to the solvent alone because a number of additional factors, including the type of food, the method of extraction, the temperature and length of the extraction process, the impact of the food matrix, etc., may contribute to a decrease or increase in antioxidant activity (Zlotek *et al.*, 2015).

2.4.3 Effect of storage on anti-oxidant activity

Antioxidants may be destroyed during storage due to physical and biological causes such as temperature rise and enzymatic activity (Kader *et al.*, 2002; Rossi *et al.*, 2003). According to (Song *et al.*, 2006) the antioxidant capacity of irradiation (3 and 5 kGy) kale juice reduced during storage at 10 °C for one to three days despite an increase in total phenolic content.

2.5 Description of sensory attributes of tea

The description of sensory attributes of tea is shown in Table 2.10 as

Table 2.10 Description of sensory attributes of tea

S.N.	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" flavour typical of dried grass or dried herbs.
	Earthy—boiled potatoes, damp potting soil	Aromatics associated with damp soil, wet foliage, and damp potting soil.
	Woody cinnamon, dry dusty, bark	Aromatics associated with dry fresh-cut wood; bark, cinnamon, dust.
2	Taste-Flavour	
	Bitter—quinine, caffeine	Flavours associated with the taste on the tongue stimulated by solutions of caffeine, quinine.
	Sweet—caramel, maple syrup	Flavours associated with materials that also have a sweet taste, such as molasses, caramelized sugar, and maple syrup.
	Honey—sweet typical honey	Flavours associated with the sweet, caramelized flora and woody aromatic associated with honey.

S.N.	Attributes	Description
	Rooibos	Flavours associated with a combination of honey, woody and herbal-floral notes with a slightly sweet taste and subtle astringency.
	Fruity—peach, mango-like	Flavours associated with a mixture of peach-mango like fruits.
	Perfume—floral, lavender	Flavours associated with a light fragrant aromatic characteristic of lavender.
	Medicinal	Flavours 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.
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, all spice).

Source: Malongane *et al.* (2020)

2.6 9-point hedonic scale rating

The 9-point hedonic scale has been used routinely in food science since 60 years (Wichchukit and Omahony, 2015). Since its development, the 9-point hedonic scale has been the most commonly used scale for testing consumer preference and acceptability of foods (Lim, 2011). The original 9-point scale, developed by the U.S. army for menu planning for their

canteens, consisted of a series of nine verbal categories representing degrees of liking from ‘dislike extremely’ to ‘like extremely’ (Nicolas *et al.*, 2010).

Traditionally, the ‘words’ on the 9-point hedonic scale are reassigned as ‘numbers’, while other ‘9-point hedonic scales’ are purely numerical; the two are not interchangeable (Wichchukit and Omahony, 2015). The 9-point hedonic scale is a balanced bipolar scale around neutral at the center with four positive and four negative categories on each side (Lim, 2011). For subsequent quantitative and statistical analysis, the verbal categories are generally converted to numerical values: ‘like extremely’ as ‘9’, ‘dislike extremely’ as ‘1’. Yet, sometimes what is termed a 9- point hedonic scale is an unstructured numerical scale, labeled at the ends with ‘dislike extremely’ and ‘like extremely’. The former scale requires consumers to categorize foods according to how much they are liked or not; the latter requires the consumers to differentiate numerically between the foods in terms of the relative degree of liking for each (Nicolas *et al.*, 2010).

2.7 Chemometric analysis

The principal component analysis and correlation was carried out for multivariate analysis of data.

2.7.1 Principal Component Analysis

One of the most significant and effective techniques in chemometrics as well as a variety of other fields is Principal Component Analysis (PCA). The weights required to create the new variable that, in a particular sense, best explains the variation in the entire dataset are provided by principal component analysis. The first principal component refers to this new variable and its defining weights. If the scale and offset differences are not taken into account, the PCA model will only pay attention to variables that are measured in large quantities. To ensure that all variables have an equal chance of being modeled, there is a preprocessing tool called autoscaling that will give each column the same "size." With autoscaling, the mean value of each variable is subtracted before the variable is divided by the standard deviation. In autoscaling, it is crucial to remember that each variable is scaled to be the same size, and as a result, each variable will experience both positive and negative values because its mean has been removed. Take note that a sample average now equates to a set of zeros. Therefore, zero now denotes an average "signal" rather than the lack of a

"signal." PCA can be carried out using this pre-processing of the data (Bro and Smilde, 2014).

2.7.2 Correlation Plot Analysis

Correlation plot shows matrix chart of phytochemical content and pharmacological parameters. The upper panels show the Pearson's correlation coefficients while the lower panels report the scatter plots. *, **, *** indicates significance at $p<0.05$, <0.01 and 0.001 respectively (Batubara *et al.*, 2020). A correlation analysis begins with the construction of a scatter plot or scatter diagram [a graphical representation of the data] with one variable on the X-axis and the other on the Y-axis. A line is usually drawn through the points on a scatter plot to identify linearity in the relationship. This line is called the regression line or the least squares line, because it is determined such that the sum of the squared distances of all the data points from the line is the lowest possible.

Correlation also called as correlation analysis, is a term used to denote the association or relationship between two (or more) quantitative variables. This analysis is fundamentally based on the assumption of a straight line [linear] relationship between the quantitative variables. Similar to the measures of association for binary variables, it measures the "strength" or the "extent" of an association between the variables and also its direction. The end result of a correlation analysis is a correlation coefficient whose values range from -1 to +1 which is shown in Figure 2.2. A correlation coefficient of +1 indicates that the two variables are perfectly related in a positive [linear] manner, a correlation coefficient of -1 indicates that two variables are perfectly related in a negative [linear] manner, while a correlation coefficient of zero indicates that there is no linear relationship between the two variables being studied.

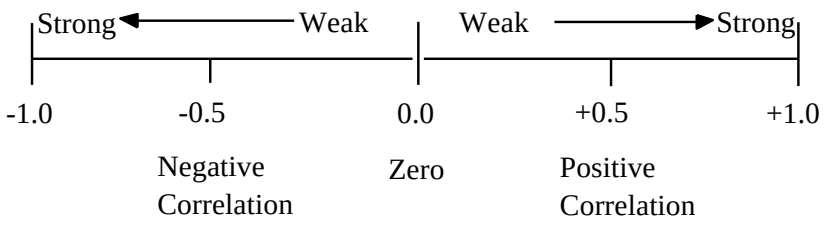

Fig 2.2 The spectrum of the correlation coefficient (-1 to +1)

Part III

Materials and Methods

3.1 Collection and identification of raw materials

3.1.1 Collection and identification of green tea

For this study, the leaves of *Camellia sinensis* were chosen as the plant material. The plant leaves were collected from the Central Campus of Technology, Dharan, Sunsari during August month. The plant was five-years old and from the gumti variety. The plant was identified by the Botanist of Central Campus of Technology, Dharan.

3.1.2 Collection and identification of gurjo

One and half year-old stem of Gurjo (*Tinospora sinensis*) with a diameter of 1.4–1.6 cm was harvested from Bijyapur, Dharan of Sunsari district. The stem was collected during the month of August (mid-month). The plant was identified by the Botanist of Central Campus of Technology, Dharan.

3.1.3 Packaging material

Glass jars were used for packaging green tea samples during storage study. Similarly, the *Tinospora sinensis* plant was sorted, cleaned, and packed in a polyethylene bag.

3.2 Materials, equipment and chemicals used

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

3.3 Methodology

3.3.1 Preparation of green tea powder

The preparation of green tea powder is shown in Figure 3.1 as

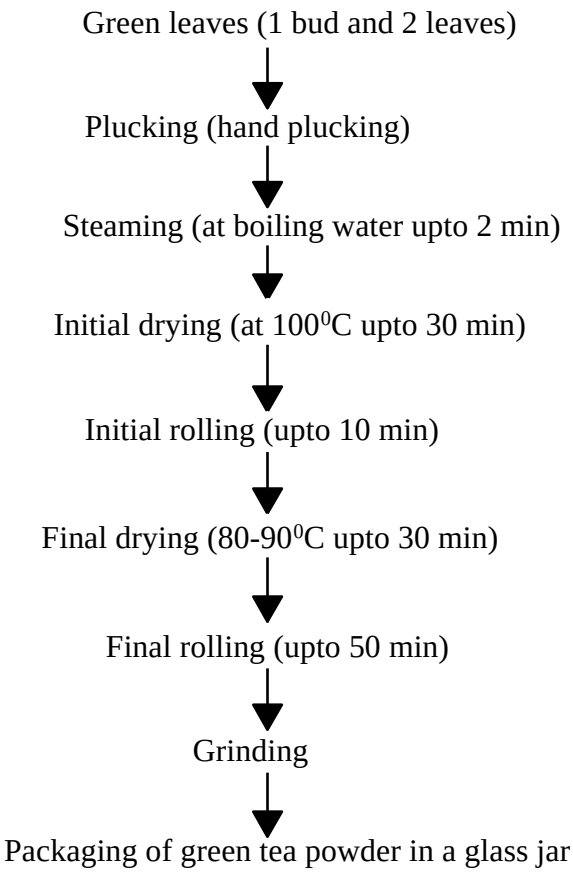

Fig. 3.1 Flowchart of green tea powder

Source: Singh *et al.* (2014)

3.3.2 Preparation of gurjo stem powder

The preparation of gurjo stem powder is shown in Figure 3.2 as

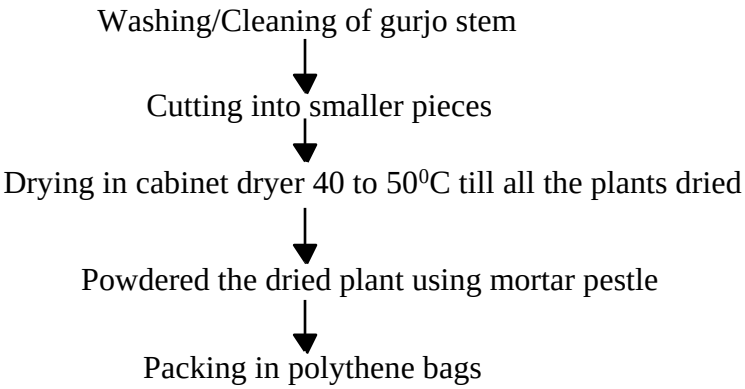

Fig. 3.2 Flowchart of gurjo stem powder

Source: Katuwal (2017)

3.4 Experimental design

Design Expert v. 13.0.1.0 software was used to determine different gurjo stem incorporated green tea (blend) powder formulations. Simple mixture design was used to determine the different blend formulations. A total of four blend formulations were obtained for the two components i.e., gurjo and green tea extract each with a lower level of 35% and upper level of 65% of yield of green tea. Based on the output, five different formulations were obtained which are shown in Table 3.1.

Table 3.1 Different formulations of gurjo stem powder added to green tea powder

Product	Formulation
Control	100% green tea
A	35% gurjo + 65% green tea
B	17.5% gurjo + 82.5% green tea
C	26.25% gurjo + 73.25 % green tea
D	8.75% gurjo + 91.25% green tea

All these formulations were carried out after preparation of green tea powder and gurjo powder.

3.5 Analysis of proximate

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 nitrogen free extractives (NFE)

The NFE of the samples was determined by difference method as described by AOAC (2005).

NFE (%) = 100 - (%protein content + %crude fat + %crude fiber + % ash content)

3.6 Preparation of plant extracts for phytochemical analysis

The plant extracts were performed using methanol solvent extraction. The organic extraction was performed by the Soxhlet extraction method. This extraction was done by taking 20 g of dried plant powder and was placed into a glass thimble then extracting with 250 ml of methanol. The extraction processes were carried on till the solvent in the siphon tube of Soxhlet apparatus become colorless. After that, the extract was heated in a hot water bath at 35 ºC until all the solvent evaporated. The dried plant crude extract was kept in a refrigerator at 2-8 ºC for their future use (Jaradat *et al.*, 2015).

3.7 Analysis of phytochemicals

3.7.1 Determination of total phenolic content

Total phenolic content (TPC) in the plant methanolic extract was determined using the spectrophotometric method (Waterhouse, 2002; Mythili *et al.*, 2014) with some modifications. 1 mg/ml aqueous solutions for methanolic extract were prepared for the analysis. The reaction mixture was prepared by mixing 0.5 ml of plant extract solution, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of sodium carbonate (Na_2CO_3) aqueous solution. The samples were thereafter incubated in a thermostat at 45 ºC for 45 min. The absorbance was determined using a spectrophotometer at wavelength of 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured

absorbance, the concentration of gallic acid equivalent is expressed in terms of (mg of GA/g of extract).

3.7.2 Determination of total flavonoid content

Total flavonoid content was determined using a modified aluminum chloride assay method as described by (Barek and Hasmadi, 2015). 2 ml of solution was pipetted out in a test tube in which 0.2 ml of 5% Sodium Nitrate ($NaNO_3$) was mixed and stood for 5 min. 0.2 ml Aluminum Chloride ($AlCl_3$) was pipetted out mixed in the tube and allowed to stand for 5 min. This was followed the addition of 2 ml of 1N Sodium Hydroxide (NaOH) in the tube and finally the volume was made up to 5ml. The absorbance was measured after 15 min at 510 nm against a reagent blank. The test result was correlated with the standard curve of Quercetin (20, 40, 60, 80, 100µg/ml) and the total flavonoid content was expressed as mg quercetin equivalents (QE).

3.7.3 Determination of total tannin content

Tannin was determined by the Folin-Ciocalteu method. About 0.1 ml of the sample extracts were added toa volumetric flask (10ml) containing 7.5 ml distilled water and 0.5 ml Folin-Ciocalteu reagent, 1 ml 35% Sodium Carbonate (Na_2CO_3) solution and dilute to 10 ml distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of Gallic acid (20, 40, 60, 80, 100µg/ml) was prepared in the same manner as described earlier. Absorbance for test and the standard solution was measured against blank at 725 nm with a UV/visible spectrophotometer. The tannin content was expressed in terms of mg of GAE/g of extract (Mythili *et al.*, 2014).

3.8 Determination of DPPH radical scavenging activity

The DPPH radical scavenging activities (anti-oxidant activities) of the extracts were determined as per the method described by (Singh *et al.*, 2008). 1 ml of the sample extract was taken in a test tube and 2 ml of 0.004% methanolic solution of DPPH was added. Then the test tube was incubated at room temperature 28°C for 30 min in the dark and absorbance was measured at 517 nm using a UV-vis spectrophotometer. Similarly, control was also run using methanol instead of the sample. The DPPH scavenging activity was calculated as follows:

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

Where,

Ac represents for absorbance of control;

As represents for absorbance of test sample

3.9 Statistical analysis

Data were expressed as the mean ± SD from three replicates. Analysis of variance 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 $p<0.05$. R version 4.02 was used for multivariate analyses using correlation and principal component analysis. The Pearson correlation coefficients between phytochemicals and DPPH radical scavenging activity were generated using Performance Analytics packages in R. The FactoMineR packages in R was used to create PCA analysis using a data matrix of phytochemicals and pharmacological variable (DPPH).

3.10 Sensory analysis of gurjo stem incorporated green tea

Sensory evaluation of tea samples was conducted to establish preference rating of tea for flavor, aroma, appearance, and overall acceptability using a 9-point hedonic scale. The hedonic rating test is used to measure the consumer acceptability of food products. This method can be used with untrained panelists as well as with experienced ones (Rangana, 1986). The tea samples were examined and scored by semi-expert panelists of Central Department of Food Technology, Hattisar, Dharan. The total semi-expert panelists were 15 in number.

Blended tea 3 g was brewed using the same water volume of 150 ml freshly boiled water for 5 min. All samples were served in 150 ml cups coded with random alphabets under warm conditions. The panel room was completely free of food/chemical odors, unnecessary sound, and mixing of daylight. Each panelist was provided with an evaluation card (Appendix B) to record their opinion on sensory observations. They were provided with potable water for rinsing between the samples. Verbal communication among the panelist was prohibited. They were asked to evaluate the samples individually using a scorecard. Sensory parameters based on brew color, aroma, taste, mouthfeel, and overall acceptance were analyzed. The grading system was based on a 9-point hedonic rating test which was indicated as, 9 = Like

extremely; 8 = Like very much; 7 = Like; 6 = Like slightly; 5 = Neither like nor dislike;4 = Dislike slightly;3 = Dislike moderately;2 = Dislike;1 = Dislike extremely.

Part IV

Results and Discussion

Green tea is most widely used beverages in the world after water and it has generated a lot of interest among researchers and the general public. Combining green tea with medicinal plants is one strategy for enhancing the health-promoting properties of green tea to compensate for poor diets. So, this work was carried out for the evaluation of phytochemicals, anti-oxidant and sensory parameters of gurjo stem incorporated green tea (blend) formulations. At first raw materials green leaves and gurjo stem were hand plucked and harvested from Central campus of Technology, Dharan. Then green tea and gurjo stem powder were prepared for sensory evaluation and then best tea blend was analyzed among four samples of formulations.

4.1 Sensory evaluation

Sensory evaluation was carried out for: color, aroma, taste, mouthfeel and overall acceptability of infusion by 15 panelists using a 9-point hedonic scale. The statistical analysis (One-way ANOVA) was done ($p<0.05$). There was a significant difference for three of the sensory attributes evaluated viz., of infusion. The result of the sensory evaluation and statistical analysis is listed in Table 4.1.

Table 4.1 Average Sensory Score

	Mean score				
Samples	Color	Aroma	Taste	Mouthfeel	Overall acceptability
A	5.933 ± 0.711^{a}	6.6 ± 1.019^{a}	6.133 ± 0.805^{b}	6.2 ± 0.653^{b}	6.133 ± 0.498^{b}
B	6.667 ± 0.596^{a}	6.333 ± 0.596^{a}	6.867 ± 0.498^{ab}	6.667 ± 0.971^{ab}	6.933 ± 0.442^{a}
C	6.667 ± 0.596^{a}	6.4 ± 0.8^{a}	6.867 ± 0.618^{ab}	7 ± 0.632^{ab}	6.8 ± 0.742^{ab}
D	6.667 ± 1.192^{a}	6.733 ± 0.711^{a}	7.067 ± 1.123^{a}	7.067 ± 0.928^{a}	7.333 ± 1.074^{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.

4.1.1 Color

The mean sensory score for the color of samples A, B, C and D was found to be 5.933, 6.667, 6.667, and 6.667 respectively. The highest score was obtained same for sample B, C and D and the least obtained for sample A. There was no significant difference among the samples ($p<0.05$) as shown in Table 4.1. Sample B, C and D was found superior based on color from statistical analysis.

Among all formulation samples, B, C and D had the similar best appealing color because it may be of light yellowish white creamy color of gurjo in combination with green tea. And it might be due to dark yellowish color in appearance, Sample A was least score by panelist since the color is a very important parameter for selecting any food, as man eats with his eyes. According to Yan *et al.* (2018), color is an important quality indicator for the taste of green tea infusions, and the desired infusion color is mainly yellowish green. The temperature and drying time are the main factors affecting the color change of dried green tea. Long time drying at high temperature could result in non-enzymatic browning reactions, which are important phenomena that occur during food processing. Non-enzymatic browning includes a wide number of reactions such as Maillard reaction, caramelization, maderization, and ascorbic acid oxidization (Lin *et al.*, 2010).

According to Adnan *et al.* (2013) reported results regarding color scores of tea samples which revealed significant variation ($p<0.05$) among different tea samples. The color scores assigned to green tea samples ranged from 3-7 in comparison to 5-8 for black tea samples, which revealed that highest color scores were found in black tea as compared to green tea due to oxidation and fermentation processes during tea processing. The amino acids in tea have a significant role in color production which may be oxidized by catechins resulting in tea liquor color (Ying *et al.*, 2005). In addition to this, other tea components such as thearubigins and theaflavins are also reported to affect the sensory characteristics of the tea especially brightness of tea color (Owuor and Obanda, 2001).

4.1.2 Aroma

The mean sensory score for the color of samples A, B, C and D was found to be 6.6, 6.333, 6.4 and 6.733 respectively. The highest score was obtained for sample D and the least obtained for sample A, B and C. There was no significant difference among the samples ($p<0.05$) as shown in Table 4.1. Sample D was found superior based on aroma from statistical analysis.

In comparison to other formulations, the formulation having 8.75% gurjo had an acceptable aroma. This may be due to the pleasant aroma. Since the aroma of green tea is very delicate, slight mistakes or problems in the manufacturing process or storage may affect the aroma even if no changes are observed in the major constituents (Horie *et al.*, 1993). The aroma was affected due to high temperature of tea during processing. Thus, high temperature leads to Maillard reactions which causes burnt aroma in panelist (Lin *et al.*, 2010).

According to Adnan *et al.* (2013) results regarding aroma scores of tea samples which revealed significant variation ($p<0.05$) among different tea samples. The highest aroma scores were observed in green tea (8.5 scores) in comparison to black tea (4 scores) which may be due to use of more young tea leaves as well as controlled fermentation during processing. The difference in aroma score of tea samples may also be due to variations in thearubigins, caffeine and catechin compounds among green and black tea samples (Ying *et al.*, 2005). The results obtained from the present study are in line with study of Owuor and Obanda (2001) who observed better aroma scores in commercial tea containing more amounts of caffeine and thearubigins.

4.1.3 Taste

The mean sensory score for the taste of four samples A, B, C and D was found to be 6.133, 6.867, 6.867 and 7.067 respectively. The least score for taste was obtained for sample A while the highest mean score was obtained for sample D. The taste of the brew was significantly different ($p<0.05$) as shown in Table 4.1. Here sample B and C are similar to sample A and D. There was significant difference in the sample A and D while sample B and C was no significantly different from all other samples in brew taste. Sample D was superior based on the flavor of infusion from statistical analysis.

In this study, Formulation D had the best acceptable taste in comparison to other formulation. This may be because of astringent, bitter and pungent taste and after digestion taste of this herb gurjo with green tea was sweet. Polyphenol-amino acid ratio is a good indicator of astringent taste of tea. The high ratio of polyphenol to amino acid causes a strong and bitter taste. The green tea with low phenol-amino acid ratios has a good taste. The lowest phenol-amino acid ratio of 5.86 scores was obtained by microwave vacuum drying; while the highest ratio of 7.87 scores was found in green tea from hot air drying. Results indicated that the green teas produced from microwave drying not only retained more nutrient but also had a less astringent taste and better quality than green teas from hot air drying (Lin *et al.*, 2010).

According to Adnan *et al.* (2013) results regarding taste scores of tea samples revealed significant variation ($p<0.05$) among different tea samples similar to our analyzed data. The scores assigned to taste ranged from 4-8.5 for both green and black tea samples indicating better taste in both types of tea. Caffeine is regarded as important parameter for commercial tea sensory evaluation having significant contribution in the development of taste (Ying *et al.*, 2005). The amounts of other components such as thearubigins, theaflavins, amino acids, and catechins also have a significant contribution in the sensory characteristics of tea (Kato *et al.*, 2008).

4.1.4 Mouthfeel

The mean sensory score for the taste of four samples A, B, C and D was found to be 6.2, 6.667, 7 and 7.067 respectively. The least score for mouthfeel was obtained for sample A while the highest mean score was obtained for sample D. The mouthfeel of the brew was significantly different ($p<0.05$) as shown in Table 4.1. Here sample B and C are similar to sample A and sample D. There was significant difference in the sample A and D while sample B and C was no significantly different from all other samples in brew taste. Sample D was superior based on the mouthfeel of infusion from statistical analysis.

In comparison to other formulations, Formulation D had the best acceptable mouthfeel. This may be because of juice of stem with green tea. The mouthfeel of formulation D was little bitter, pleasing and acceptable to the sensory panelist. According to studies of Yan *et al.* (2018), the intensities of bitterness and astringency increased with longer infusion time, higher water temperature, smaller particle size and reduced water/tea ratio. Some high-

quality teas are perceived to be bitter in the mouth and sweet in the throat. For other high quality teas, bitter and astringent have negative connotations while aromatic, sweet and delicate are desired characteristics (Ahmed and Stepp, 2012).

4.1.5 Overall acceptance

The mean sensory score for the taste of four samples A, B, C and D was found to be 6.133, 6.933, 6.8 and 7.333 respectively. The least score for overall acceptance was obtained for sample A while the highest mean score was obtained for sample D. The overall acceptance of the brew was significantly different ($p<0.05$) as shown in Table 4.1. There was significant difference in the sample A & B and A & D while samples B and D was no significantly different from all other samples in overall acceptance. Sample D was superior based on the overall acceptance of infusion from statistical analysis. This may be due to the better eye-appealing color, better aroma, taste and mouthfeel of the formulation.

According to Yan *et al.* (2018), the scores of overall acceptances varied from 3.81 to 6.38 in green tea infusion samples. The overall acceptance was evaluated based on the taste and color (except the aroma) of taste of green tea infusions. The standard references of bitterness, astringency, umami, and sweetness were due to quinine monohydrochloride dihydrate, tannic acid, sodium glutamate, and sucrose, respectively.

According to Adnan *et al.* (2013) results regarding overall acceptance scores of tea samples revealed significant variation ($p<0.05$) among different tea samples similar to our analyzed data. In both green tea and black tea samples, overall acceptability scores ranged between 4-8 scores. Quality evaluation of commercial tea depends up on number of factors such as caffeine, amino acids, catechins, thearubigins and theaflavins. Tea samples with high amount of both chemical and volatile compounds have positive association with respect to sensory attributes of tea including overall acceptability. The results of present study are in line with the results of Owuor and Obanda (2001) who observed better sensory quality of tea samples having high quality of raw material with maximum amounts of chemical and volatile components used during processing.

4.1.6 PCA-Biplot for different formulated samples using sensory parameters

Principal Component Analysis (PCA) was conducted to select the best variety of gurjo incorporated green tea among four samples. The first principal component (Dim. 1) with

eigen value 2.83 (Table C.2.I) was responsible for 70.7% of the variation while the second principal component (Dim. 2) with eigen value 0.83 (Table C.2.I) was reported for 20.7 % of the variation. So, together, they accounted for about 91.4% of the total variation as shown in Figure 4.1.

The principal component analysis plot for sample A, B, C and D using five different sensory parameters is shown in PCA Biplot. As we can see Samples D and C in the same quadrant shows that there is strong correlation between Sample D and C. Also, the closest proximity of mouthfeel and overall acceptance shows that sample D possess high value of these parameters. Likewise Sample B is close to taste, mouthfeel and overall acceptance but it shows that Sample B has low correlation with mouthfeel and overall acceptance as compared to sample D and C. The farthest proximity of aroma with sample A shows that it has better aroma than others. But sample A is far from taste, mouthfeel, overall acceptance and taste that means Sample A have the lowest value in terms of taste, mouthfeel, overall acceptance and taste. Hence in terms of mouthfeel, overall acceptance Sample D was superior followed by Sample C. Likewise, Liang *et al.* (2008) PCA results obtained that the sensory reference of his selected best sample were depended on the factors of color, taste and volatiles of green tea.

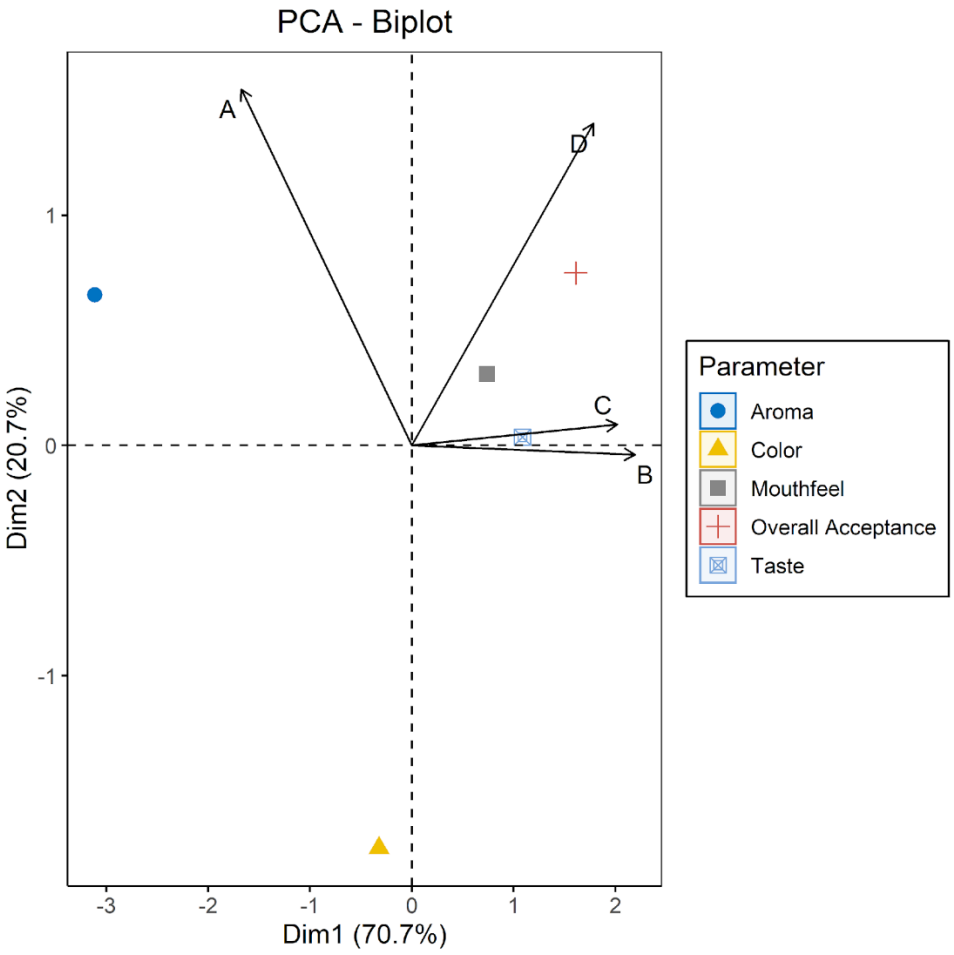

Fig 4.1 PCA Bi-plot distribution of dimension 1 and 2 for selecting the best variety of blend.

4.1.7 Choosing of best sample

The mouthfeel and overall acceptance were highest in the sample containing 8.75 parts of *Tinospora sinensis* stem powder and 91.25 parts of green tea powder i.e., sample D. This may be due to the better color, aroma, taste and mouthfeel of the formulation. Also, from PCA, the closest proximity of mouthfeel and overall acceptance shows that sample D possess high value of these parameters.

4.2 Proximate analysis

4.2.1 Proximate analysis of *Tinospora sinensis* (gurjo) stem

The proximate composition of gurjo stem powder is shown in Table 4.2.

Table 4.2 Proximate composition of gurjo stem powder

Proximate analysis	Gurjo stem (%)
Moisture content	5.133±0.047
Protein content	5.657±0.099
Crude fat	2.389±0.049
Crude fiber	14.897±0.049
Ash content	6.043±0.099
Nitrogen free extractives (NFE)	71.009±0.049

*Values are the means of triplicates on a dry basis (db) except moisture content and figures in the parenthesis are standard deviation of the triplicates.

The moisture content in gurjo (*Tinospora sinensis*) stem was found to be 5.133%. Modi *et al.* (2021) also found 10.01% moisture content in *Tinospora cordifolia* stem. Tyagi *et al.* (2020) obtained the moisture content in *Tinospora sinensis* stem 9.12%. The variations in the result may be due to different species, genetic origin, geographical location, source, handling, extraction solvent, time of extraction, and conditions of cultivation.

The protein content in gurjo (*Tinospora sinensis*) stem was found to be 5.657%. Modi *et al.* (2021) also found 8.06% protein content in *Tinospora cordifolia* stem. Rahal *et al.* (2014) reported protein content in *Tinospora cordifolia* in dry basis was found to be 7.740%. Nile and Khobragade (2009) also reported similar values of *Tinospora cordifolia* which was found to be 4.5%. Similarly, Tyagi *et al.* (2020) obtained the protein content 5.12% in *Tinospora sinensis* stem. These values are very similar to our analyzed values. The results showed that collection time, geographical and environmental differences play a key role in protein composition.

The crude fat in gurjo (*Tinospora sinensis*) stem was found to be 2.389%. Modi *et al.* (2021) found 1.77% crude fat in *Tinospora cordifolia* stem. Rahal *et al.* (2014) reported that crude fat in *Tinospora cordifolia* stem in dry basis was found to be 2.49%. Nile and

Khobragade (2009) also reported similar values of *Tinospora cordifolia* which was found to be 3.1%. Similarly, Tyagi *et al.* (2020) obtained the crude fat 3.49% in *Tinospora sinensis* stem. These values are very similar to our analyzed values. A slight variation may be due to the varietal differences, agro climatic conditions, maturity and the time of the harvesting.

The crude fiber in gurjo (*Tinospora sinensis*) stem was found to be 14.897%. Modi *et al.* (2021) also found 26.99% crude fiber in *Tinospora cordifolia* stem. Rahal *et al.* (2014) reported crude fiber in *Tinospora cordifolia* in dry basis was found to be 56.420%. Nile and Khobragade (2009) also reported similar values of *Tinospora cordifolia* which was found to be 15.9%. The difference in studies may be due to differences in species, agro climatic conditions, maturity and the time of the harvesting. Similarly, Tyagi *et al.* (2020) obtained the crude fiber 4.34% in *Tinospora sinensis* stem which is very low than our analyzed data. Similarly, having high fiber softens the stools, preventing constipation.

The ash content in gurjo (*Tinospora sinensis*) stem was found to be 6.043%. Modi *et al.* (2021) also found 7.05% ash content in *Tinospora cordifolia* stem. Rahal *et al.* (2014) reported ash content in *Tinospora cordifolia* in dry basis was found to be 7.960%. Nile and Khobragade (2009) and also reported similar values of *Tinospora cordifolia* which was found to be 12.40%. These high ash contents suggest that the samples are good sources of minerals (Mohammed and Sulaiman, 2009). Similarly, Tyagi *et al.* (2020) obtained the ash content 6.26% in *Tinospora sinensis* stem which is near to our analyzed value. A slight variation may be due to difference in species of plant.

The nitrogen free extractives (NFE) in gurjo (*Tinospora sinensis*) stem was found to be 71.009%. Modi *et al.* (2021) also found 46.11% carbohydrates (NFE) in *Tinospora cordifolia* stem. Likewise, Sood (2015) reported that NFE in *Tinospora cordifolia* stem was found to be 50.01%. Also, NFE/carbohydrate was determined by difference method which may have shown such variation it is easy but not accurate method for the NFE or carbohydrate determination. Conclusively, environmental conditions, variety of plants, experimental errors, can be considered as the causes of the variations (Santana *et al.*, 2015).

4.2.2 Proximate analysis of green tea and blend

The proximate analysis of green tea (Control) and blend (product D or gurjo stem incorporated green tea) is shown in Table 4.3.

Table 4.3 Proximate composition of green tea and blend powder

Proximate composition	Green tea powder (%)	Blend powder (%)
Moisture content	$6.467^{a} \pm 0.124$	$5.267^{b} \pm 0.047$
Protein content	$23.555^{a} \pm 0.101$	$21.534^{b} \pm 0.086$
Crude fat	$5.884^{a} \pm 0.101$	$2.709^{b} \pm 0.049$
Crude fiber	$9.265^{a} \pm 0.050$	$14.214^{b} \pm 0.049$
Ash content	$5.488^{a} \pm 0.101$	$5.946^{b} \pm 0.049$
Nitrogen free extractives (NFE)	$55.840^{a} \pm 0.219$	$55.590^{a} \pm 0.049$

*Values are the means of triplicates on a dry basis(db) except moisture content 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.

4.2.2.1 Moisture content

The moisture content of green tea and blend have significant difference P (<0.05) following the decrement from 6.467 % to 5.267%. Ahmad *et al.* (2014) also obtained similar value 4.88% of moisture content in green tea. Likewise, Rubab *et al.* (2020) also found the similar value of moisture content 5.43% in green tea. The higher moisture content in green tea samples may be due to exclusion of fermentation process during processing of green tea as compared to black tea because during this process much of the polyphenols are destroyed that retain moisture content (Ahmad *et al.*, 2014).

Another important factor may be use of packaging material (Mohammed and Sulaiman, 2009) to maintain a constant moisture level during storage of commercial tea samples, so moisture content in commercial tea is an essential parameter of quality (Modi *et al.*, 2021). Yao *et al.* (2006) also observed 70% of commercial tea samples having moisture content of 6.6% or less and 30% sample containing more moisture percentage up to 8% which can have negative effect on shelf life of the product, so for the better quality of the product moisture percentage should be controlled between 2.5-6.5%.

4.2.2.2 Protein content

It was observed that protein content is also significantly decreased ($P<0.05$) in blend compared to that of green tea. The protein content ranged from 23.555% in green tea to 21.427 % in blend at 5% level of significance. The highest amount of protein content in green tea may be due to no fermentation of green tea during processing. According to Ahmad *et al.* (2014) and Rubab *et al.* (2020) the protein content in green tea was 18.06% and 14.32%, which was near to our analyzed value. The compositional variations in green tea may be due to differences in variety, season, geographic origin, agronomic practices (soil, water, minerals, and fertilizers), age, and position of leaf on harvested shoot (Ahmad *et al.*, 2014).

4.2.2.3 Crude fat

There was significance difference in fat content ($P<0.05$) from 5.844% green tea to 2.709% blend. Likewise Ahmad *et al.* (2014) also found very similar value of fat which was found to be 2.49%. The lower fat content in blend may be due to higher amount of fiber in blend comparable to green tea. The highest amount of fat content in green tea may be due to no fermentation of green tea during processing. These results are in line with the study of Rehman *et al.* (2002) who suggested 0.95-1.62% fat content for better quality of the commercial tea samples. Rubab *et al.* (2020) also found 0.99% fat content in green tea leaves. The results showed that collection time, geographical and environmental differences play a key role in fatty acid composition.

4.2.2.4 Crude fiber

It was observed that crude fiber content is also significantly increased ($P<0.05$) in gurjo incorporated green tea which was due to higher crude fiber content in gurjo than that of green tea. The crude fiber ranged from 9.265% in green tea to 14.214% in blend. Since the blend has higher crude fiber which stimulates the movement of the bowel and helps to prevent the constipation (Mohammed and Sulaiman, 2009).

Rubab *et al.* (2020) found very high value of crude fiber in green tea which was 49.36%. Likewise Ahmad *et al.* (2014) found 15.35% fiber in green tea which was near to our analyzed value. Higher fiber content of tea may be by using the stems like impurities during its processing while low fiber content in tea samples due to use of younger leaves of tea

plant. Moreover, the process of curling, tearing, and crushing also destroyed the structure of tea leaf and thus fiber content might be effected (Bashir *et al.*, 2019). Previous researchers also indicated positive association between fiber content and keeping quality of the tea and proposed fiber content of less than 16.5% in order to maintain high quality of tea during storage (Venkatesan *et al.*, 2006).

4.2.2.5 Ash content

The ash content was found to be significantly higher ($P<0.05$) in blend then that of control product green tea due to higher fiber content and maturity in gurjo stem than a bud and leaves of *Camellia sinensis*. These high ash contents suggest that the samples are good sources of minerals (Mohammed and Sulaiman, 2009).The ash content was ranged from 5.487% in green tea to 5.946% in blend. Likewise, Ahmad *et al.* (2014) and Rubab *et al.* (2020) also found very similar result of green tea which was found to be 5.60% and 5.02% respectively. Ash content of tea is a significant factor because less moisture content is the reason of higher ash content in tea. Since, extracted raw material used for the production of tea, this is the main cause for less ash content in tea (Bashir *et al.*, 2019).

4.2.2.6 Nitrogen free extractives (NFE)

The nitrogen free extractives (NFE) of green tea and blend have no significant difference following the decrement from 55.840 % to 55.590%. The difference in value may be due to presence of different carbohydrates in plants. But Ahmad *et al.* (2014) also found the similar value of nitrogen free extractives (NFE) in green tea which was found to be 53.68%. Also, NFE/carbohydrate was determined by difference method which may have shown such variation it is easy but not accurate method for the NFE or carbohydrate determination. Conclusively, environmental conditions, variety of plants, experimental errors, can be considered as the causes of the variations (Santana *et al.*, 2015).

4.3 Quantitative analysis of phytochemicals in samples

The measured concentrations of total phenols, total flavonoids, and tannins using solvent as methanol, the methanolic extract of green tea, *Tinospora sinensis* stem and best sample (blend) are shown in Figure 4.2, 4.3 and 4.4.

4.3.1 Total Phenol Content (TPC)

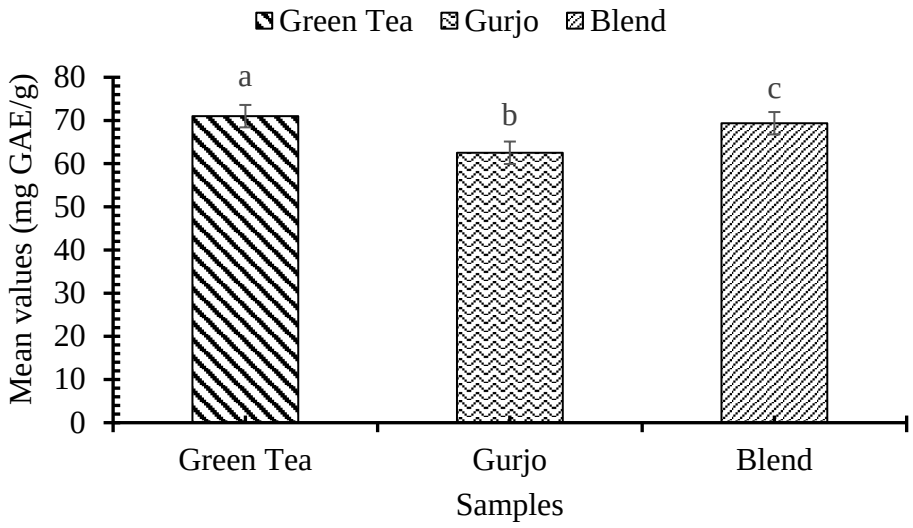

Fig 4.2 Bar diagram for TPC of different samples [Values in the figure having different alphabets at top are significantly different (P<0.05)]

The TPC of green tea was found to be 70.996 ± 0.564 mg GAE/g dry weight which was calculated by using the calibration curve and absorbance values (Figure E.1). According to Sumathi *et al.* (2015), the TPC of methanolic extract of green tea were found to be 76 mg GAE/g dry weight in green tea. The variations in data may be due to different maturity level, cultivar effect and weather conditions.

The TPC of methanol extract of *Tinospora sinensis* stem was found to be 62.518 ± 0.00047 mg GAE/g which was calculated by using the calibration curve and absorbance values (Figure E.1). According to study done by Praveen *et al.* (2012), TPC of methanol leaf extract of *Tinospora cordifolia* was found to be 44.36 ± 0.65 mg GAE/g respectively. The high difference in TPC values between all these studies might be due to difference in plant species, difference in plant parts used, their maturity and season during analysis and extraction method used. According to Usano-Alemany *et al.* (2014) who found that TPC of methanol 66.037 mg GAE/g, thus indicating it as the best month (June-July) for harvesting of gurjo. Exposure of plants to different temperature levels due to seasonal variations can have profound effect in their phytochemical compositions. Sivakumar *et al.* (2010) found that TPC of methanol stem extract of *Tinospora cordifolia* was 72.130 mg/g dried extract. The difference may be due to analysis of the plant material during different season or of different geography or maturity stage or even due to difference in species of plant.

Environmental edaphic factors like rainfall, soil type and sun exposure can affect polyphenol content of plant and plant foods according to Rajbhar *et al.* (2015).

The TPC was found to be 69.353±0.009 mg GAE/g dry weight for the blend which was calculated by using the calibration curve and absorbance values (Figure E.1). Namdev and Gupta (2015) also obtained that herbal green tea formulation containing *Withania somnifera* stems, *Terminalia arjuna* bark, *Cinnamon bark* and *Tinospora cordifolia* stems was 18.43 ug GAE/mg sample with *Withania somnifera* stems, *Terminalia arjuna* bark, *Cinnamon bark, Tinospora cordifolia* stems and green tea yielding 7.8, 41.25, 3.43, 4.68 and 30.31ug GAE/mg sample. The results shows that herbal green tea formulations have lower value when comparing with green tea. The differences in the value of TPC may be due to difference in plant parts used, maturity, season, extraction method, environmental conditions, post-harvest storage conditions, etc. during analysis.

4.3.2 Total Flavonoid Content (TFC)

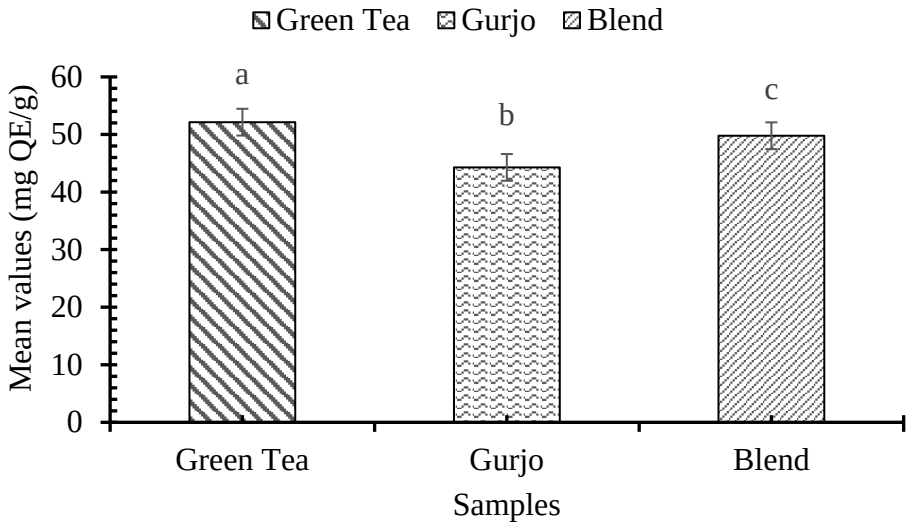

Fig 4.3 Bar diagram for TFC of different samples [Values in the figure having different alphabets at top are significantly different (P<0.05)]

The total flavonoid content (TFC) of green tea was found to be 52.134 ± 0.00047 mg QE/g which was quantified by using the calibration curve as well as the absorbance values (Figure E.2). The flavonoid content of old leaves of *Camellia sinensis* green tea using methanol extraction was 70.3 ± 4.428 mg QE/g dry weight (Acharya *et al.*, 2013). The differences in

TFC studies may be due to different maturity, growth conditions, post-harvest factors, cultivar effect, extraction methods, etc. during analysis.

The TFC of *Tinospora cordifolia* stem methanol extract is 44.283 ± 0.009 mg QE/g which was quantified by using the calibration curve as well as the absorbance values (Figure E.2). Praveen *et al.* (2012) have reported that TFC of methanol extract of leaf of *Tinospora cordifolia* was found to be 10.31±1.20 mg QE/g. The differences in the value of TFC may be due to difference in plant parts used, maturity and season during analysis.

The TFC of blend was found to be 49.781±0.00047 mg QE/g which was quantified by using the calibration curve as well as the absorbance values (Figure E.2). Namdev and Gupta (2015) also obtained that herbal green tea formulation containing *Withania somnifera* stems, *Terminalia arjuna* bark, *Cinnamon* bark and *Tinospora cordifolia* stems was 50 ug CE/mg sample with *Withania somnifera* stems, *Terminalia arjuna* bark, *Cinnamon* bark, *Tinospora cordifolia* stems and green tea leaves yielding 24, 84, 120, 42 and 58 ug CE/mg sample. The results shows that herbal green tea formulation have lower TFC in comparison with green tea. The differences in the value of TFC may be due to difference in plant parts used, maturity, season, cultivar effect, geographic conditions, extraction methods, post-harvest storage conditions, etc. during analysis.

4.3.3 Tannin Content (TC)

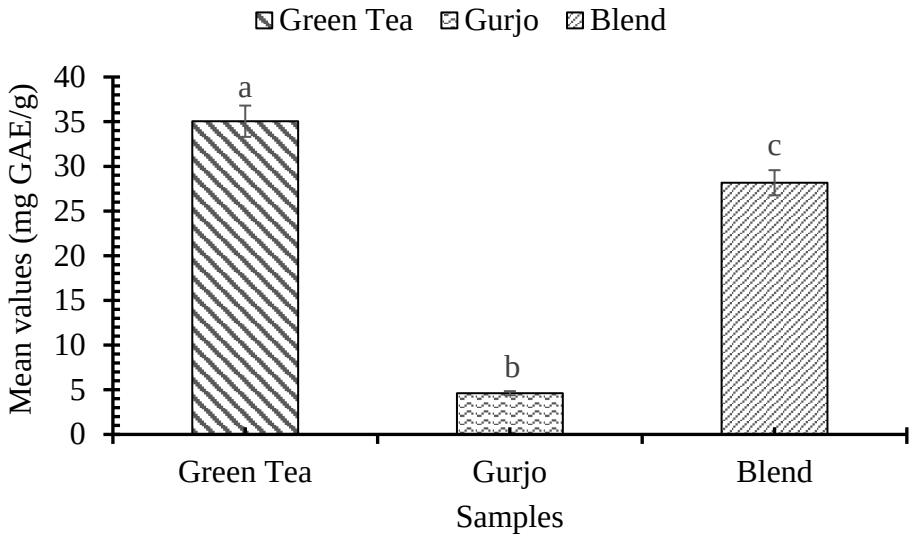

Fig 4.4 Bar diagram for TC of different samples [Values in the figure having different alphabets at top are significantly different (P<0.05)]

The tannin content of green tea was found to be 35.05 ± 0.047 mg GAE/g quantified by using the calibration curve as well as the absorbance values (Figure E.3). The tannin content in green tea has been reported as 37 ± 2.6 mg GAE/g dry weight and the same in roasted coffee beans as 18 ± 1.7 mg GAE/g dry weight (Savolainen, 1992). The difference in tannin content (TC) may be due to various factors, including the variety, genotype, climate, soil, vegetative stage of the plant, harvest time, storage, processing, and treatment.

The tannin content of *Tinospora sinensis* (gurjo) stem was found to be 4.612 ± 0.217 mg GAE/g quantified by using the calibration curve as well as the absorbance values (Figure E.3). *Tinospora cordifolia* stem contained tannin in the range of 13.8 ± 0.5 mg GAE/g which is comparable to that occurring in several common fruits and coffee beans (Sivakumar *et al.*, 2010). The differences may be due to different species, maturity stage, geographical locations and other factors.

The tannin content of blend was found to be 28.167±0.094 mg GAE/g dry weight quantified by using the calibration curve as well as the absorbance values (Figure E.3). Namdev and Gupta (2015) also obtained that herbal green tea formulation containing *Withania somnifera* stems, *Terminalia arjuna* bark, *Cinnamon* bark and *Tinospora cordifolia* stems was 0.271 ug TAE/mg sample with *Withania somnifera* stems, *Terminalia arjuna* bark, *Cinnamon bark, Tinospora cordifolia* stems and green tea leaves were found to 0.1235, 0.0785, 0.2985, 0.2895 and 0.2335 ug TAE/mg sample. The differences in the value of TC may be due to difference in plant parts used, maturity and season during analysis.

4.4 Anti-oxidant property

The percentage inhibition of DPPH radical scavenging activity of green tea, *Tinospora sinensis* stem and blend is shown in Figure 4.5.

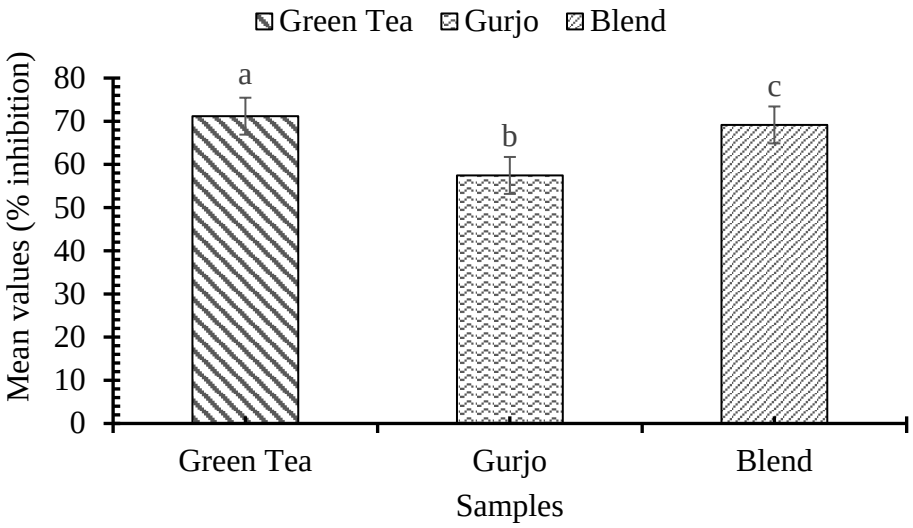

Fig 4.5 Bar diagram for DPPH of different samples [Values in the figure having different alphabets at top are significantly different (P<0.05)]

The DPPH radical scavenging activity of green tea was found to be 71.173% inhibition. Namdev and Gupta (2015) obtained the methanolic extract of *Camellia sinensis* green tea by DPPH radical scavenging activity was found to be 94.10 % inhibition. The differences in the value of DPPH may be due to difference in plant parts used, maturity and season during analysis.

The DPPH radical scavenging activity of gurjo was found to be 57.451% inhibition. Upadhyay *et al.* (2013) obtained the DPPH radical scavenging activity of methanolic bark extract of *Tinospora cordifolia* 62.14% inhibition. The difference in values may be due to the use of different parts of plant. The methanolic bark extract demonstrated the highest absorbance value (0.612 ± 0.0025) at 10 mg/ml and lowest (0.385 ± 0.0006) at 1 mg/ml and in similar manners, the absorbance value decreased with the decrease in the concentration of the extract. Similarly the DPPH radical scavenging activity of *Tinospora cordifolia* stem was found to be 45.46 % inhibition suggested by Namdev and Gupta (2015). The difference in studies may be due to different maturity stage or even use of different species of plant.

The DPPH radical scavenging activity of blend was found to be 69.143% inhibition. Namdev and Gupta (2015) also obtained that herbal green tea formulation containing *Withania somnifera* stems, *Terminalia arjuna* bark, *Cinnamon bark* and *Tinospora cordifolia* stems was 93.29% inhibition with *Withania somnifera* stems, *Terminalia arjuna*

bark, *Cinnamon bark*, *Tinospora cordifolia* stems and green tea leaves yielding 51.95, 87.93, 38.95, 45.46 and 94.10 % inhibition. The results shows that herbal green tea formulation have lower DPPH in comparison with green tea. The differences in the value of DPPH may be due to plant parts used, maturity and season during analysis.

4.5 PCA-Biplot for different methods using varieties of sample extracts

Principal Component Analysis (PCA) was conducted to select the best methods for prepared extracts among phenols, flavonoids, tannin and DPPH. The first principal component (Dim. 1) with eigen value 3.98 (Table F.1) was responsible for approx. 99.47% of the variation while the second principal component (Dim. 2) with eigen value 1.43 (Table F.1) was reported for approx. 0.35% of the variation. So, together, they accounted for about 99.82% of the total variation as shown in Figure 4.6.

As we can see the location of TFC and TC in the same quadrant shows that there is strong correlation between flavonoid content and tannin content. Also, the closest proximity of green tea extracts (green circles) with TFC and TC shows that they possess high value of these parameters. The figure also shows that green tea extracts have better correlation with TFC but low correlation with TC. Similarly, the green tea extracts have good correlation with TPC and DPPH but while comparing green tea extracts to TFC and TC there seems to be poor correlation with TPC and DPPH. The farthest proximity of gurjo extracts (blue circle) with TFC, TC, TPC and DPPH shows that they have lowest TFC, TC, TPC and DPPH radical scavenging activity. The closet proximity of best sample (blend) extract with TPC and DPPH have better correlation. The best sample has also good correlation with TC compared to TFC.

Likewise, Batubara *et al.* (2020) used principal component analysis (PCA) to evaluate the phytochemical and pharmacological properties of the *Orthosiphon aristatus*. They also obtained similar result i.e., the antioxidant activity (DPPH & FRAP) was found to be positively correlated with the total phenolic and total flavonoid contents. Tea polyphenols have been found to possess the stronger antioxidant activity (Dufrense and Farnworth, 2001). Shrestha *et al.* (2010) also claimed good correlation between total TPC and antioxidant activity by the ferric reducing antioxidant power assay.

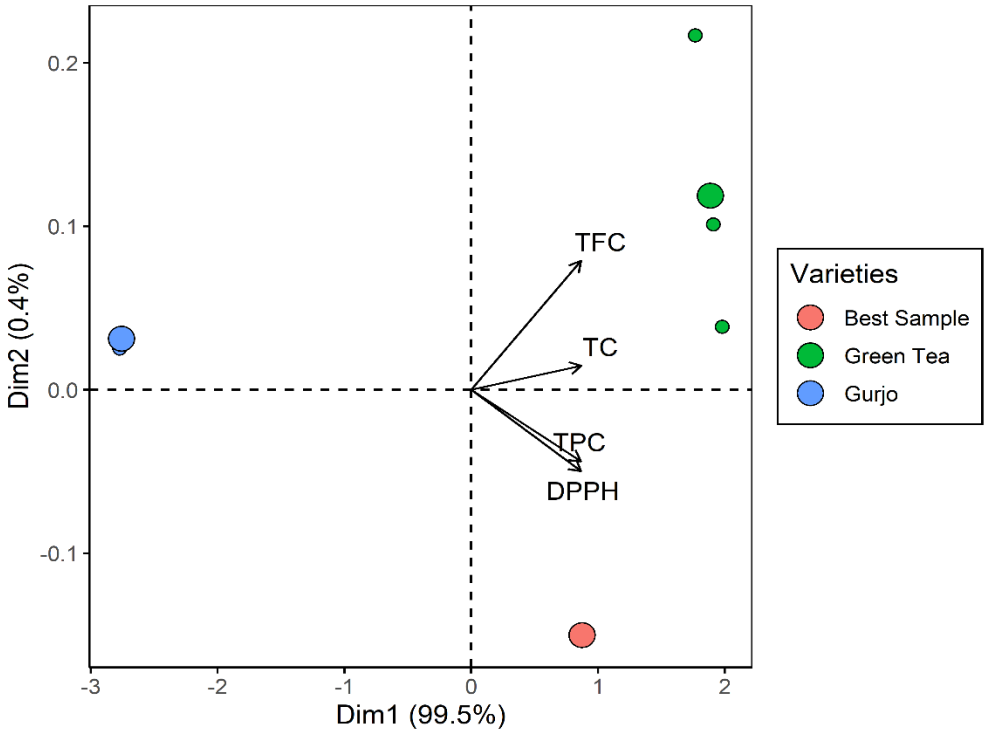

Fig. 4.6 PCA-Biplot for different methods using varieties of sample extracts

4.6 Cluster dendrogram of different parameters

The cluster dendrogram of different parameters (TPC, TFC, TC and DPPH) are shown in Figure 4.7.

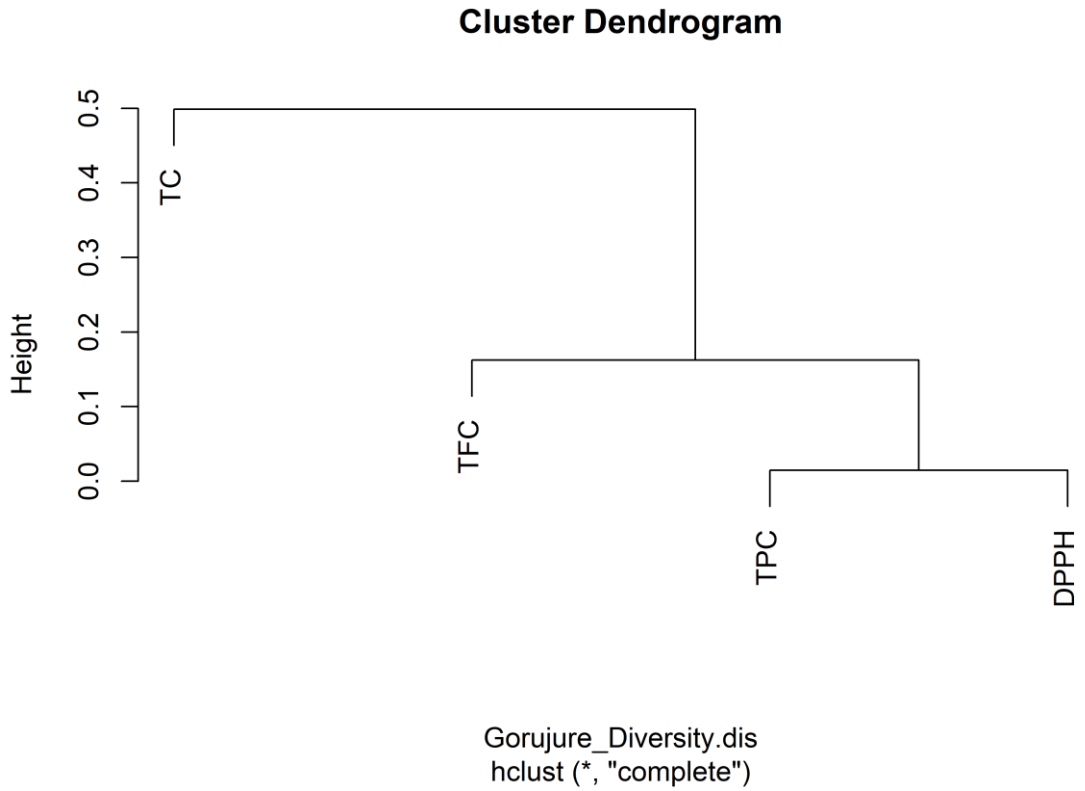

Fig 4.7 Cluster dendrogram of different parameters

Cluster dendrogram shows that TC is higher among the phytochemicals and DPPH. It also shows that TC is significantly different in proportion than others phytochemicals and DPPH. Likewise, TFC is different from TPC and DPPH and it is significantly different than others. TPC and DPPH are correlated to each other and similar in proportion. Thus, among all TC showed it had maximum height and was better, different and superior than rest of samples.

4.7 Correlation between different parameters

The correlation between different parameters TPC, TFC, TC and DPPH radical scavenging activity is shown in Figure 4.8.

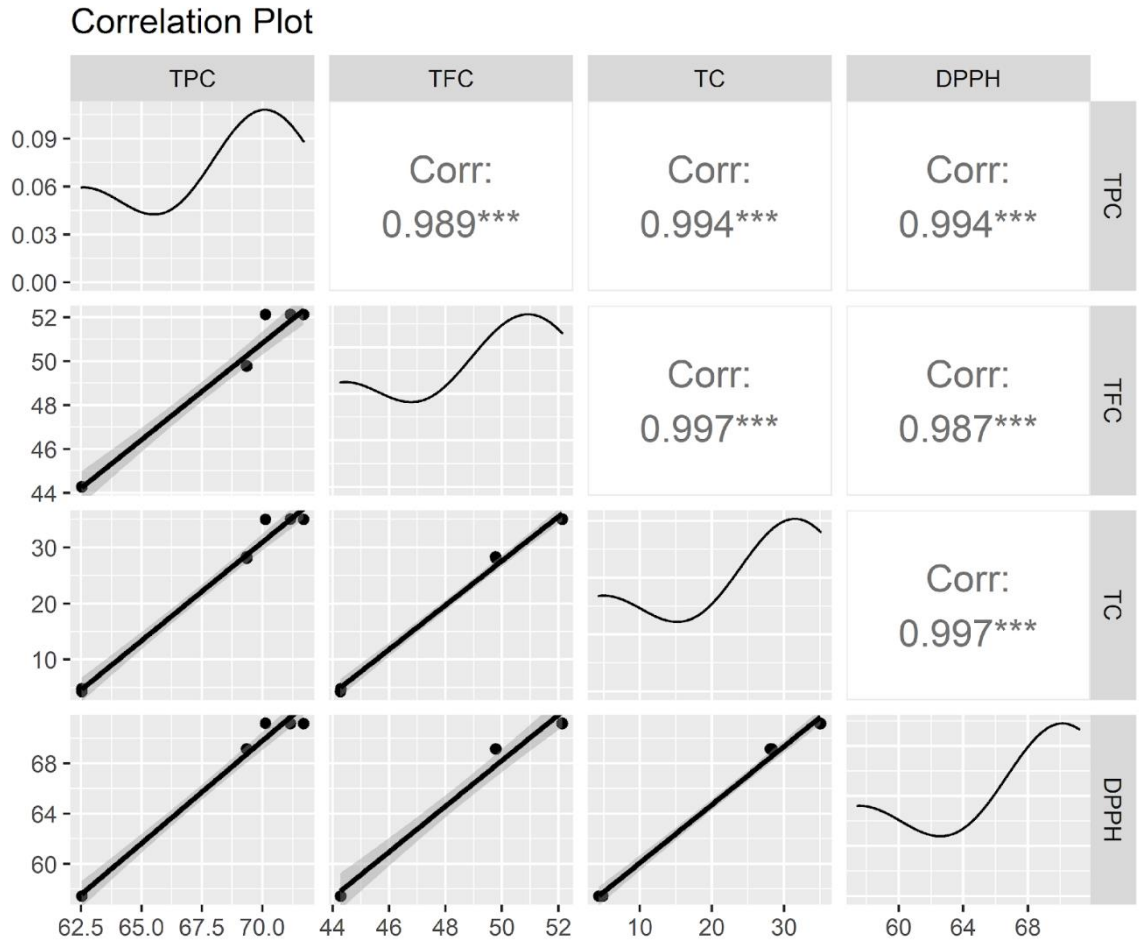

Fig 4.8 Correlation between different parameters

4.7.1 Correlation between TPC and DPPH radical scavenging activity

There was good positive correlation (99.4%) between those two parameters as shown in Figure 4.8. This result showed that with increase in TPC of extracts the scavenging activity also increased indicating increase in antioxidant activity. Similar to our work Sultana *et al.* (2007) has found strong positive correlation (r=0.940) between TPC and DPPH radical scavenging activity. Dudonne *et al.* (2009) have also reported correlation coefficient (r) of 0.939 between these two parameters. Also Moein *et al.* (2008) found significant positive correlation of R= 0.60 and R = 0.65 respectively between TPC and DPPH radical scavenging activity (% inhibition). Kim (2012) has also reported good positive correlation (r^2 =0.8623) between TPC and DPPH scavenging activity in methanol extracts of boxthorn fruit. These results indicate the antioxidant activity of dried boxthorn fruit extracts based on DPPH scavenging activity could be attributed to the presence of phenolic compounds as major components in both extracts. Plant phenolics constitute one of the major groups of

compounds acting as primary antioxidants or free radical terminators (Wang *et al.*, 1996; Cao *et al.*, 1997).

Plant polyphenols have long been reported as source of natural antioxidants from plant sources. Presence of such high positive correlation between TPC and DPPH radical scavenging activity in our study helps us to understand that polyphenols present in our extracts might be good scavengers of DPPH radical and can thus probably be good source of natural antioxidants.

4.7.2 Correlation between TFC and DPPH radical scavenging activity

There was moderate positive correlation (98.7%) between those two parameters is shown in Figure 4.8. Our study showed that with increase in TFC of extracts, the DPPH radical scavenging activity was increased to some extent. This result could be interpreted as flavonoids in the extract possess considerable ferric ion reducing activity. In the work done by Maisuthisakul *et al.* (2007), a moderate positive correlation of 0.79 was found between TFC (mg of RE/g) and DPPH antiradical activity ($1/IC_{50}$) value hence showcasing fair contribution of radical scavenging activity by flavonoids. Similarly, Sultana *et al.* (2007) has found high positive correlation (r=0.875) between TFC and DPPH radical scavenging activity.

On the other hand, Moein *et al.* (2008) reported no correlation (r= -0.172) between TFC and DPPH radical scavenging activity. Meda *et al.* (2005) have also found a low correlation between TFC and radical scavenging activity. There are various groups of flavonoids and their antioxidant capacity/potential is highly dependent upon their molecular structure, particularly the number and position of hydroxyl groups (Bhaigyabati *et al.*, 2014). Different flavonoid compound can show different affinity towards DPPH radical scavenging activity (Hirano *et al.*, 2001). Thus, the presence/absence and amount of certain specific flavonoids in plants and the plant extracts is highly important regarding the antioxidant effect they exert upon particular compounds under different antioxidant activity assays (Hirano *et al.*, 2001; Firuzi *et al.*, 2004).

4.7.3 Correlation between TC and DPPH radical scavenging activity

There was strong positive correlation (99.7%) between those two parameters is shown in Figure 4.8. The correlation between tannins(X) and free radical scavenging activity (Y) is found to have a correlation coefficient of r^2 =0.690. In this case also, among the phenolic compounds also, the contribution of tannin is found to be 69% in these eight selected plants of *Lamiaceae* found in Manipur. The present study shows that among the phenolic compounds also, tannins show high free radical scavenging activity and are good antioxidants which are also found to use as anti-carcinogenic, anti-mutagenic and in treatment of cancer patients (Ramakrishnan *et al.*, 2006).

4.7.4 Correlation between TPC and TFC

There was moderate positive correlation between TPC and TFC (98.9%) in our study is shown in Figure 4.8. Since flavonoids is the subcomponent of polyphenols there is expected to be some kind of good relation between TPC and TFC in the extracts or juices of plants or herbs. Maisuthisakul *et al.* (2007) reported high correlation coefficient (r= 0.92) between TPC (mg of GAE/g) and TFC (mg of RE/g). But some studies have reported low correlation between these two parameters. In a study conducted by Moein *et al.* (2008), a low correlation (R = 0.048) was found between phenolic compounds and flavonoids. Such low correlation was also found by other researchers such as Meda *et al.* (2005). Presence of other chemical groups such as amino acids and proteins can also interfere with TPC determination by FC reagent method thus resulting in higher content/concentration (Moein *et al.*, 2008). Although flavonoids represent the largest part of dietary polyphenols and flavonoids being the subcomponents of polyphenols, naturally there is expected to have high correlation between TPC and TFC content (Gonzalez *et al.*, 2013). But the different solvent used, extraction method and processing can highly affect the amount of these phytochemicals in the extracts (Rababah *et al.*, 2010; Waleed *et al.*, 2014; Dhanani *et al.*, 2017).

4.7.5 Correlation between TPC and TC

There was good positive correlation between TPC and TC (99.4%) in our study is shown in Figure 4.8. Polyphenolic compounds as determined by the uncorrected vanillin assay are highly correlated (0.1% level) with tannins determined as protein precipitable phenols (r = 0.920) and the modified vanillin assay (r = 0.882) and at the 1% level with total phenols (r

= 0.780). Tannin determined as protein precipitable phenols is highly correlated (0.1%) to tannin as determined by the modified vanillin assay (r = 0.810). The correlation between. total phenols and tannin by the modified vanillin (r =.0.549) and the precipitation methods (r = 0.661) is significant only at the 5% level (Bressani *et al.*, 1983).

4.7.6 Correlation between TFC and TC

There was strong positive correlation between TFC and TC (99.7%) in our study is shown in Figure 4.8. According to Baldwin *et al.* (1987), the total phenolic content was positively correlated with condensed tannin content ($r = 0.62$, $P < 0.05$) but negatively correlated with hydrolysable tannin content ($r = -0.70$, $P < 0.01$) and condensed and hydrolysable tannin contents were negatively correlated ($r = -0.78$, $P < 0.01$) .

Part V

Conclusions and Recommendations

5.1 Conclusions

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

1. Green tea (91.25%) powder in combination with gurjo (8.75%) stem powder i.e., selected best tea blend powder was found highly acceptable to consume.

2. The Sample D (blend) was found to be superior than others samples on the basis of color, aroma, taste, mouthfeel and overall acceptance of infusion from statistical analysis at $P<0.05$. Also, from principal component analysis (PCA), the closest proximity of mouthfeel and overall acceptance shows that sample D possess high value of these parameters.

3. The moisture, protein, fat, crude fiber and ash content in blend were found to be 5.267%, 21.534%, 2.709%, 14.214% and 5.946% and were significantly different ($P<0.05$) than that of control product green tea. As there was no significance difference in nitrogen free extractive (NFE) of blend and green tea, the NFE in blend was found to be 55.590%.

4. The TPC, TFC, TC of blend having 69.353±0.009 mg GAE/g, 49.781±0.00047 mg QE/g and 28.167±0.094 mg GAE/g were found to be significantly different ($P<0.05$) from green tea.

5. The DPPH scavenging activity of blend having 69.143±0.00047% inhibition was found to be significantly different from green tea.

6. According to principal component analysis (PCA), the closet proximity of blend extract with TPC and DPPH have better correlation. Likewise, among the correlation between different parameters, TC & DPPH and TC & TFC was found to be strongly correlated (99.7%) to each other followed by TPC & DPPH and TPC & TC (99.4%) respectively.

5.2 Recommendations

The following recommendations can be used to continue the experiment:

1. Among four tea blends, green tea in combination with *Tinospora sinensis* stem at a proportion of 91.25:8.75 was found highly acceptable to consume, thus, can be used for commercial production of a new tea blend.

2. Different parts of *Tinospora sinensis* can be used for analysis of phytochemicals and anti-oxidant activity.

3. Effect of different drying methods on the quality of green tea can be studied.

Part V

Summary

Tea from *Camellia sinensis* is a popular beverage that is consumed all over the world after water. Teas have long attracted scientific interest due to their biological effects and chemical composition. *Tinospora sinensis* is well known in Ayurveda and traditional medicine for its admirable therapeutic efficiency. The stem is a more widely used and beneficial component of the plant and its extract has been shown to be a good source of antioxidant for nutraceutical purposes.

For the preparation of blend, simple mixture design was used. Five different formulations of blend powder namely Control (100% green tea), A (65% green tea + 35% gurjo), B (82.5%green tea + 17.5% gurjo), C (73.75% green tea + 26.25% gurjo) and D (91.25% green tea + 8.75% gurjo) were prepared following cup testing process of tea. Sensory evaluation was carried out based on color, aroma, taste, mouthfeel and overall acceptability. The data obtained was statistically analyzed using one-way ANOVA at 5% level of significance. The product D got the highest mean sensory score after the control product. Also, from PCA, the closest proximity of mouthfeel and overall acceptance shows that product D possess high value of these parameters.

The proximate composition of product D (blend) for moisture, crude protein, crude fat, crude fiber, total ash and NFE were found to be 5.267%, 21.534%, 2.709%, 14.214%, 5.946% and 55.590%. The moisture, protein, fat, fiber and ash content except NFE in blend was found to be significantly different ($P<0.05$) from green tea. The TPC, TFC, TC and DPPH radical scavenging activity of blend were found to be 69.353 ± 0.009 mg GAE/g, 49.781 ± 0.00047 mg QE/g, 28.167 ± 0.094 mg GAE/g dry weight and 69.143% inhibition and were found to have significantly different from green tea. By PCA, the closet proximity of blend with TPC and DPPH had better correlation. The TPC increases with increase in DPPH radical scavenging activity. The blend has also good correlation with TC as compared to TFC. All parameters were found to be positively correlated to each other but among all TC & DPPH and TC & TFC were found to be strongly correlated (99.7%) to each other followed by TPC & DPPH and TPC & TC (99.4%) respectively.

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Appendices

Appendix A

Materials, Equipments 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. Equipments 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.H_2SO_4, Conc. HCl, etc.

Appendix B

Sensory Analysis Score Card

Name of the panelist: Date:

Name of the product: Gurjo stem incorporated green tea

Dear panelist, you are provided with 4 samples of gurjo stem incorporated green tea on each proportion with variation on gurjo stem powder content. 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

Parameters	Product code			
	A	B	C	D
Color				
Aroma				
Taste				
Mouthfeel				
Overall Acceptability				

Any comments:

……..…….

……..

Signature:

Appendix C

Sensory analysis output

C.1 ANOVA for sensory parameter

C.1.I One-way ANOVA for color

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	3	6.050000	2.01667	2.7590	0.0506
Error	56	40.933333	0.73095		
C. Total	59	46.983333			

Connecting Letters Report

Level		Mean
B	a	6.6666667
C	a	6.6666667
D	a	6.6666667
A	a	5.9333333

Levels not connected by same letter are significantly different.

C.1.II One-way ANOVA for aroma

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	3	1.516667	0.505556	0.7173	0.5458
Error	56	39.466667	0.704762		
C. Total	59	40.983333			

Connecting Letters Report

Level		Mean
D	a	6.7333333
A	a	6.6000000
C	a	6.4000000
B	a	6.3333333

Levels not connected by same letter are significantly different.

C.1.III One-way ANOVA for taste

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	3	7.600000	2.53333	3.7203	0.0164*
Error	56	38.133333	0.68095		
C. Total	59	45.733333			

Connecting Letters Report

Level			Mean
D	a		7.0666667
B	a	b	6.8666667
C	a	b	6.8666667
A		b	6.1333333

Levels not connected by same letter are significantly different.

C.1.IV One-way ANOVA for mouthfeel

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	3	7.066667	2.35556	3.4115	0.0235*
Error	56	38.666667	0.69048		
C. Total	59	45.733333			

Connecting Letters Report

Level			Mean
D	a		7.0666667
C	a	b	7.0000000
B	a	b	6.6666667
A		b	6.2000000

Levels not connected by same letter are significantly different.

C.1.V One-way ANOVA for overall acceptance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	3	11.200000	3.73333	6.4527	0.0008*
Error	56	32.400000	0.57857		
C. Total	59	43.600000			

Connecting Letters Report

Level			Mean
D	a		7.3333333
B	a		6.9333333
C	a	b	6.8000000
A		b	6.1333333

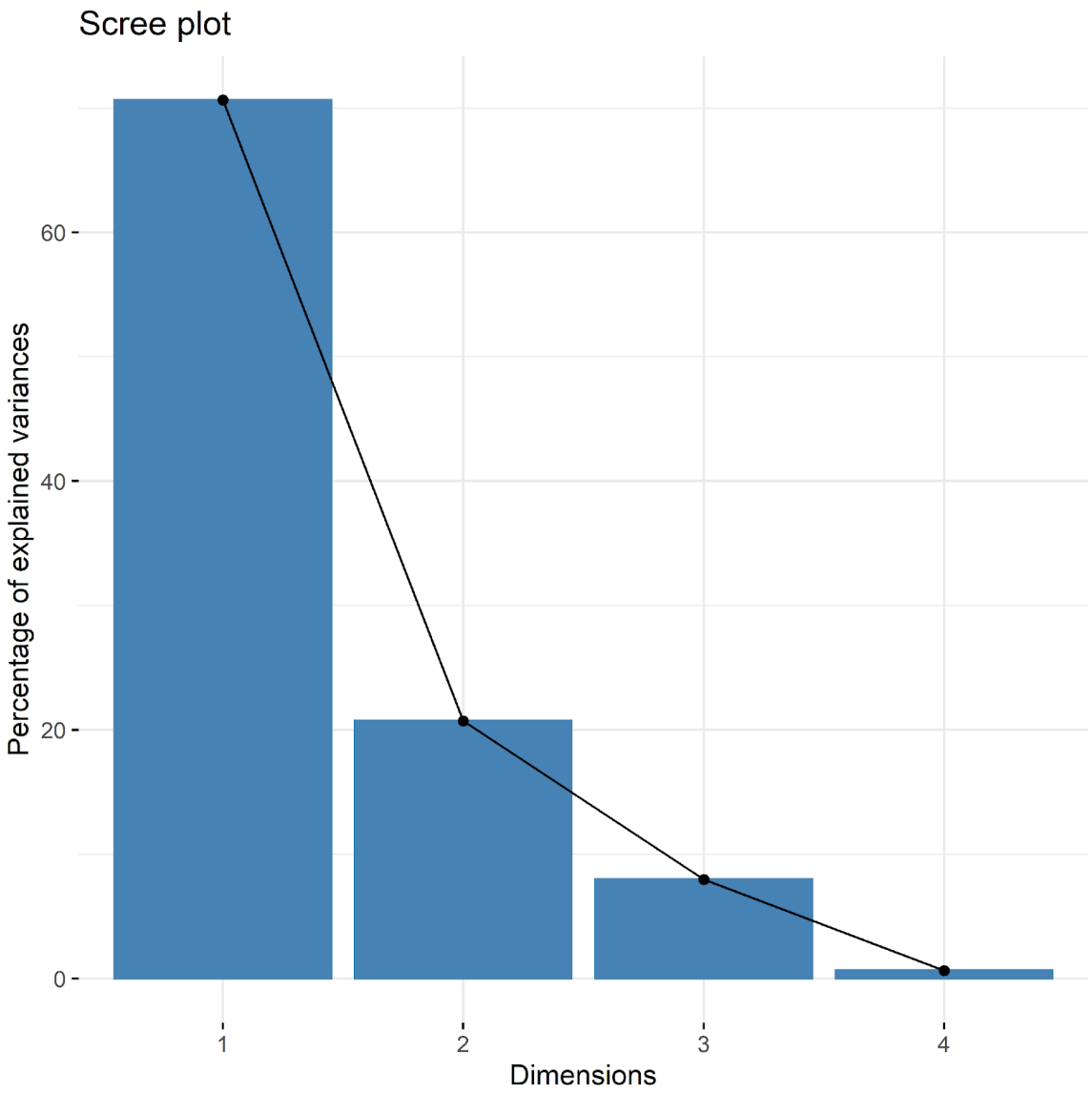
Levels not connected by same letter are significantly different.

C.2 PCA for sensory

C.2.I Eigen value for PCA-Biplot of selecting best tea blend

Dimensions	Eigen value	Variance percent	Cumulative variance percent
1	2.82647964	70.6619910	70.66199
2	0.82906308	20.7265771	91.38857
3	0.31889698	7.9724246	99.36099
4	0.02556029	0.6390074	100.00000

C.2.II Scree plot for PCA-Biplot of selecting best tea blend



Appendix D

Table D.1 t-Test (two- product assuming unequal variance) for moisture content of the optimum product D with control green tea

	Green tea	Blend
Mean	6.466666667	5.266666667
Variance	0.023333333	0.003333333
Observations	3	3
Hypothesized Mean Difference	0	
d.f.	3	
t Stat	12.72792206	
P(T<=t) one-tail	0.000523119	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.001046238	
t Critical two-tail	3.182446305	

Table D.2 t-Test (two- product assuming unequal variance) for protein content of the optimum product D with control product green tea

	Green tea	Blend
Mean	23.55498533	21.53261553
Variance	0.015238567	0.011141235
Observations	3	3
Hypothesized Mean Difference	0	
d.f.	4	
t Stat	21.56678628	
P(T<=t) one-tail	1.36704E-05	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	2.73407E-05	
t Critical two-tail	2.776445105	

Table D.3 t-Test (two- product assuming unequal variance) for crude fat of the optimum product D with control product green tea.

	Green tea	Blend
Mean	5.844202117	2.709168947
Variance	0.015238562	0.003713744
Observations	3	3
Hypothesized Mean Difference	0	
d.f.	3	
t Stat	39.44316263	
P(T<=t) one-tail	1.79276E-05	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	3.58551E-05	
t Critical two-tail	3.182446305	

Table D.4 t-Test (two- product assuming unequal variance) for crude fiber of the optimum product D with control product green tea

	Green tea	Blend
Mean	9.265198483	14.21434097
Variance	0.00380964	0.003713749
Observations	3	3
Hypothesized Mean Difference	0	
d.f.	4	
t Stat	-98.82887027	
P(T<=t) one-tail	3.1426E-08	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	6.28521E-08	
t Critical two-tail	2.776445105	

Table D.5 t-Test (two- product assuming unequal variance) for total ash of the optimum product D with control product green tea

	Green tea	Blend
Mean	5.487848333	5.94609809
Variance	0.015238562	0.003713744
Observations	3	3
Hypothesized Mean Difference	0	
d.f.	3	
t Stat	-5.765431825	
P(T<=t) one-tail	0.00518563	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.010371259	
t Critical two-tail	3.182446305	

Table D.6 t-Test (two- product assuming unequal variance) for NFE of the optimum product D with control product green tea

	Green tea	Blend
Mean	55.84063857	55.5907395
Variance	0.072383177	0.003713742
Observations	3	3
Hypothesized Mean Difference	0	
d.f.	2	
t Stat	1.569067859	
P(T<=t) one-tail	0.128594598	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.257189197	
t Critical two-tail	4.30265273	

Appendix E

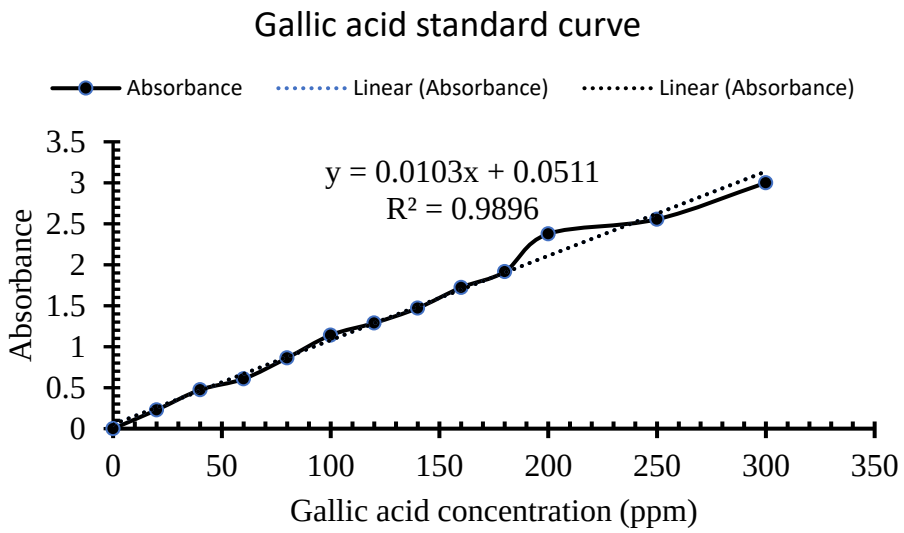


Figure E.1 Gallic acid standard curve of phenol

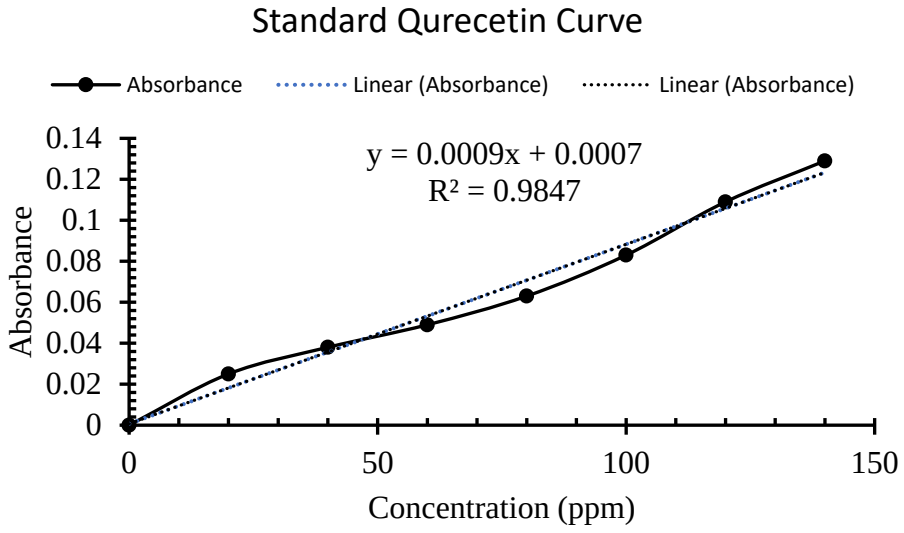


Figure E.2 Standard Qurectin curve of flavonoid

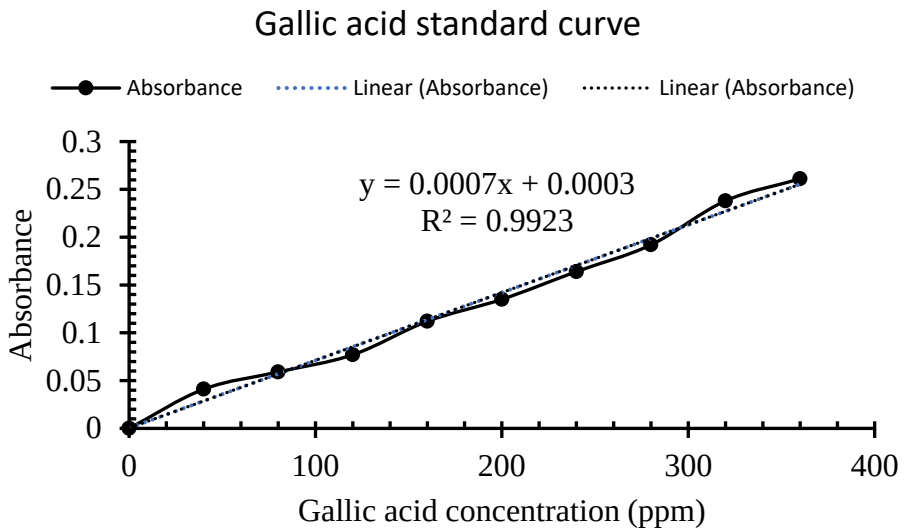


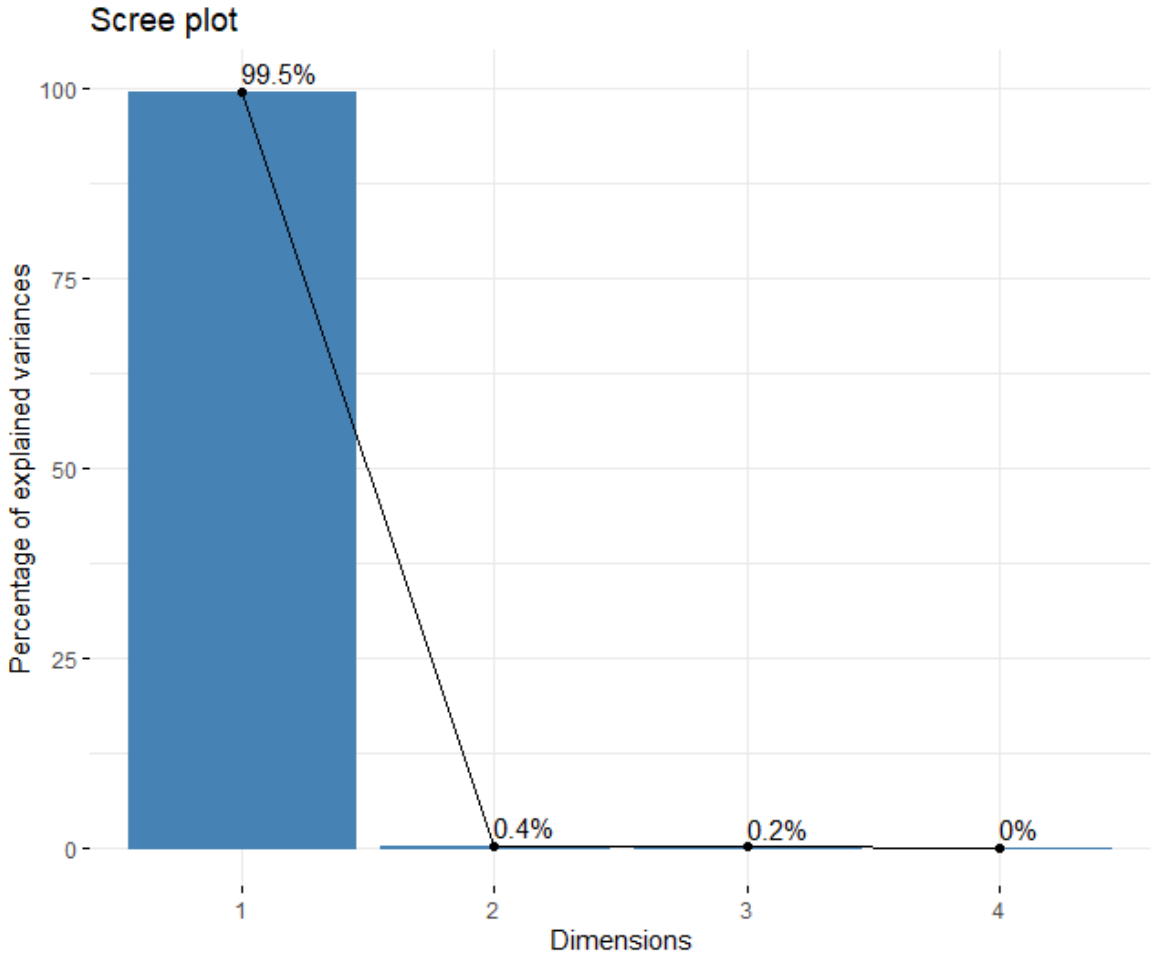
Figure E.3 Gallic acid standard curve of tannin

Appendix F

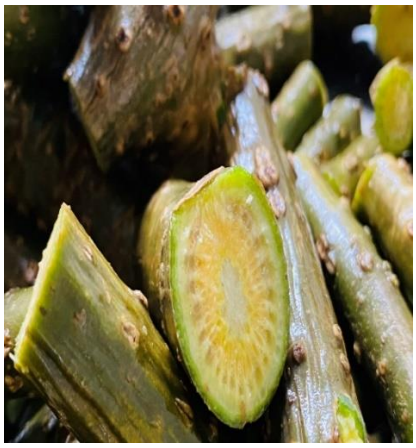
F.1 Eigen value of TPC, TFC, TC and DPPH

Dimensions	Eigen value	Variance percent	Cumulative variance percent
1	3.978735e+00	99.468380490	99.46838
2	1.435735e-02	0.358933754	99.82731
3	6.841356e-03	0.171033895	99.99835
4	6.607443e-05	0.001651861	100.00000

F.2 Scree plot of TPC, TFC, TC and DPPH



Color plates



P1: Gurjo stem cut pieces

P2: Plucking of green tea leaves

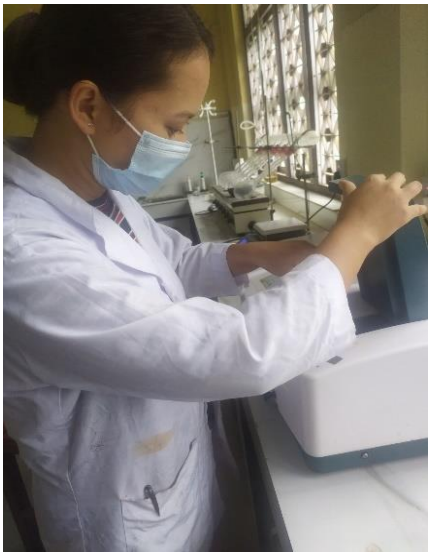
P3: Green tea leaves

P4: Kjeldahl for protein

P5: Crude fiber determination

P6: Beaker containing methanolic extract

P7: Phytochemicals work



P8: Use of spectrophotometer