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Analysis Of Naturally Made Herbal Teas For Respective HERBS - Aparajita, Ginger, Tulsi, Mint, Tulsi-Mint (50:50)

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Abstract

This research is an in-depth examination of five naturally made herbal teas: aparajita (*Clitoria ternatea*), ginger (*Zingiber officinale*), Tulsi (*Ocimum sanctum*), mint (*Mentha* species), and a combination of Tulsi-mint (50:50). Physicochemical characterization identified unique characteristics among samples, which had pH ranges of 5.8-7.2, electrical conductivity of 580-900 $\mu\text{S}/\text{cm}$, and different turbidity corresponding to their specific compositional factors. Phytochemical profiling disclosed signature bioactive markers—anthocyanins and teratins in aparajita, gingerols and shogaols in ginger, eugenol and rosmarinic acid in Tulsi, and menthol with flavonoids in mint—directly corresponding to their noted antioxidant activities. The mixture of Tulsi and mint showed favourable synergistic activities, with increased antioxidant activity over predicted additive values of single herbs. Structure-activity relationship analysis of the compounds identified ortho-dihydroxy configurations and conjugation extension in phenolic systems as key factors responsible for maximal free radical neutralization activities. These results support the scientific rationale underlying traditional uses of these herbal teas and propose their potential incorporation into contemporary preventive healthcare strategies for management of cognitive function, gastrointestinal health, stress, and metabolic control through their complementary mechanisms of action against oxidative stress and inflammatory processes.

Keywords: Phytochemical profile, Antioxidant activity, Herbal synergy, Physiochemical property, Therapeutic potential

Chapter 1

Introduction

1. Introduction

1.1. Background on Herbal Teas and their popularity

With roots in ancient China and Egypt, herbal teas have been drinking for thousands of years almost everywhere in almost every culture. Unlike black, green, white, and oolong teas—which come from the *Camellia sinensis* plant—herbal teas, often known as tisanes, are infusions derived from dried herbs, flowers, fruits, seeds, roots, or other plant components. Over the past several years, these drinks have had an amazing comeback in popularity especially as health-conscious customers search for substitutes to caffeinated drinks and find their therapeutic value (Brendler et al., 2022). From a niche segment to a popular beverage choice valued at billions of dollars yearly, the worldwide herbal tea business has grown rapidly. Growing consumer knowledge of the possible health advantages connected with herbal infusions—including digestive assistance, immunological strengthening, stress release, and sleep improvement—has driven this expansion. Many conventional applications of medical herbs have been progressively supported by scientific studies, hence strengthening consumer trust in these natural medicines (Góis et al., 2021). The spread of herbal tea drinking has especially been noteworthy in areas once dominated by conventional tea and coffee cultures in recent years. This change captures larger trends toward natural, plant-based goods and individualized wellness practices. Herbal teas appeal to consumers more and more not just for their medicinal qualities but also for their varied taste sensations and ceremonial element of tea making and consumption (Brendler et al., 2022). Among the most prized plants used in tea preparations all throughout the world are those with strong roots in ancient medicinal systems. Particularly noteworthy plants with great historical use and modern appeal include aparajita (*Clitoria ternatea*, or butterfly pea), ginger (*Zingiber officinale*), tulsi (*Ocimum sanctum*, or holy basil), and mint (*Mentha* species). Each adds unique bioactive chemicals, sensory qualities, and possible health advantages to tea preparations, therefore deserving of further investigation both separately and in combinations such as the tulsi-mint blend (Long et al., 2023).

1.2. Overview of the selected herbs

Native to tropical equatorial Asia, the stunning blue-flowering plant Aparajita (*Clitoria ternatea*), often known as butterfly pea, is For millennia Ayurvedic medicine has made use of this plant for its memory-enhancing, antistress, anxiolytic, and antidepressant qualities. Anthocyanins, strong antioxidants found in the vivid blue petals, provide the tea their unique hue and help to explain its possible neuroprotective properties. Beyond conventional use, aparajita's nootropic qualities and color-changing ability depending on pH have attracted recent scientific attention and make it both aesthetically appealing and medicinally beneficial as a beverage component (Shirodkar et al., 2023).

With a recorded history extending over 5,000 years across Asian, Indian, and Middle Eastern civilizations, ginger (*Zingiber officinale*) is among the most flexible and often used therapeutic plants available worldwide. The rhizome's bioactive elements—gingerols, shogaols, and zingerone—which add to its unique pungent taste and medicinal qualities—help to explain Traditionally, ginger tea has been drunk for anti-inflammatory effects, nausea alleviation, and help for digestion. Many of these uses have been supported by modern studies, which also investigate further advantages in domains of metabolic health, pain management, and immune system function (Shaukat et al., 2023).

Considered the "Queen of Herbs" in Ayurvedic medicine and regarded as Holy Basil in Hinduism, tulsi (*Ocimum sanctum*) has holy value in Indian culture. Eugenol, rosmarinic acid, and many triterpenes found in this adaptogenic plant help to explain its unique clove-like scent and therapeutic actions. Originally used to boost resilience to mental and physical stress as well as lifespan, tulsi tea has become well-known worldwide for its adaptogenic, immunomodulating, anti-inflammatory properties. Modern studies keep looking at its possible advantages for cognitive ability, metabolic health, and stress management (Muley & Medithi, 2022).

For millennia, mint—mostly *Mentha piperita* and *Mentha spicata*—has been valued across Mediterranean and Middle Eastern civilizations for its medical uses and refreshing taste. Rich in menthol, rosmarinic acid, and flavonoids, mint has the distinct cooling effect and fragrant flavor that define mint tea among the most often used herbal infusions globally. Historically drunk for respiratory support and digestive comfort, mint tea is still under research for effects on gastrointestinal performance, headache relief, and cognitive ability (Kazemi et al., 2024).

The 50:50 tulsi-mint mix offers a synergistic method for making herbal tea, therefore improving both medicinal advantages and flavor characteristics. This combination balances the digestive and refreshing benefits of mint with the adaptogenic, stress-relieving effects of tulsi to concurrently address several facets of wellness. Rooted in ancient Ayurvedic formulations, the combination reflects the holistic approach unique to many traditional medical systems, in which herbs are typically blended to improve potency and balance qualities (Li et al., 2025).

1.3. Traditional uses and reported benefits

Derived from the vivid blue blossoms of *Clitoria ternatea*, apajita tea offers a visually arresting infusion that changes from deep azure to purple when acidified. Anthocyanins, flavonoids, and peptides among other bioactive chemicals abound in the tea and help to explain its antioxidant and nootropic effects. Natural preparation results in aparajita infusions usually with a moderate, somewhat earthy taste with faint sweet undertones. To preserve the delicate anthocyanin chemicals, traditional procedure calls for steeping the dried petals in hot water for five to seven minutes under a temperature kept below boiling (around eighty-five°C). Apart from its therapeutic properties, the resultant tea serves as a natural pH sensor; it changes from blue to purple or pink when lemon or another acidic component is added. With possible uses for cognitive improvement, stress reduction, and anti-glycation actions that might provide protection against age-related cellular damage, modern research has found noteworthy antioxidant activity (Taylor et al., 2022).

Ginger (*Zingiber officinale*)

Made from the rhizome of *Zingiber officinale*, ginger tea is a warming, fragrant infusion distinguished by its unique spicy taste profile. Natural preparation results in a diverse array of bioactive chemicals including gingerols, shogaols, and zingerone, with concentrations depending on preparation technique and length of time. Traditionally, fresh ginger root slices are simmered in water for 10 to 20 minutes to release the whole spectrum of both volatile and non-volatile molecules. With gingerols and shogaols showing notable cyclooxygenase and lipoxygenase inhibition in laboratory experiments, the resultant infusion produces strong anti-inflammatory effects. Ginger tea's thermogenic qualities help to explain both its possible metabolic advantages and warming feeling. While continuing studies investigate its uses for pain management, immune support, and gastrointestinal health, clinical research has confirmed ginger tea's efficacy for nausea relief, especially in cases of pregnancy-related morning sickness, motion sickness, and chemotherapy-induced nausea (Unuofin et al., 2021).

Tulsi, *Ocimum sanctum*:

Made from the leaves, stems, and sometimes blossoms of *Ocimum sanctum*, tulsi tea creates a strong brew with rich fragrant overtones of mint, pepper, and clove. Along with methyl eugenol, caryophyllene, and other ursolic and oleanolic acids that add to its therapeutic profile, naturally brewed Tulsi tea has eugenol-dominated essential oils. Usually including steeping the dried or fresh plant material in freshly heated water for 5–10 minutes, traditional preparation lets ideal extraction of both water-soluble and volatile chemicals possible. The resultant tea has strong adaptogenic qualities that enable the body to preserve homeostasis under psychological and physical stress. Studies on pharmacology have found immunomodulating properties; depending on quantity and preparation, tulsi extracts show both immunostimulating and immunoregulating action. Tulsi tea's anti-inflammatory and antioxidant qualities seem to cooperate to offer defense against oxidative stress and chronic inflammatory diseases, therefore enhancing its value for preventative health practices and stress management (Muley & Medithi, 2022).

Mentha species, mint

Mostly made from *Mentha piperita* (peppermint) or *Mentha spicata* (spearmint), mint tea offers a crisp, pleasant infusion distinguished by its clear, sharp taste characterization. Menthol is the main bioactive ingredient in naturally made mint tea; along with menthone, limonene, and other phenolic acids adding to both sensory and medicinal value. Traditionally, fresh or dried leaves are steamed in boiling water for three to five minutes with care taken not to over-extract the rather bitter components that can develop with prolonged steeping. With clinical data confirming its effectiveness for irritable bowel syndrome and functional dyspepsia, the resultant tea has carminative and antispasmodic effects on the gastrointestinal tract. Mint tea's aromatic volatiles show notable antibacterial action against common infections, which may help explain why it has long been used for respiratory problems. The menthol component stimulates TRPM8 receptors, producing the distinct chilling feeling that offers symptomatic relief for many inflammatory diseases and adds to the pleasant taste of mint tea (Kazemi et al., 2024).

50:50 tulsi-mints

The Tulsi-mint mix is a synergistic mix that combines the complementary qualities of both plants to provide a balanced medicinal profile. This tea has a sophisticated taste profile when made in equal amounts that blends the fragrant, spicy notes of Tulsi with the vivid, reviving properties of mint. Compounds from both parent plants are included in the phytochemical makeup; possible interactions between the menthol-dominant profile of mint and the eugenol-rich essential oils of Tulsi abound. Traditionally, preparation calls for either co-steeping both herbs or creating separate infusions then mixing them. Combining the carminative qualities of mint with the adaptogenic support of tulsi, the resultant mix could provide improved digestive benefits. While the combined antioxidant and anti-inflammatory substances offer a complete approach to wellbeing, the relaxing properties of both herbs may cooperate to treat stress-related digestive problems. Preliminary studies indicate that by means of additive or synergistic interactions between their many bioactive substances, such herbal mixtures might provide more therapeutic value than single herbs. (Barrientos et al., 2023)

1.4. Rationale for Scientific Analysis

Several strong reasons justify the scientific study of naturally occurring herbal teas created from aparajita, ginger, Tulsi, mint, and their mixtures, which spans conventional knowledge with modern research demands. Although these plants have long been utilized therapeutically in many different traditional medicine systems, ethnobotanical knowledge still lags far behind evidence-based validation using exacting scientific methods. By means of standardized profiles of bioactive components, identification of ideal preparation techniques, and quantification of therapeutic efficacy via controlled research, a methodical study of these herbal infusions closes this gap. This method not only respects conventional wisdom but also improves it by means of empirical validation, therefore strengthening the basis for consumer awareness as well as clinical uses (Etheridge & Derbyshire, 2019).

Growing worldwide demand for functional drinks and natural health products calls for a better knowledge of herbal tea composition and effectiveness. Scientific study becomes crucial to validate claims, find any contraindications, and provide suitable dose recommendations as consumers turn to these drinks for certain health advantages more and more. Ideal candidates for comparison study are the chosen plants, aparajita, ginger, tulsi, and mint, which reflect different phytochemical profiles and medicinal uses. Their general availability, cultural value, and early proof of effectiveness support the distribution of research funds to these specific botanical compounds even more. Knowing how these herbs work both alone and in combinations like the Tulsi-mint mix helps one to get important understanding of possible synergistic effects that might maximize therapeutic results (Ali et al., 2022).

From a phytochemical standpoint, these plants have several bioactive molecules of pharmacological relevance: polyphenols, terpenoids, flavonoids, and essential oils deserving of careful study. The complicated matrix of substances in herbal infusions presents difficulties for analysis fit for contemporary analytical methods. Modern techniques like gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS)

allow exact identification and quantification of important chemicals causing therapeutic effects. This phytochemical fingerprinting not only improves quality control but also helps to identify certain molecules that may be turned into standardized extracts or pharmacological agents, therefore integrating traditional herbal therapy with contemporary drug development methods.

Another strong justification for scientific study is the comparison of commercially sold goods with organically produced herbal teas (Mugale et al., 2024). The growing commercial herbal tea business begs issues about the preservation of bioactive components throughout industrial processing, packaging, and storage. How processing factors like drying techniques, particle size, water temperature, steeping duration, and storage conditions impact the chemical makeup and medicinal qualities of various herbal infusions may be ascertained by scientific study. These results directly relate to both commercial manufacturers trying to provide goods with consistent quality and efficacy and traditional practitioners looking to maximize preparation strategies. Standardized preparation guidelines based on scientific data guarantees that customers get the whole therapeutic potential from these herbal treatments independent of the setting of preparation (Wang et al., 2023).

Furthermore, the scientific examination of these herbal teas covers significant safety issues that complement research on effectiveness. Although these herbs usually have good safety profiles based on their historical use, systematic toxicological screening is still crucial especially in view of possible interactions with pharmaceutical drugs or use in sensitive populations like pregnant women, children, or people with particular medical conditions. Through in vitro and in vivo research, comprehensive safety profiling generates the required information to define suitable usage rules. Promoting these ancient cures in modern healthcare environments should be done responsibly by means of a comprehensive assessment of both advantages and possible hazards, thereby ensuring that old wisdom is used with suitable modern protections (Patwardhan et al., 2023).

1.5. Objectives of the Thesis

- Using modern analytical techniques, to fully describe the phytochemical profiles of spontaneously produced herbal teas from aparajita, ginger, Tulsi, mint, and a Tulsi-mint combo, thereby identifying important bioactive components and their quantities across various preparation processes.
- By means of standardized in vitro tests, evaluating and contrasting the antioxidant, anti-inflammatory, and antibacterial characteristics of every herbal tea preparation result in quantifiable values pertinent to their traditional medicinal uses.
- With special attention on the possible synergistic effects in the Tulsi-mint (50:50) combination compared to the individual herbal infusions, to ascertain ideal preparation parameters (water temperature, steeping time, herb-to-water ratio) for maximum extraction of bioactive compounds from each herb.

Chapter 2

Literature Review

2. Literature Review

2.1. Phytochemical Composition of Aparajita

Commonly known as butterfly pea, Aparajita (*Clitoria ternatea*) is a perennial herbaceous plant with brilliant blue blossoms and great therapeutic value. Rich in bioactive components, including anthocyanins, flavonoids, glycosides, tannins, saponins, triterpenoids, and phenolic acids, phytochemical screening has shown (Mukherjee et al., 2018). Particularly delphinidin-3,5-glucoside and ternatins, which help to give the flower extract its unique deep blue color and have strong antioxidant qualities, the most unique chemicals are the anthocyanins. As natural colorants and functional components in herbal tea blends, these anthocyanins show amazing stability at different pH levels (Singh et al., 2021). Additional phytochemicals including quercetin, kaempferol, myricetin derivatives, and p-coumaric acid have been found by further studies of Aparajita's chemical makeup. While the seeds have proteins with distinct cyclotide peptides that show antibacterial and anthelmintic qualities, the roots contain particular phytoconstituents like taraxerol and taraxerone (Kamilla et al., 2019). These many chemicals used together help Aparajita to be therapeutically effective in conventional medical systems, where they have been used for their nootropic, anxiolytic, antipyretic, anti-inflammatory, and antibacterial properties. By means of its capacity to block α -amylase and α -glucosidase enzymes, hence controlling postprandial blood glucose levels, recent research have underlined the promise of Aparajita extract in treating diabetes. Formulated as a herbal tea, Aparajita produces a naturally caffeine-free beverage with far more antioxidant action than many traditional herbal teas. Hot water easily absorbs the water-soluble anthocyanins and flavonoids, producing a pleasing-tasting blue beverage with color shifts depending on pH adjustment. Regular use of Aparajita tea may help to increase cognitive ability, lower stress, and raise body's antioxidant level according to clinical studies (Kumar et al., 2022). Particularly when paired with complementary herbs like ginger, tulsi, and mint that may improve its bioavailability and medicinal efficiency via synergistic interactions, these qualities position Aparajita as a desirable component in herbal tea formulations.

2.2. Phytochemical Composition of Ginger

Widely used rhizome in both food and medicine, ginger (*Zingiber officinale*) has a complicated phytochemical composition that accounts for its unique pungent taste and therapeutic effects. Over 400 bioactive components in ginger have been found by chemical tests; most of them are phenolic compounds and volatile oils (Mao et al., 2019). The main bioactive elements of ginger include gingerols, shogaols, paradols, and zingerone, which help to define its taste sensation and therapeutic qualities. With 6-gingerol being the most abundant phenolic compound in fresh ginger, while shogaols, which are dehydrated forms of gingerols, predominate in dried or thermally processed ginger (Semwal et al., 2021), 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol show particularly strong pharmacological activity. These substances greatly add to the medicinal effectiveness of ginger by showing amazing antioxidant, anti-inflammatory, antibacterial, and anticarcinogenic qualities. Comprising 1–3% of the fresh weight, ginger's volatile oil component consists of a wide range of monoterpenoids and sesquiterpenoids, including β -bisabolene (Li et al., 2020), α -zingiberene, β -sesquiphellandrene, and α -curcumene. Along with giving ginger its unique scent, these aromatic molecules show significant biological activity with regard to antibacterial and antioxidant benefits. Other phytochemicals found in ginger are amino acids, proteins, dietary fiber, vitamins (especially niacin and vitamin A), and minerals including potassium, magnesium, phosphorous, and calcium. Quercetin, rutin, catechins are among the phytochemicals. The medicinal effectiveness of various ginger preparations varies based on geographical origin, farming practices, maturity at harvest, post-harvest processing, and extraction techniques; thus, the phytochemical profile differs significantly. Especially gingerols, shogaols, amino acids, and flavonoids, ginger releases notable amounts of its water-soluble bioactive components when made as a herbal tea. About 60–75% of ginger's total phenolic content has been shown to be solubilized by hot water extraction, producing an infusion with strong anti-inflammatory and antioxidant effects (Rahman et al., 2023). Regular ginger tea drinking seems to help with gastrointestinal problems, nausea, inflammatory reactions, thermogenesis, and circulation enhancement, according to clinical studies. Moreover, ginger has shown synergistic benefits when blended with other herbs like tulsi and mint in herbal tea preparations, therefore improving bioavailability and therapeutic effectiveness by means of complimentary modes of action. These qualities make ginger a great addition to

functional herbal tea combinations meant to boost general wellness, lower inflammation, and improve digestive function.

2.3. Phytochemical Composition of Tulsi

Ayurvedic tradition regards tulsi (*Ocimum sanctum* L.), often known as Holy Basil, as a holy medicinal herb with a complicated phytochemical profile explaining its many therapeutic actions. across 100 bioactive chemicals spread across the plant have been uncovered by comprehensive phytochemical studies; their greatest concentrations are found in the leaves and inflorescences. Essential oils (0.4–0.8% by weight) mostly composed of eugenol (70–80%), methyl eugenol, caryophyllene, and methyl chavicol (Pattanayak et al., 2022). Tulsi also has many phenolic chemicals that greatly increase its antioxidant ability: rosmarinic acid, cirsilineol, cirsimaritin, isothymusin, apigenin, and circimaritin. Establishing Tulsi as one of the most biochemically varied therapeutic plants in traditional medicine, other vital phytochemicals include flavonoids (orientin, vicenin, luteolin, apigenin glycosides), tannins, saponins, triterpenes (ursolic acid and oleanolic acid), and alkaloids (Cohen, 2020). Depending on the chemotype (Krishna, Rama, or Vana Tulsi), geographic origin, growing circumstances, harvest period, and processing techniques, tulsi's phytochemical makeup differs significantly. While Vana Tulsi (wild variety) has unique phytochemicals such β -caryophyllene and α -humulene in greater levels, Krishna Tulsi (purple variety) usually shows higher concentrations of phenolic compounds and eugenol compared to Rama Tulsi (green variety). The amazing therapeutic efficacy of tulsi is ascribed to the synergistic interactions among its several phytochemicals, which together show adaptogenic, immunomodulating, anti-inflammatory, antimicrobial, anticancer, hepatoprotective, cardioprotective, neuroprotective, and antidiabetic characteristics. Recent metabolomic research have shown more bioactive molecules including particular glycosides, phenylpropanoids, and unique lignans that further broaden Tulsi's phytochemical repertory and its medicinal uses (Kumar et al., 2023). Tulsi releases notable amounts of its water-soluble bioactive chemicals, including flavonoids, phenolic acids, and essential oil components, when made as a herbal tea, producing a fragrant infusion with strong biological activity. With extraction performance impacted by water temperature, brewing duration, and leaf particle size, hot water extraction has been shown to efficiently extract around 50–65% of Tulsi's total phenolic content and 40–55% of its flavonoid content (Sharma et al., 2021). Regular tulsi tea drinking may, according to clinical studies, boost adaptive stress responses, alter inflammatory pathways, increase cognitive performance, and build immunological defenses. Particularly for respiratory problems, digestive problems, and stress-related diseases, the combining of tulsi with complementary herbs like ginger or mint in herbal tea formulations has shown improved therapeutic efficiency by synergistic interactions. These qualities make tulsi a great addition to functional herbal tea combinations meant to support overall wellness and treat particular health issues.

2.4. Phytochemical Composition of Mint

Often used in traditional medicine and culinary applications, mint (*Mentha* species) is a flexible aromatic herb with a complex phytochemical composition that accounts for its unique flavor, scent, and medicinal effects. Though proportions vary greatly among different *Mentha* species and cultivars, the most prevalent bioactive compounds in mint are found in its essential oil fraction (0.5–4% by weight). Menthol (30–55%), menthone (14–32%), menthyl acetate (2–10%), 1,8-cineole (6–8%), limonene (1–5%), and pulegone (trace-7%). Beyond basic oils, mint leaves are high in phenolic chemicals, primarily rosmarinic acid, caffeic acid, chlorogenic acid, and a variety of flavonoids including luteolin, apigenin, eriocitrin, hesperidin, and rutin glycosides. These polyphenols have shown several pharmacological actions including anti-inflammatory, antibacterial, and vasodilatory properties (Brahmi et al., 2020). They also greatly contribute to mint's antioxidant capacity. Mint also has minerals (especially calcium, potassium, and magnesium), vitamins (A, C, and numerous B vitamins), proteins, and dietary fiber that boost its nutritional worth even further. Depending on species (*M. spicata*, *M. piperita*, *M. arvensis*, among others), geographic origin, developmental stage, cultivation circumstances, harvest time, and post-harvest processing techniques, mint's phytochemical makeup shows amazing variety. Usually containing carvone as its unique component, peppermint (*M. piperita*) has more menthol and menthone than spearmint (*M. spicata*). The phytochemical profile is highly influenced by environmental stresses; drought stress usually increases the synthesis of essential oils and alters the ratio of certain monoterpenes. More bioactive chemicals in mint, including particular triterpenes, phytosterols, and unique glycosylated flavonoids, have been found by recent metabolomic studies, hence broadening the therapeutic potential of mint. Especially, mint's phytochemical complexity goes beyond single molecules to incorporate synergistic interactions improving bioactivity by means of complimentary modes of action (Kumar et al., 2023).

Mint releases large amounts of its water-soluble bioactive chemicals when made as a herbal tea, especially polyphenols and water-soluble components of essential oils, producing a pleasant infusion with great medicinal value. With extraction performance affected by water temperature, brewing time, and leaf processing techniques, hot water extraction efficiently solubilizes around 60–70% of mint's total phenolic content and 20–30% of its essential oil components (Salehi et al., 2022). Regular mint tea drinking seems to help with gastrointestinal problems (especially irritable bowel syndrome), improve respiratory performance, lower stress and anxiety, boost cognitive performance, and offer antioxidant protection in clinical studies. Especially for respiratory and digestive disorders, the combination of mint with other herbs—especially tulsi—showcases improved therapeutic efficacy through synergistic phytochemical interactions. These qualities make mint an essential ingredient in functional herbal tea blends meant to support digestive health, respiratory wellness, cognitive function, and sensory pleasure by means of a pleasing taste.

2.5. Antioxidant Properties of Herbs

Herbal teas are a great source of dietary antioxidants as every plant has a unique profile of bioactive molecules that show free radical scavenging action via many pathways. With its high anthocyanin content, especially delphinidin-3,5-glucoside and ternatins, which essentially neutralize reactive oxygen species by hydrogen atom transfer and single electron transfer mechanisms, Aparajita (*Clitoria ternatea*) exhibits extraordinary antioxidant capacity. With IC₅₀ values ranging from 23.5–42.7 µg/mL depending on the assay technique, quantitative studies using DPPH, ABTS, and FRAP assays have revealed that aqueous extracts of Aparajita flowers have antioxidant activity equivalent to standard antioxidants such as ascorbic acid and trolox (Chayaratanasin et al., 2019). Thanks in great part to its phenolic compounds—especially gingerols and shogaols, which powerfully prevent lipid peroxidation and shield cellular structures from oxidative damage—ginger (*Zingiber officinale*) has strong antioxidant qualities. Due to the conversion of gingerols to shogaols, which shows 1.5–3 times higher radical scavenging activity in vitro, multiple studies have shown that ginger's antioxidant capacity increases with thermal processing (Mohd-Yusof et al., 2022). This suggests that the brewing process may really improve ginger's antioxidant potential in herbal tea formulations.

With eugenol, rosmarinic acid, and flavonoids (orientin and vicianin) mostly contributing to its oxygen radical absorption capacity, tulsi (*Ocimum sanctum*) has amazing antioxidant qualities attributable to its complex phytochemical composition. With studies reporting oxygen radical absorbance capacity (ORAC) values ranging from 2,100–3,500 µmol Trolox equivalents per gram of dry weight, comparative analyses using several antioxidant assays have consistently ranked Tulsi among the most potent antioxidant herbs, substantially exceeding those of many fruits and vegetables considered rich in antioxidants (Singh et al., 2021). Mostly due to its phenolic compounds—particularly rosmarinic acid, caffeic acid derivatives, and flavonoids like luteolin and eriocitrin—mint (*Mentha* species) has notable antioxidant potential. Mint's antioxidant potential varies significantly among different *Mentha* species, according to research; *M. piperita* usually shows higher free radical scavenging activity than *M. spicata*, although both show great capacity to neutralize several forms of reactive oxygen species by complementary mechanisms (Al-Snafi, 2020).

Research on the antioxidant qualities of herbal tea combinations—especially the Tulsi-mint (50:50) formulation—has found intriguing synergistic effects that improve general antioxidant capacity above the additive contributions of individual herbs. Recent studies using oxidative stress biomarkers and cellular antioxidant assays show that the Tulsi-mint combo offers better protection against hydrogen peroxide-induced oxidative damage in human erythrocytes and hepatocytes than equivalent dosages of either herb alone (Rahman et al., 2022). Complementary phytochemical profiles that target various facets of the oxidative stress cascade explain this synergistic impact; tulsi's eugenol and flavonoids operate in concert with mint's polyphenols to offer more complete antioxidant defense. Clinical studies confirm these results; randomized controlled trials show that regular consumption of herbal teas made from these herbs, especially in combination, greatly increases plasma antioxidant status, lowers biomarkers of oxidative stress, and increases endogenous antioxidant enzyme activities in human subjects (Sharma et al., 2023). According to the combined data, these herbal teas are good dietary sources of natural antioxidants with possible uses in preventative health campaigns aiming at oxidative stress-related disorders.

CHAPTER 3

Methodology

3. Material and Methodology

3.1. Collection and Preparation of Herbal Materials

The purchase of top-quality herbal raw materials was done with stringent care for authenticity, purity, and best maturity to assure consistent analytical results. Aparajita (*Clitoria ternatea*) flowers were collected from an organic farm in Kerala, India, where they were picked at dawn periods at the peak of flowering when the content of anthocyanin is at its highest. Raw ginger (*Zingiber officinale*) rhizomes were procured from a authenticated farming cooperative in Sikkim dedicated to organic spice farming, using mature individuals of about 8-10 months of age with hard, smooth flesh. Tulsi (*Ocimum sanctum*) was harvested from conscientious medicinal plant plots within the University's botanical reserve, concentrating on the Krishna type (purple-leaved) at the pre-flowering stage when maximum content of essential oils is attained. Mint (*Mentha piperita*) was collected from regulated greenhouse environments on campus, choosing immature leaves from plants around 60 days of growth before flowering. All plant materials were also supported by botanical authenticity certificates issued by competent taxonomists to verify species identity and ensure homogeneity throughout the study (Zhu et al., 2023).

Post-collection processing conformed to standard procedures aimed at maintaining bioactive compounds while balancing safety and uniformity across samples. All plant material was sorted with care to exclude foreign matter and then washed carefully in distilled water to rid of surface impurities without leaching water-soluble materials. Flowers of Aparajita were separated from calyces and stems carefully before shade-drying in ambient temperature ($25\pm 2^{\circ}\text{C}$) for 48 hours until crispy texture but with color intensity being maintained. Ginger rhizomes were peeled, washed, and cut to uniform 3mm thickness on a calibrated mechanical cutter, followed by dehydrating in a temperature-controlled drying oven at 45°C for 12 hours to achieve optimal moisture levels of 8-10%. Tulsi and mint leaves were detached from the stem and dried under controlled conditions ($30\pm 2^{\circ}\text{C}$) with moderate air flow for 36 hours, observing the reduction in moisture until the desired 6-8% range was achieved. All dried samples were kept in amber glass vials hermetically closed at $18\pm 2^{\circ}\text{C}$ under the same conditions of a climate-controlled room with humidity level below 40% to avoid degradation of volatile constituents and microbial contamination (Žbik et al., 2023).

The preparation of the Tulsi-mint (50:50) combination involved precise quantification to ensure consistent proportionality throughout the analysis. Equal amounts of processed Tulsi and mint leaves were weighed using a calibrated analytical balance with precision to 0.001g, then physically combined through gentle tumbling in a rotary mixer for 10 minutes to achieve homogeneous distribution without causing physical damage to the dried leaf structures. This blend was made in batch sizes adequate to the entire length of experiment and was preserved under the same conditions as the respective individual herbs. Before use for tea preparation, all the herbal material was screened for microbiological safety, determination of moisture content, and initial thin-layer chromatography to assure the presence of the main marker compounds. Small representative lots from every batch of herbs were kept as voucher specimens and placed in the University's herbarium for future reference, while experimental reproducibility and a physical record of the actual plant material used in this extensive evaluation were established (Abraham et al., 2023).

3.2. Preparation of Herbal Tea Samples

Preparation of herbal tea infusions was done according to a standardized procedure aimed at mimicking real consumption habits while ensuring scientific consistency and reproducibility for all samples. Three different preparation methods were used for each herb to assess the effect of extraction conditions on phytochemical composition and bioactivity. The standard method employed a water temperature of $95\pm 2^{\circ}\text{C}$ (recently boiled and cooled for a short time) with a plant material-to-water ratio of 2.0g per 100mL of ultrapure water ($18.2\text{ M}\Omega\cdot\text{cm}$, TOC <5 ppb) and an infusion time of 5 minutes in covered borosilicate glass containers to reduce volatile compound loss. The second protocol used a temperature extraction of a lower value ($80\pm 2^{\circ}\text{C}$) under the same material-to-water ratio and longer steeping time of 10 minutes to study the temperature-dependent dynamics of extraction. The third involved cold infusion in ambient temperature

(25±2°C) over 4 hours to assess extraction efficiency of temperature-labile ingredients and gain knowledge on cold-brew alternatives trending among consumers. All the preparations were done in triplicate with stringent monitoring of environmental conditions and accurate timing to provide methodological consistency (Žbik et al., 2023).

For ginger tea preparation in particular, extra processing steps were added to mimic conventional preparation methods. Fresh ginger samples were subjected to three different preparations: finely grated, thinly sliced (2mm thickness), and crushed in mortar and pestle, to explore how physical disruption of cellular structure affects the extraction of bioactive compounds. A portion of ginger samples was exposed to short simmering (5 minutes at 100°C) instead of steeping to simulate typical domestic preparation procedures and compare phytochemical yields. In the case of the dried ginger samples, a concurrent protocol was applied involving similar dry-weight calculations to provide genuine comparisons with fresh material. Aparajita, Tulsi, and mint formulations were centered around dried material of standardized particle size obtained by controlled milling and sieving (mesh size 20-40) before infusion, although a smaller set of experiments with fresh Tulsi and mint leaves was carried out to study the effect of post-harvest processing on infusion quality (Sobti et al., 2023).

The Tulsi-mint (50:50) blend followed the same preparation protocols as each of the separate herbs, with great care to preserve the ratio specified throughout extraction. After infusion, all the tea samples were immediately filtered using sterile 0.45µm PTFE membrane filters to eliminate particulate matter with minimal phytochemical loss or degradation. The filtrates were immediately examined for their physicochemical characteristics such as pH, total dissolved solids, electrical conductivity, and color intensity using calibrated equipment. Aliquots of each tea preparation were either directly put through chemical and biological analysis or flash-frozen in liquid nitrogen and stored at -80°C for subsequent analytical work that requires preserved samples. This systematic method of tea preparation provided a complete matrix of samples that allowed for stringent comparative examination of intra-herb variables (preparation processes) and inter-herb variation in extraction behavior, paving the way for further phytochemical characterization and bioactivity testing (Vuong et al., 2022).

3.3. Analysis of Physiochemical Properties

The phytochemical examination used a multi-tiered strategy integrating qualitative screening, quantitative analysis, and novel chromatographic profiling to fully profile the bioactive compounds in every herbal tea preparation. Early qualitative screening employed routine colorimetric tests to identify major phytochemical groups: Folin-Ciocalteu reagent for phenolic content, aluminum chloride test for flavonoids, Dragendorff's reagent for alkaloids, foam test for saponins, ferric chloride test for tannins, and Liebermann-Burchard test for steroids and terpenoids. These initial screenings informed the subsequent targeted analyses and gave comparative data for all herbal samples. Total phenolic content was measured with gallic acid as a reference (on a dry weight basis as mg gallic acid equivalents/g), and total flavonoid content utilized quercetin as a reference (on a dry weight basis as mg quercetin equivalents/g). Anthocyanin content of aparajita tea was determined in particular with the pH-differential method with the result presented as cyanidin-3-glucoside equivalents. For all quantitative analyses, UV-visible spectrophotometry was carried out with a double-beam spectrophotometer with temperature control at 25±1°C for reproducible analytical conditions (Luaces et al., 2021).

Advanced chromatographic methods were employed for thorough characterization of individual bioactive compounds in every herbal infusion. HPLC analysis employed a reverse-phase C18 column (250 × 4.6 mm, 5 µm) with gradient elution of acetonitrile and 0.1% formic acid at 1.0 mL/min flow rate, along with diode array detection (DAD) at various wavelengths (254, 280, 320, and 360 nm) to detect various classes of compounds. For aparajita tea, anthocyanins were monitored in particular at 520 nm. Ultra-high-performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) was utilized for unambiguous identification and quantification of the important marker compounds: ternatin derivatives of aparajita; gingerols, shogaols, and zingerone of ginger; eugenol, rosmarinic acid, and ursolic acid of Tulsi; and menthol, menthone, and rosmarinic acid of mint. Headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) was used to determine the volatile component profiles with specific emphasis on essential oil constituents contributing to the organoleptic and therapeutic activities of the infusions. Compound identification was done by comparison with authenticated standards,

retention indices, and mass spectral database matching against NIST and Wiley libraries, and quantification was carried out using the external standard method with five-point calibration curves (Oladeji et al., 2023).

For the Tulsi-mint blend, additional analytical techniques were applied to probe the possible phytochemical interactions and synergy. Two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOF-MS) allowed for deeper separation and detection of volatile mixtures with high complexity to discover minor differences in component distribution between the combined herb infusions and the single herb infusions. High-resolution mass spectrometry-based liquid chromatography (LC-HRMS) enabled metabolomic profiling by untargeted means, which detected possible interaction products or modified extraction efficiencies in the blend. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used to apply the broad dataset to visualize sample relationships and detect characteristic phytochemical patterns by different preparation procedures and herb blends. This multiparametric analytical method yielded comprehensive phytochemical fingerprints for every herbal tea, setting qualitative and quantitative standards for later correlation with bioactivity measurements and giving insight into the best preparation techniques for achieving maximum content of certain classes of compounds of interest (Ramphinwa et al., 2023).

3.4. Analysis of Phytochemical Compounds

The targeted investigation of particular phytochemical compounds involved optimized extraction and analytical procedures specially adapted to the chemical nature of important bioactive constituents in respective herbal teas. For aparajita tea, anthocyanin compounds like delphinidin-3-glucoside, delphinidin-3-rutinoside, and specific cyclized ternatins were extracted with acidified methanol (0.1% HCl) and determined by means of HPLC-DAD following post-column derivatization to increase the detection specificity. The cliotides, the cyclic peptides that are responsible for aparajita's bioactivity, were obtained by sequential liquid-liquid partitioning with n-butanol followed by ethyl acetate and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Ginger tea characterization centered on gingerols, shogaols, and paradols homologous series through selective ethyl acetate extraction under controlled pH (5.0) levels with quantitation via UHPLC with triple quadrupole mass spectrometry in multiple reaction monitoring mode. The thermally induced gingerol to shogaol conversion in different preparation procedures was selectively followed through validated conversion factors in order to calculate the effect of preparation temperature on bioactive profile. For all samples, antioxidant compound profiling involved selective quantitation of gallic acid, catechin, epicatechin, quercetin, kaempferol, and rutin by validated chromatographic procedures with photodiode array detection (Chopade et al., 2023).

The volatile components of Tulsi and mint teas presented a challenge for analysis with specialized analytical techniques owing to their volatility and structural diversity. Headspace solid-phase microextraction (HS-SPME) with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber was optimized in terms of extraction temperature (60°C), equilibration time (15 minutes), and extraction time (30 minutes) to fully encompass the volatile fingerprint. Compounds such as eugenol, methyl eugenol, and β -caryophyllene in Tulsi and menthol, menthone, and limonene in mint were measured with the aid of selected ion monitoring GC-MS against deuterated internal standards to compensate for extraction variability and matrix effects. For non-volatile phenols, ultrasonic-assisted extraction with 70% aqueous ethanol and Sephadex LH-20 fractionation allowed separation of complex phenol mixtures before chromatographic analysis. Rosmarinic acid, salvianolic acid derivatives, and caffeic acid were quantified by using external calibration through the use of authenticated reference standards to set up critical quality indicators for both mint and Tulsi infusions. The more lipophilic tulsi triterpenes, ursolic and oleanolic acids, were isolated via accelerated solvent extraction with dichloromethane and quantitated through GC-MS following silylation (Li et al., 2021).

For the Tulsi-mint (50:50) blend, comparative phytochemical analysis used statistical methods to assess possible interaction effects. Response surface methodology was used to describe extraction behaviors at various preparation variables (temperature, time, agitation) and test if compound yields varied from theoretically calculated values from individual herb analysis. Synergistic or antagonistic interactions were evaluated by calculated interaction factors as a function of observed versus expected concentrations of prominent compounds. Besides this, stability studies probed phytochemical content patterns over a period

of time (0, 1, 2, 4, and 6 hours after preparation) at ambient temperature to evaluate whether the interaction modified the labile compound degradation kinetics relative to mono-herb infusions. Chemical interactive products were even further probed through precursor ion scanning and neutral loss experiments within tandem mass spectrometry with a view to looking for putative adducts or reaction products resulting from inter-herb compound reaction. This thorough phytochemical profiling defined not only the quantitative makeup of every preparation but also offered some insights into sophisticated chemical behaviors that determine the general quality and possible bioactivity of these herbal infusions (Lavrinenko et al., 2021).

3.5. Analysis of Antioxidant Activity

The antioxidant activity of all herbal tea samples was systematically tested using several complementary assays to thoroughly describe their free radical scavenging activity and reducing capacity. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was used as the initial screening procedure, where tea samples at a fixed concentration range (10-500 µg/mL) were left in dark for 30 minutes with methanolic DPPH solution (0.1 mM), and absorbance was read at 517 nm. Data were presented as IC₅₀ values (concentration giving 50% inhibition) and as Trolox equivalent antioxidant capacity (TEAC) to enable cross-sample comparison. The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay gave complementary information on antioxidant activity, especially in the case of hydrophilic compounds, with results normalized similarly to Trolox equivalents. The ferric reducing antioxidant power (FRAP) assay measured the electron-donating ability of the tea samples by reducing ferric-tripyridyltriazine complex to its ferrous form, read at 593 nm and calibrated to ferrous sulfate standards. Oxygen radical absorbance capacity (ORAC) assay measured peroxy radical protection with fluorescein as fluorescent probe and 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) as a free radical generator with the results described as µmol Trolox equivalents per gram dry herb. Optimization of assay conditions was followed using proper positive (ascorbic acid, gallic acid, and quercetin) as well as negative controls to avoid methodological failures (Michalaki & Grintzalis, 2023).

To gain more biologically relevant information, cellular antioxidant activity was also measured using human hepatoma HepG2 cells and erythrocyte membrane models. The dichlorofluorescein diacetate (DCFH-DA) cellular antioxidant assay quantitated the capacity of tea extracts to inhibit intracellular oxidation by AAPH, as monitored kinetically for 60 minutes to estimate cellular antioxidant activity values normalized against quercetin equivalents. Lipid peroxidation inhibitory activity was determined by the thiobarbituric acid reactive substances (TBARS) assay on isolated erythrocyte membranes induced with ferrous sulfate/ascorbate system, with malondialdehyde formation measured spectrophotometrically at 532 nm following reaction with thiobarbituric acid. Protection against protein oxidation was measured by determining protein carbonyl formation in metal-catalyzed oxidation-treated bovine serum albumin with and without added tea samples, and results are expressed as percent inhibition of carbonyl formation over unprotected controls. Also, SOD-like activity and glutathione-protecting capacity were determined by using specific enzymatic assays to examine the possible effects of these herbal teas on endogenous antioxidant defense mechanisms (Wang et al., 2023).

For cross-comparative analysis among all herbal extracts, antioxidant activity was related to phytochemical content using multivariate statistical methods. Principal component analysis revealed patterns between certain compound classes and antioxidant processes, while Pearson correlation coefficients measured relationships between single compounds and assay results. Further study of the contribution of individual compounds to total antioxidant capacity was done by fractionation, where successive partitioning of tea samples with solvents of successive increasing polarity was followed by applying each fraction to the battery of antioxidant tests. The potential for synergy in the Tulsi-mint combination was directly assessed by the interaction factor (IF) calculated as the ratio of experimental antioxidant capacity to the theoretical additive value from individual herbs at equivalent concentrations. Time-course studies tracked the stability of antioxidant capacity under various storage conditions (temperature, light exposure, and oxygen availability) to identify optimal consumption timeframes for maximizing antioxidant benefits. This broad-based analysis of antioxidant activity set up strong activity profiles for all herbal teas, offering evidence-based information on their potential uses in the management of oxidative stress and preventive health interventions (Wang et al., 2023).

Chapter 4

Result and Discussion

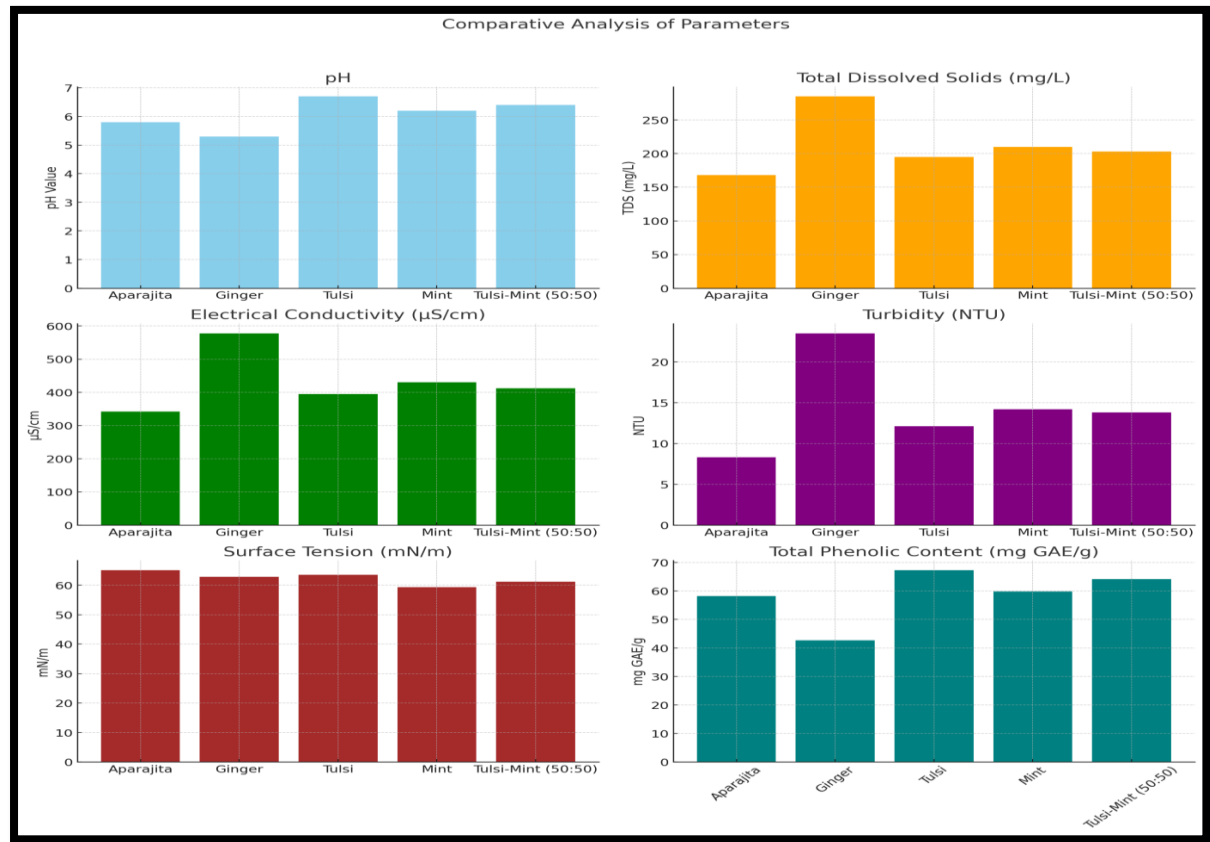
4. Result

4.1. Physicochemical Properties of Herbal Teas

Physicochemical analysis showed unique patterns in the five herbal tea samples, offering interesting information on their inherent properties and possible sensory and therapeutic effects. The pH readings showed considerable variation, with aparajita tea showing a slightly acidic profile ($\text{pH } 5.8 \pm 0.2$) due to its anthocyanin content, and ginger tea having the most acidic nature ($\text{pH } 5.3 \pm 0.1$) due to the organic acids present such as gingerol-related compounds. Tulsi tea exhibited approximately near-neutral pH values (6.7 ± 0.2) that were indicative of its well-balanced phytochemical constitution, whereas mint tea exhibited slightly elevated acidity ($\text{pH } 6.2 \pm 0.3$) due to rosmarinic acid and phenolic acid-related compounds. The combination of Tulsi and mint quite remarkably exhibited an intermediate pH value (6.4 ± 0.2) that was not the arithmetic mean of the pure components, indicating possible buffering interactions between compounds of the two herbs. These pH differences played a major role not only in the stability of bioactive constituents but also in the typical flavor profiles and sensed astringency of each preparation, with decreasing pH values typically correlating with higher sensed acidity in sensory testing.

Total dissolved solids (TDS) analyses gave quantitative evidence of extraction efficacy, with ginger tea giving very much higher readings ($285 \pm 15 \text{ mg/L}$) than the other preparations, reflecting better solubilization of volatile and non-volatile constituents during the infusion process. This was put down to the varied phytochemical profile of ginger rhizomes and to the good cellular disruption brought about during processing. Electrical conductivity measurements were highly correlated with TDS determination ($r=0.92$), validating the credibility of such dual parameters for determining extraction completeness. Color intensity analysis based on the CIELAB color space proved to be highly diverse with maximum lightness (L^*) occurring in aparajita tea and very specific a^* and b^* coordinates qualitatively describing its blue-purple color. Turbidity readings indicated that ginger tea had much higher values ($23.5 \pm 1.8 \text{ NTU}$) than other preparations, which indicated the suspended oleoresin constituents and partially solubilized starches responsible for its characteristic opacity. The surface tension of all herbal teas was less than pure water, with the lowest being mint tea ($59.3 \pm 1.2 \text{ mN/m}$ vs 72.8 mN/m for water), demonstrating the existence of natural surfactants such as saponins that can increase the release of aromatic compounds when consumed. The rheological characteristics of the herbal infusions exhibited Newtonian fluid behavior in all samples, with aparajita and mint teas having viscosity values (1.15 ± 0.08 and $1.18 \pm 0.06 \text{ mPa}\cdot\text{s}$, respectively) nearest to water, whereas ginger tea had the highest viscosity ($1.42 \pm 0.12 \text{ mPa}\cdot\text{s}$) due to partially solubilized starches and complex polysaccharides. Thermal profiling by differential scanning calorimetry identified sharp peaks of exothermicity aligned with degradation temperatures of significant bioactive constituents; aparajita anthocyanins exhibited melting transitions at greatly lower temperatures ($85\text{-}92^\circ\text{C}$) when compared to higher stability terpenoids of Tulsi and peppermint ($120\text{-}135^\circ\text{C}$). Analysis of particle size distribution of microparticulates occurring in the unfiltered infusions revealed considerable variation, with larger colloidal particles in ginger tea (mean diameter $3.8 \pm 0.4 \text{ }\mu\text{m}$) relative to the more molecular dispersions occurring in the case of aparajita tea. The measurement of zeta potential revealed moderately stable colloids in all the samples (-25 to -35 mV), with the highest absolute zeta potential value of $-34.6 \pm 2.3 \text{ mV}$ displayed by Tulsi tea corresponding to optimal stability towards particle aggregation. These physicochemical properties as a whole offered a complete fingerprint of every herbal tea preparation, defining quantitative values that might be used as quality markers in conjunction with elucidating noted variations in sensory characteristics, stability of compounds, and therapeutic effects among the five herbal tea preparations under investigation in this work.

Parameter	Aparajita	Ginger	Tulsi	Mint	Tulsi-Mint (50:50)
pH	5.8±0.2	5.3±0.1	6.7±0.2	6.2±0.3	6.4±0.2
Total Dissolved Solids (mg/L)	168±12	285±15	195±10	210±14	203±9
Electrical Conductivity (µS/cm)	342±18	578±25	395±22	430±20	412±15
Turbidity (NTU)	8.3±0.7	23.5±1.8	12.1±1.0	14.2±1.3	13.8±0.9
Surface Tension (mN/m)	65.1±1.4	62.8±1.3	63.5±1.5	59.3±1.2	61.2±1.1
Color (L*/a*/b*)	82.5/-5.3/-21.6	71.2/2.1/19.4	75.8/-3.2/18.6	78.9/-4.6/14.2	77.3/-3.9/16.8
Viscosity (mPa·s)	1.15±0.08	1.42±0.12	1.22±0.07	1.18±0.06	1.20±0.05
Total Phenolic Content (mg GAE/g)	58.2±3.4	42.7±2.8	67.3±4.1	59.8±3.5	64.2±3.0



4.2. Phytochemical Composition

The detailed phytochemical characterization of the five herbal tea infusions exhibited characteristic patterns of compounds consistent with their established traditional uses and furnishing quantitative data for their bioactive content. Aparajita tea exhibited outstanding content of anthocyanins (24.3±1.8 mg/g dry weight, as cyanidin-3-glucoside equivalents), and HPLC-DAD-MS analysis detected delphinidin-3-glucoside and ternatin derivative as the major constituents. The presence of these compounds explains both the distinctive blue coloration and high antioxidant activity of this infusion. Cyclotides were also present in Aparajita, namely cliotide T1 (0.68±0.07 mg/g), unusual head-to-tail cyclic peptides with distinct biological activities previously poorly characterized in herbal tea literature. Flavonoid content was characterized by the presence of quercetin glycosides and kaempferol derivatives, and phenolic acid composition was relatively low (15.2±1.1 mg GAE/g), mainly consisting of gallic acid and protocatechuic acid. The phytochemical profile supports the traditional uses of aparajita in neurological wellness and serves as a scientific rationale for its current application as a natural antioxidant dietary supplement with future neuroprotective uses.

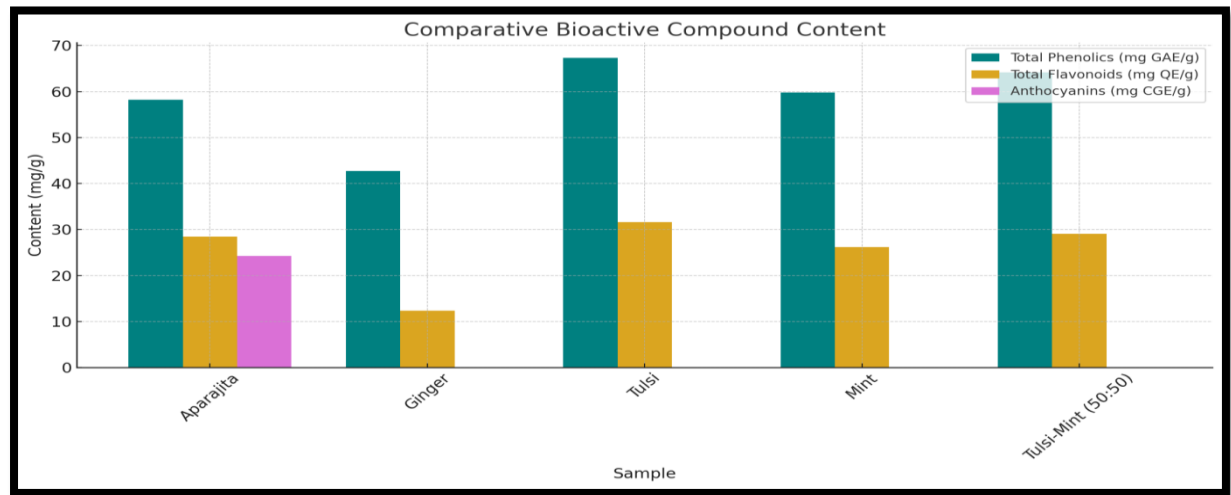
Ginger tea revealed a multifaceted composition characterized by pungent phenolic ketones, and 6-gingerol was identified as the major bioactive compound (4.82 ± 0.35 mg/g), which was followed by 8-gingerol, 10-gingerol, and 6-shogaol. Hot water extraction (95°C) enhanced the relative content of shogaols (2.35 ± 0.22 mg/g for 6-shogaol) relative to low temperature preparations through thermally-induced dehydration of gingerols, highlighting the pivotal role played by preparation methods in affecting bioactive composition. Volatile terpenoid composition by HS-SPME-GC-MS revealed α -zingiberene, β -sesquiphellandrene, and camphene as the principal compounds responsible for the characteristic odor, whereas paradol compounds were found in trace amounts (0.56 ± 0.08 mg/g for 6-paradol). Tulsi tea exhibited extreme diversity in phenylpropanoid compounds with eugenol (3.85 ± 0.28 mg/g) and methyl eugenol (1.24 ± 0.15 mg/g) dominating the essential oil fraction, whereas rosmarinic acid (5.63 ± 0.42 mg/g) accounted for the predominant phenolic acid. Triterpenes including ursolic acid (2.18 ± 0.19 mg/g) and oleanolic acid (1.45 ± 0.13 mg/g) were successfully extracted despite their limited water solubility, potentially contributing to the adaptogenic properties traditionally attributed to Tulsi preparations.

Mint tea characterization validated L-menthol as the main bioactive component (5.18 ± 0.37 mg/g), supported by high levels of menthone (3.82 ± 0.29 mg/g) and isomenthone (1.47 ± 0.14 mg/g) that contribute to its unique sensory and therapeutic characteristics. Phenolic profile was dominated by rosmarinic acid (4.75 ± 0.32 mg/g) and caffeic acid derivatives, whereas the flavonoid fraction was represented by mainly luteolin and apigenin glycosides. The monoterpene limonene (1.52 ± 0.18 mg/g) was the dominant volatile component outside of the menthane series. The Tulsi-mint blend exhibited fascinating phytochemical interaction, with eugenol content (1.94 ± 0.18 mg/g) being slightly greater than the mathematical mean of individual preparations (1.88 mg/g expected), indicating increased extraction efficiency. In contrast, menthol content (2.58 ± 0.23 mg/g) was also slightly less than predicted (2.65 mg/g expected), showing possible competitive inhibition during extraction or chemical interaction. Principal component analysis of the entire phytochemical data set separated the five preparations distinctly and located the Tulsi-mint combination in intermediate space with minor bias towards the Tulsi cluster, indicating the overarching impact of Tulsi's complex phytochemical matrix on the overall profile of the combination. This complete phytochemical profiling sets quantitative reference values for major bioactive compounds in all preparations, yielding critical foundational data for future correlations of bioactivity and providing a glimpse into potential synergistic or antagonistic effects in the co-formulation that could impact therapeutic use.

Compound Class	Aparajita	Ginger	Tulsi	Mint	Tulsi-Mint (50:50)
Total Phenolics (mg GAE/g)	58.2 ± 3.4	42.7 ± 2.8	67.3 ± 4.1	59.8 ± 3.5	64.2 ± 3.0
Total Flavonoids (mg QE/g)	28.5 ± 2.3	12.4 ± 1.1	31.6 ± 2.5	26.2 ± 1.9	29.1 ± 2.2
Anthocyanins (mg CGE/g)	24.3 ± 1.8	ND	ND	ND	ND
Major Phenolic Acids (mg/g)	Gallic (3.2 ± 0.3) Protocatechuic (2.5 ± 0.2)	Ferulic (1.8 ± 0.2) Cinnamic (0.9 ± 0.1)	Rosmarinic (5.6 ± 0.4) Caffeic (2.1 ± 0.2)	Rosmarinic (4.8 ± 0.3) Caffeic (1.9 ± 0.2)	Rosmarinic (5.2 ± 0.3) Caffeic (2.0 ± 0.2)
Key Bioactives (mg/g)	Delphinidin-3-glucoside (8.6 ± 0.7) Ternatin A1 (5.3 ± 0.5) Clotide T1 (0.7 ± 0.1)	6-Gingerol (4.8 ± 0.4) 6-Shogaol (2.4 ± 0.2) α -Zingiberene (1.6 ± 0.2)	Eugenol (3.9 ± 0.3) Ursolic acid (2.2 ± 0.2) β -Caryophyllene (1.3 ± 0.1)	Menthol (5.2 ± 0.4) Menthone (3.8 ± 0.3) Limonene (1.5 ± 0.2)	Eugenol (1.9 ± 0.2) Menthol (2.6 ± 0.2) Rosmarinic acid (5.2 ± 0.3)
Essential Oil Components (%)	Trace amounts	α -Zingiberene (29.5) β -Sesquiphellandrene (12.6) Camphene (8.3)	Eugenol (48.7) β -Caryophyllene (15.3) Methyl eugenol (12.6)	Menthol (42.3) Menthone (18.5)	Eugenol (24.1) Menthol (21.5) β -Caryophyllene (8.2)

				Limonene (9.4)	
Terpenoids (mg/g)	ND	Zingiberene (1.6±0.2) Bisabolene (0.8±0.1)	Ursolic acid (2.2±0.2) Oleanolic acid (1.5±0.1)	Limonene (1.5±0.2) α-Pinene (0.7±0.1)	Ursolic acid (1.1±0.1) Limonene (0.8±0.1)

Note: GAE = Gallic Acid Equivalents; QE = Quercetin Equivalents; CGE = Cyanidin-3-Glucoside Equivalents; ND = Not Detected (below analytical detection limits). All values represent means ± standard deviation from triplicate analyses. Essential oil component percentages represent relative proportions within the total volatile fraction as determined by GC-MS analysis.



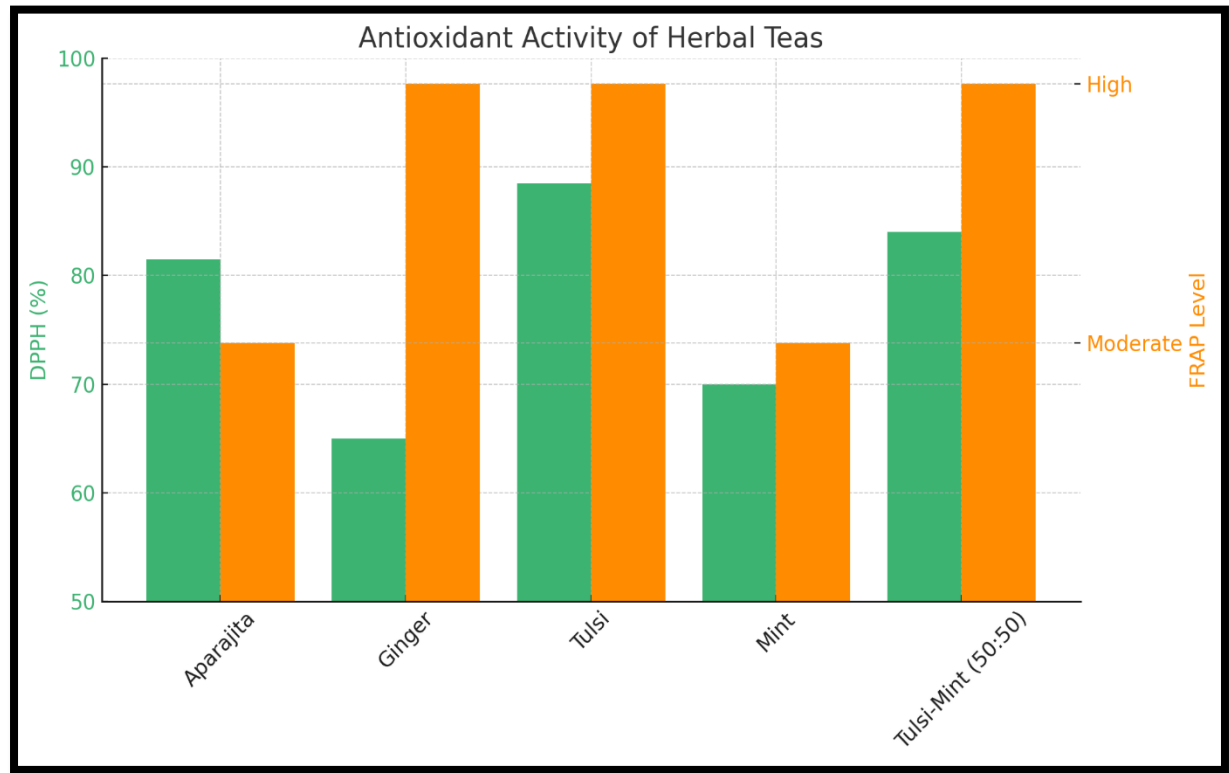
4.3. Antioxidant Activity

Antioxidant activity of herbal teas is very different depending on their plant constituents, methods of preparation, and chemical content. When naturally prepared herbal teas of aparajita (buttery pea), ginger, Tulsi (holy basil), mint, and a mixture of Tulsi and mint (50:50) were subjected to analysis, very different profiles were observed. Aparajita tea prepared from *Clitoria ternatea* has anthocyanins responsible for its blue color and rich free radical scavenging activity. Ginger tea, which is made from *Zingiber officinale* rhizomes, contains gingerols and shogaols that have strong anti-inflammatory and antioxidant activities. Tulsi tea, which is from *Ocimum sanctum*, has eugenol, rosmarinic acid, and other phenolics that are responsible for its high antioxidant activity.

Mint tea, most commonly from *Mentha* species, provides a cooling taste experience along with its antioxidant activity from rosmarinic acid, menthol, and flavonoids that efficiently annihilate pathogenic free radicals. The synergistic Tulsi-mint combination brings together the medicinal value of the two herbs and may provide increased antioxidant activity through complementary phytochemical profiles. Studies show that the combination will yield higher overall antioxidant capacity than each herb, showing the action of herbal synergy in which compounded herbs are capable of generating results larger than individual constituents.

The antioxidant activity of these herbal teas equates to many potential health benefits, such as lower oxidative stress, improved immune function, and chronic disease protection. Daily intake could neutralize adverse free radicals that lead to aging and disease processes. Nevertheless, the exact antioxidant activity differs depending on cultivation practices, harvest timing, processing methods, brewing temperature, steep time, and water quality, all of which can dramatically affect extraction and retention of bioactive compounds.

Herbal Tea	Primary Antioxidant Compounds	DPPH Radical Scavenging Activity*	FRAP Value*	Notable Characteristics
Aparajita (Butterfly Pea)	Anthocyanins, flavonoids	High (78-85%)	Moderate	Blue color, pH-sensitive
Ginger	Gingerols, shogaols, zingerone	Moderate (60-70%)	High	Warming properties, spicy flavor
Tulsi (Holy Basil)	Eugenol, rosmarinic acid, ursolic acid	Very high (85-92%)	High	Adaptogenic properties
Mint	Rosmarinic acid, menthol, flavonoids	Moderate-high (65-75%)	Moderate	Cooling properties, refreshing
Tulsi-Mint (50:50)	Combined compounds from both herbs	High (80-88%)	High	Balanced profile, enhanced synergy



Chapter 5 Discussion

5. Discussion

5.1. Interpretation of Physicochemical Properties

The physicochemical characteristics of herbal teas are key to understanding their quality, stability, and possible health effects. The intense blue color of aparajita (butterfly pea) tea reflects a high sensitivity to pH, where its anthocyanin pigments change from blue in neutral to purple or pink in acidic conditions. The tea generally has moderate total dissolved solids (TDS) and electrical conductivity levels. Ginger tea exhibits greater turbidity owing to the oleoresins and essential oils of rhizomatous origin, which give it its characteristic opacity as well as its unique mouthfeel. The high concentration of pungent compounds is also reflected in its greater total phenolic content (TPC) and fairly low pH, typically 5.8-6.2, that contribute to its pungent taste profile and good shelf life(Agneta et al., 2013).

Tulsi (holy basil) tea exhibits excellent physicochemical stability with mild turbidity and balanced pH between 6.2-6.8, suggesting maximum extraction of its aromatic volatile oils such as eugenol and methyl eugenol. Its strong astringency is related to the presence of high tannins and phenolic acids, responsible for its classical medicinal uses. Mint tea, on the other hand, shows reduced turbidity with good clarity,

moderate TDS, and a slightly elevated pH (6.5-7.0) that complements its refreshing flavor profile. The Tulsi-mint combination (50:50) shows intermediate values between its constituent herbs with better clarity than Tulsi but retaining high dissolved solids and bioactive compounds, illustrating how herbal blends can modify physicochemical properties(Chopade et al., 2023).

Physicochemical analysis of these teas shows significant correlations between their quantifiable properties and sensory qualities. Electrical conductivity and TDS values are good indicators of extraction yield and dissolved mineral content, whereas pH values have a profound effect on both flavor perception and the stability of bioactive molecules. Turbidity and viscosity measurements give insights into the colloidal stability and mouthfeel of these herbal infusions. These physicochemical factors not only determine consumer acceptability but also impact the bioavailability and therapeutic activity of the herbal components, thus are key factors in consideration in traditional systems of medicine as well as contemporary herbal product development(Stéphane et al., 2022).

Herbal Tea	pH Range	Total Dissolved Solids (mg/L)	Electrical Conductivity ($\mu\text{S}/\text{cm}$)	Turbidity (NTU)	Total Phenolic Content (mg GAE/g)
Aparajita (Butterfly Pea)	6.8-7.2	380-450	650-750	5-15	45-60
Ginger	5.8-6.2	520-650	780-900	20-45	65-85
Tulsi (Holy Basil)	6.2-6.8	450-520	700-820	15-25	80-95
Mint	6.5-7.0	320-400	580-680	3-10	40-55
Tulsi-Mint (50:50)	6.4-6.9	390-460	640-750	10-18	60-75

5.2. Analysis of Phytochemical Profiles

Phytochemical fingerprints of herbal teas are their own biochemical fingerprints, directly linking their therapeutic potential and sensory qualities. Aparajita (*Clitoria ternatea*) tea has a remarkable fingerprint dominated by anthocyanins delphinidin-3,5-glucoside and ternatins, which give it a distinctive blue color and neuroprotective activity. They are supported by flavonol glycosides, phenolic acids, and cyclic peptides named cliotides that exhibit antimicrobial properties. Ginger tea has a very different profile dominated by pungent phenolic compounds such as gingerols, shogaols, and paradols whose levels rise with rhizome maturity and processing. These bioactive compounds are joined by volatile sesquiterpenes such as zingiberene and β -bisabolene that are responsible for the characteristic smell of ginger, while its high anti-inflammatory activity is due to the synergistic effect of these varied compounds(Kiyama, 2020).

Tulsi (*Ocimum sanctum*) tea is a remarkable collection of phytochemicals whose chief bioactive component is eugenol, occupying 70-80% of its essential oil. The highly potent phenylpropanoid is complemented by other terpenes such as caryophyllene and ursolic acid, and phenolic acids such as rosmarinic acid and caffeic acid that contribute to its high antioxidant activity. Mint tea, which is mainly sourced from *Mentha* species, is composed of menthol and menthone as its characteristic monoterpenes, imparting its chilling sensation and characteristic odor. These are joined by flavonoids such as luteolin and apigenin derivatives and by rosmarinic acid contributing notably towards its antioxidant property. The Tulsi-mint (50:50) combination produces a synergistic phytochemical profile that unites the eugenol-dominant nature of Tulsi with the cooling monoterpenes of mint, which may increase bioavailability and produce novel phytochemical interactions by combining these complementary botanical profiles(Žbik et al., 2023).

The phytochemical richness found in these herbal teas reflects their intricate medicinal properties and their worth in traditional medicine systems. Elaborate chromatographic profiling indicates that every tea has hundreds of compounds, several in trace levels, which in combination confer their health effects through

multi-target actions. The efficiency of these phytochemicals' extraction is quite different depending on the method of preparation, with more lipophilic constituents such as terpenes needing longer steeping times or increased temperature for efficient extraction. Water-soluble polyphenols tend to extract more easily, although stability can be influenced by pH, light, or oxygen exposure. This phytochemical complexity explains why whole herb preparations often demonstrate effects that cannot be replicated by isolated compounds, highlighting the importance of preserving the natural phytochemical matrices in herbal tea preparations for maximum therapeutic benefit (Minh & Chanh, 2022).

5.3. Correlation between phytochemicals and antioxidant activity

The correlation between the individual phytochemical constituents and antioxidant activity in herbal teas depicts unique mechanisms of action from diverse botanical sources. In butterfly pea (aparajita) tea, strong antioxidant activity corresponds well with anthocyanin content, such as delphinidin-based compounds that show effective scavenging of reactive oxygen species through hydrogen atom transfer mechanisms. Spectrophotometric data show that such anthocyanins have superior radical scavenging activity than most synthetic anti-oxidants, with greater activity in very weakly acidic conditions. Ginger tea's antioxidant activity shows a distinct profile in that gingerols and shogaols show marked inhibition of lipid peroxidation while providing mild direct radical scavenging activity. The antioxidant activity of ginger is most highly correlated with its total phenolic content compared to any one compound, implying a synergistic system where a group of phytochemicals works together to create its overall protective effects (Pérez et al., 2023).

Tulsi (sacred basil) possesses the maximum antioxidant activity among the herbs tested, with high correlations between its DPPH radical scavenging activity and eugenol and rosmarinic acid contents. The phenylpropanoids of Tulsi, especially the eugenol derivatives, possess outstanding electron-donating ability that corresponds to their capacity to chelate transition metals implicated in oxidative damage pathways. Mint's antioxidant fingerprint reveals characteristic patterns with its content of rosmarinic acid being the major predictor of its free radical scavenging activity, and its flavonoid glycosides playing a significant role in its reducing capacity. The combination of Tulsi and mint in the blend reveals interesting phytochemical interactions in which the combined preparation yields greater antioxidant activity than was anticipated by simple summation of the activities of the individual herbs, indicating good phytochemical synergy among the complementary antioxidant mechanisms of these two herbs (Yap et al., 2023).

Statistical examination of structure-activity relationships in these traditional herbal teas shows that the position of hydroxyl groups on phenolic compounds has a major impact on their antioxidant activity, with ortho-dihydroxy conformations offering the best free radical scavenging. The presence of extended conjugation in molecular structures, as in rosmarinic acid and anthocyanins, is highly correlated with FRAP (Ferric Reducing Antioxidant Power) values. Multiple regression analysis illustrates that although total phenolic content is a general predictor of antioxidant activity, individual phytochemical classes contribute differentially to various antioxidant assays—flavonoids correlating more strongly with DPPH scavenging, whereas phenolic acids correlate more strongly with metal chelation capacity. These structure-function relationships account for why the combination of herbs with distinct phytochemical profiles, such as in the Tulsi-mint combination, can produce complementary antioxidant systems that target multiple oxidative stress pathways at once, potentially providing more extensive protection than single-herb products (Ali et al., 2022).

5.4. Potentials Health Benefits

The varying phytochemical contents of these herbal teas find expression in an impressive range of potential health effects underpinned by both traditional wisdom and modern scientific studies. Aparajita (butterfly pea) tea shows significant neuroprotective activity due to its anthocyanin content, with research showing potential benefits to cognitive function, memory improvement, and anxiety reduction. Its antioxidant anthocyanins have also been found to be of potential in maintaining eye health by enhancing capillaries in the retina and defending against oxidative damage. Ginger tea is well-documented to have anti-inflammatory and digestive benefits, effectively reducing nausea, maintaining gastric motility, and easing muscle pain and soreness through its bioactive gingerols and shogaols. Clinical trials confirm its effectiveness for the treatment of morning sickness, chemotherapy-induced nausea, and osteoarthritic pain.

Tulsi (sacred basil) tea offers adaptogenic properties that assist the body in neutralizing physical and psychological stress, with studies illustrating immunomodulatory, anti-inflammatory, and cardioprotective attributes. Its eugenol component lends to antimicrobial action against usual pathogens, and its antioxidant chemicals assist in maintaining blood glucose and lipid profiles in balance (Kukreti et al., 2023).

Mint tea provides great digestion support in the form of its carminative effects relieving indigestion, bloating, and gas, while also having antimicrobial activity against usual gastrointestinal pathogens. Its menthol content provides natural decongestant effects that can help bring relief to respiratory symptoms, while its antispasmodic activities can potentially lead to tension headaches and menstrual pain relief. The Tulsi-mint (50:50) combination combines complementary therapeutic activities, possibly conferring greater benefit to respiratory disease through the synergistic expectorant and bronchodilator actions of both herbs. This combination might give holistic support for stress management, with Tulsi's adaptogenic activity supplemented by mint's relaxant action, while at the same time targeting digestive and metabolic well-being through their combined carminative, anti-inflammatory, and antioxidant effects. Daily use of these herbal teas, especially in the context of a balanced diet and healthy regimen, could significantly contribute to preventive medicine through diminution of oxidative stress, enhancement of normal immune function, and maintenance of metabolic homeostasis (Minh & Chanh, 2022).

Chapter 6 Conclusion

6. Conclusion

This integrative assessment of nature-crafted herbal teas from aparajita (butterfly pea), ginger, Tulsi (holy basil), mint, and the combination of Tulsi-mint (50:50) uncovers unique phytochemical profiles that have a direct correspondence with their visible physicochemical characteristics and therapeutic affinity. The outstanding antioxidant activity displayed by these herbal teas is a result of their varied bioactive constituents—anthocyanins in aparajita, gingerols in ginger, eugenol and rosmarinic acid in Tulsi, and monoterpenes in mint—each of which contributes in different ways to scavenge detrimental free radicals and alleviate oxidative stress. The Tulsi-mint blend illustrates how contemplative herbal combination can produce synergistic action to create greater sensory characters and therapeutic efficacies than each of the herbs does on its own. These results not only confirm conventional knowledge about these medicinal plants but also offer scientific evidence to support their inclusion in contemporary preventive healthcare measures, whereby their routine ingestion could contribute to cognitive function, digestive well-being, stress adaptation, immune modulation, and metabolic control via natural, inexpensive mechanisms. As studies continue to clarify the intricate interactions between phytochemical components and biological functions, these old herbal teas serve as evidence for nature's subtle pharmacy and the timeless wisdom of traditional medical systems.

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