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"CHARACTERIZATION OF MEDICINAL PROPERTIES IN HERBAL TEA EXTRACTION FROM MEDICINAL PLANT"

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ABSTRACT

Herbal tea is fundamentally a blend created from the leaves, seeds, and roots of healing plants. The primary value of herbal teas lies in their therapeutic properties, which mainly arise from the phytochemicals and pharmacological traits found in these medicinal plants. This research centers on analyzing the medicinal attributes of Brahmi (Centella asiatica), lemongrass (Cymbopogon citratus), black pepper (Piper nigrum), clove (Syzygium aromaticum), cinnamon (Cinnamomum verum), mint (Mentha spicata), rose (Rosa damascena), cardamom (Elettaria cardamomum), and fennel seeds (Foeniculum vulgare). The aim of the experiment involves evaluating the phytochemicals and measuring key in vivo assays, such as antioxidant and anti-inflammatory effects. Aqueous extracts of the medicinal plants are prepared using established infusion methods. The phytochemical analysis indicates the presence of tannins, alkaloids, saponins, cardiac glycosides, flavonoids, steroids, terpenoids, and proteins. Quantitative assessments revealed considerable amounts of flavonoids, which correlated with substantial antioxidant activity assessed by hydrogen peroxide in a phosphate buffer. At a concentration of 200 µg/mL, the extract demonstrated a 75.5% scavenging effect, highlighting its significant antioxidant capability, likely due to the synergistic effects of phenolic-rich spices. Additionally, quantitative analysis indicated notable levels of tannins, flavonoids, saponins, terpenoids, and proteins, which were linked to prominent anti-inflammatory activity as observed using phosphate buffer and albumin, showing a scavenging activity of 73.6%. The results support the concept that herbal plants in tea can serve as a natural source of health-enhancing compounds, providing possible advantages for well-being and disease prevention. This research offers both a scientific and traditional foundation for creating plant-based therapeutic drinks. The methodology employed in this study with medicinal plants and aqueous extracts is straightforward, quick, and environmentally friendly. Future investigations will further explore cytotoxicity, bioavailability, pharmacokinetics, consistent dosages, and cultural and ethnopharmacological significance.

KEY WORDS: Herbal tea, Medicinal plants, Phytochemicals, Antioxidant activity, activity, Anti-inflammatory properties, Plant-based therapeutic beverages, Natural health products.

1. Introduction

Before modern pharmaceuticals, humans depended on medicinal plants for healing. Many still value these plants due to long-standing beliefs about their natural role in human health. Approximately 80% of the 5.2 billion people in developing countries rely on traditional medicine for primary healthcare [1], with over 3.3 billion using plant-based remedies. Nearly all of the world's 2,000 ethnic groups have traditional medical knowledge [2]. Historically, nature provided essentials like medicine, food, shelter, and transport [3]. Herbal medicine remains dominant in healthcare, especially in developing nations, but is also gaining recognition in developed countries for its therapeutic and economic value [4]. Ancient systems of medicine are deeply rooted in plant use, with evidence dating to 2600 BC in Mesopotamia, where oils from *Commiphora*, *Cedrus*, *Glycyrrhiza glabra*, *Papaver somniferum*, and *Cupressus sempervirens* were used for ailments like inflammation and parasitic infections [5]. Traditional medicine is widely practiced in China, India, Japan, Pakistan, Sri Lanka, and Thailand. In China, over 40% of medicinal use is tribal, and Thai remedies include legumes from Mimosaceae, Fabaceae, and Caesalpiniaceae families. The herbal market was worth $2.5 billion in the mid-1990s. Japan shows a preference for herbal over pharmaceutical drugs. Plants are vital across industries—cosmetics, pharmaceuticals, and chemicals—and essential to drug discovery. They've helped treat cancer, hepatitis, and AIDS. From 1950–1970, about 100 U.S. drugs were plant-derived, including vincristine, reserpine, and vinblastine. From 1971–1990, new plant-based drugs like artemisinin, guggulsterone, and teniposide emerged, followed by irinotecan, paclitaxel, and gomishin between 1991–1995. The 1953 discovery of serpentine from *Rauwolfia serpentina* improved hypertension treatment. *Catharanthus roseus* yielded vinblastine for childhood cancers, while *Nothapodytes nimmoniana* is used in Japan for cervical cancer [6]. Despite modern drugs, only a third of diseases have effective treatments. Herbal remedies remain relevant due to low side effects and compound synergy [7]. Many groundbreaking drugs are plant-derived, and WHO encourages integrating herbal medicine into healthcare for affordability and safety. Investigating active plant compounds has led to crucial new drugs [8]. Recent research on plants like *Artemisia annua*, *Taxus spp.*, *Lantana camara*, and *Bacopa spp.* has identified valuable compounds. Some previously overlooked plants are now vital medicinal resources. Bioassay-guided methods using traditional plants have produced many new therapeutic agents. The industrial use of morphine in 1826 by E. Merck marked the beginning of modern plant-based pharmaceuticals. By 1991, nearly half of top drugs originated from natural products [9].

1.1 GENERAL CONSTITUENTS OF MEDICINAL PLANTS: -

Therapeutic plants contain bioactive phytochemicals that produce specific physiological effects in the human body [10]. Key components include alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, and phenolic compounds [11]. These naturally occurring compounds, found in fruits, vegetables, herbs, flowers, leaves, and roots, work with nutrients and fiber to help defend against diseases. Phytochemicals are classified as primary (e.g., sugars, amino acids, chlorophyll) and secondary (e.g., alkaloids, terpenoids, phenolics) based on their role in plant metabolism [12].

1.2 APPLICATIONS OF MEDICINAL PLANTS IN AYURVEDA: -

Ayurveda emphasizes diagnosing a patient's condition before recommending internal remedies, diet, and lifestyle changes. Herbal preparations play a key role in healing. Anything in nature may be used medicinally [13], but classical texts caution against using substances not fully understood [14]. Substances from plants, animals, or minerals can be included in the Ayurvedic pharmacopoeia if their identity, properties, and uses are well known. Even poisons can heal if used properly, while misused medicines can harm. Of around 10,000 medicinal plants in the Indian subcontinent, only 1200–1500 have been officially adopted into Ayurveda over 3000 years, with each thoroughly studied before inclusion [15].

1.3 ACTIONS OF SPICES AND MEDICINAL PLANTS IN AYURVEDA

Spices significantly influence doshas, second only to food. Derived from roots, seeds, bark, and other plant parts, spices are rich in active phytochemicals—secondary metabolites that defend plants and impact human health. Mostly found in tropical areas, spices are dried to preserve potency and shelf life. Their effects depend on usage and individual constitution. While spices like cumin, ginger, and coriander aid digestion, not all are

suitable for everyone—particularly those with gastric issues. Indian cuisine, though flavorful and often healthy, isn't inherently Ayurvedic unless tailored to one's health needs.
About 90% of Ayurvedic preparations come from plants, which act more potently than food or spices to restore doshic balance. These are used in carefully crafted formulations called "yogas," refined over generations. Polyherbal remedies, combining 3 to 30 plants, are often more effective than single herbs. In such blends, key herbs provide primary action while others support absorption, transport, and reduce toxicity.

1.4 THE ROLE OF FOOD IN AYURVEDA

In Ayurveda, food plays a key role in healing and is closely linked to metabolic and gastrointestinal health, as well as conditions affecting the skin, muscles, joints, and mind. While occasional intake of unsuitable foods may not harm, regular consumption can worsen or trigger illness. Dietary advice is tailored to the illness stage and the dosha involved, evolving as the condition progresses. Food and medicine interactions are important, so diet plans must consider ongoing treatments to avoid adverse effects.

1.5 HERBAL TEAS IN GENERAL

Herbal teas, or *tisanes*, are not true teas as they don't come from the *Camellia sinensis* plant [16]. Made from dried leaves, seeds, fruits, flowers, and other plant parts, they are caffeine-free and enjoyed for both taste and health benefits [17]. Each blend is typically crafted for specific effects like relaxation or revitalization. Common benefits include stress relief, detoxification, immune and heart support, antioxidant supply, better sleep, and overall wellness—all without caffeine [18].

1.6 TYPES OF HERBAL TEA

Herbal tea enriches tea culture and supports health and wellness. Most teas are non-fermented, made like green tea, while semi-fermented teas include oolong, and fermented teas include black tea [19]. Non-fermented tea is produced by heating, rolling, compressing, and drying fresh leaves to preserve color and nutrients [20]. Semi-fermented tea undergoes harvesting, drying, rolling, further drying, screening, and baking [21]. Fermented tea's main biochemical is catechin (~20% dry weight), which oxidizes during fermentation to form theaflavins and thearubigins, giving fermented tea its distinctive aroma and orange-red color [22].

1.7 NON-FERMENTED TEA

Most herbal teas are unfermented, made by picking, withering, blanching, rolling, and drying. Castanopsis lamontii buds are dried to make a tea used in southwest China for freshening breath and reducing oral inflammation [23]. Combretum micranthum, known as "long-life herbal tea," is used in West African traditional medicine, especially for kidney ailments [24]. Sonchus glutinosa (rock tea) from the Mediterranean is used for digestive issues [25]. Chamomile tea, from Matricaria species, is popular for its anti-inflammatory effects [26]. Yacon (Smalanthus sonchifolius) from the Andes is traditionally used for diabetes and digestive and kidney problems [27].

1.8 FERMENTED TEA

Fermented herbal teas, a smaller segment, are made by picking, withering, rolling, fermenting, and drying. Rooibos, from *Aspalathus linearis* in South Africa, is a popular example rich in polyphenols linked to health benefits [28]. Honeybush tea (*Cyclopia intermedia*) is dried and fermented after harvest [29]. Vine tea, from fermented *Ampelopsis grossedentata* leaves, has been used as a beverage and remedy in southern China for centuries [30].

AIMS:

To characterize the medicinal properties of herbal tea extracts derived from selected medicinal plants, with a focus on identifying and quantifying bioactive compounds, antioxidant activity, and anti-inflammatory properties, in order to provide a scientific basis for the development of plant-based therapeutic beverages.

OBJECTIVES:

- To extract and characterize the phytochemical composition of herbal teas derived from selected medicinal plants.
- To evaluate the antioxidant activity and anti-inflammatory properties of the herbal tea extracts.
- To identify and quantify the bioactive compounds responsible for the medicinal properties of the herbal teas.
- To provide a scientific basis for the development of plant-based therapeutic beverages using the herbal tea extracts.

2. METHODOLOGY

2.1 MATERIALS USED

- ***Herbs and spices*: -** Brahmi (Centella asiatica), lemongrass (Cymbopogon citratus), black pepper (Piper nigrum), clove (Syzygium aromaticum), cinnamon (Cinnamomum verum), mint (Mentha spicata), rose (Rosa damascena), cardamom (Elettaria cardamomum), and fennel seeds (Foeniculum vulgare),
- Masculine cloth
- Chemicals Used: - $FeCl_3$, Wagner's reagent, iodine- potassium iodide, H_2SO_4, NaOH, HCL, Conc.H_2SO_4, Chloroform, Ninhydrin Reagent, Molisch Reagent, Phosphate Buffer, BCG Solution, Aluminum chloride, sodium nitrate, Methanol, vanillin, Ethanol, $K_3[Fe(CN)_6]$, FCR Reagent, Sodium Carbonate, Oxide Reducing Agent, Tannic Acid, Saturated Sodium Bicarbonate Solution, Gallic Acid, Hydrogen Peroxide, H_2o, Albumin,
- Glassware used: - Testubes, Conical flask, Ball Flask, Micro pipette, Microtips,

Equipment: - Weigh balance, P^H Meter, UV- Spectrophotometer, Soxhlet Extraction Unit, Incubator, Water Bath,

2.2 SELECTION AND COLLECTION OF MEDICINAL PLANT MATERIAL: -

- **Plant selection:** Utilizing conventional wisdom, ethnobotanical sources, or Ayurvedic documents we have chosen medicinal herbs like Brahmi (Centella asiatica), lemongrass (Cymbopogon citratus), black pepper (Piper nigrum), clove (Syzygium aromaticum), cinnamon (Cinnamomum verum), mint (Mentha spicata), rose (Rosa damascena), cardamom (Elettaria cardamomum), and fennel seeds (Foeniculum vulgare), recognized for their healing properties.
- **Collection:** Brahmi (Centella asiatica) is collected from local vendor in Krishnarajendra market and lemongrass (Cymbopogon citratus) is collected from agricultural field in Hoskote. Black pepper (Piper nigrum), clove (Syzygium aromaticum), cinnamon (Cinnamomum verum), mint (Mentha spicata), rose (Rosa damascena), cardamom (Elettaria cardamomum), and fennel seeds (Foeniculum vulgare) is collected from Grocery store in Krishnarajendra market. (Figure 1)

2.3 SENSORY ANALYSIS: -

Sensory analysis of herbal tea is the systematic evaluation of the organoleptic properties such as appearance, aroma, flavor, mouthfeel, and aftertaste of herbal tea using human sensory perception. This analytical approach combines standardized sensory methodologies to assess quality, consumer acceptance, and product differentiation, Sensory analysis plays a critical role in optimizing formulation, ensuring consistency, and aligning herbal tea products. (Figure 2)

Table Chart

Table 1: - Sensory Analysis chart

SI NO	Sample's	Sweet	Sour	Bitter	Fragrant	Woody	Fruity	Peppermint	Spicy
1	Brahmi			✓					
2	Lemongrass			✓	✓			✓	
3	Mint	✓			✓			✓	✓
4	Rose petals				✓				
5	Cinnamon	✓				✓			
6	Pepper							✓	✓
7	Clove			✓		✓			✓
8	Cardamom	✓			✓			✓	✓

2.4 PREPARATION OF HERBAL TEA AND EXTRACTS

- **Cleaning and drying**: we have taken Brahmi leaves, lemon grass and rose petals we have washed it in hot water for 2 times then we have cutted leaves into small pieces measuring 0.3cm. then we have left leaves for shade drying to retain active compounds for 4 days under shade between the newspapers.
- **Powdering:** we have taken black pepper (Piper nigrum), clove (Syzygium aromaticum), cinnamon (Cinnamomum verum), mint (Mentha spicata), rose (Rosa damascena), cardamom (Elettaria cardamomum), and fennel seeds (Foeniculum vulgare), and then powdered them into flakes using mortar and pestle.
- **Tea infusion (Aqueous extract):-** we have prepared 100gms of formulation as table follows.

Table 2: - 100gms of Formulation.

S.I No	Samples	Grams
1	Brahmi	18g
2	Lemongrass	18g
3	Mint	6g
4	Rose	11g

5	Clove	6g
6	Cinnamon	4g
7	Cardamom	11g
8	Pepper	15g
9	Fennel seeds	10g
Total		100g

Aqueous extraction of herbal tea

We have taken 50gms of formulation in ball flask then added 500ml of distilled water then we have kept for boiling at 90^0C degree in Soxhlet extraction unit for 90 mins to form a decoction the we have used this decoction for phytochemical analysis by qualitative, quantitave methods and In vitro assays. (Figure 3)

2.5 PHYTOCHEICAL ANALYSIS

Qualitative Analysis:

1. **Test for Tannins Ferric Chloride Test:** 2.0 ml of extracts were taken then 2.0 ml of FeCl3 was added. Intense blue or brownish green colour precipitate indicates the presence of tannin in plant extracts.
2. **Test for Alkaloids Wagner's Test:** 2.0 ml of extracts were taken, add 2.0 ml of Wagner's reagent (2.5 gm of iodine is dissolved in 12.5gm of potassium iodide) solution in it. If intense brownish colour forms, this indicates the presence of alkaloids in plant extract.
3. **Test for Saponins Foam Test:** 2.0 ml of plant extracts were taken, add 20 ml distilled water. The test tube was then shaken for 15 minutes. The formation of layer of foam indicates the presence of saponins in plant extracts.
4. **Test for Cardiac Glycoside Keller-Killani Test:** 5 ml of extract is treated with 2 ml of glacial acetic acid, containing one drop of FeCl3 solution and 1 ml of conc. H2SO4. Browning of the interface indicates a deoxysugar which is the characteristic of cardiac glycosides. Below the brown a violet ring was observed while in the acetic acid layer a greenish ring was observed.
5. **Test for Phenol Ferric Chloride Test:** In the extract add few drops of FeCl3 solution. Appearance of intense brown colour indicates the presence of phenol in plant extract.
6. **Test for Flavonoid Alkaline Reagent Test:** 2.0 ml of extracts were taken then add 0.5 ml of NaOH solution in it. If intense yellow colour that becomes colorless on addition of few drop of diluted HCI indicated the presence of flavonoid in the plant extract.
7. **Test for Steroids:** In 2.0 ml of aqueous plant sample were taken then and 2 ml of chloroform solution was added in it and add 2ml of conc. H2SO4 from the side of the test tube. Formation of intense yellow colour with green fluorescence colour indicates the precipitate of steroid.
8. **Test for Terpenoid Salkowski test:** In 2.0 mL of extracts add 2 ml of chloroform and 0.5 ml of conc. H2SO4. Formation of intense reddish-brown colour indicates the precipitate of terpenoid.
9. **Test for Protein**: In 2.0 ml of plant extracts add 2.0 ml of Ninhydrin reagent and boiled for 5 to 10 minutes in boiling water bath. Formation of dark purple colour indicates the presence of protein in plant extract.

Quantitative Analysis:

1. **Quantitative Estimation of Alkaloids** [Gravimetric Method]:

- To 1ml of test extract sample and 1ml of NH4OH (ammonium hydroxide)
- *Concentration:* concentrate the filtrate to one-quarter of its original volume using a water bath.

- *Precipitation:* *Add conc.NH_4OH dropurise to the concentrated extract until the solution become alkaline & precipitation is complete.
  *Allow the mixture to settle.
- *Collection & drying:* *Filter the precipitate using pre-weigh filter paper.
  *Wash the precipitate with dilute NH_4OH.
  *Dry the filter paper with the precipitate in an over at 60-80^{0}C until a constant weight is achieved.
- *Calculation:* Determine of the dried alkaloid precipitate.
- *Formula:* Total alkaloid content (%) = $\frac{\text{Weight of alkaloid ppt}}{\text{Weight of plant sample}} \times 100$

2.Quantitative Estimation of Phenolic Compounds:

Determination of total phenolic by Folin-ciocalteu's method

The total phenolic content in different solvent extracts was determined with the Folin-Ciocalteu's reagent (FCR). In the procedure, different concentrations of the extracts were mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min 4 ml of sodium carbonate solution was added. The final volume of the tubes were made up to 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer, a calibration curve was constructed using catechol solutions as standard and total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight and the standard graph.

1. Quantification of tannin total content:

Folin-Ciocalteu method was used to quantify the tannin total content. About 0.1ml of plant extract was added in 10 ml of volumetric flask containing the distilled water of 7.5ml and Folin-Ciocalteu phenol reagent of 0.5ml, 35% Na2 CO3 solution of 1 ml and diluted to 10ml using distilled water. The reagent mixture was well shaken and kept at 30°C temperature for 30min. A set of Gallic acid solutions (20, 40, 60, 80 and 100µg/ml) were prepared as mentioned earlier. Absorbance of standard and test solutions was analyzed with blank at 725nm.

2. Quantitative Estimation of Flavonoid's

Total flavonoid content was determined by Aluminum chloride method using catching as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminum chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 544 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g, dried extract).

2.6 PHARMACOLOGICAL ANALYSIS:

1. Antioxidant Assays (Hydrogen Peroxide Scavenging Assay)

The ability of the extract to scavenge hydrogen peroxide was determined according to the method of Ruch et al., (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 680nm in a spectrophotometer (8500 II, Bio- Crom GmbH, Zurich, Switzerland). Extracts (200–1000 µg) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 680 nm was determined after ten minute against a blank solution containing in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of extract and standard was calculated using the following equation:

- **% of Inhibition = (A of control – A of Test)/A of control * 100**

2. Anti-inflammatory Test (Albumin Denaturation Assay)

1ml of test sample of different concentration in 2.8 ml phosphate buffer. 0.2ml Albumin mixed in First testube. Well-kept a side then standard solution is made up of composition take 1ml of sample 0.2ml albumi, 2.8ml phosphate buffer mixed well in Second testube. Then control is made up of 2.8ml phosphate buffer. 1ml distilled water, 0.1 albumin mixed well in test tube 3 incubate the Third testubes at room temperature for 15 mins. Kept in water bath at 70^0C for 5 mins then cooled at room temperature and testubes were estimated spectrophotomerically at 660nm.

- **% of Sample inhibition = (1-Optical density of test sample / Optical density of control) X 100**
- **% of Std. Solution = (1-Optical density of Std. Solution / Optical density of control) X 100**

3. RESULTS

Qualitative & Quantitative Analysis Test Results:

Table 3: - Qualitative Analysis

TESTS	OBSERVATION	INFERENCE
1) **Test for Tannin's** Ferric Chloride test	Brownish green colour appeared	+ve Test presence of Tannins [Figure 4]
2) **Test for Alkaloids** Wagner's test	Brownish colour appeared	+ve Test presence of Alkaloids [Figure 5]
3) **Test for Saponin's** Foam test	Formation of Layer of foam indicates	+ve Test presence of Saponins [Figure 6]
4) **Test for Cardiac Glycoside** keller-Killani test	Brown a Violet ring was observed	+ve Test presence of Cardiac Glycoside (deoxysugar) [Figure 7]
5) **Test for Phenol** Ferric Chloride test	No Appearance of intense brown colour	-ve Test Absence Phenol [Figure 8]
6) **Test for Flavonoid** Alkaline Reagent test	Intense yellow colour that becomes colorless on addition of few drop of diluted HCL	+ve Test presence of Flavonoid [Figure 9]
7) **Test for Steroids**	Formation of intense yellow colour with green fluorescence colour indicates	+ve Test presence of the precipitate of Steroids [Figure 10]
8) **Test for Terpenoid** Salkowski test	Formation of reddish-brown colour indicates	+ve Test presence of the precipitate of Terpenoid [Figure 11]

9) Test for Protein	Formation of dark purple colour indicates	+ve Test presence of the precipitate of Protein [Figure 12]

This indicates that the herbal tea is rich in a variety of bioactive compounds such as alkaloids, flavonoids, saponins, glycosides, terpenoids, and steroids, which are known for various therapeutic and health benefits. Only phenols were found to be absent.

1. Quantitative Estimation of Alkaloids

Calculation:

Determine of the dried alkaloid precipitate.

Formula: Total alkaloid content (%) = $\frac{\text{Weight of alkaloid ppt}}{\text{Weight of plant sample}} \times 100$

Weight of filter paper = 0.60

Weight of sample dry filter paper = 0.64

$$(\%) = \frac{0.04}{2} \times 100$$

Total alkaloid content = 2%

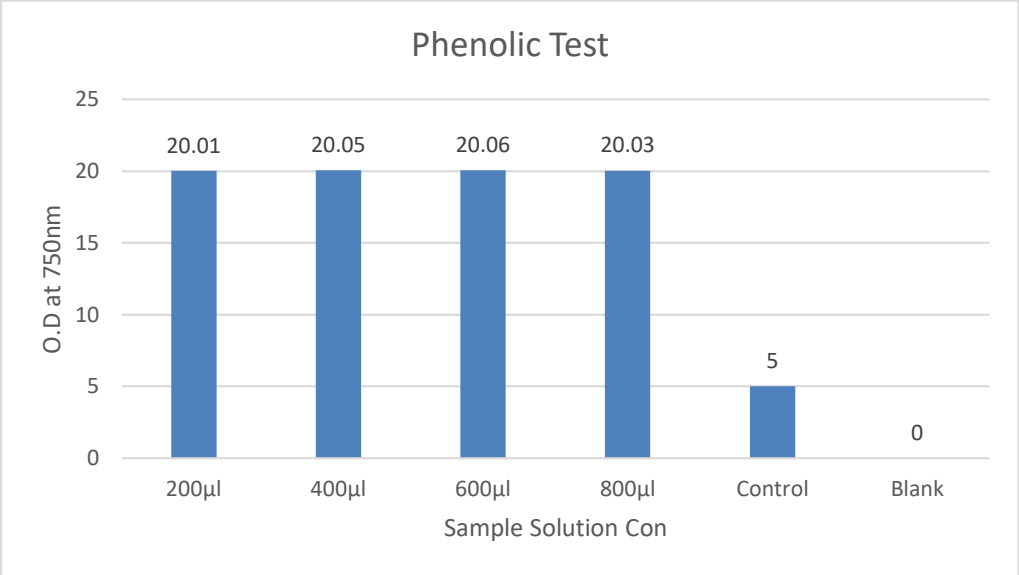
The herbal tea aqueous extract's total alkaloid content, as determined by quantitative measurement, was 2% (w/w). This (figure 13) indicates a moderate alkaloid concentration and is within the usual range reported for medicinal plant preparations (0.5-3%). Alkaloids are secondary metabolites that contain nitrogen and have a variety of pharmacological properties, such as analgesic, anti-inflammatory, antibacterial, and effects on the central nervous system. The level of alkaloids found in the herbal tea raises the possibility that it may support physiological advantages like pain management, increased immunity, and alertness. Given that consuming too many alkaloids can be toxic, the moderate content also suggests a balance between therapeutic efficacy and safety.

2. Quantitative Estimation of Phenolic Compounds:

Determination of total phenolic by Folin-ciocalteu's method

Table 4: - Total phenolic by Folin-ciocalteu's

Sample Solution	Distilled water (H_2O)	O.D at 750nm
1) 200µl	800µl	20.01Abs
2) 400µl	600µl	20.05Abs
3) 600µl	400µl	20.06Abs
4) 800µl	200µl	20.03Abs
5) Control	0	5.00Abs
6) Blank	1000µl	0.00



Calculation:

Total Phenolic content (mg/g) = C x V / m

C = Concentration of calibration curve (µg/ml)

V = Volume of extract used (ml)

M = Mass of Sample (g)

$$= \frac{0.208 \times 0.6}{21}$$

= 0.005µg / g

= 0.005 TPC / g.

Using the Folin-Ciocalteu colorimetric method, the herbal tea aqueous extract's total phenolic content (TPC) was calculated to be 0.005 mg of phenolic compounds per g of dried material. In comparison to the phenolic-rich herbal teas commonly reported in the literature (5-50 mg GAE/g), this very low number indicates that, despite the presence of phenolic compounds, their concentration in the aqueous extract is limited. Because of their strong antioxidant properties, phenolic compounds are well-known for their ability to scavenge free radicals, reduce inflammation, and improve general health. The extraction solvent (water), temperature, extraction duration, and the particular blend composition could all be responsible for the low TPC value found in this investigation. The addition of solvents like ethanol or methanol or the improvement of extraction conditions may raise the phenolic compound yield in further research. (Figure 15)

3. Quantification of tannin total content:

Calculation:

Absorbance (A) =m × C + b

Where:

A = absorbance (O.D. = 3.99)

C = concentration of tannins in µg/mL

m = slope of the calibration curve

b = y-intercept of the calibration curve

A = 0.039 x C + 0.05

3.99 = 0.039 x C + 0.05

C = $\frac{3.99 - 0.05}{0.039} = \frac{3.94}{0.039}$ = **101.03µg/mL**

The total tannin content of the herbal tea aqueous extract was found to be 101.02 µg/mL, which is higher than the typical range reported for many herbal infusions (20–80 µg/mL). Elevated tannin levels suggest strong antioxidant, antimicrobial, and astringent potential. Tannins are polyphenolic compounds known to stabilize proteins, inhibit microbial growth, and scavenge free radicals, thereby contributing to reduced oxidative stress. This high tannin concentration likely enhances the overall medicinal efficacy of the herbal tea and supports its use in traditional and functional health beverages. (Figure 14)

4. Quantitative Estimation of Flavonoid's

Calculation:

Absorbance = 0.027 x C + 0.02

Where: C (catechin concentration)

- C is the concentration in **µg/mL**
- Measured absorbance = **1.370**

1.370 = 0.027 x C + 0.02

C = $\frac{1.370 - 0.02}{0.027} = \frac{1.35}{0.027}$ = 50µg/mL

1 mL of extract and measured 50µg/mL = 0.05mg/mL

Flavonoid content = $\frac{0.05\ mg}{0.1\ g}$ = 0.5 mg CE/g dried extract

Final Result:

Flavonoid content = 0.5mg catechin equivalents per gram of dried extract

The herbal tea extract's flavonoid content, as determined quantitatively, was 0.5 mg catechin equivalents (CE) per gram of dried extract. Important polyphenolic substances called flavonoids are well-known for their cardioprotective, anti-inflammatory, and antioxidant properties. The tea's ability to scavenge free radicals may be greatly enhanced by the moderate level of flavonoids found, which would support its potential to lower oxidative stress and improve health. Flavonoids, which are naturally abundant in ingredients like Brahmi, lemongrass, clove, and mint, are probably what caused the observed amount. The outcome is consistent with values reported in the literature for polyherbal teas, indicating a balanced concentration of flavonoids for therapeutic advantages without being overly bitter. (Figure 16)

Antioxidant Assays

1. Antioxidant Assays (Hydrogen Peroxide Scavenging Assay)

Calculation:

% of Inhibition = (A of control – A of Test)/A of control * 100

1. 200µl = 0.614Abs = $(\frac{2.509-0.614}{2.509}) \times 100$

= **75.52%**

2. 400µl = 0.976Abs = $\frac{(2.509-0.976)}{2.509} \times 100$

= **61.09%**

3. 600µl = 1.437Abs = $\frac{(2.509-1.437)}{2.509} \times 100$

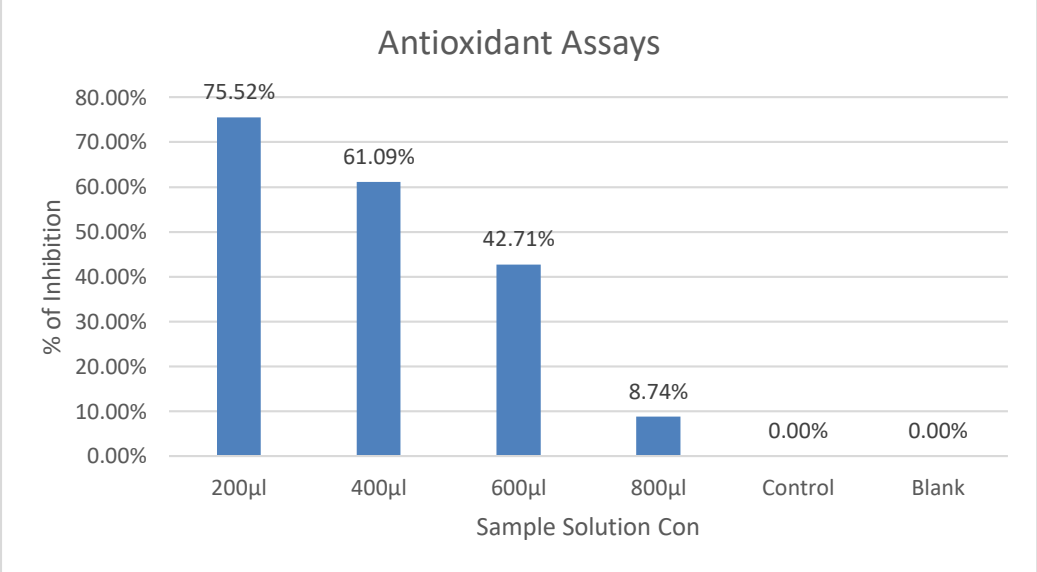
= **42.71%**

4. 800µl = 2.290Abs = $\frac{(2.509-2.290)}{2.509} \times 100$

= **8.74%**

Table 5: - Hydrogen Peroxide Scavenging Assay

Sample Solution	Distilled water (H_2O)	O.D at 680nm	% of inhibition
1) 200µl	800µl	0.614Abs	75.52%
2) 400µl	600µl	0.976Abs	61.09%
3) 600µl	400µl	1.437Abs	42.71%
4) 800µl	200µl	2.290Abs	8.74%
5) Control	0	2.509Abs	0
6) Blank	1000µl	0.00	0



Technique for assessing a substance's antioxidant potential is the hydrogen peroxide scavenging assay. Hydrogen peroxide is scavenged and neutralized by antioxidants in the sample, which stops it from degrading and producing radicals. Because there is less hydrogen peroxide available to react with a chromogen, this scavenging action is usually indicated by a drop in absorbance. larger antioxidant activity, shown as a larger percentage of inhibition, is indicated by test samples having lower absorbance readings than the control.

However, Table data indicates a result that deviates from the dose-response relationship that antioxidant assays are supposed to demonstrate. When the herbal tea sample's volume rises from 200 ml to 800 ml, the proportion of Inhibition drops to 8.74% from 75.52%. Because of this inverse connection, poorer antioxidant activity is produced by higher sample concentrations (assuming that a bigger volume of the same solution equals to a higher concentration of active chemicals).

Anti-inflammatory test

2. Anti-inflammatory Test (Albumin Denaturation Assay)

% of Sample inhibition = (1-Optical density of test sample / Optical density of control) X 100

% of Sample inhibition = (1-0.816 / 0.250) x 100

= 73.6%

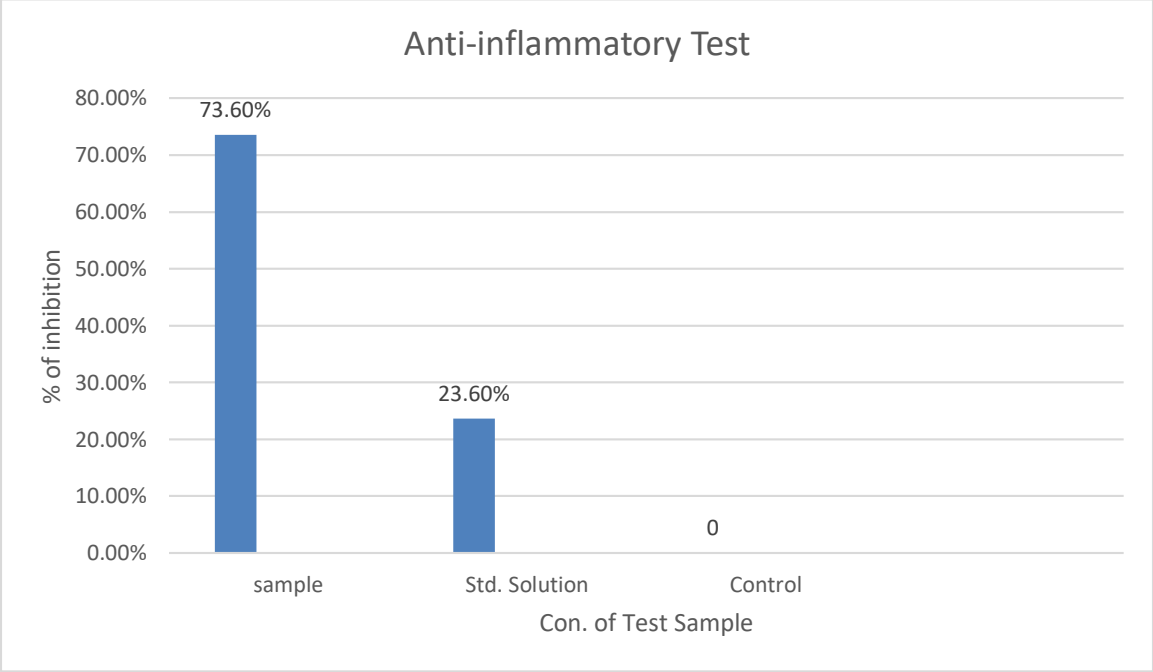
% of Std. Solution = (1-Optical density of Std. Solution / Optical density of control) X 100

% of Std. Solution = (1-0.191 / 0.250) x 100

= 23.6%

Table 6: - Anti-inflammatory

Test samples	O.D at 660nnm	% of inhibition
Sample	0.816Abs	73.6%
Std. Solution	0.191Abs	23.6%
Control	0.250Abs	0



The herbal tea extract's 73.6% suppression of protein denaturation points to powerful anti-inflammatory properties.High inhibition suggests the extract can stabilize proteins and stop inflammatory damage. The albumin denaturation experiment simulates protein denaturation in inflammatory settings.Bioactive substances including phenols, flavonoids, and tannins found in fmedicinal plants like clove, cinnamon, brahmi, and mint are probably what cause the effect.In the albumin denaturation assay, the herbal tea extract showed 73.6% suppression of protein denaturation, suggesting potent anti-inflammatory effect in vitro. This implies that the phytochemicals in the tea might protect proteins from heat-induced denaturation, a process important for the regulation of inflammation. The synergistic effects of phenolic-rich plants including brahmi, clove, and cinnamon are responsible for the activity. (Figure 20)

4.CONCLUSION

This research offers a scientifically grounded and traditionally inspired exploration into the medicinal potential of herbal tea extracts derived from selected medicinal plants. The phytochemical screening revealed the presence of a diverse range of bioactive compounds, including alkaloids, flavonoids, saponins, glycosides, terpenoids, and steroids, all of which are known to exhibit a wide spectrum of therapeutic effects. While phenols were absent, the extract demonstrated notable antioxidant activity, achieving a 75.5% scavenging effect at a concentration of 200 µg/mL. This potent antioxidant capability is likely due to the synergistic interactions among other phytoconstituents, particularly those contributed by phenolic-rich spices used in the formulation.

Quantitative analysis further confirmed significant levels of tannins, flavonoids, saponins, terpenoids, and proteins compounds associated with anti-inflammatory, antimicrobial, and immune-modulating effects. The anti-inflammatory potential of the extract was validated through a protein denaturation assay, showing a 73.6% inhibition rate, thereby underscoring its possible application in managing inflammation-related health conditions. Crucially, the water extraction technique used is easy to use, economical, quick, and ecologically benign. This is in line with the worldwide movement toward sustainable research methods and green chemistry. Additionally, it maintains the conventional method of water-based formulations, which are frequently employed in herbal therapy, increasing the findings' cultural and usefulness.

This work supports the use of herbal teas as possible medicinal agents as well as health-promoting beverages by combining ethnopharmacological knowledge with scientific validity. The findings provide a framework for additional investigation, which ought to concentrate on elaborating on the pharmacological profile of the extract. The development of standardized dosages for reliable efficacy, research into bioavailability and pharmacokinetics to comprehend absorption and metabolism, cytotoxicity tests to guarantee safety, and further examination into the studies of pharmacokinetics and bioavailability to comprehend absorption and metabolism, the development of standardized dosages for reliable effectiveness, and a more thorough examination of the traditional and cultural functions of such herbal products.

In conclusion, this study opens the door for the development of standardized, safe, and efficient therapeutic beverages based on both tradition and science, while also highlighting the great potential of medicinal plant-based herbal teas as multipurpose health supplements.

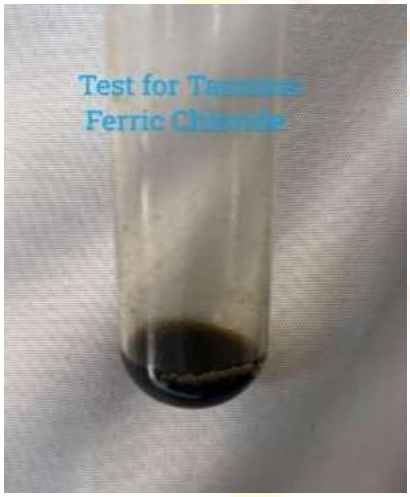
5.LIST OF FIGURES

[Figure 1] Collection of Spices

[Figure 2] Tea Samples

[Figure 3] Filtration of Tea Sample

Qualitative Analysis Figures:



[Figure 4]

[Figure 5]

[Figure 6]

[Figure 7]

[Figure 8]

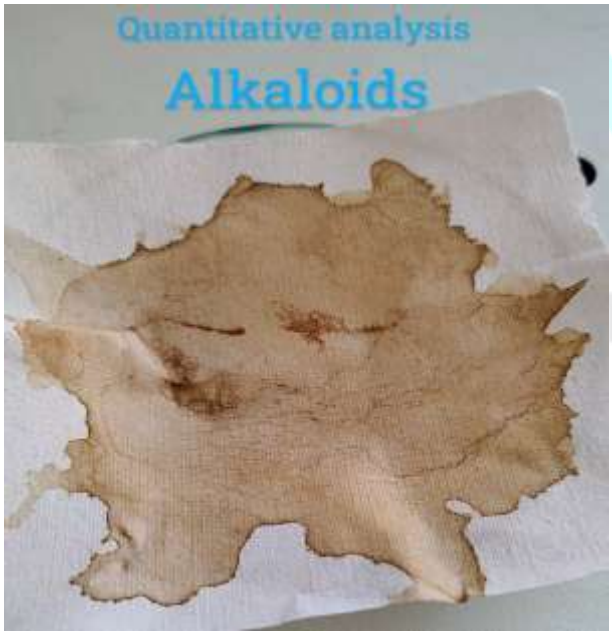
[Figure 9]

[Figure 10]

[Figure 11]

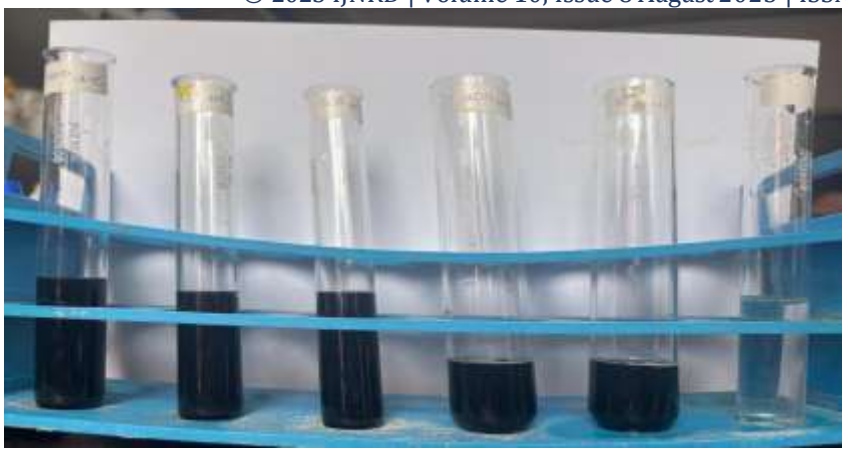
[Figure 12]

Quantitative Analysis Figure



[Figure 13] Alkaloids

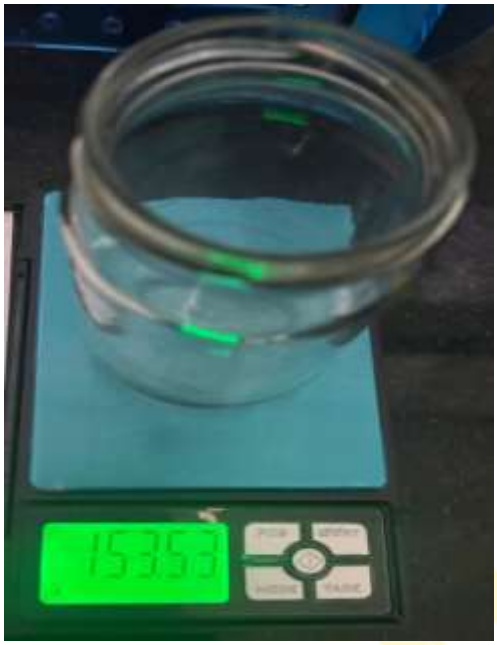
[Figure 14] Tannin's Test



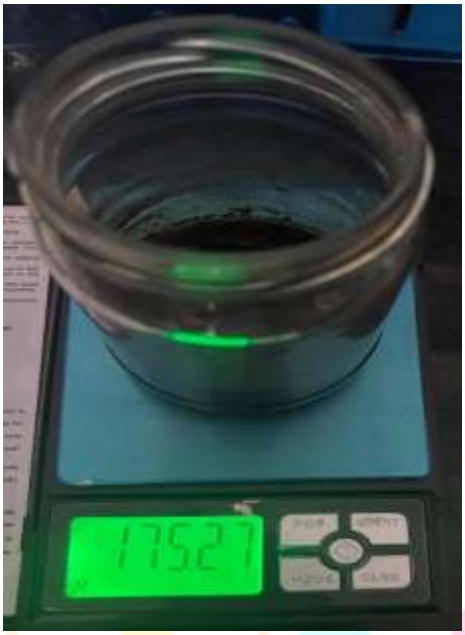
[Figure 15] Phenolic Test

[Figure 16] Flavonoid Test

[Figure 17] Sample Boiling at 90^0 C



[Figure 18] Empty Weighing Jar



[Figure 19] Sample Weighing Jar

Anti-inflammatory Assay

[Fig 20] Anti-inflammatory Test

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