COMPARATIVE *IN-VITRO* EVALUATION OF ORGANOLEPTIC, PHYSICOCHEMICAL, AND ANTIOXIDANT PROPERTIES OF SELECTED HERBAL TEAS.

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A PROJECT SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICS AND PHARMACEUTICAL TECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DOCTOR OF PHARMACY (PHARM.D) DEGREE OF THE UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA.

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CERTIFICATION

This is to certify that this work was done by **ERUORE OROBOSA EKPOH**, in the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, in partial fulfillment for the award of the Pharm. D degree from the University.

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DEDICATION

This project is dedicated to God Almighty, whose loving kindness and infinite mercy have sustained me throughout pharmacy school, and also to my beloved parents and siblings, whose love, prayers, and support have been my strength throughout this academic journey.

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ABSTRACT

Background: Herbal teas, also known as tisanes, are beverages prepared by infusing plants or plant parts such as seeds, bark, leaves, flowers, dried nuts, fruits, and grasses into hot water, producing a distinct taste with various health benefits. Insufficient comparative analysis exists for commonly consumed herbal teas in Nigeria, regarding antioxidant activity, organoleptic, and physicochemical parameters.

The purpose of this study is to conduct an *in vitro* evaluation of organoleptic, physicochemical, and antioxidant properties of selected herbal teas.

Method: Ten herbal tea brands (Ginseng tea, Adams Moringa tea, Organic Immune System Booster, 3 Ballerina Tea, Legend Tea and Herbs, Qualitea, Top Tea, Lipton, Costa Tea, and Richmond) were selected from supermarkets and local markets in Edo state, Nigeria. The selected herbal teas were assessed for their organoleptic and physicochemical properties (pH, moisture content, and specific gravity). In the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, the absorbance at 517 nm of the herbal teas brewed at different temperatures (95, 28, and 10°C) was determined using an ultraviolet spectrophotometer. The percentage inhibition was calculated, and the results were recorded.

Result: The organoleptic properties of the herbal tea brew were fresh and within acceptable sensory limits. The pH determination shows that the herbal teas have pH values within the acidic range (<7), from 4.83 to 6.03. From the test carried out, the moisture content ranged from 3.4% to 8.5% (<10%). The specific gravity determination showed that the teas have specific gravity similar to that of water (1.000 g/mL), ranging from 0.92 to 1.02 g/mL. The DPPH radical scavenging assay shows that the percentage inhibition of the teas brewed at 95°C ranged from 0 to 83.46%. The percentage inhibition of the teas brewed at 28°C ranged from 59.04 to 82.02%, and the percentage inhibition of teas brewed at 10°C ranged from 65.52 to 84.62%.

Conclusion: The findings suggest that the analyzed herbal teas met the basic quality requirements. The majority of the herbal teas exhibited high antioxidant activity (high percentage inhibition) when brewed at lower temperatures. Costa tea did not show activity at 95°C.

CHAPTER ONE

1.0. Introduction and Background of the Study

Herbal medicines are defined by the World Health Organization (WHO) as herbs, herbal materials, herbal preparations, and complete herbal products that comprise plant parts, other plant materials, or mixtures of these as active ingredients (World Health Organization, 2023). Plant materials may include leaves, stems, roots, seeds, fruit, wood, and fibers.

Herbal teas, also known as tisanes, are beverages prepared by infusing plants or plant parts, such as seeds, bark, leaves, flowers, dried nuts, fruits, and grasses into hot water, producing a distinct taste with various health benefits. Herbal teas are different from traditional teas. Traditional teas are derived from the leaves and buds of the plant Camellia sinensis or Camellia assamica. Its origin is dated approximately 2700 B.C., and was discovered by the Chinese culture (Pan *et al*., 2022). Herbal teas do not contain caffeine but do contain a variety of bioactive compounds that contribute to their antioxidant activity, which play vital roles in neutralizing free radicals.

The use of herbal teas can be traced back to ancient civilizations in China, Egypt, and India, where they were used for medicinal and ceremonial purposes. It is believed that Shen Nong was the first person to discover tea in 2737 BCE (Pan *et al*., 2022)

To prepare herbal teas, dried herbs, flowers, fruits, and/or spices are steeped in hot water for consumption (Anderson and Zagorski, 2023). There are various herbal teas, including ginger, ginseng, chamomile, moringa, peppermint, and hibiscus. Herbal infusions differ from other types of tea, despite sharing a similar name. The major difference lies in the origin of the plants used to prepare them. Traditional tea originates from the leaves of the Camellia sinensis plant; examples include green tea, white tea, yellow tea, and black tea. Herbal teas are prepared using plant parts (flowers, bark, leaves, roots, etc.) and are caffeine-free, making them suitable for consumption at any time of the day.

Herbal teas contain a variety of bioactive compounds, including polyphenols. Polyphenols are a large group of natural chemicals produced by plants. They are secondary metabolites that generally act as a defense mechanism against ultraviolet radiation or pathogen aggression (Sen *et al*., 2020). Examples include phenolic acids, flavonoids, lignans, curcumin, flavonol, and stilbenes. Other examples of bioactive compounds include alkaloids, amino acids, vitamins (A, C, E), terpenoids, beta-carotene, and polysaccharides. These compounds are responsible for their antioxidant properties, which offer a variety of therapeutic benefits when consumed.

Consuming herbal teas is beneficial as they are known to be rich sources of bioactive compounds. These bioactive compounds possess antioxidant, antiviral, anti-inflammatory, antidiabetic, antibacterial, and anticancer activities. It is also known to be useful for preventing various human diseases, including the maintenance of cardiovascular and metabolic health (Khan and Mukhtar, 2013). Herbal teas are also beneficial for relieving stress, improving digestion, enhancing cognitive function, soothing nausea, supporting immunity, modulating and detoxifying steroid metabolism, and regulating enzyme activity (Lampe, 2003).

1.1. Antioxidants and Free Radicals

Antioxidants are substances that help combat the potential damage to cells caused by free radicals (Roberts, 2023). They are also referred to as molecules that protect the body's cells from damage caused by unstable molecules called free radicals. Free radicals are chemical species that contain one or more unpaired electrons (Robert, 2023). These radicals are called reactive oxygen/nitrogen species (ROS/RNS), and are produced during cellular metabolism and other functional activities. They play important roles in cell signalling, apoptosis, and gene expression. There are situations in which the production of reactive oxygen/nitrogen species exceeds the body's capacity to counter them, leading to oxidative stress and cellular damage. Antioxidants function as an off valve for free radicals by donating electrons, thereby neutralizing them (Harvard Health Publishing, 2019). This leads to the production of new free radicals that are less active and dangerous, thereby living longer than the neutralized radicals. In the body, a balance between reactive oxygen/nitrogen species and antioxidants is important for optimal cellular health.

Antioxidants can be found in foods, such as fruits, herbs, spices, and vegetables. Natural antioxidants are preferred over synthetic antioxidants because the latter are prone to cause skin allergies, fatty liver, and gastrointestinal distress (Gulcin and Alwasel, 2023). The sources of natural antioxidants include plants such as garlic, ginger, tea, lemon grass, cinnamon, cloves, fennel, and rosemary. They work via various mechanisms, including decreasing localized oxygen concentrations, preventing chain initiation by scavenging initiating radicals, binding catalysts (metal ions) to prevent radical generation, and chain breaking to prevent continuous hydrogen abstraction by active radicals.

The efficacy of antioxidant compounds depends on a variety of factors, including structural properties, concentration, characteristics of the substrate susceptible to oxidation, the physical state of the system, presence of prooxidants, and temperature (Munteanu and Apetrei, 2021). In recent times, there has been increased scientific interest in the antioxidant activity of herbal teas, given that oxidative stress is linked to the development of several disease conditions, which they help combat.

1.2. Effect of Temperature on Antioxidant Activity

During the preparation of herbal teas, the water temperature required for extracting bioactive compounds during brewing is of optimum importance. The brewing temperature increases solubility and affects the extraction efficiency of bioactive compounds. Previous studies have shown that extraction efficiency increases proportionally with water temperature, and the highest yield was obtained at 100°C (Cheng *et al*., 2023). However, extreme temperatures can degrade the leaves, and prolonged brewing can make the infusion dark and bitter (Lee and Chambers, 2009).

Temperature is a critical factor in the preparation of herbal teas; it influences the extractive potential of bioactive compounds and their stability, which in turn affects antioxidant activity. It is therefore important to investigate how brewing temperatures (95°C, 28°C, 10°C) affect the antioxidant activity, and organoleptic and physicochemical properties of selected herbal teas.

1.3. Evaluation of Antioxidant Activity

1.3.1. DPPH Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is one of the most widely used methods for evaluating the antioxidant activity of food products, pure compounds, plant or herbal extracts, including herbal tea infusions. It is widely used for its simplicity and the speed with which the tests are conducted. This study highlights how different brewing temperatures affect the release of bioactive compounds in tea preparations.

The basis of this assay is the spectrophotometric measurement of the capacity of antioxidants to scavenge DPPH radicals (Gulcin and Alwasel, 2023). It measures a sample's ability to donate hydrogen atoms or electrons to neutralize DPPH free radicals, thereby indicating the sample's potential to act as a natural antioxidant. The single electron of the nitrogen atom in DPPH is reduced by taking the hydrogen atom from the antioxidant, and this leads to the formation of the corresponding hydrazine. The radical is stable due to the presence of stearic crowding on the divalent nitrogen atom as well as the push-pull effect caused by the diphenylamino group and picryl, which are electron donors and acceptors, respectively. The DPPH radical has an intense violet colour. When in contact with a substance with antioxidant potential, it changes from a deep violet to a yellow or light yellow. Hydrazine (DPPH-H) is formed as a result of the radical reduction by the hydrogen atom transferred from the antioxidants.

The result of this analysis is expressed as percentage inhibition, which is calculated using the absorbance of the blank (negative control) and the absorbance of the samples at 517nm.

1.3.2. Interpretation of Result

A high absorbance value indicates low antioxidant activity, meaning the herbal tea infusion scavenged few radicals. A low absorbance value indicates high antioxidant activity, which means that the bioactive compounds present in the herbal tea infusions donated sufficient hydrogen atoms to neutralize the DPPH free radicals. Therefore, herbal tea samples with low absorbance values would demonstrate high percentage inhibition, and high absorbance values would have low percentage inhibition (Brand-Williams *et al.,* 1995).

1.3.3. Strengths and Limitations of DPPH Radical Scavenging Assay

Table 1.3.3: Strengths and limitations of DPPH Assay

S/N	Aspect	Strengths	Limitations
1	Ease of use	Simple and quick method suitable for routine analysis.	It requires methanol/ethanol as a solvent, which may not accurately represent aqueous systems.
2	Detection mechanisms	Visible colour change from deep purple to yellow.	Coloured plant extracts interfere with the absorbance reading at 517nm.
3	Applicability	Suitable for assessing the antioxidant activity of polyphenol-rich herbal tea extract.	Not suitable for hydrophilic compounds that react poorly in organic solvents.
4	Stability of radical	DPPH is a stable free radical that can be handled safely and easily.	DPPH radical is synthetic and is not found in biological systems, thereby limiting physiological relevance.
5	Reproducibility	Provides comparable results among different herbal tea samples under standard conditions.	The rate of reaction can vary depending on concentration, affecting the comparability of results.
6	Quantitative capacity	Enables numerical expression of antioxidant activity (% inhibition).	Does not distinguish between antioxidant mechanisms (Hydrogen atom transfer or electron transfer).

1.4. Overview of Organoleptic and Physicochemical Properties

Organoleptic and physicochemical parameters, including pH, moisture content, and specific gravity, are vital quality control tests to ensure the safety, stability, and efficacy of herbal teas.

1.4.1. Organoleptic Properties

Organoleptic properties refer to sensory characteristics of a product that can be perceived by our sense organs (eyes, nose, mouth, and skin), such as colour, taste, odour, appearance, and texture. Evaluation of organoleptic properties is vital because it shapes how we perceive substances, including herbal products. These sensory qualities provide preliminary information about the chemical composition and processing quality of herbal teas. They are the first indicators of the quality of herbal products; for instance, herbal materials that have been subjected to microbial degradation will show discolouration compared to when they are fresh. Organoleptic properties also affect consumers' willingness to purchase and consume products.

The organoleptic properties we evaluate include appearance (colour, finish), aroma (odour before/after brewing), texture (feel), and taste (sweet, bitter, sour). Evaluating these properties is an essential step in assessing the overall quality of herbal teas.

Importance of Organoleptic Property Evaluation

Evaluation of organoleptic properties is crucial for

I. Enhancement of consumer satisfaction, as sensory appeal affects consumer purchasing decisions.

II. It serves as an indicator of a product's quality and acceptability, which influences consumers' preferences.

III. It aids in detecting adulteration, contamination, and spoilage, especially in the absence of advanced analytical tools.

IV. It is useful for quality control and standardization, ensuring uniformity across product batches.

V. It is useful for the initial identification and differentiation of herbal teas, based on taste and aroma.

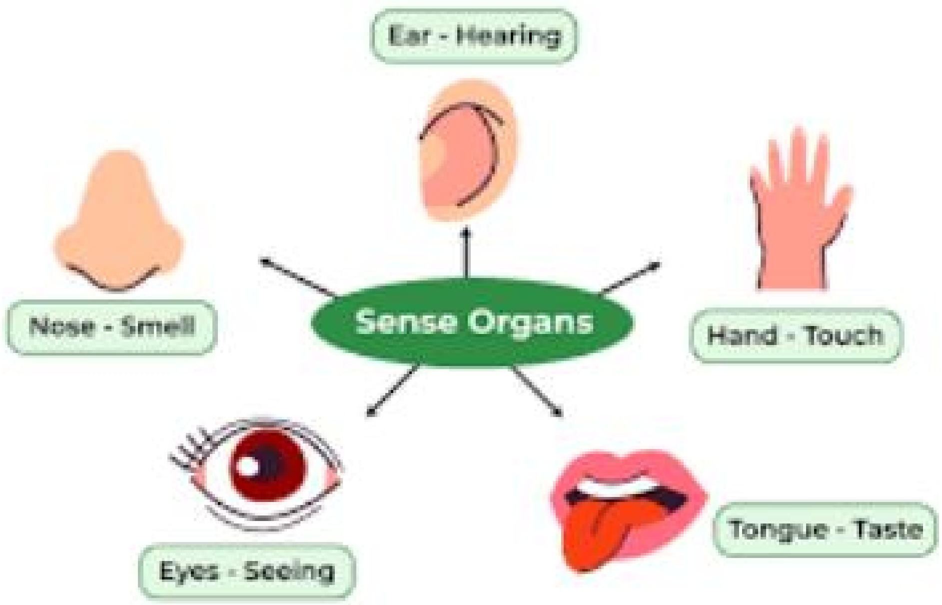

Figure 1.1. Sense Organs and Their Functions

1.4.2. Physicochemical Properties

Physicochemical properties are fundamental characteristics that give insight into a substance's physical and chemical nature and influence its appearance, solubility, bioavailability, and shelf life. They provide information about the safety, efficacy, and stability of herbal products, pharmaceutical formulations, and food formulations. The evaluation of these parameters is vital because variations in the formulation can affect the composition and performance of the final product. They are important for standardization and quality control. The following physicochemical parameters are carried out in this study:

1.4.2.1. pH

pH determination is important for detecting toxicity in aqueous solutions. It measures the acidity or alkalinity of a substance, which can significantly affect the preservation and bioavailability of its active constituent. On a scale of 0 to 14, a pH of 7 is neutral, a pH above 7 is alkaline, and a pH below 7 is acidic. For herbal tea preparations, it is important to maintain an appropriate pH range, as extreme acidity or alkalinity can degrade bioactive compounds and reduce therapeutic efficacy. The pH value can also serve as an indicator for contamination or chemical instability during storage. In herbal tea, the pH value also affects the taste and colour of infusions.

Importance of pH Evaluation

pH evaluation is crucial for;

I. **Indication of Stability:** It determines the chemical stability of herbal preparations; extreme values can accelerate the degradation of active compounds.

II. **Consumer Acceptability:** It affects the taste and palatability of herbal products, which are crucial for consumer compliance.

III. **Shelf life and safety**: A suitable pH helps prevent microbial growth, prolonging shelf life.

IV. **Bioavailability of active compounds**: It influences the solubility and absorption of phytochemicals, thereby affecting therapeutic efficacy.

1.4.2.2. Moisture Content

Moisture content refers to the amount of water present in a sample. This is an important physicochemical parameter because moisture can promote microbial growth and enzymatic reactions. In herbal preparations, excessive moisture can promote microbial growth and chemical degradation, while insufficient moisture can affect the texture and extractability of the bioactive compounds. It also provides insight into the effectiveness of the drying process during production. Therefore, it is important to assess the moisture content of herbal tea to ensure efficacy and adequate storage.

Importance of Moisture Content Evaluation

Moisture content evaluation is crucial for;

I. **Prevents degradation of active compounds:** Excess water can trigger hydrolysis and oxidation of phytochemicals, lowering therapeutic efficacy.

II. **Improves storage and handling** – Moisture control reduces clumping, stickiness, and weight variability in herbal powders and teas, ensuring uniformity

III. **Maintains organoleptic properties** – Balanced moisture helps preserve taste, aroma, and texture, enhancing the acceptability of herbal teas.

1.4.2.3.Specific Gravity

Specific gravity measures the ratio of the density of a substance to the density of water at a specific temperature. It provides information about the concentration, purity, and quality of formulations. In herbal preparations, specific gravity serves as an indicator of solute concentration and the presence of dissolved solids (sugar and minerals). This is vital as it reflects the consistency and uniformity of formulations. Variations in the specific gravity of herbal formulations arise from differences in plant metabolite concentration, extraction efficiency, and solvent composition.

Importance of Specific Gravity Evaluation

Specific gravity evaluation is crucial for;

I. **Quality control**: It helps ensure batch-to-batch uniformity in herbal formulations.
II. **Detection of adulteration**: Abnormal values may indicate dilution, contamination, or addition of foreign substances in finished herbal preparations.
III. **Indicator of concentration**: It reflects the relative content of dissolved solids, giving an estimate of extract strength.
IV. **Predicts stability**: It helps monitor changes during storage that may affect product integrity.

1.5. Statement of the Problem

Herbal teas are consumed worldwide and are known for their numerous health benefits, although limited scientific data exist on how the method of preparation (brewing temperature) affects the extractive potential of antioxidants and their physicochemical properties. Furthermore, there is insufficient comparative analysis of commonly consumed herbal teas in Nigeria, regarding antioxidant activity, organoleptic, and physicochemical parameters. Addressing this is vital for establishing quality standards and providing evidence-based recommendations.

1.6. Significance of the Study

The significance of this study is to:

I. To provide scientific evidence on the effect of brewing temperature on the antioxidant activity of herbal teas, which helps consumers maximize their health benefits.

II. To contribute to quality assurance, which serves as an indicator of the purity, safety, and stability of herbal teas.

III. To add to the existing literature by providing comparative data on various herbal teas, which may be useful for researchers, nutritionists, and other healthcare professionals

1.7. Aim and Objectives of Study

This study aims to conduct an *in vitro* evaluation of organoleptic, physicochemical, and antioxidant properties of selected herbal teas.

This study is specifically designed to:

I. Evaluate the effect of different brewing temperatures on the antioxidant activity of the selected herbal teas using the DPPH method of analysis.

II. Evaluate the organoleptic properties of herbal teas.

III. Evaluate the physicochemical properties (pH, moisture content, specific gravity).

CHAPTER TWO

2.0. Materials and Methods

2.1. Materials

DPPH powder, buffer (pH 4 and pH 9), and methanol were sourced from the Pharmaceutical Chemistry Laboratory in the University of Benin, Benin City.

2.2. Sample Collection

Ten samples of different selected brands of herbal teas commonly consumed were bought from supermarkets and local markets in Edo state, Nigeria.

They include: Ginseng tea, Moringa tea, Organic Immune System Booster, 3 Ballerina tea, Legend Tea and Herbs, Qualitea, Top Tea, Lipton, Costa Tea, and Richmond. The samples were selected based on accessibility, affordability, and popularity.

2.3. Sample Preparation

One tea bag of each selected herbal tea was brewed in 100 - 200 mL of hot water (95°C). It was left to infuse for 5 minutes, and the tea bag was removed afterwards. The freshly brewed tea was then allowed to cool down and filtered. The same procedure was followed for brewing the teas at other temperatures (28 and 10°C). After cooling, the various tests were then carried out.

2.4. Label Claims and Organoleptic Properties

After the purchase of these samples, information on manufacturing and expiry dates, ingredients, batch numbers, manufacturer's information (name, address, country of origin of the brand), registration status by the National Agency for Food and Drugs Administration and Control (NAFDAC), net quantity, storage conditions, directions for use and warnings were obtained and recorded from the products label.

An assessment of organoleptic properties was performed on all selected samples. Parameters such as appearance (colour, shape/size), odour, infusion colour, and taste. This assessment was carried out under natural light in a well-ventilated room. This is done to prevent external interference.

2.5. Physicochemical Properties

Physicochemical properties are fundamental characteristics that describe the physical and chemical natures of a substance. They give an insight into the quality and stability of the selected herbal teas. The method for carrying out the parameters is discussed below;

2.5.1 pH

The pH of the formulations was measured using a pH meter and buffer solutions with pH 4.0 and 9.0. The pH meter was calibrated using the buffer solutions.

20-30 mL of the cooled, filtered tea was poured into a clean beaker. The pH electrode was inserted into the solution, and after 10-20 seconds, the pH value was recorded. The electrode was rinsed after every use.

2.5.2 Moisture Content

Ten porcelain dishes were dried and weighed. 2-3 g of the powdered herbal teas were weighed into a porcelain dish, and the weight was recorded. The porcelain dishes were then placed in an oven at 105°C for 3 hours. Afterwards, it was allowed to cool, and the final weight of the porcelain dish with the sample was recorded. The drying was repeated until a constant weight was achieved. The percentage moisture content was calculated using the formula:

$$\% \text{ moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where, W_1= Weight of empty porcelain dish

W_2= Weight of porcelain dish + powdered sample before drying

W_3= Weight of porcelain dish + powdered sample after drying

2.5.3 Specific Gravity

The specific gravity of the herbal teas was determined using a beaker and an analytical weighing balance (0.001 g sensitivity). A clean, dry beaker was weighed and its weight recorded (W_1). The beaker was then filled with a known volume (50 mL) of distilled water, and the weight was recorded (W_2). The weight of water was calculated as (W_2 - W_1). Afterwards, the beaker was emptied, dried, and refilled with an equal volume (50 mL) of brewed tea infusion, and the weight was recorded (W_3). The weight of the sample was calculated as (W_3 - W_1). Specific gravity is calculated with the formula:

$$\text{Specific Gravity} = \frac{\text{Weight of Sample (equal volume)}}{\text{Weight of Water (same volume)}}$$

Note: This method is less precise than when a pycnometer is used, but it provides a reliable estimation for liquid herbal samples.

2.6 Antioxidant Assay using DPPH Method of Evaluation

0.004 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was dissolved in 100 mL of methanol to produce a 0.004% w/v solution. The resultant solution is light-sensitive and kept away from light to prevent degradation. Vitamin C is used as the standard for this test. 100 mg of vitamin C was diluted in 100 mL of distilled water, and the resultant solution was used to carry out the analysis.

10 mL of each herbal tea infusion, brewed at different temperatures, and 2 mL of DPPH solution were pipetted into test tubes wrapped in aluminum foil, which were then incubated in the dark for 30 minutes, to allow the reaction to proceed. The same procedure was repeated using a vitamin C (standard).

Using an ultraviolet-visible spectrophotometer, the absorbance was measured at 517nm, and the results were recorded. The percentage radical scavenging capacity is calculated for each sample using the formula:

$$\% \text{ Inhibition} = \frac{Abscontrol - Abssample}{Abscontrol} \times 100$$

Where: $Abs_{control}$ is the absorbance of the DPPH + solvent (Blank test)

Abs_{sample} is the absorbance of the DPPH + sample

CHAPTER THREE

3.0. Results and Discussion

3.1. Organoleptic Properties

Table 3.1 below shows the organoleptic properties of the selected herbal teas, including appearance, shape/size, odour, colour of infusion, and taste.

The result shows that the tea leaves in the tea bag are finely ground, with most being fannings. The majority had a rich black colour, but Moringa, Qualitea, and Legend Tea and Herbs had a pale green appearance. 3 Ballerina Tea had a bright green colour, while Ginseng tea appeared light yellow. The odour was fresh, but Richmond had a fruity smell. Moringa, Legend Tea, and Herbs had a pungent characteristic odour. Organic immune booster and Costa Tea had an earthy aroma.

The infusion colour for all selected samples was dark brown after brewing, except Moringa Tea, which had a light yellow appearance. The taste of the selected herbal teas ranged from bitter to bland, sweet, and characteristic.

Studies have shown that variations in the plant source, drying method, and storage conditions can significantly affect the organoleptic properties of herbal formulations. Overall, the organoleptic properties of the herbal tea samples are within acceptable sensory limits and provide useful information for further physicochemical evaluations.

Table 3.1: Organoleptic properties

Product Name	Colour	Shape/Size	Odour	Colour of Infusion	Taste
3 Ballerina Tea	Bright green	fannings	Fresh	Dark brown	Bland
Organic Immune Booster	Black	Finely grounded	Earthy	Dark brown	Bitter
Top Tea	Black	Finely grounded	Fresh	Dark brown	Bland
Ginseng Tea	Light yellow	Finely grounded	Fresh	Dark brown	Sweet
Legend Tea and Herbs	Pale green	Fannings	Pungent	Dark brown	Characteristic
Richmond	Black	Finely grounded	Fruity	Dark brown	Sweet
Costa Tea	Black	Fannings	Earthy	Dark brown	Characteristic
Moringa	Pale green	Fannings	Pungent	Light yellow	Bitter
Qualitea	Pale green	Fannings	Fresh	Dark brown	Characteristic
Lipton	Black	Finely grounded	Fresh	Dark brown	Characteristic

3.1.1. Label Claims and Product Details

Table 3.1.1. below provides information on the contents of the selected herbal teas. Information on manufacturing and expiry dates, ingredients, batch numbers, registration status by the National Agency for Food and Drugs Administration and Control (NAFDAC), number of tea bags, and estimated net weight.

From the table, it can be seen that the ingredients are listed on the product's package except for Qualitea, Legend Tea and Herbs, and Ginseng Tea. The manufacturing date was noted in most of the selected teas, except for 3 Ballerina and Ginseng Tea. All products had an expiry date and a batch number, except for Organic Immune Booster, Ginseng, Legend Tea, and Herbs. The batch number for Richmond was stated, but it was unclear. The NAFDAC registration number was also noted on all the products' packages, except for Legend Tea, and Herbs, and Moringa.

The number of tea bags on the product's package matched the number in the package for all the selected brands of herbal tea except Top Tea, which had 23 tea bags instead of 25.

The estimated net weight was also determined, and it was found that the net weight varied by no more than ± 3 g. Organic Immune Booster did not have a labeled net weight; the estimated net weight was 32.94 g. The variation in net weight could result from various conditions, such as storage conditions (temperature and moisture), packaging errors (calibration issues, machine variability), and particles that have fallen out during handling.

Table 3.1.1: Label Claims and Product Details

Product Name	Ingredients	Man. Date	Exp. Date	Batch No.	NAFDAC No.	No. of Tea bags	Estimated Net weight(g)
3 Ballerina Tea	Cassia angustifolia (Senna)	Nil	11/11/2025	2303F01	D1-2659	18	52.21
Organic Immune Booster	Vitamins B6 and E, Ginger, Garlic, Cinnamon, Astragalus Root, Chamomile, Oregon Grape Root, Lemon Balm	10/04/2024	12/04/2028	Nil	A1-8662	16	32.94
Top Tea	100% pure Black tea	07/06/25	01/12/26	158258	01-1286	23	46.79
Ginseng	Nil	Nil	05/2027	Nil	D-13365	12	14.35
Legend Tea and Herbs	Nil	08/2024	08/2027	Nil	Nil	25	51.18
Richmond	Ceylon, Indian & Kenyan Black tea	05/2025	05/2028	Not clear	B1-8241	25	50.17
Costa Tea	Black tea	08/2023	12/2025	04-D5	A8-9240	25	51.53
Moringa	Powdered Moringa	01/2024	01/2027	171/23	Nil	25	32.79
Qualitea	Nil	10/2023	10/2026	D23284	01-7026	25	51.91
Lipton	Black tea	27/02/25	26/08/26	25-09-4	01–132	25	53.80

3.2. Physicochemical Properties Result

Table 3.2 provides information on the results of the physicochemical tests carried out on the selected herbal teas, including pH determination, percentage moisture content as well and specific gravity determination.

pH

The pH is an important quality control parameter that reflects the level of acidity or alkalinity in herbal preparations and affects the stability, taste, and shelf life of herbal teas. The pH scale ranges from 0-14; values below 7 indicate an acidic solution, values above 7 indicate an alkaline solution, and a value of 7 indicates a neutral solution (neither acidic nor basic). In this study, the pH values of the herbal tea samples ranged between 4.83 and 6.03, indicating a mildly acidic nature. Previous studies state that the safe pH level of herbal teas ranges from 4.5 to 6.5 (Brusie, 2017), and the result reflects that the teas are safe for consumption. These values fall within the acceptable pH range (<7), which indicates they are chemically stable under normal storage conditions. It also mimics the pH of the gastrointestinal tract (stomach), which will aid in adequate digestion. Furthermore, pH values within this range indicate minimal risk of microbial contamination and ensure bioactive compounds are preserved, thereby enhancing therapeutic efficacy.

Moisture Content

Moisture content is essential for assessing the quality and stability of herbal teas. It provides information on the amount of water present in a sample and directly influences the growth of microbes and the overall physical stability of herbal teas. From the test carried out, the percentage moisture content ranged between 3.4% and 8.5% (<10%). It was found that Top Tea and Legend Tea and Herbs had the lowest percentage moisture content at 3.4% and 4.4% respectively, while Richmond Tea had the highest percentage moisture content of 8.5%. The result indicates that the selected herbal teas were adequately dried and, therefore, it is less likely to support microbial growth during storage. The moisture content should be as low as possible, as it offers better stability against degradation (Kunle *et al.,* 2012). Low moisture content prevents mold growth, enzymatic degradation, and oxidation of active constituents. The variations seen in the percentage moisture content can be attributed to differences in drying techniques and environmental humidity.

Specific Gravity

The specific gravity of a substance indicates the density of a liquid sample relative to that of water. It includes information on the concentrations of dissolved solids and extractable constituents in herbal teas. The specific gravity of water is 1.000 g/ mL, so values greater than 1.000 g/mL indicate the tea is denser than water (contains dissolved solids like sugar), while values less than 1.000 g/mL indicate that the tea is less dense than water. Based on the test results, the specific gravity ranged from 0.92 to 1.02 g/mL. Values close to that of water indicate that the herbal infusions will mix well with water rather than float or sink. These values suggest that the selected herbal teas contain adequate levels of soluble bioactive compounds, which contribute to their potential therapeutic and antioxidant activities. The variations in specific gravity could be due to differences in plant species used, extraction efficiency, and dissolved-solid concentration.

Table 3.2: Physicochemical Properties Result

Product Name	pH	Moisture Content (%)	Specific gravity g/ mL
3 Ballerina Tea	5.37	7.5	0.99
Organic Immune Booster	5.25	6.5	0.92
Top Tea	5.02	3.4	1.01
Ginseng Tea	4.83	5.8	1.02
Legend Tea and Herbs	5.59	4.4	1.00
Richmond	5.04	8.5	1.02
Costa Tea	4.91	5.9	1.00
Moringa	6.03	5.7	1.01
Qualitea	5.58	7.2	1.01
Lipton	5.00	5.0	0.98

3.3. DPPH Radical Scavenging Assay

Figures 3.1, 3.2, and 3.3 provide information on the percentage inhibition/radical scavenging activity at 517nm of the selected herbal teas brewed at different temperatures, including 95, 28, and 10℃. All these were compared to the percentage inhibition of the standard (Vitamin C), which is 81.25%.

The assay revealed notable differences in antioxidant activity among the herbal teas brewed at 95℃. Moringa herbal tea brew had the highest inhibition at 83.46%, surpassing the standard (81.25%), followed by Legend Tea and Herbs (65.19%), Ginseng (61.44%), Qualitea (59.23%), and Richmond (51.7%), while Costa Tea showed no activity (0%). Top Tea (7.69%), 3 Ballerina Tea (13.95%), Lipton (18.75%), and Organic Immune Booster (22.88%) had low inhibitions, which indicates low antioxidant activity when brewed at high temperatures. The variations reflect diverse polyphenolic profiles, with high-temperature brewing enhancing the extraction of bioactive compounds in herbal teas like Ginseng and Moringa, although heat-sensitive compounds may degrade in certain blends. The superior performance of Moringa is consistent with other brewed Moringa Tea infusions achieving 70 to 80% inhibition, exceeding ascorbic acid at equivalent concentrations (Pakade *et al.,*2013).

The herbal teas brewed at 28 and 10℃ exhibited a higher percentage inhibition than 95℃, therefore demonstrating higher antioxidant activities. Recently, cold or room temperature water extraction has become a new way of preparing herbal teas for consumption. It is highly aromatic, and there is also adequate reduction in caffeine levels and in bitter taste (Lin *et al.,* 2013). This suggests that brewing of herbal teas at room temperature and temperatures lower than it preserves heat-sensitive antioxidants.

In Moringa, Ginseng, and Legend Tea and Herbs, there was no significant change in the antioxidant activity when brewed at different temperatures. In the other teas, there was a significant change in the antioxidant activity, which suggests that they are better consumed when brewed at lower temperatures.

These findings align with evidence that low-temperature brewing preserves antioxidant potency in phenolic-rich herbs, often outperforming hot extraction for sensitive compounds (Lantano *et al.*, 2015)

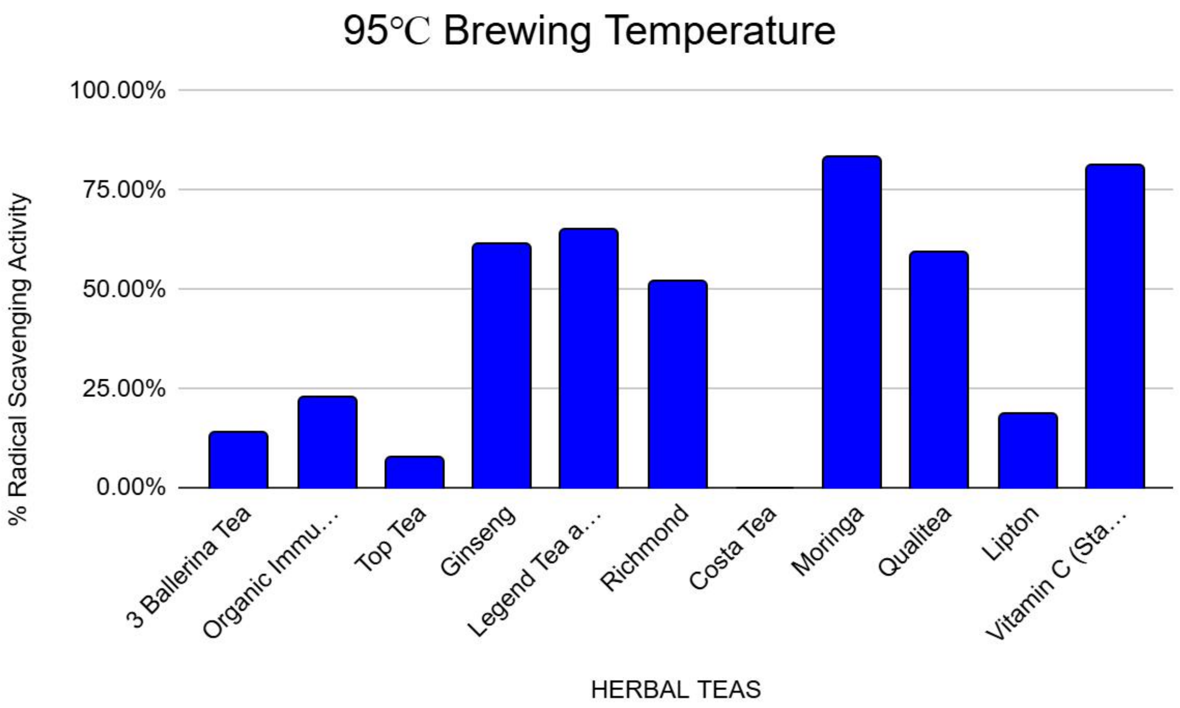

Figure 3.1. % Radical Scavenging Activity at 95℃ for selected Herbal Teas

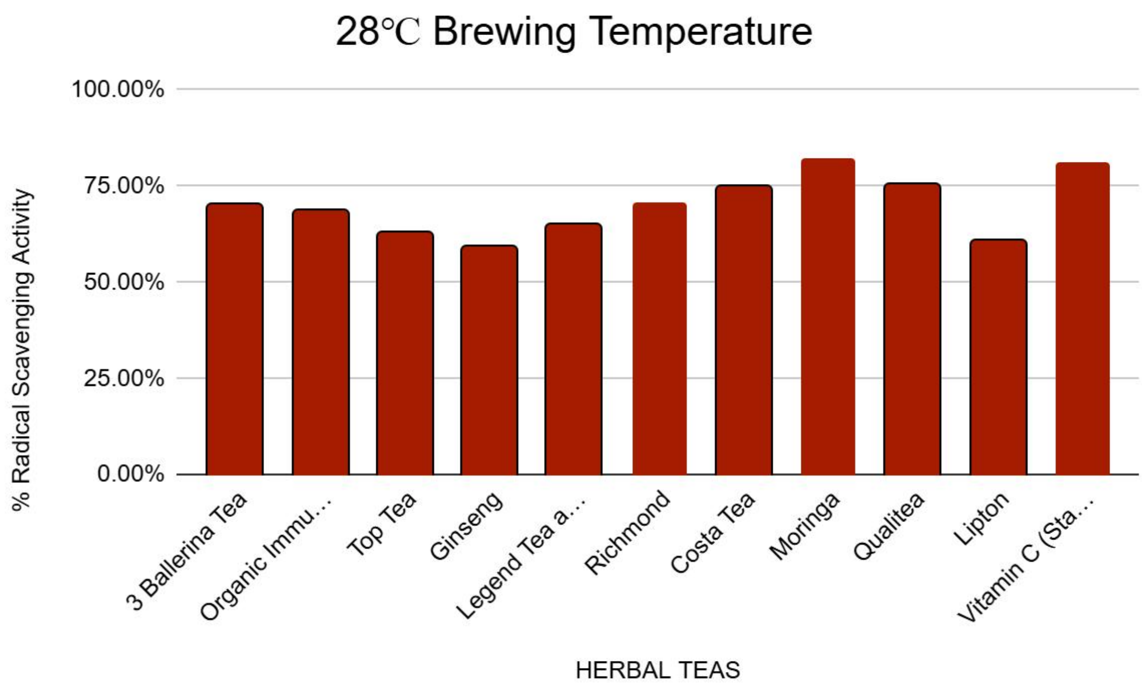

Figure 3.2. % Radical Scavenging Activity at 28℃ for selected Herbal Teas

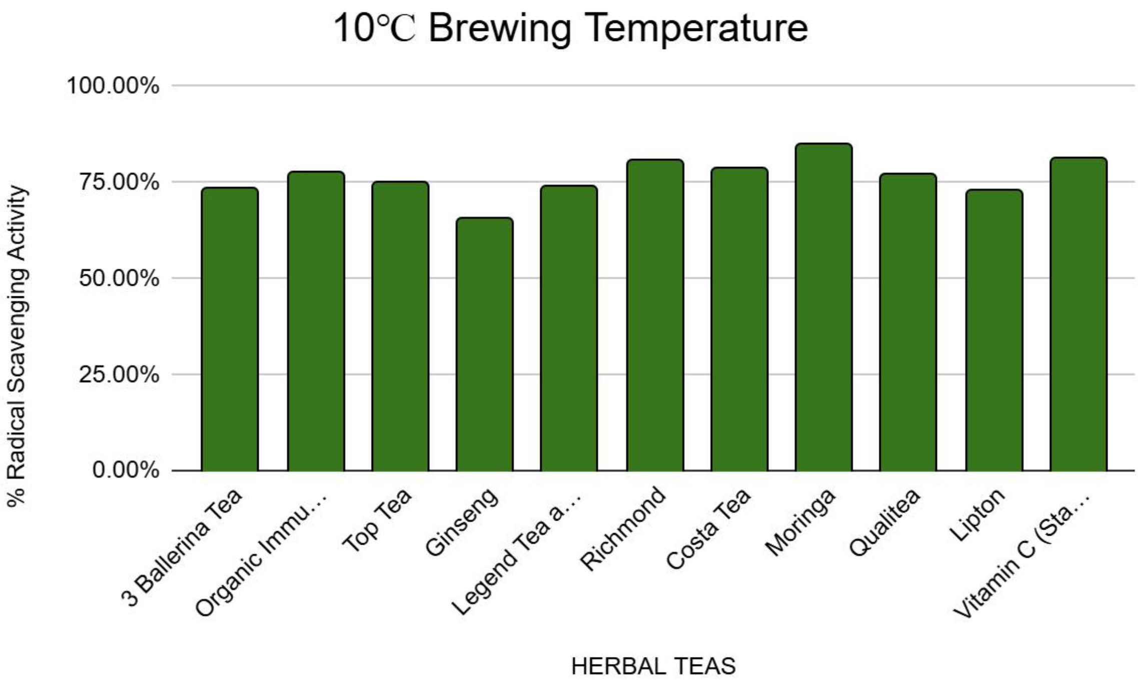

Figure 3.3. % Radical Scavenging Activity at 10°C for selected Herbal Teas

CHAPTER FOUR

4.0 Conclusion

The moisture content of the selected herbal teas was below 10%. This means it is of optimal quality, and the likelihood of microbial growth is very slim. pH analysis indicates that all selected sample infusions are acidic, suggesting they will be well digested in the gastrointestinal tract. The results of the specific gravity analysis show that the density of the selected herbal teas is similar to that of water. In the DPPH assay, the antioxidant activity of some selected herbal teas was higher when brewed at lower temperatures.

Overall, the findings suggest that the analyzed herbal teas met the basic quality requirements and may contribute beneficial antioxidant effects when consumed as part of a healthy diet. Further studies involving microbial analysis, heavy metal assessment, and standardization of herbal formulations are recommended to ensure safety, reproducibility, and regulatory compliance.

4.1 Limitations of the Study

The limitations of this study include:

1. Batch-to-batch variability: Chemical composition may vary across batches, affecting consistency.
2. Laboratory constraints, such as equipment availability, calibration, and sensitivity, may affect the accuracy of results.
3. Storage conditions: Factors such as light, humidity, and packaging during storage may alter phytochemical stability before testing of selected teas.
4. Limited antioxidant assay: Using only the DPPH method may not capture the full antioxidant profile.

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APPENDICES

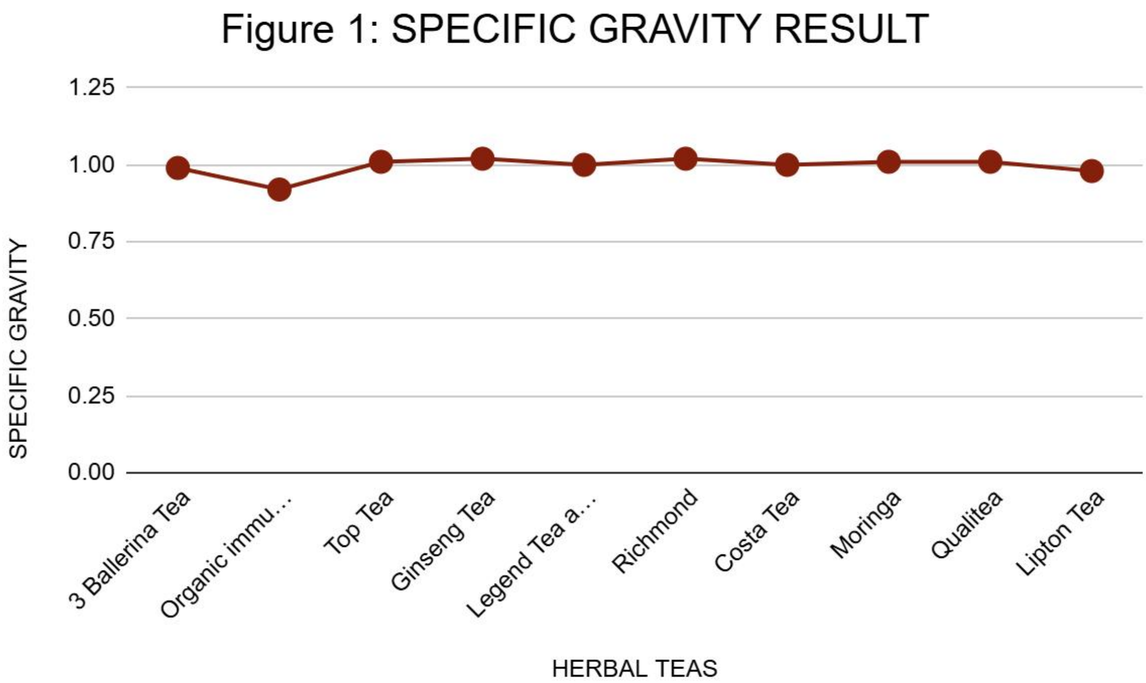

Figure 1: SPECIFIC GRAVITY RESULT
SPECIFIC GRAVITY
1.25
1.00
0.75
0.50
0.25
0.00
3 Ballerina Tea
Organic immu...
Top Tea
Ginseng Tea
Legend Tea a...
Richmond
Costa Tea
Moringa
Qualitea
Lipton Tea
HERBAL TEAS

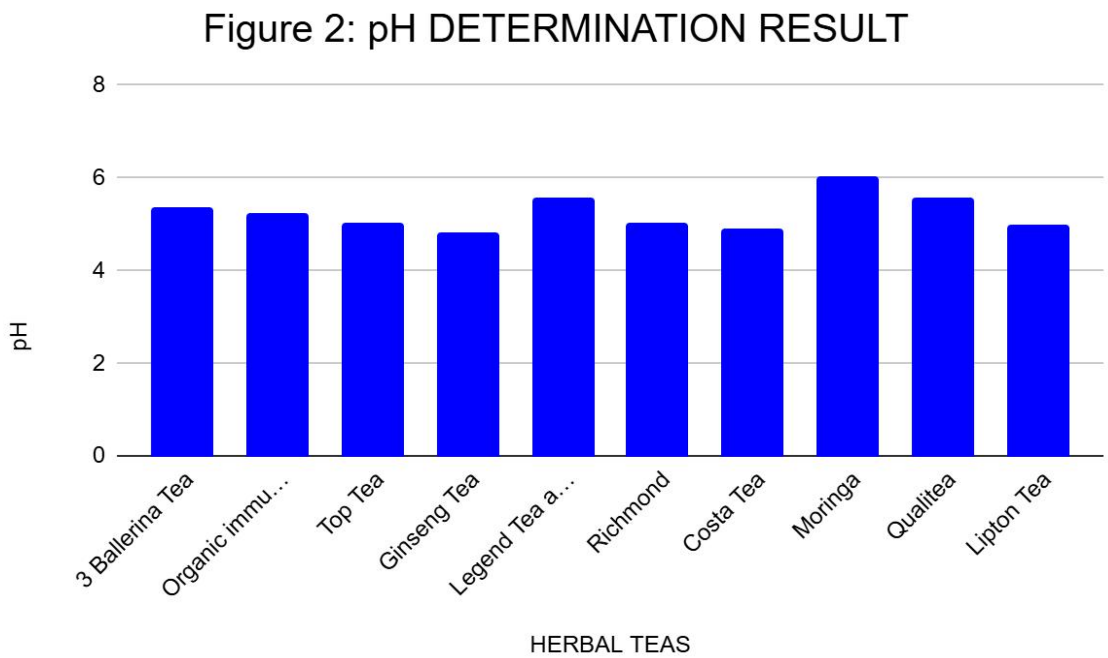

Figure 2: pH DETERMINATION RESULT
pH
8
6
4
2
0
3 Ballerina Tea
Organic immu...
Top Tea
Ginseng Tea
Legend Tea a...
Richmond
Costa Tea
Moringa
Qualitea
Lipton Tea
HERBAL TEAS

Figure 3: % MOISTURE CONTENT RESULT

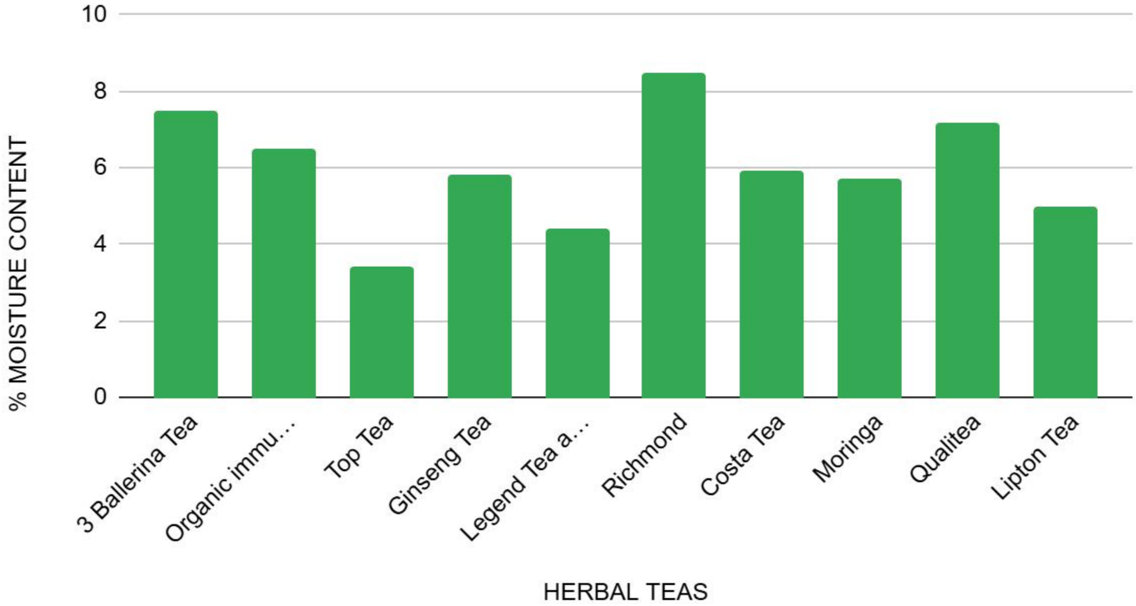

Table 3.3: % Radical Scavenging Activity Result

Product Name	95°C	28°C	10°C
3 Ballerina Tea	13.95	70.19	73.26
Organic Immune Booster	22.88	68.56	77.60
Top Tea	7.69	62.88	75
Ginseng	61.44	59.04	65.52
Legend Tea and Herbs	65.19	65	74.04
Richmond	51.7	70.67	80.48
Costa Tea	-	75.10	78.36
Moringa	83.46	82.02	84.62
Qualitea	59.23	75.19	76.92
Lipton	18.75	60.67	72.98
Vitamin C (Standard)	-	81.25	-

Table 3.3.1: Absorbance at 517nm of Selected Herbal Teas at Different Temperatures

Product Name	95°C	28°C	10°C
3 Ballerina Tea	0.895	0.310	0.278
Organic Immune Booster	0.802	0.327	0.233
Top Tea	0.960	0.386	0.260
Ginseng	0.401	0.426	0.317
Legend Tea and Herbs	0.362	0.364	0.270
Richmond	0.502	0.305	0.203
Costa Tea	1.040	0.259	0.225
Moringa	0.172	0.187	0.160
Qualitea	0.424	0.258	0.240
Lipton	0.845	0.409	0.281
Vitamin C (Standard)	-	0.195	-