Chapter 1: Introduction

Many hundreds of Millions of people throughout the world drink tea every day because it is delicious, affordable, widely accepted socially, and completely risk-free. Tea's fame has persisted through the ages. *Camellia sinensis* is a shrub whose leaves are used to make this beverage (Siro and Young,2000).

Originating in southeast Asia, the *Camelia sinensis* shrub has been utilized by humans ever since they figured out how to use steam to boil water. Emperor Shen Nung (2737 BC) is credited for introducing tea (tcha in Chinese) to the Chinese imperial court for the first time. Dutch traders brought tea to western nations about 1610, and since then its popularity has spread from the aristocracy to the commoners, to the people of neighboring countries, and eventually to the rest of the world.

Camellia sinensis, the species responsible for making tea and a member of the family Theaceae, spread from Southeast China to India, Sri Lanka, and other tropical and subtropical regions. About 30 nations throughout the world cultivate the tea plant. It does well in warm, humid climates (tropical and subtropical) with good drainage and a mildly acidic soil.

The way in which tea is consumed nowadays reflects regional norms and cultural norms. The majority of the world's green tea supply is consumed in China and Japan, with smaller amounts eaten in several North African and Middle Eastern nations. As reported by Pastore and Fratellone (2006). On average, people drink around 0.12 liters of soda each day, making it the second most popular beverage after water. This explains why Oolong tea is so popular in China and Taiwan, green tea in many parts of Asia and North Africa, and black tea in the West. Northern Ireland has the most dedicated tea drinkers, drinking 3.16 kilograms per person year, followed by the United Kingdom 2.53 kg/person and Kuwait 2.52 kg/person.

Green tea is a "non-fermented" tea. In the 17th century, India began supplying Japan with green tea. An estimated 2.5 million tons of tea leaves are harvested annually across the world, with 20% of the total being green tea, which is mostly eaten in Asia, certain regions of North Africa, the United States, and Europe.

Green tea's health benefits, in particular, have been recognized for quite some time (Weisburger, 2000) comparatively, it has more catechins than either black or oolong tea. Catechins have been shown to be powerful antioxidants in both in vitro and in vivo. The antioxidant capacity of this tea is further enhanced by the presence of a number of essential minerals and vitamins. At the moment, it is grown in at least 30 different nations. Trials show that it has significant therapeutic potential, thus it's utilized extensively in traditional medical practices across India and China, including Ayurveda, Unani, and Homoeopathy. Ancient Indian, Chinese, Japanese, and Thai civilizations all shared a love of green tea. This was proven by (Sato & Miata, 2000).

Many people like drinking tea for its many health advantages (Sairo, 2000) and because of its distinctive aroma and flavor (Cabrera,2006). Moreover, teas can be transformed into instant teas through the use of water extraction, concentration, and spray/freeze drying. The popularity of instant teas has skyrocketed over the past several decades due to their portability and simplicity of usage in the food business. The scent of tea and instant tea is a crucial factor in the goods' overall appeal, popularity, and price (Du et al., 2019). Furthermore, aromatic volatiles have been shown to play critical functions in human health control (Kuroda et al., 2005; Tomi et al., 2019). Water extraction, concentration, and drying are standard methods used to create instant tea. The popularity of instant teas has grown in recent decades due to their portability and simplicity of usage in the food business (Ni et al., 2020). There is evidence that instant sweet tea has a higher total phenolic content, total flavonoid content, antioxidant capacity, phloridzin and trilobatin content than traditional sweet tea (Liu et al., 2021). Therefore, consumers can benefit from including the instant sweet tea as part of their diet.

Camellia sinensis, often known as tea, is a kind of evergreen shrub that is native to East Asia and, more specifically, the border regions of southwestern China and northern Burma. As an additional rarity, *Camellia taliensis* tea is prepared from the plant's leaves. (Nadkarni, 1976; Fuglie, 2001) It is the second most drank beverage on the planet, behind water (Ramaa et al. (2006). Tea comes in a wide variety of flavors and aromas, from sweet and nutty to flowery and grassy. Some teas have a cooling, somewhat bitter and astringent flavor (Ni et al.,2020), while others have completely distinct qualities. The caffeine in tea is responsible for its stimulating impact on humans.

Moringa oleifera first discovered in India, the medicinal moringa tree has now spread across Asia, South America and even to certain regions of Africa. Moringa, or *Moringa oleifera* according to its scientific name, is a relatively new addition to Western medicine despite its long history of usage in other parts of the world.

Moringa leaves are highly beneficial to one's health. Vitamins, minerals, antioxidants, proteins, and more may all be found in abundance in Moringa oleifera. The leaves of the moringa tree are often ground into a powder and used as a nutritional additive. However, many of moringa's nutrients are still being uncovered by experts.

One of these is moringa, a tree of 14 different species from the genus Moringa and the family Moringaceae that bears the name "wonder tree" (Nadkarni, 1976). *Moringa oleifera* Lam, often known as the drumstick tree, is the most widely grown and well-known variety (Ramachandran et al., 1980). Moringa is a life-saving plant that provides nourishment to the underprivileged and is beneficial to individuals of all ages (Fuglie, 2001). The best defense against these diseases was found to be eating meals that contained plant components high in bioactive chemicals (Ramaa et al., 2006).

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1.1 Health benefits of moringa leaves:

Plants' naturally produced antioxidants, especially polyphenols, are the primary defense against oxidative damage to tissues. They do this in two ways: either by directly boosting a cell's defenses or by scavenging free radicals (Du et al., 2010). The extract of fully ripe Moringa Oleifera leaves was shown to block higher levels of both enzymatic and non-enzymatic antioxidants in a comparative investigation.

Type 2 diabetes mellitus is a metabolic disease that lasts a long time. Impairment in glucose tolerance and persistently high blood sugar levels characterize the diabetic state (Tiwari and Roa, 2002). Moringa oleifera has traditionally been used to treat diabetes mellitus due to its pharmacological effects (Bhishagrantna,1991; Babu and haudhuri,2005).

Cancer, rheumatoid arthritis, diabetes, and maybe other factors may all be treatable with moringa. In South Asian traditional medicine, it is used for the treatment of a variety of illnesses (Mehta et al., 2003; Karadi et al., 2007; Roy et al., 2007)

The scientific community is beginning to take notice of Moringa oleifera as a feasible development platform because of the growing body of research showing that Moringa is a rich natural resource for phytochemicals. Rumalaya and Septilin (the Himalaya Drug Company, Bangalore, India), Orth herb (Walter Bushnell Ltd, Mumbai, India), Kupid Fort (Pharma Products Pvt. Ltd, Thayavur, India), and Livospin (Herbals APS Pvt. Ltd, Patna, India) are just some of the commercially available health formulations that contain M. oleifera (Mehta et al.,2003).

1.2 Objectives:

1. Our primary goal was to combine green tea with moringa powder to create a revitalizing, beneficial beverage and emerging subtype.
2. To analyse and contrast the brewed tea's proximate analysis, sensory attributes, antioxidant activity, vitamin C and E, polyphenol and flavonoid content, caffeine level, and microbiological properties.
3. To determine the overall drinkability and quality of its constituent parts and soaring interest from consumers both at home and abroad.

Chapter 2: Review of Literature

2.1 Overview of tea production and consumption:

More than 2.50 billion kilograms of tea (including 0.56 billion kilograms of green tea varietals made by eight nations) are produced annually from 2.56 million hectares of planting by thirty countries. About 1.32 billion kg of tea is exported annually from 28 countries once local demand is met (ITC,2001). Table 1 displays the annual tea output of the world's 12 largest tea-producing countries. More than 54 million kg of tea is being produced each year in Bangladesh, over an area of around 49,000 hectares. Roughly 18 million kilos of tea may be exported each year, bringing in foreign currency equivalent to about 1775 million Taka (Taka 63 = US $ 1). (BTRI,2003). There has been a yearly growth of 3% in global tea output (International Tea Committee report 2001), with Bangladesh exhibiting a 1.84% increase in production and contributing 1.37% of global tea exports. The eastern hilly regions of Bangladesh are where the majority of the country's tea is grown. These regions include the districts of Sylhet, Moulvibazar, Habigonj, and Chittagong. Greater Sylhet accounts for almost 96% of yearly output (63% of which is of Moulvibazar district) from 93% of planted land (62% of which is of Moulvibazar district). It is important to remember that Sterling firms account for only 42% of total plantation land, while they generate roughly 50% of the annual output.

Table 2.1: Country wise Productivity of Tea (kg/ha)- 2000 AD (International Tea Committee report 2001).

Country	Production (kg/ha)
India	1743
Japan	1745
Sri Lanka	1450
Bangladesh	1102
China	627

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Approximately 1.5 lakh people are employed by the 167 commercial Tea Production Estates and Tea Gardens spread across 2,79,507.88 acres of land in Bangladesh. In addition, Bangladesh is responsible for producing 3% of the world's tea. The value of Bangladesh's tea market was estimated at BDT 3500 crore in 2021. As reported by the Bangladesh Tea Board, the country of Bangladesh is home to 167 commercial tea

plantations and gardens. Approximately 6 crores 74 lakh kg of tea is produced every year from these tea estates and tea gardens, which cover a total area of 2,79,507 acres (2,79,506,88). The year 2021 saw Bangladesh produce 9.65 million metric tons of tea, an all-time record.

2.2 Scientific Classification:

I. *Camellia sinensis*

Kingdom: Plantae

Order: Ericales

Family: Theaceae

Genus: Camellia

Species: *Camellia sinensis*

II. *Moringa oleifera*

Kingdom: Plantae,

Division: Magnoliphyta,

Class: Magnoliopsida,

Order: Brassicales,

Family: Moringaceae,

Genus: Moringa,

Species: *Moringa oleifera*

Figure 2.2: *Camellia sinensis & Moringa oleifera*

2.3 Varieties of tea:

a) Green tea: Green Tea is made from freshly plucked, unfermented leaves. Green tea's wide range of beneficial compounds has been shown to have the greatest possible impact on human health.

b) Black tea: More than seventy-two percent of the world's tea is black tea. Black tea preserves a significant amount of antioxidant polyphenols such as flavonoids, however, most of the EGCG antioxidants are oxidized during the fermentation process. Toxins in the body can be flushed out with the aid of these antioxidants.

c) White tea: In white Tea, the tea buds and young leaves are plucked just as they begin to unfold. Next, the leaves undergo minimal processing by being steamed and dried. As a result, white tea from the *C. sinensis* plant keeps more of its beneficial compounds, including its high antioxidant and low caffeine content (green, black or oolong).

d) Oolong tea: Oolong tea, a partly fermented tea with flavor and health benefits resembling those of both green and black teas. Antioxidants are abundant, and their protection of healthy skin cells and slowing of the aging process are both benefits.

e) Pu'erh Tea: This tea is made from a large-leaf form of the tea plant and may be harvested at any time. It's made in a manner analogous to that of black tea. What sets this tea apart is the lengthy aging process anywhere from 50 to 100 years that follows harvesting.

f) Roobios or Red tea: The South African bush from which Roobios or Red Tea is extracted. Because it naturally has no caffeine, it is safe for nursing mothers and pregnant women to use. Antioxidant levels are particularly strong in Rooibos or Red Tea.

Black tea is the most popular kind in the west, whereas green and oolong tea is more popular in Asia. The fermentation process reduces the polyphenol content of the leaves while increasing the caffeine concentration. Moringa tea, sunflower tea, ginger tea, tulsi tea, and other herbal teas have gained popularity in recent years due to the interest people have shown in their purported health benefits (Sharangi,2009).

2.4 Moringa leaves:

Leaves of the Indian medicinal plant moringa (Moringa oleifera Lam) are widely used across the tropics and subtropics. The Moringaceae family includes the plant species *Moringa oleifera*, which is a member of the Brassica genus of vegetables. The Moringaceae family consists of a single genus, of which there are 13 species (Khawaja et al., 2010). Although Moringa is a common food in these areas, it is also revered for the health benefits it provides. Due to it's remarkable healing qualities for a wide range of disorders, including certain chronic conditions, it is sometimes referred to as "the miracle tree" among the general public (Abdul Razis et al., 2014). Vitamins, minerals, beta carotene, amino acids, antioxidants, anti-inflammatory elements, omega 3 and omega 6 fatty acids, and many more have all been shown to be present in Moringa thanks to extensive scientific investigation (Fahey 2005; Hsu et al., 2006; Kasolo et al., 2010).

2.4.1 Nutritional composition:

The moringa leaves are nutritionally very rich, leaving behind carrots, oranges and even milk in terms of nutritional value.

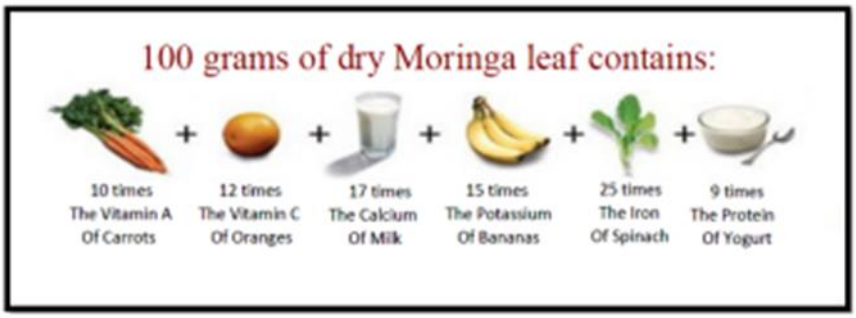

Figure 2.4: Comparison of nutrients in Moringa with various food items

(Fuglie, 1999)

2.4.2 Moringa leaves contain a significant bioactive component:

Moringa leaves are said to have a high concentration of nutrients such as vitamins and beta carotene, as well as potassium and protein. It's a great way to get some natural antioxidants into our system. For this reason at they include antioxidants such as

flavonoids, ascorbic acid, carotenoids, and phenolics (Dillard and German, 2000; Siddhuraju and Becker, 2003).

Plant part	Medicinal Uses	References
Root	Antilithic, rubefacient, vesicant, carminative, antifertility, anti-inflammatory, stimulant in paralytic afflictions; act as a cardiac/circulatory tonic, used as a laxative, abortifacient, treating rheumatism, inflammations, articular pains, lower back or kidney pain and constipation,	*The Wealth of India*, 1962; Padmarao *et al.*, 1996; Dahot, 1988; Ruckmani *et al.*, 1998
Leave	Purgative, applied as poultice to sores, rubbed on the temples for headaches, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh; leaf juice is believed to control glucose levels, applied to reduce glandular swelling	Morton, 1991; Fuglie, 2001; Makonnen *et al.*, 1997; *The Wealth of India*, 1962; Dahot, 1988
Stem bark	Rubefacient, vesicant and used to cure eye diseases and for the treatment of delirious patients, prevent enlargement of the spleen and formation of tuberculous glands of the neck, to destroy tumors and to heal ulcers. The juice from the root bark is put into ears to relieve earaches and also placed in a tooth cavity as a pain killer, and has anti-tubercular activity	Bhatnagar *et al.*, 1961; Siddhuraju and Becker, 2003
Gum	Used for dental caries, and is astringent and rubefacient; Gum, mixed with sesame oil, is used to relieve headaches, fevers, intestinal complaints, dysentery, asthma and sometimes used as an abortifacient, and to treat syphilis and rheumatism	Fuglie, 2001
Flower	High medicinal value as a stimulant, aphrodisiac, abortifacient, cholagogue; used to cure inflammations, muscle diseases, hysteria, tumors, and enlargement of the spleen; lower the serum cholesterol, phospholipid, triglyceride, VLDL, LDL cholesterol to phospholipid ratio and atherogenic index; decrease lipid profile of liver, heart and aorta in hypercholesterolaemic rabbits and increased the excretion of faecal cholesterol	Nair and Subramanian, 1962; Bhattacharya *et al.*, 1982; Dahot, 1998; Siddhuraju and Becker, 2003; Mehta *et al.*, 2003
Seed	Seed extract exerts its protective effect by decreasing liver lipid peroxides, antihypertensive compounds thiocarbamate and isothiocyanate glycosids have been isolated from the acetate phase of the ethanolic extract of *Moringa* pods	Faizi *et al.*, 1998; Lalas and Tsaknis, 2002

Figure 2.4.2: Medical uses of Moringa plant (Anwar et al.,2009)

2.4.3. Good source of Protein:

There are claims that Moringa's protein is of excellent quality and very digestible because of the amino acid profile it contains (Foidl et al., 2001). While previous research has shown between 18 and 16 amino acids in dried Moringa leaves (Foidl et al.,2001; SanchezMachado et al.,2009).

The dried leaves have the potential as a supplemental protein source for both animals and humans. As it has been indicated that amino acid supplementation is crucial in satisfying a significant amount of an animal's protein and energy requirements, this protein content is of special nutritional value (Brisibe et al., 2009). Amino acid-rich diets are protective against gastrointestinal parasites (Kyriazakis and Houdijk, 2006). Proteins are also crucial for the constant restoration of cellular protein depleted by gastro-intestinal helminth infections (Coop and Holmes, 1996).

Table 2.4.3: Minerals content of dry moringa leaves: (Moyo et al., 2011)

Macro nutrients	Dry leaves (%)
Calcium	3.65 ± 0.036
Magnesium	0.50 ± 0.005
Potassium	1.50 ± 0.019
Sodium	0.164 ± 0.017
Micronutrients	**Dry leaves (mg/kg)**
Iron	490 ± 49.649
Copper	8.25 ± 0.143
Manganese	86.8 ± 3.940

It's also worth noting that dried Moringa leaves have a remarkably high deposit of mineral components.

- It's also worth noting that dried Moringa leaves have a remarkably high deposit of mineral components. Bones and teeth can't grow or stay healthy without it, and that's why it's so important in warding off osteoporosis. it is also needed for normal blood clotting and the nervous system.
- Fe, which is typically inadequate in many plant-based diets, was found in abundance in the leaves of the plant. For oxygen transport and cellular functions including development and division, iron is a crucial component of hemoglobin and myoglobin (Kozat, 2007). The oxidation of carbohydrates, proteins, and lipids, as well as the proper functioning of the central nervous system, both require iron as a trace element (Umar et al., 2007).
- The vitamin C content of moringa reportedly aids in the body's absorption of iron (Anwar et al., 2007).

2.5 Green Tea and Health Benefits:

- Green Tea has anti-inflammatory effects among its other biological effects (Chung, 2003). According to research green tea can reduce inflammation by blocking the production of the inflammatory cytokine TNF-alpha (Sueoka et al., 2001). This action is mediated by green tea's ability to dampen the activity of the transcription factor NF-kB.
- Green tea's protective effects against inflammatory diseases like rheumatoid arthritis and multiple sclerosis are the result of its ability to block the production

of a protein called tumor necrosis factor-alpha. (Aneja et al., 2004; Aktas et al., 2004; Li et al., 2004).

- While oral administration of green tea polyphenols to mice was observed to reduce UV-induced increases in epidermal activity (Agarwal et al., 1993). Topical application of green tea reduced phorbol ester-induced elevations in epidermal COX and lipoxygenase activity (Katiyar et al., 1992).

- Green tea's preventive effect was discovered in research (Setiawan et al.,2001) that included 133 stomach cancer patients, 166 chronic gastritis cases, and 433 healthy controls. In case-control research included 141 people with esophageal cancer and 223 healthy controls, Gao et al. found that frequent tea intake was associated with a lower risk of developing esophageal cancer. (Zhong, et al., 2001)

2.6 Advantages to Your Health from Ginger:

Most cultures across the world utilize ginger (Zingiber officinale Roscoe, Zingiberaceae) as a seasoning (Surh et al. 1999). The principal pungent element thought to exhibit a range of noteworthy pharmacological and physiological effects is the oleoresin (oily resin) from the rhizomes (roots) of ginger, which includes several bioactive components. The absence of a thorough knowledge of ginger's mechanisms of action advises caution in its therapeutic usage, despite the fact that ginger is usually thought to be safe (Kaul and Joshi, 2001; Wilkinson 2000a).

- Ginger's antioxidant capabilities are commonly cited as the method by which the spice exerts its healthful effects. The presence of oxidative stress has been linked to a wide range of ailments (Aeschbach et al. 1994).
- Ginger has been shown to reduce indicators of oxidative stress associated with aging (Topic et al., 2002), and it has been proposed that it can protect against ethanol-induced hepatotoxicity by dampening the oxidative effects of the drug in rats (Mallikarjuna et al., 2008).
- Ginger has been linked to a number of health benefits, including reduced inflammation, edema, and discomfort. Both a dried ginger extract rich in gingerol (Young et al. 2005) and a dry ginger extract rich in gingerol (Young et al. 2005) were shown to have analgesic and powerful anti-inflammatory effects.

- Several research institutions, including our own, are now examining ginger and its constituents for their potential cancer-preventive and therapeutic uses. Gingerol and zerumbone's antioxidant properties are linked to their cancer-fighting effects (Bode et al. 2011).

2.7 Types of bioactive compound found in Moringa and Green tea:

a) Flavonoids:

Polyphenol molecules with 15 carbon atoms are known as flavonoids. They consist mostly of flavonoids, flavanols, and flavanones. Flavonoids have several biological functions, including antioxidant, antibacterial, anticarcinogenic, and neuroprotective actions. Quercetin, a flavonoid of the flavanol type found in garlic, tea, and apples, is ingested on an almost daily basis. It is estimated that between 0 and 30 mg of quercetin is consumed daily in the typical Western diet (D'Andrea, 2015). Hesperidin, a flavanone found in citrus fruits, has poor bioavailability, poor water solubility, and short biological life (Parthasarathy et al., 2009). Nigenin, a natural flavanone also found in citrus fruits like grapefruit and oranges, improves insulin signaling in the brain and memory (Ghofrani et al., 2015).

b) Polyphenols:

Many hydroxyl groups are linked to benzene rings in polyphenols. Phenolic chemicals have attracted interest because of their prevalence in food, antioxidant activity, and their function in protecting against numerous disorders related to oxidative stress. Fruits and drinks (fruit juice, tea, and coffee) are the main sources of phenols in the diet, with cereals, vegetables, and legumes providing smaller quantities. In comparison to the about 100 mg found in a cup of red wine, tea, or coffee, fruits including apples, grapes, pears, cherries, and other berries can contain up to 200-300 mg of phenolic compounds per 100 g of fresh weight. On average, humans ingest around 1 g of phenolics each day (Scalbert et al., 2005).

2.7.1 Health benefits of Bioactive compunds:

Because of the presence of functional groups in their structure, bioactive substances exhibit antioxidant, free radical scavenging, and chelating activities. They were also responsible for most of the beneficial effects of flavonoids on health. In addition to preventing mutations, flavonoids also fight tumors. Flavonoids are known to block a wide variety of enzymes, including oxygenises (prostaglandin synthase), that play a pivotal role in the production of eicosanoids. As a result, flavonoids can reduce hyaluronidase activity and promote the health of proteoglycans found in connective tissue. The treatment described here would prevent germs from spreading or cancer from metastasizing (Havsteen, 2002).

2.8 Antioxidants:

Natural substances known as antioxidants prevent damage from oxidation despite their relatively modest concentration compared to the dominant oxidizable substrate (Halliwell, 2007). More than 170 antioxidants have been described in recent research (Zhou, 2012). According to research by Boxin et al. (2002), reactive oxygen species (ROS) have a positive effect on the immune system and can work as agents with anticancer, immunity-boosting, antibacterial, antimicrobial, antifungal, cholesterol-lowering, antiparasitic, and anti- 9 inflammatory effects (Bub et al., 2003). Fiber, polyphones, conjugated dimers of linolenic acid, limonene, epigallocatechin, soy protein, isoflavones, and vitamins A, B, C, and E are all antioxidants found in fruits and vegetables. Calcium, chlorophyllin, alipharin, sulfur compounds, tetrahydro curcumin, glutathione, lipoic acid, indoles, thiocyanates, protease inhibitors, and marine aminols are only some of the ingredients (Karakaya et al., 2001).

2.8.1 Source of antioxidants:

Antioxidants are found primarily in vitamins C and E as well as in polyphone, lycopene, copper, a-carotene, cysteine, and sialic acid. Juices, beverages, and even heated liquids can contain high levels of antioxidants such as polyphenols, vitamin C, vitamin E, carotene, and lycopene (Ramadan-Hassanien, 2008).

2.8.2 Functions of antioxidants:

Antioxidants have a crucial role in the protective benefits of plant-based diets. Consistent fruit and vegetable eating lowers the risk of acquiring chronic illnesses (Dembinska et al., 2008). There are long-term advantages to eating foods high in antioxidants (Sin et al., 2013). Antioxidants have recently been linked to free radicals, which are thought to be responsible for cell damage, cancer prevention, and increased longevity (Kalcher et al., 2009). All antioxidants function through the antioxidant system, which is responsible for protecting the body against free radicals and the hazardous by-products of their metabolism. Antioxidants go through a series of modifications, the last of which is the creation of a complex with lipids. Photo chemicals, compounds found in plants with persistent antioxidant activity, are receiving a lot of attention as dietary components that fight chronic illnesses (Peter, 2007).

2.8.3 Mechanism of antioxidant activity in the human body:

Oxidative stress causes an increase in levels of reactive oxygen species (ROS), which in turn affects cellular function when ROS generation and detoxification are in equilibrium. Damage from reactive oxygen species (ROS) may be seen in a variety of biological macromolecules, such as lipid peroxidation acid and protein. However, as ROS levels are beyond this threshold, ROS production increases. This might lead to an overload of impulses being transmitted to the cell, potentially compromising key components of the cell's signaling pathways. ROS causes irreversible damage to vital macromolecules. The principal cytosolic low molecular weight sulfhydryl compounds that serve as cellular reductants are protein-bound thiol and non-protein thiol. This has led to thiol being utilized extensively as an initial line of defense against oxidative damage. Long-term damage to cellular macromolecules caused by oxidative stress has been demonstrated to have a role in the onset of illnesses such as atherosclerosis, coronary heart disease, liver cancer, diabetes, and carcinogenesis. Antioxidants can inhibit the formation of free radicals and reactive oxygen species (Shahidi and Ambigaipalan, 2015). Greater resistance to oxidative stress and the diseases it might cause can be attained by eating a diet high in organic foods rich in antioxidants (Adwas et al., 2019).

Chapter 3: Materials and Methods

3.1 Study areas and Sample collection:

The duration of the study was from March 1, 2022, to July 27, 2022, a span of five months. *Moringa oleifera* leaf samples were gathered from the Kutupalong region, also known as the Rohingya refugee camp 5. Specifically, this part of Cox's bazaar is located in the Ukhiya Upazila. The moringa leaves were picked up in brand-new bags, so they were completely pristine and unspoiled. They were subsequently delivered to the Department of Food Science and Technology at Chattogram Veterinary and Animal Sciences University (CVASU) in Khushi, Chattogram, Bangladesh.

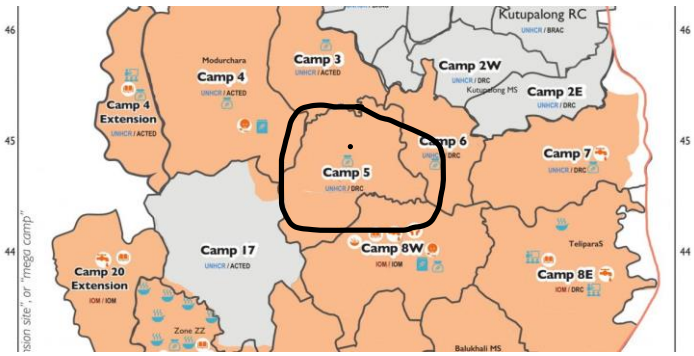

Figure 3.1: Location of Camp 5

3.2 Design of Experiment:

At first, moringa leaves were gathered right. Once it was done, we collected green tea, ginger, and black seed. Once samples were collected, they were ground into a powder. Then, the components were blended together in various ratios to create a nourishing tea (Fig 3.4). The three types of Tea such as Type A, Type B, and Type C were prepared using different portions of herbs (Table 3.6).

Tea's proximate composition (moisture, ash, crude fat, protein, and crude fiber), vitamins (C & B2), and minerals (Fe, K, Mg, Ca) were examined after processing, as were the amounts of polyphenols, flavonoids, antioxidant activity, and caffeine present. Consumers were asked to rate how satisfied they were with two different versions of the same product (samples A and B). Proximate analysis, nutrient content, microbial load, and customer acceptability testing were conducted for each product type. Similar testing was done on one of the controls.

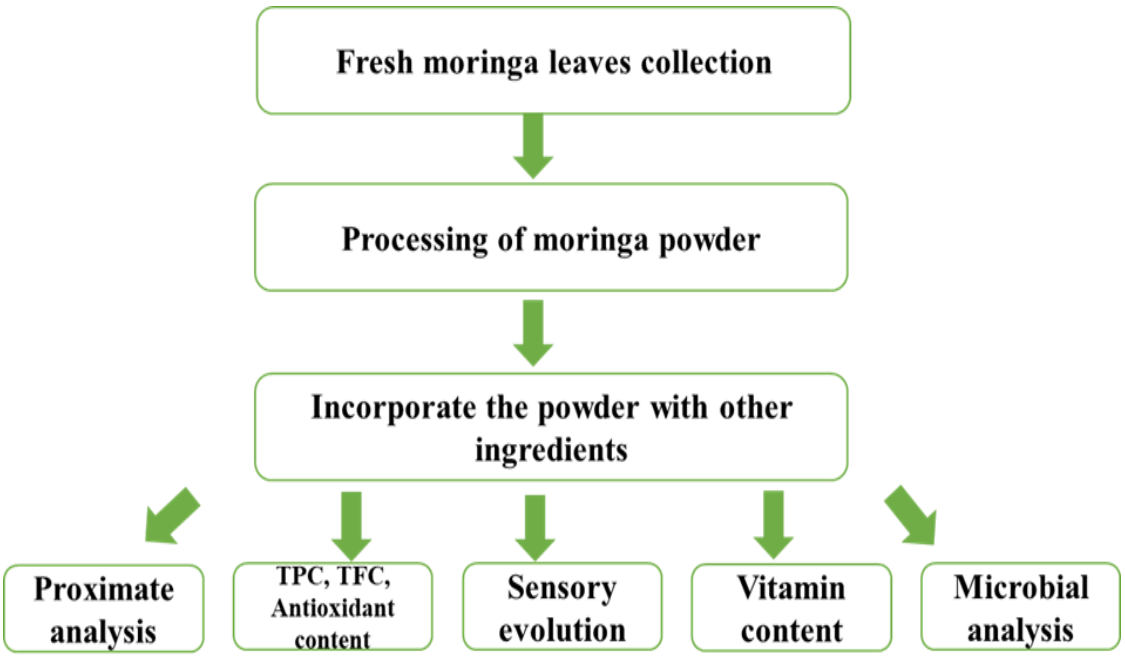

Figure 3.4: Design of experiment

3.3 Powder preparation:

3.3.1 Moringa Powder preparation:

The moringa leaves have a thorough washing to remove sand and other debris. After harvesting the leaves, they spent around 24 hours drying at 60 ° C in a cabinet dryer. The powdered material was filtered through a fine (2 mm mesh) sieve to remove any lingering particles. The powder was kept in labelled plastic containers until it was ready to be used.

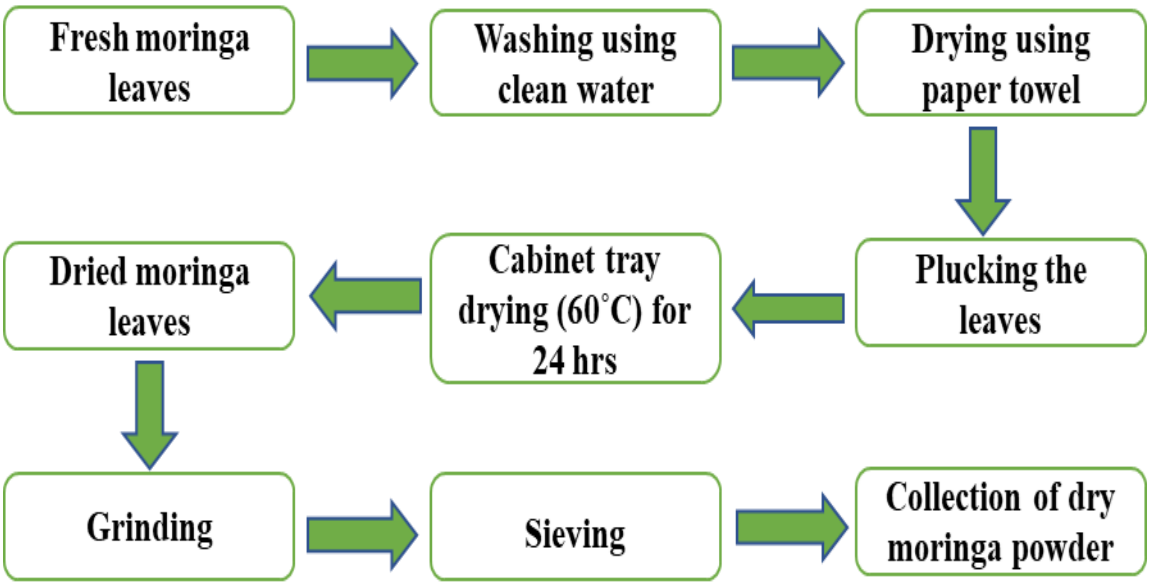

Figure 3.5.1 Moringa powder Preparation

3.3.2 Ginger powder preparation:

Ginger was peeled and sliced paper thin, no more than an eighth of an inch thick. It was dried for three to four hours in the oven. Some of the ginger will dry sooner than others since it was sliced at a little varied thickness. About two hours later, ginger were examined and discarded any dry bits. Repeatedly after twenty to thirty minutes, the portions that have dried were taken out. After dry everything it was taken it out of the oven and allowed to cool completely. A blender were used to finely chop the ginger.

Figure 3.5.2: Ginger powder preparation

3.4 Tea processing:

Table 3.4: Composition of Experimental Tea:

Type A		Type B		Type C
Green tea	**90%**	Green tea	**80%**	Commercial Green Tea
Moringa powder	**8%**	Moringa powder	**15%**	
Ginger powder	**2%**	Ginger powder	**5%**	

Each component was measured and weighted separately as instructed in the recipe. After processing all of the constituents into powder form and blending in the powder in two formulation ratios, the produced tea's nutritional value and sensory attributes were evaluated.

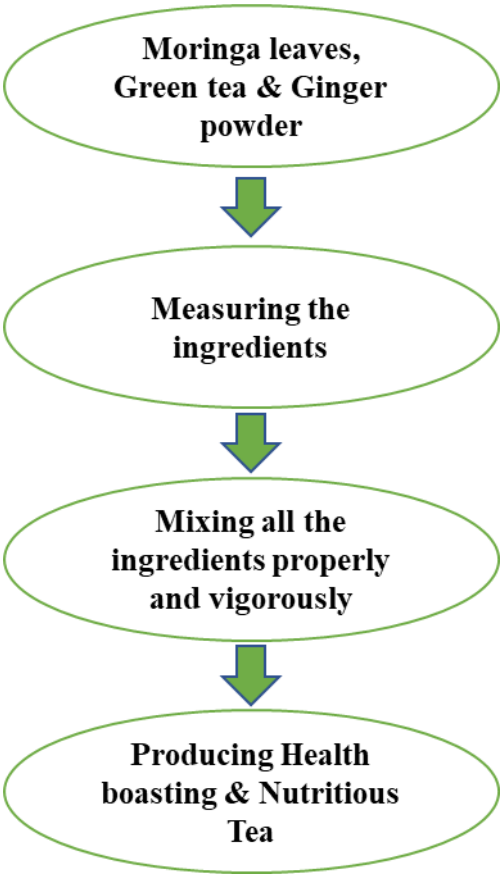

Figure 3.4: Tea processing

3.5 Determination of Antioxidant capacity by DPPH scavenging method

The Making of an Extract

One gram (1g) sample and put it in a Felcon tube. After waiting 72 hours, 10 ml of 100% methanol was added to the mixture. Continuous straining was done every 4 hours. It was filtrate after 72 hours and found methanoic extract.

Procedure

Extract antioxidant activity was evaluated using a modified version of the DPPH assay first established by Azlim et al (2010). Methanoic DPPH solution was made by dissolving 6 mg of DPPH into 100 mL of 100% methanol. The methanoic extract was then diluted with 2 mL of DPPH solution. The ingredients were combined, shaken gently, and then let to rest for 30 minutes at room temperature in complete darkness. The absorbance was determined using a UV-VIS spectrophotometer with a 517 nm setting (UV-2600, Shimadzu Corporation, USA). The control was made by adding two milliliters of DPPH solution to one milliliter of methanol, which served as the blank. Scavenging activity was calculated by comparing absorbance readings of test samples with DPPH standard solution. The ability of an extract to scavenge DPPH free radicals is calculated using the following equation:

$$\% \text{ of inhibition} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100$$

Trolox was utilized as the standard, and the validation standard curve was TEAC composite (Trolox equivalent antioxidant mobility). The results were reported in milligrams (mg) per 100 grams of powder on a dry weight (DW) basis.

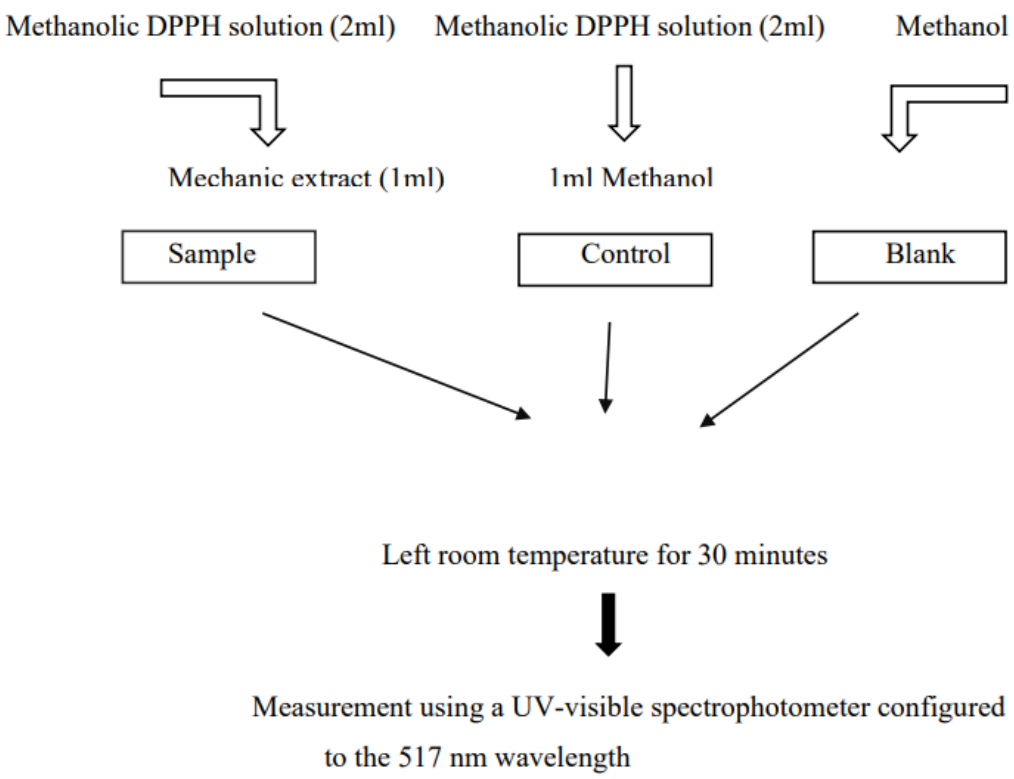

Figure 3.5: Antioxidant capacity determination

3.6 Determination of bioactive compounds

The Making of an Extract

In Felcon tube, 5 gm of TAC sample and 1 gm of additional TPC and TFC sample were collected. After that, 10 mL of 100% ethanol was added and the mixture was left for 72 hours. Continuous straining was done every 4 hours. After 72 hours, the filtrate was collected, and an ethanoic extract was identified.

3.6.1 Total phenolic content (TPC):

The Folin-Ciocalteu reagent procedure, with minor adjustments, was employed to quantify TPC of the extracts (Al-Owaisi et al., 2014). The Folin-Ciocalteu procedure, as published by Vergani et al. (2016), was employed to assess the total polyphenol content (TPC) of sample. 1 ml of ethanoic extract and 1.5 ml of FC reagent were mixed in a falconer tube and kept at room temperature for 3 minutes. After that, the mixture was exposed to 1.5 ml of 7.5% Na_2CO_3 for 60 minutes. The absorbance at 765 nm was measured using a UV-VIS Spectrophotometer (UV2600, Shimadzu Corporation, USA)

with C_2H_5OH serving as the blank. In terms of gallic acid equivalents (GAE), TPC was determined to be mg GAE/g of extract.

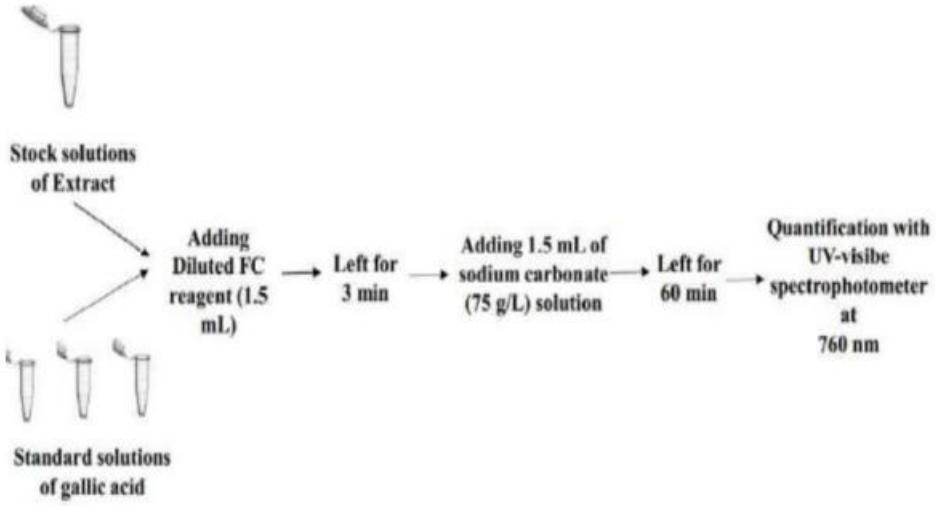

Figure 3.6.1: Determination of Total phenolic content (TPC)

3.6.2 Total flavonoid content (TFC)

Total flavonoid content (TFC) was calculated by applying a modified version of the aluminum chloride colorimetric method reported by Chang et al. (2002) to the fruit samples. Extract stock solutions (1 mg/mL) were added to a cuvette along with aliquots of 5 mL of diluted extract and 1.5 mL of 95% ethanol. After that, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of distilled water were added to the cuvette. The mixture was allowed to come to room temperature for 30 minutes. Absorbance was measured at 415 nm using a UV-visible spectrophotometer; the blank was 10% aluminum chloride replaced with roughly the same amount of pure water (UV-2600, Shimadzu Corporation, USA). The amount of flavonoids was determined by dividing the absorbance of the sample extract by the value from a quercetin standard curve. The effectiveness of TFC is measured in terms of quercetin equivalents per 100 grams of extract (mg QE/100g).

The procedure of total phenolic content:

- A falcon tube containing 0.5 ml of an ethanoic stock solution was refilled with 5 ml of % ethanol.
- To 100 ml of distilled water, 2.8 ml of $AlCl_3$ solution, and 10% $AlCl_3$ solution were added to make a 10% $AlCl_3$ solution.
- After waiting 30 minutes, a yellow color developed after adding 100 ml of potassium acetate solution.
- Spectrophotometer readings was taken in the 415 nm UV-visible range
- "Blank water" was a term used to describe distilled water that contains 10% $AlCl_3$.

3.7 Proximate analysis of tea:

3.7.1 Moisture Content:

The moisture content of enhanced tea powder was determined using the AOAC method 925.09 (2000). After washing, drying for three hours at 105°C and cooled in desiccators, the crucibles were ready for use. After that, the crucibles were subjected to a weight check. Around 2 grams were added to the crucible. After placing the sample in the crucible, the sample was dried for 48 hours at 105°C. Desiccators were used to cool the crucibles once they were dried. The crucibles were weighed and recorded once they cooled down. The percentage of moisture was then calculated using the formula in the equation.

$$\%\ of\ Moisture\ Content = \frac{W1-W2}{W2}$$

Where,

$W1$= weight of the sample (g) before drying and

$W2$ = weight of the sample (g) after drying

3.7.2 Protein content:

In order to determine the amount of crude protein present in the enhanced tea powder, the macro Kjeldahl method, number 920.87, was used. A portion of sample material weighing about 1g was weighed on oiled filter paper. After being carefully packaged, the materials were inserted into a Kjeldahl digestive tube of 100 ml capacity. Each 100 ml digestive tube was given 5.0 ml of concentrated sulfuric acid and 2 g of Kjeldahl catalyst, and a blank was prepared by putting a piece of filter paper inside the tube. After initially producing a clear blue solution, the samples were digested further to release the nitrogen contained in the heterocyclic ring. It was chilled before 20 cc of distilled water was added to dissolve the components in the digest. weaker version. Ammonia production was sped up by adding 40 ml of 40% sodium hydroxide to 50 cc of the digest. The resulting vapors of steam distillation were collected in a 50 mL flask containing 4% boric acid. Using a bromocresol green/methyl red mixture as a reagent, the distillate was titrated with 0.1520 N HCl standard solution. The nitrogen content was determined using the following formula.

$$\% \text{ of Nitrogen} = \frac{\text{Titre (blank)in ml} \times \text{Conc.of acid N/mol}}{\text{weight of sample (g)}} \times 100$$

Protein content was determined by multiplying the nitrogen content by a factor of 6.25 for plant sources (equation).

$$\%of\ CP = \%N \times Factor \ (6.25)$$

3.7.3 Fiber Content:

The dietary fiber content of enriched tea powder was determined using the 3.7.3 Fiber Method 920.86. One gram was taken from each sample for analysis of crude fiber. After being soaked for 30 minutes in 0.125M diluted sulfuric acid, the materials were rinsed three times in hot water. The residue was rinsed three times with hot water after being digested in a moderate alkaline solution (0.125M KOH) for an additional 30 minutes. The remainder was digested after five hours of cooking, then refrigerated and weighed. The ash was weighed again after being burned for two hours at 525°C in a muffle furnace. The total quantity of fiber was determined by the following formula:

$$\%\ of \text{ fibre} = \frac{W1 - W2}{W}$$

Where,

$W1$= weight of the sample (g) before drying

$W2$ = weight of the sample (g) after drying

W = weight of dry sample taken for determination (g)

3.7.4 Crude Fat:

The AOAC (2000) method employs Soxhlet apparatus to calculate the total crude fat content of the samples.

Procedure

After being dried, samples were placed in thimbles topped with fat-free cotton fiber. The thimble was inserted into the fat extraction tube, which was connected to a Soxhlet apparatus. Anhydrous petroleum ether, about 75ml, was poured through the tube's sample into the flask. The condenser was connected to the fat extraction tube's cap. The sample was extracted for at least 16 hours in a water bath maintained at (70-80) °C. After the feature extraction process was complete, the thimble was removed from the apparatus and the petroleum ether was distilled or collected in a Soxhlet tube. After the petroleum had been refined into a fine consistency, it was purified by pouring it through a small funnel into a dry (previously weighted) beaker. A lot of petroleum ether was used to thoroughly clean the flask. The petroleum ether was dried for 1 hour at 100°C, refrigerated, and weighed after being evaporated in a steam bath at room temperature. The sample's ether-soluble components were identified by their relative density shift. The percentage of fat in the crude food was written as follows:

$$\% \text{ Of Crude fat} = \frac{\text{Weight of ether soluble material}}{\text{Weight of sample taken}} \times 100$$

3.7.5 Carbohydrate:

By deducting the measured amounts of protein, fat, ash, and moisture from 100 in accordance with the Person approach, we may calculate the total carbohydrate content of the sample.

3.8 Vitamin C Concentration Test:

Vitamin C's antioxidant characteristics serve as the basis for its chemical analysis. Vitamin C's dye-diminishing abilities Evaluation of 2, 6-dichloride phenol indophenols in plant or animal extracts is common practice. In this scenario, vitamin C was altered into dehydroascorbic acid by the pigment. At the same time, the dye is made, a molecule without color is created. That the reaction's terminal state can be precisely determined. Rapid excretion and filtering are preferred due to the potential for oxidized Vitamin C, which is nearly destroyed during testing and grinding, to contribute excess into plant products. The oxidation that occurs during the extraction process is due to the usage of metaphosphoric acid. A solution with a very high acidity level will produce the most precise results. The titration may be finished in under a minute. The dye turns a brilliant shade of blue when diluted in water. If you put it in an acidic solution, the color will go away. (AOAC, 2016).

3.8.1 Reagent required:

a) The formula for the dye is as follows:

1. 260 ml of 2, 6-dichlorophenol indophenols

2. 100 ml of distilled water and 210 mg of NaHCO3.

b) Concentration of metaphosphoric acid (3%)

1. Metaphosphoric acid (15/7.5 mg).

2. Glacial acetic acid is diluted with distilled water to create 500/250 ml from 40/20 ml. The ascorbic acid combination as is normal 500 ml of metaphosphoric acid solution was combined with 50/25 mg of crystalline ascorbic acid.

3.8.2 Procedure

There was a dye solution in the burette. Then, 5 mL of vitamin C solution was added to a conical flask. The burette was lowered gradually into the conical flask containing the dye. It was determined that the titration was complete when a pinkish hue arose, persisted for 20 seconds, and then faded away. There were at least three separate readings collected. A similar process was applied to a solution of ascorbic acid whose concentration was not known. Specifically, the result was presented as a percentage of milligrams (mg percent).

3.9 Caffeine Content determination:

Introduction

Caffeine, a central nervous system (CNS) stimulant with a particular effect on blood vessels, is extensively taken as a beverage across the world (Rogers and Dernoncourt, 1998). A component is extracted from its matrix. Extracting caffeine from a particular sample involves using water as the primary solvent. While solubility in room temperature water is low for caffeine (2 g/100 mL), it increases to 66 g/100 mL when heated to 100^0C (Subila and Shirley,2016). Also, at room temperature, caffeine dissolves well in chloroform. Caffeine may therefore be readily extracted using chloroform from a sample of an aqueous solution. The density differences between chloroform and caffeine allow for their separation. The caffeine that has crystallized may be extracted and its concentration determined by UV/ Vis Spectrometry and chromatographic techniques using following procedure and steps.

a) Tea powder should be dissolved in water, therefore step:

First, place 25 grams of sample W0 into a 1000 mL beaker using an analytical balance. Add 250 mL of distilled water to the beaker before adding anhydrous sodium sulfate. Stirring frequently during the 30 minutes of boiling, bring the solution to a temperature of 100^0C. Remove the beaker from the fire and place it somewhere cold when the designated amount of time for boiling has passed. Using Whatman filter paper and a vacuum filter, the particles were separated from the solution.

b) Caffeine Chloroform Extraction:

Place the above solution in a 500 mL separatory funnel and stir for 5 minutes while adding 100 mL of chloroform gradually. Let the bottom of the chloroform extract accumulate. Pour the chloroform layer into the beaker carefully. Under vacuum, run a backward filter paper through the chloroform-caffeine combination. Chloroform may get through in this method, but water and debris are kept. The mixture should be placed in a 125 mL Erlenmeyer flask.

c) Caffeine crystallization:

Set up a water bath at 620 degrees Celsius in a fume hood, then add the chloroform solution. Chloroform may be brought to a boil at a temperature of 620 °C. Reduce the volume of the solution to around 20 mL by evaporating it. After then, take it away from the stove or oven. Put a clean watch crystal on a scale and note the reading (W1). Add some powerful caffeine solution to the watch glass. A water bath can be used to evaporate the watch glass. To use up the entire concentrated solution, simply repeat the steps above. Take the watch crystal out of the water and set it somewhere to cool down. Dry off the wet spot at the base of the watch crystal. Caffeine's mass may be calculated by reweighing the watch glass (W2). Caffeine concentration can be determined at this time.

d) Calculation:

Weight of caffeine, W3= W2-W1

Percent yield of caffeine, Y can be calculated as:

$$Percent\ Yield, Y = \frac{(\text{weight of caffeine,W3})}{(\text{weight of sample,WO})} \times 100$$

Where:

Wo = Weight of sample

W1 = Weight of watch glass

W2 = Weight of watch glass with caffeine

W3 = Weight of caffeine

3.10 Mineral Content:

Minerals are absorbed from the food matrix and then absorbed again after digestion. A mixture of HNO_3 and $HClO_4$ in a 2:1 ratio was used to break down a tea sample acidically. One gram of material was weighed and placed in a conical flask. After adding 7 ml of HNO_3 and 3 ml of $HClO_4$, the flask was set on a hot plate at 200W for 3 minutes to ensure thorough digestion. After being allowed to cool, the solution was filtered into a 100 ml standard flask lined with filter paper and further diluted with distilled water until it reached the desired level. The mineral concentration was

calculated using this solution. The Atomic Absorption Spectroscopy analyzer was used to identify the levels of individual minerals (potassium, magnesium, calcium and iron) (Humalyzer 3000). The biochemical analysis was performed using a commercial kit (Radox). The analysis was presented as a percentage in mg/100g.

3.10.1 Iron (Fe) content analysis:

Ascorbic acid is used to convert the ferrous ion that has been freed up in a somewhat acidic environment back to its native, bivalent state. When combined with iron ions, ferrozine produces a vibrant molecule. The amount of iron in the sample determines the vibrancy of the resulting hue. Pipettefuls of 1 ml reagent were used to make up blank solutions, whereas 200 L of standard and 1 ml of reagent were used to make up the standards. For the production of the sample solution, 200 L of the sample extract and 1 mL of the reagent were added. A 10-minute incubation period at room temperature was performed after the mixing. Absorbance was determined by comparing the standard and sample to a blank. Results for iron concentration were reported in micrograms per deciliter (g/dl).

3.10.2 Determine calcium (Ca) levels:

In an alkaline environment, calcium ions combine with O-Cresolphthalein to generate a violet complex. Into a cuvette, 25 L of distilled water and 1 ml of working reagent were placed to make a reagent blank solution. One milliliter of the working reagent was mixed with 25 milligrams of the Ca^{++} standard. The sample solution was made by combining 25 L of sample extract with 1 mL of the working reagent. Absorbance measurements were taken, both of the sample and the reference. Calcium concentration is given in milligrams per milliliter, and the standard concentration is multiplied by the ratio of sample absorbance to the standard absorbance.

3.10.3 Potassium (K) determination:

Sodium tetraphenylboron interacts with potassium to create a fine turbidity of potassium tetraphenylboron. The level of turbidity is directly relation to the content of the potassium in the sample. For the manufacture of blank solution, 1ml potassium reagent and 0.02ml deionized water put into cuvette by pipette. For standard solution, 1ml potassium reagent and 0.02ml sample extract were placed into cuvette. After mixing this solution were incubated at retention for 5 minutes. The absorbance of

Standard and sample measured against blank within 15 minutes. The ratio of sample absorbance to standard absorbance is multiplied by standard concentration (mg/dl) and potassium concentration was obtained in mg/dl.

3.10.4 Magnesium (Mg) determination:

The technique is based on the specific binding of calmagite, a metallochromic indicator, to magnesium at an alkaline pH, resulting in a shift in the complex's absorption wavelength. The intensity of the chromophores generated is related to the concentration of magnesium in the sample. For the manufacture of the reagent blank solution, 1ml reagent was taken in the cuvette. 1ml reagent and 10µL sample extract were put into the cuvette for the produced sample solution. For the standard solution preparation, 1 ml reagent and 10L magnesium standard were taken in the cuvette. After mixing let these cuvettes rest for 2 minutes at room temperature. The absorbance of the sample and standard at 520nm was measured against the reagent blank. The ratio of the sample absorbance to standard absorbance is multiplied by standard concentration (mg/dl) and the concentration was obtained in mg/dl.

3.11 Microbial analysis:

3.11.1 Aerobic plate count/ Bacterial plate count/ Bacterial plate count:

The number of viable bacteria present in a given sample may be determined by doing an aerobic plate count. Other names for the aerobic plate count (SPC), total plate count (TPC), and mesophilic count include standard plate count and aerobic colony count, respectively (APC). The Average Number of Plates (SPC) method were employed to count how many bacteria were still alive (TVC). Each cell is expected to form a distinct colony when cultured on nutrient-rich agar, the basis of the test. The full bacterial environment is not addressed; rather, it only examines microorganisms that can grow aerobically at mesophilic temperatures (25 to 40°C). Despite its limitations in determining the type of bacteria present, APC may be used to assess general acceptability, sanitary quality, compliance with excellent requirements, and as a safety indication. APC may reveal insights regarding a food's longer storage or expected organoleptic improvement (Banwart, 2012).

3.11.2 Sample Preparation:

The approach employed to obtain the sample has a considerable influence on the accuracy of the analysis and interpretation of the findings. The sample size should accurately reflect the whole amount. Thus, the material was carefully mixed to guarantee that the sample was representative of the current lot. Twenty-five grams of this tea was transferred to a conical flask with a capacity of 250 milliliters. To dilute the samples, phosphate buffer saline (0.6 M KH_2PO_4 at pH 7.2) was applied. The pipette received 100 mL of buffered saline, which was then intensively agitated while moving back and forth. With roughly the same buffer water, the volume biscovered. To sterilize all of the equipment, solutions, and other supplies, they must always be heated to 1210 C for 15 minutes. After being diluted to a concentration 10 times lower than its original (1-10-1 dilution), the generated sample was employed as a stock solution (Andrews, 1992).

3.11.3 Dilution:

We used 9 ml blanks to make the following dilutions.

a) The first step was to dilute the solution (1 ml in 9 ml).
b) A vortex mixer was used for the mixing
c) One milliliter of the well-blended was transferred to the next tube. It was 10-2 times less concentrated. The final dilution after this process was around 10-6.

3.11.4 the Average number of plates:

The number of microorganisms present in the preserved samples was estimated using an SPC. This data may be used for a variety of purposes, including estimating the longevity of a product's shelf life and gauging the safety and freshness of food at 45°C, using a pipette, place 1ml of the dissolved material in any of the sterile, empty Petri dishes with nourishing agar medium (Plate count agar). On a flat surface, the dishes were crammed together. Instead of leaving the plates in an incubator at 37 °C for 24 hours to let the media develop, the plates were simply exchanged. (AOAC, 1990).

3.11.5 Molds and Yeasts:

Research on molds and yeasts was carried out using the international standard operating procedure (ISO), which included plating a 1 mL sample on Rose-Bengal

chloramphenicol agar (DRBC, Oxoid, Thermo Fisher Scientific Inc., Waltham, MA, USA), and incubating the plates at 22°C for 72 hours. As a whole, the colonies are tallied. Multiple subcultures of colonies with distinct morphologies are grown on new Rose-Bengal agar and sent out for genetic testing.

3.11.6 Counting and Recording Data:

When deciding which plates to incubate for bacterial colony counting, we considered both the number of colonies and how easy they would be to count. It was determined to not use the plate where the colonies were scattered, overlapping, and confusing. Plates with colonies of 30–250 were chosen because they were visually appealing, distinct, and countable. CFU/g or ml is the standard dilution factor for CFU plates. The viable total bacteria test followed all the usual protocols: a diluted sample was taken, standard plate counts were employed, and volumes were recorded and kept. The incubation period was 24 hours at 37 degrees Celsius (AOAC, 1990).

3.12 Sensory Evaluation:

The amount of consumer approval was measured by sensory analysis. The created product's potential market success was determined by a taste-testing panel. A panel examination was placed at CVASU. There was a total of 10 people on the panel, and they all got the product to try. Samples A, B, and C each have their unique formula encoded. The panelists randomly selected samples from the three sets without being given any instructions. The panelists evaluated the tea based on its appearance, color, flavor, consistency, flavor, sweetness, and overall acceptability. With this tactic, you're making it evident that you think your product is of higher quality than it real (Sing et al., 2008). Four different items were tested, and each one was given its own score four samples were subjected to a comprehensive sensory evaluation, including evaluations of their taste, look, flavor, mouth feel, sweetness, and overall acceptability, using nine-point Hedonic assessments (Larmond, 1977).

3.13 Statistical Analysis:

Information was recorded in a Microsoft Excel 2019 spreadsheet for further statistical evaluation. Each sample was used three times. Descriptive statistics (mean and standard deviation) were used in the sensory analysis of enhanced cookies and banana peel. The information was organized, coded, and documented using Minitab 19 for Windows. Then, the results were analyzed statistically. Data on proximate composition, phytochemicals, antioxidant capacity, and sensory evaluation were analyzed using Minitab's one-way ANOVA methods to establish a 95% confidence interval for the degree of significant variation. A post hoc "Fisher" test was performed to determine the differences in variance between the groups in the samples. The statistical analysis used a 5% level of significance $P<0.05$.

Chapter 4: Result

4.1: The nutritive values of Tea:

The nutritional value of tea is shown in **Table 4.1**. All samples showed large variations. Type B significantly the higher concentrations of crude protein (23.54%), crude fat (5.68%), and crude fiber (14.06%) compared to Type A and Type C. Type A had the lowest crude fiber content (12.81%) and fat content (4.75%). Contrarily, the crude protein content of sample C is the lowest which is (22.40%).

Table 4.1: Nutritional properties of tea:

	A	B	C	P value
Dry matter%	90.14 ± 0.015^{b}	90.05 ± 0.01^{c}	91.58 ± 0.0058^{a}	0.00
Carbohydrate %	43.47 ± 0.0321^{b}	40.55 ± 0.0289^{c}	45.083 ± 0.197^{a}	0.00
Moisture %	9.86 ± 0.015^{b}	9.96 ± 0.0058^{a}	8.42 ± 0.0058^{c}	0.00
Crude fiber %	12.81 ± 0.010^{c}	14.06 ± 0.0100^{a}	13.27 ± 0.0058^{b}	0.00
Fat %	4.75 ± 0.0100^{c}	5.68 ± 0.0100^{a}	4.98 ± 0.0100^{b}	0.00
Ash %	6.01 ± 0.0058^{b}	6.20 ± 0.0100^{a}	5.98 ± 0.0100^{c}	0.00
Crude protein %	$23.10 \pm 0{,}0100^{b}$	23.54 ± 0.0058^{a}	22.40 ± 0.0100^{c}	0.00

Legends: Means ± SD and values in the same column with the same superscripts are statistically significant ($P<0.05$).

Type A – Green tea with 8% moringa powder

Type B – Green tea with 15% moringa powder

Type C – Commercial green tea

4.2 Total phenolic, flavonoid, and antioxidant capacity:

4.2.1 The antioxidant power of tea

The DPPH assay was used to calculate the total antioxidant capacity of tea. Table 4.2 displays the antioxidant concentration of the samples. A calibration curve was constructed by plotting the percentage of inhibition against the concentration of the Trolox test, which was given in milligrams per one hundred grams. In this study, the maximum antioxidant capacity in Type B was significantly higher than Type A and

Type C. In Type B (3.35±0.0006[a] mg/100g) the antioxidant content is highest while the lowest can be found in Type C (3.13±0.0026[c] mg/100g).

4.2.2 Bioactive Compounds:

Polyphenols, flavonoids, and anthocyanins are the most abundant bioactive chemicals found in fruits, vegetables, tea, meat, and other animal proteins, and they offer a wide variety of health benefits for humans. Bioactive chemicals were isolated from physicochemical extracts of sample material and then evaluated against established criteria. The following are the outcomes of using bioactive substances.

4.2.2.1 Amount of Total Polyphenol Compounds (TPC):

The total polyphenol content (TPC) of the tea samples was determined using the Folin-Ciocalteu technique. A complete breakdown of the tea's polyphenol content is shown in Table 4.2. The outcome was reported in milligrams of GAE per gram of sample. Between 4.14 and 4.22 milligrams of gallic acid equivalent per gram, the polyphenol content of the extract was very variable (gallic acid equivalent). Sample C had the highest total polyphenol content (TPC) in this study at (19.045±0.0100[a] mg/100g), whereas samples A and B had the lowest TPC's at (14.81±0.0156[c] mg/100g) and (15.88±0.0217[b] mg/100g), respectively.

4.2.2.2 Total flavonoid concentration (TFC) of tea:

Using a modified aluminum chloride colorimetric method, we determined the total flavonoid content (TFC) of the fruit extract. The whole flavonoid count is shown in Table 4.2. This particular result was expressed as mg QE/100g extract. Type A has 8.75 mg QE/100g of TFC, whereas Type B contains 9.62 mg QE/100g, and Type C contains 8.28 mg QE/100g.

Table 4.2 Total phenolic, flavonoid, and antioxidant capacity of experimental teas:

Type	Total phenolic content (mg GAE/100g)	Total flavonoid content (mg QE/100g)	Total antioxidant capacity (mg/100g)
A	14.80 ± 0.0156[c]	8.75 ± 1.248[b]	3.26 ± 0.0015[b]
B	15.88 ± 0.0217[b]	9.62 ± 0.640[a]	3.35 ± 0.0006[a]
C	19.045 ± 0.0100[a]	8.28 ± 1.108[c]	3.13 ± 0.0026[c]
P value	0.00	0.001	0.00

Legends: Means ± SD and values in the same column with the same superscripts are statistically significant (P<0.05).

Type A – Green tea with 8% moringa powder

Type B – Green tea with 15% moringa powder

Type C – Commercial green tea

4.3 Total Caffeine content:

Caffeine is a bitter, white crystalline purine. It is most commonly found in pharmaceuticals as caffeine sodium benzoate, and caffeine citrate, in combination with pain relievers such as aspirin and acetaminophen (Sama et al., 1994). Caffeine, a central nervous system (CNS) stimulant with a particular effect on blood vessels, is extensively taken as a beverage across the world (Rogers and Dernoncourt, 1998). Here, in table 4.3 Type A contains the highest amount of caffeine whereas Type B contains the lowest amount of caffeine. Type C contains less amount of caffeine than other types.

Table 4.3: Caffeine content of experimental tea:

Type	Caffeine content (mg/1gm)
A	10.08 ± 0.0100^{a}
B	8.033 ± 0.0577^{b}
C	12.00 ± 0.0560^{b}
P value	0.00

Legends: Means ± SD and values in the same column with the same superscripts are statistically significant (P<0.05).

Type A – Green tea with 8% moringa powder

Type B – Green tea with 15% moringa powder

Type C – Commercial green tea

4.4 Total Minerals content:

Significant components of tea also include important minerals such as manganese, iron, magnesium, calcium, and potassium. Among the three types we can see from table 4.4 type B contains highest amount of Iron, Magnesium, calcium and Potassium and lowest amount of minerals contains by Type C.

Table 4.4 Total Minerals content of different Teas:

Type	Iron mg%	Magnesium mg%	Calcium mg%	Potassium mg%
A	5.67 ± 0.1528^{c}	1.67 ± 0.058^{a}	7.13 ± 0.1528^{b}	2.43 ± 0.1528^{b}
B	8.63 ± 0.551^{a}	1.80 ± 0.100^{a}	9.13 ± 0.1528^{a}	2.83 ± 0.1528^{a}
C	7.00 ± 0.100^{b}	1.33 ± 0.1528^{b}	5.13 ± 0.1528^{c}	2.60 ± 0.00^{ab}
P value	0.003	0.005	0.022	0.001

Legends: Means ± SD and values in the same column with the same superscripts are statistically significant (P<0.05).

Type A – Green tea with 8% moringa powder

Type B – Green tea with 15% moringa powder

Type C – Commercial green tea

4.5 Total Vitamin content:

Vitamin C is an essential nutrient, with a recommended daily intake of 60 mg, and higher intakes have been associated with numerous health benefits. Vitamin C, also called ascorbic acid or ascorbate, is an essential vitamin and antioxidant that occurs in nearly all fresh fruits and vegetables. From the table 4.5 type B contains the highest amount of vitamin C than type A and type C.

Table 4.5 Total Vitamin content of experimental teas:

Type	Vitamin C %
A	3.27 ± 0.058^{c}
B	6.0 ± 0.100^{a}
C	5.47 ± 0.058^{b}
P value	0.00

Legends: Means ± SD and values in the same column with the same superscripts are statistically significant (P<0.05).

Type A – Green tea with 8% moringa powder

Type B – Green tea with 15% moringa powder

Type C – Commercial green tea

4.6 Microbial analysis:

Table 4.6 shows the microbial count of type A, B and C. total bacterial count of these three types are under the safety level. There also found no mold and yeast growth in the teas.

Table 4.6 Microbial analysis of different type teas:

Type	Total bacterial count (TBC)	Mold & Yeast Count
A	5.3×10^3 CFU/ml	0
B	6.5×10^3 CFU/ml	0
C	7.0×10^3CFU/ml	0

Type A – Green tea with 8% moringa powder

Type B – Green tea with 15% moringa powder

Type C – Commercial green tea

4.7 Sensory evaluation:

Results for sensory attributes, including appearance, smell, taste, touch, and general approval, are shown in Table 4.7. The flavor and texture were greatly enhanced by the use of moringa powder. The Smell, color, texure and overall acceptability of Type B tea was significantly higher compared to Type A and Type C.

Table 4.7: Sensory evaluation of teas:

Type	Smell	Taste	Colour	Texture	Overall acceptability
A	7.01 ± 0.667^{bc}	7.20 ± 0.78^{ab}	5.41 ± 1.430^{c}	5.800 ± 1.317^{b}	6.79 ± 1.075^{b}
B	8.21 ± 1.155^{a}	8.10 ± 0.87^{a}	6.83 ± 0.789^{b}	5.500 ± 1.524^{bc}	7.02 ± 1.054^{ab}
C	6.10 ± 1.197^{c}	5.90 ± 1.66^{c}	6.27 ± 1.054^{a}	5.600 ± 0.843^{c}	6.20 ± 0.919^{b}
P value	0.001	0.022	0.07	0.005	0.006

Legends: Means ± SD and values in the same column with the same superscripts are statistically significant ($P<0.05$) for smell, taste and overall acceptability. Not statistically significant for colour and texture ($P>0.05$).

Type A – Green tea with 8% moringa powder

Type B – Green tea with 15% moringa powder

Type C – Commercial green tea

Chapter 5: Discussion

5.1 Proximate composition

Moringa-induced green tea's chemical composition is listed in Table 4.1. Samples A, B, and C were found to have drastically varying amounts of moisture, total carbohydrate, crude fiber, ash, fat, and crude protein. Type A and B tend to have higher nutrient concentrations than Type C as we induced moringa leaf powder in it.

5.1.1 Carbohydrates:

Type A had a carbohydrate content of 43.34%, type B was 40.55%, and type C was 45.08%. As anticipated, the incorporation of moringa leaves into types A and B decreased the concentration of carbohydrates. Carbohydrate content decreased with the increased moringa percentage in the tea. Type B tea had a low glycemic index which may be attributed to the use of higher level of moringa leaves.

5.1.2 Moisture:

Type B has the greatest percentage compared to type A and C (Table 4.1). Based on this result, we may conclude that adding moringa leaves powder to green tea increases its hydration levels. Some hypothesized causes for the discrepancy include geographical and varietal variations. Moringa leaves have a moisture content of 9.00 (2.30%) when dried (Sodamade et al., 2013). The body's rate of food absorption and assimilation is directly related to the amount of moisture present in the diet. It's also a factor in how well food will keep. Based on the stated value, it seems that protein concentrates extracted from moringa oleifera leaves should not be kept at room temperature for an extended amount of time. The level of moisture in food is often used to predict how long it will keep. Tea that had a greater moisture content than average was easily spoiled.

5.1.3 Ash Content:

Type B had the highest ash concentration (6.20 %) out of the three types. Type B has 6.01 %, whereas type C has 5.97 % ash. Ash content in food is mostly predicted by the amount of ash present. When compared to values published for other vegetable species, such as Talium triangulare (waterleaf) (0.62%), Rosselle (java jute) (0.46%), and Cochorus Olitorius (jute mallow) (0.32%), this one is much higher. Amaranthus

hybridus (green amaranth) 0.49 percent and Telfaira oceidentalis (fluted gourd) 0.68 percent (Saidu and Adunbarin 1998). According to the ash content, they are a valuable mineral element source (Sodamade et. Al, 2013).

5.1.4 Fat Content:

The fat percentages in samples A and C are 4.75% and 4.98% correspondingly. Sample B, on the other hand, had the highest proportion of moringa oleifera and, hence, the highest fat content, at 5.68%. Moringa oleifera has a crude fat percentage of 2.43± 0.47% (Sodamade et al., 2013). The calories is obtained from food are directly proportional to the quantity of fat it contains. The American Heart Association recommends that adults consume no more than 2% of their daily calories from fat to reduce their risk of developing cardiovascular disease, cancer, and senescence (Davidson et al., 1975).

5.1.5 Protein content:

Table 4.1 reveals that the protein content of types A and B is 23.10% and 23.54%, respectively. The protein content of type C is lower, which is 22.24%. The protein content of moringa leaves increases as their percentage grows. Protein concentrations made from moringa leaves have a relatively high crude protein content of 39.13%, which is greater than the 17.01% found in moringa leaves as reported by Ogbe and John (2012). Moringa's high crude protein content suggests that protein concentrates extracted from the plant's leaves might be a useful addition to the diets of both humans and animals in need of a boost to their protein intake.

5.1.6 Fiber Content:

When comparing types A, B, and C, **Table 4.1** reveals that type B had a greater fiber content (5.1.6), with a fiber percentage of 14.06%. Incorporating fiber into one's diet has been shown to reduce the body's absorption of cholesterol by cleaning out the digestive system of harmful toxins that might cause cancer. Protecting against metabolic diseases like hypertension and diabetes mellitus, fiber also provides weight to meals and lowers the consumption of excess starchy food, which is typical of the diet of the indigenes in this region (Sodamade et al., 2013). This suggests that Sample B is preferable to Samples A and C.

5.2 Antioxidant capacity:

The primary plant chemicals that may reduce oxidative damage to tissues are those found naturally in plants and include antioxidants. **Table 4.**2 displays the results of the DPPH test on the antioxidant capacity of some commonly consumed fruits. Once it had gained an electron or proton, DPPH turned from purple to yellow, becoming a stable free radical. In the presence of antioxidants, a diamagnetic compound was discovered, as reported by Singh et al (2015). The DPPH activity levels in samples A 3.26 mg/100 g and B 3.35 mg/100 g were different. As a consequence, the moringa leaves powder added to the tea increased its antioxidant properties. These results are similar to those found by Ajibola et al. (2015), who found that increasing the quantity of Moringa oleifera leaves and cocoa powder increased the biscuits' antioxidant properties.

5.2.1 Total Polyphenol concentration:

In this study, the TPC value of Type C (19.045±0.01 mg GAE/100g) was more than that of type A (14.81%) and B (15.88%) combined. The phenolic content is proposed to be ranked as low (100 mg GAE/100g), medium (100-500 mg GAE/100g), or high (>500 mg GAE/100g). Because of this, polyphenol concentrations varied widely across the types, with the highest concentration found in type C, the second highest in type B, and the lowest in the remaining types.

5.2.2 Total Flavonoid Content:

Flavonoids, which are bioactive natural chemicals, may be found in almost every plant species. Catechins, flavonoids, flavones, and quercetin were the most common members of this family (Gadkari and Balaraman, 2015). The TFC content of types A and B is 8.75±1.248 mg QE/100g and C's is 8.28±1.108 mg QE/100g, respectively. In contrast to type C, whose TFC concentration is rather low, types B and A had much greater TFC levels (**table 4.2**).

5.3 Quantity of Caffeine:

The stimulant action of caffeine on the neurological system has been linked to an increase in blood pressure. Caffeine use beyond the recommended limit of 3–5 cups per day has also been linked to an elevated risk of cardiovascular disease. In this light, type B had the lowest caffeine content of the three types (**table 4.3**). Caffeine can be found in all three types, with the highest concentrations being found in type C (13.67±0.058 mg/1g) and type A (10.08±0.0100 mg/1g).

5.4 Vitamin Content:

5.4.1 Vitamin C:

Vitamin C is a powerful antioxidant that helps the body deal with stress by neutralizing free radicals, maintaining the reduced form of the membrane-bound antioxidant -tocopherol, and serving as a substrate in the production of oxalate and tartrate (Arrigoni and De Tullio 2002; Davey et al., 2000; Klein and Kurilich, 2000). Scurvy (Levine, 1986), the common cold (Hemila, 1992), anemia, and even infertility are all treated with it. Therefore, it is necessary to get vitamin C through food. As a strong source of vitamin C, Moringa is underutilized, even though its potential is well recognized. Here, type B had the highest vitamin C content (6.0±0.100 mg%) out of the three types. There are 3.27±0.058 mg% of it in type A, and 5.47±0.058 mg% in type C.

5.5 Mineral content:

5.5.1 Calcium content:

Calcium and phosphorous-containing compounds are essential for bone and tooth growth in adolescents, and pregnant and breastfeeding women. The calcium content rises with the proportion of moringa leaves powder used **(table 4.4),** from 7.13 mg% in type A to 9.13 mg% in type B and then to 5.13 mg% in type C. Type B, which includes 15% moringa powder, has a calcium content similar to the daily dose of 800mg advised for both adults and children (NRC 1989).

5.5.2 Magnesium content:

Compared to the 1.80 mg% found in type B and the 1.67 mg% found in type A, the 1.33 mg% found in type C (**table 4.4**) is a significant difference. These numbers are greater than those reported for leaf protein concentrates derived from the African eggplant species Solanum microcap and the spinach tree species *Cnidoscolous acinitopholis*, which are 88.60±0.21mg% and 98.30±0.61 mg%, respectively (Faboya et al,.2012). Dry moringa leaves contain 0.50% magnesium (Moyo et al., 2011).

5.5.3 Iron content

Powdered moringa leaves have an iron content of 187.00 0.03mg/100g (Sodamade et al., 2013). All three types A, B, and C have iron concentrations that are much above average. Fe concentrations in types A and B are both 5.67 mg%, whereas those in type C are both 8.63 mg% and 7.00 mg% (**table 4.4**). Dry moringa leaves contain 490mg/kg (Moyo et al., 2011). The anemia that results from an insufficient supply of iron, which is necessary for the production of hemoglobin, is particularly severe in type B. Because of this, type B is the preferred option.

5.5.4 Amount of Potassium:

People who use diuretics to manage their hypertension and whose bodies excrete too much potassium via urine or sweat may benefit from maintaining a high potassium intake, since doing so has been shown to improve iron utilization (Adeyeye, 2002; Arinathan et al, 2003). Adults should have at least 2,000 milligrams (mg) of potassium per day. (NRC 1989). A serving of Moringa Oleifera may provide 1.16 mg% of the RDI. Sample B had the greatest concentration of K 2.8mg% in this **table 4.4**, whereas type A and C have concentrations of 2.4 mg% and 2.6mg%, respectively. This suggests that type B is preferable to the other two.

5.6 Microbial analysis:

5.6.1 Total Bacterial Count:

The FDA Bacterial Analytical Manual (BAM) recommends 25-250 CFU/plate as a countable range. Here we found that sample B contains less bacterial colonies which is 6.5×10^3 CFU/ml. this rate is less than the recommended level of the FDA.

5.6.2: Mold and Yeast Growth:

There present no mold and yeast growth among these three types.

Type A, type B, and type C these three samples have no microbial growth, and we can say that these products are safe to consume.

5.7 Sensory Evaluation:

5.7.1 Distinguishing Features of the Consumer:

Eighty percent of the panelists were men, with the remaining twenty percent also being men. All of the panelists were young adults (25-32 years old). Eighty percent of consumers are obtaining a bachelor's degree, and twenty percent are pursuing a master's degree, indicating that education is a top priority.

5.7.2 Sensory assessment of Tea samples:

Table 4.7 displays the average rating given to several tea samples based on their appearance, aroma, flavor, and overall appeal. We observed statistically significant differences between the two samples on the hedonic scales measuring color, flavor, aroma, texture, and overall acceptability. The hedonic qualities of Scent Sample C, Color Sample C, and Taste Sample C were all rated as poorer than those of the other samples. Based on their sensory profiles, samples A and B scored marginally higher on the hedonic scale, indicating that they were generally more acceptable. The aroma, flavor, and overall experience of Sample B much surpassed that of Sample A. Sample C scored lower than Samples A and B, but it is still passable. However, neither the color nor the feel of the material changed. The primary difference is detectable via their gustatory and olfactory senses.

Chapter 6: Conclusion

One of the most popular drinks in the world is tea, which comes from the *Camellia sinensis* plant. On the contrary, the *Moringa oleifera* tree has been shown to have a variety of health benefits in several clinical studies. The popularity of flavored tea is expected to grow as people discover its many positive effects on their health. Green, herbal, and fruit tea are expected to see rapid sales because of their improved flavor, greater accessibility, and several additional health benefits. This research aims to find the optimal ratio of moringa to tea that will result in the most nutrient-dense beverage. In our study it was observed that the moringa leave containing tea had a higher concentration of antioxidant capacity, bioactive components, crude ash, and fiber than commercial tea. In a sensory evaluation test, the potential juror found that the tea with 15% moringa leaves (Type B) powder was more satisfying across all quality categories. As a result, the nutritional value and health benefits of moringa tea will increase significantly. It was concluded that the nutritional outcomes were adequate, which is good news for the health of the population. Consuming such nutritional food has been shown to have a protective impact against a wide range of human ailments, including cardiovascular disease, cancer, cataracts, age-related muscle degeneration, rheumatoid arthritis, and other neurological disorders. The type B that is tea containing 15% moringa, is rich in nutrients like vitamins and minerals that may help stave against conditions like these.

Limitations of the Present Research:

1. The research was very challenging to complete due to insufficient time and materials.

2. I only could detect three of the many bioactive chemicals out there: TPC, TFC, and TAC.

3. I was unable to assess the catechin concentration and kinds of flavonoids in the items owing to time constraints.

4. In addition, I employed UV visible to analyze the samples, even though GC-MS would provide more accurate results.

5. Checked only the levels of bioactive substances.

6. Small sample size.

Chapter 7: Recommendation

The nutritional value of moringa leaves is high. Moringa powder is regarded as a vital and healthy source of nutrients due to its high concentration of bioactive compounds, crude fiber, and antioxidant capacity, all of which positively impact human health. Moringa powder can be added to the tea production process to increase the tea's health benefits and nutritional content.

Based on the study's findings from the creation and evaluation of the nutritious and healthy tea, the authors suggest the following directions for future study:

a) Both moringa and green tea need to be harvested and processed.
b) Incorporating potentially organoleptically offending high-value ingredients into a product.
c) Sophisticated packaging and storage solutions will be developed to enhance current warehousing conditions.
d) It will be helpful from a therapeutic perspective, especially for people with conditions like diabetes, inflammation, and cancer, because of its medicinal relevance.
e) It's possible to use the sample size for statistical analysis of the analytical data. Even if this is the case. Our conclusion should be viewed with some care because of the small sample size, and the results should be replicated in a larger study.
f) Enough effort must be made to improve the nutritional value of commercially sold tea that boasts of health benefits and refreshments.

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Appendix A: Questionnaire for hedonic test of Tea

Sensory Evaluation Form

Consumer test for tea

Panellist No……. **Sex**…….

Age group (a) 20-30 (b) 30-40 (c) 45 and above

Time……... **Date**……....

Education level

(a) Bachelor's degree (b) Master's degree

(c) other specify…….....

Please taste each of the (4) coded products. Indicate how much you like or dislike each sample by checking the appropriate sample attribute and indicate your reference (1-9) in the column against each attribute. Put the appropriate number against each attribute.

9=Like extremely

8 = Like very much

7 = Like

6 = Like slightly

5 = Neither like nor dislike

4 = Dislike slightly

3 =Dislike moderately

2 = Dislike

1 = Dislike extremely

Attributes	9 Like extremely	8 Like very much	7 Like	6 Like slightly	5 Neither like nor dislike	4 Dislike slightly	3 Dislike moderately	2 Dislike	1 Dislike extremely
Texture									
Smell									
Taste									
Color									
Overall acceptability									

Appendix B: Picture gallery

Moringa leaf

Dried leaf

Moringa leaf powder

Green tea

Type B

Type A

Appendix C: Chemical & Microbial test

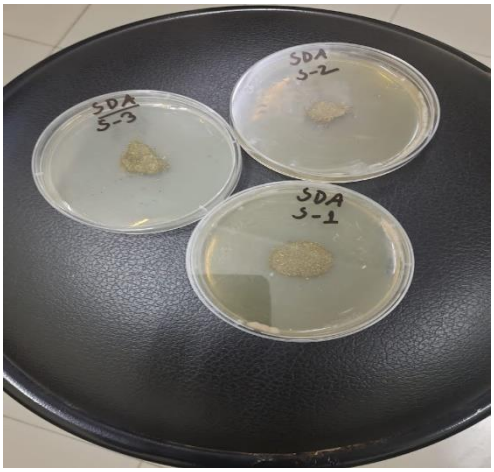
Ash content

Fiber determination

Mineral content

Fat determination

Vitamin C titration



Microbial analysis

Brief biography

Farhana Yasmin Baby received a GPA of 5 on the Secondary School Certificate Exam in 2011 and a GPA of 5 on the Higher Secondary Certificate Exam in 2013. She earned a B.Sc. (Hons.) in Food Science and Technology (BFST) with a CGPA of 3.63 from the Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University (CVASU) in Bangladesh. She is now an MS candidate in Applied Human Nutrition & Dietetics at CVASU's Faculty of Food Science and Technology's Department of Applied Food Science & Nutrition. She is very interested in working at a food testing lab doing further analysis and studies about Moringa leaves.