

A DISSERTATION ON

Development of Herbal Detox Drink Powder by Lyophilization

**SUBMITTED TO THE
DEPARTMENT OF BIOENGINEERING
FACULTY OF ENGINEERING
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IN FOOD TECHNOLOGY**

BY

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DECLARATION FORM

I, **Ishana Khan**, a student of **B.Tech-M.Tech Dual Degree Food Technology** (5th year/ 10th Semester), Integral University have completed my six-month dissertation work entitled **“Development of Herbal Detox Drink Powder by Lyophilization”** successfully from **Integral University** under the able guidance of **Mrs. Poonam Sharma**.

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

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CERTIFICATE

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I wish her good luck and a bright future.

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I wish her good luck and a bright future.

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TO WHOM IT MAY CONCERN

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I wish her good luck and a bright future.

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Place: Lucknow

Ishana Khan

Date:

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ABSTRACT

Detoxification is a process by which the body eliminates or neutralizes toxins and harmful substances that can accumulate within it. This process is essential for maintaining overall health and well-being. Toxins can enter the body through various means, such as through the foods we eat, the air we breathe, and the products we use. Coriander (*Coriandrum sativum* L) and mint (*Mentha*) leaves are rich in antioxidants and also have anti-inflammatory, anti-cancer, anti-diabetic, and anti-microbial properties. Despite the numerous health benefits of coriander and mint leaves, post-harvest losses can occur, so using them to create an herbal detox drink could be a long-term solution that benefits both human health and the environment. The various blends of herbal detox drinks using coriander juice, mint juice, lemon juice, and ginger juice along with cumin powder and salt (1:1) at varying concentrations were prepared. The physicochemical (TSS, titratable acidity, pH, antioxidant activity) analysis was performed, and sensory analysis of the various herbal detox drinks revealed that the best sample was obtained by blending mint juice, coriander juice, lemon, and ginger juice at concentrations of 50%, 30%, and 10% respectively which has TSS– 4.9°Brix, Titratable acidity– 1.76, pH– 4.06, Antioxidant activity– 34.6507%, and highest hedonic reading– 8.5. Lyophilization was done to obtain the powder of the selected sample. Further, the physicochemical (moisture content, ash content, antioxidant activity, total phenolic content, total flavonoid content) analysis and shelf-life estimation of the developed herbal detox drink powder was done.

Keywords: Coriander (*Coriandrum sativum* L), Mint (*Mentha*), physicochemical, herbal detox drink powder

CHAPTER-1

INTRODUCTION

Short-term therapies known as "Detoxification" or "Detox" diets aim to remove toxins from the body, improve health, and help with weight loss (Klein & Kiat., 2015). The use of laxatives, diuretics, vitamins, minerals, juice fasts, and/or 'cleaning meals' is frequently a part of detox diets, which also include total starvation fasts and dietary modification techniques (Allen et al., 2011). The elimination of toxins by the human body is accomplished by highly complicated procedures. Unwanted substances are partially excreted by the liver, kidneys, gastrointestinal tract, skin, and lungs (Anzenbacher & Anzenbacherova., 2001). The ecosystem on earth is becoming polluted by chemicals quicker than the human body can adjust. As contaminants accumulate, the human body transforms into a filter that traps them. Chemicals in our food, water, and air supply change our enzymes, which subsequently spread to every body function to lower our body's disease-resistance threshold (Page, L., 1998). Therefore, detoxifying our bodies on a regular basis becomes essential.

Nature has provided us with various fruits and vegetables which can detoxify our bodies if eaten in raw form like cucumber, broccoli, coriander, mint, garlic, lemon, grapefruit, watermelon, ginger, etc. To make their consumption quicker and simpler in sufficient amounts, juices are made in households and even in industries by using these naturally detoxifying fruits and vegetables. Juices can purify our bodies because they hydrate the body and flush out waste due to their high water content. Juices also contain substances like pectin, chlorophyll, citric, and malic acids, which can absorb toxins and lipids from our digestive tract. For optimum health, they also include amino acids, phytonutrients, vitamins, and minerals. They are rich in antioxidants, protect our bodies from free radicals, and lighten the stress on our liver, which helps our bodies get rid of pollutants (Bailey, C., 2011).

Coriander (*Coriandrum sativum* L) leaves have diuretic properties, aid in digestion, and help in keeping urine infection at bay. It eliminates all the toxins that have accumulated in our body, especially in the kidneys and it also aids in blood purification. Mint (*Mentha*) leaves also aid digestion, reduce stomach acid, and enhance nutrient absorption. Due to a lack of post-harvest processing facilities, a significant proportion of produce is lost in the fields or in the market because consumers do not fully utilize the excess coriander and mint production. The leaves also lose their freshness after a few days, so using them to create an herbal detox drink could

be a long-term solution that benefits both human health and the environment. Due to the perishable nature of fruits and vegetables, they typically have a relatively short shelf life. They can be transformed into ready-to-serve (RTS) beverages to preserve them (Shukla & Salve., 2023).

To address these issues, we proposed a new product development based on blending mainly the juices of mint and coriander leaves juices with other ingredients to create a powdered herbal detox drink that is ready to serve.

Coriander (*Coriandrum sativum* L.) a member of the Apiaceae (Umbelliferae) family, is mostly grown from seeds all year round (Mhemdi et al., 2011). There are two species of coriander, although only *Coriandrum sativum* L. is extensively grown, primarily in tropical regions. The other species is *C. toridylum* (Ifenzl) Bomm, which is found in the wild (M.M Sharma & R.K Sharma., 2012). With an annual production of almost three lakh tonnes, India is the world's largest producer, user, and exporter of coriander. In addition to being grown commercially in India, coriander is also grown in the following countries: Morocco, Romania, France, Spain, Italy, the Netherlands, Myanmar, Pakistan, Turkey, Mexico, Argentina, South, and Western Australia, and a lesser extent, the United Kingdom and the United State (M.M Sharma & R.K Sharma., 2012). The green leaves and dried fruits of the coriander plant are primarily responsible for its nutrients. Its leaves are a great source of iron, vitamins (Vitamin A and C), and minerals, and are very low in saturated fat and cholesterol (Bhat et al., 2014). Additionally, coriander leaves contain hepatoprotective, anticancer, anti-diabetic, anti-microbial, and anti-cholesterol properties (Ganesan et al., 2013). It is also utilized in the creation of numerous prescription drugs to treat various diseases (Bhat et al., 2014).

Mints are aromatic plants in the Lamiaceae family and *Mentha* genus. More than 30 species of *Mentha* exist worldwide, mostly in temperate and tropical/subtropical regions. *Mentha arvensis* (Peppermint/Cornmint), *Mentha spicata* (Spearmint), *Mentha piperita* (Peppermint), *Mentha pulegium* (Pennyroyal), and *Mentha requieni* (Corsican mint) are the mint species that are most readily available and utilized commercially. One of the most favorable places to find *Mentha* species is the Indian subcontinent, where at least 10 different varieties are known to exist. India currently produces and exports the most mint and its by-products, followed by China, the USA, and Brazil as the other major producers (Taneja & Chandra, 2012). Ascorbic acid, beta-carotene, iron, protein, and minerals are all abundant in mint leaves. Mint is utilized in traditional medical systems besides its culinary applications to treat biliary problems,

dyspepsia, enteritis, flatulence, gastritis, intestinal colic, bile duct, gallbladder, and gastrointestinal tract spasms. Menthol and carvone, the mint plant's main active components, have been discovered to have antioxidant, antibacterial, anti-inflammatory, and anticancer properties (Kunnumakkara et al., 2009).

The shelf life of any drink can be up to only a few days if it is stored in a refrigerator, as it contains moisture which is essential for all microbes to grow. Different techniques can be used to remove moisture from food, including sun, solar, cabinet, mechanical drying as well as adiabatic, spray, osmotic, fluidized bed, freeze, and tunnel drying. Sometimes the dried goods are ground into powder after drying to preserve them for a longer period of time, due to their high hygroscopicity, these powders should be packaged in sealed containers (Raj et al., 2016). There are significant amounts of vitamins and minerals in fresh fruit and vegetable juices. Their nutritional content may be lost during cooking and processing (Virk et al., 2021). Lyophilization, often known as freeze-drying, is a useful technique for drying things without damaging them. It involves freezing water, removing it from the sample, and then drying the sample again, first by sublimation and subsequently by desorption. After the product has been frozen, water is removed from it during the drying process known as freeze-drying (Gaidhani et al., 2015).

Objectives for the development of Herbal Detox Drink Powder are:

1. Preparation of the herbal detox drink.
2. Development of herbal detox drink powder by lyophilization from the selected composition.
3. Physico-chemical analysis and shelf-life estimation of developed herbal detox drink powder.

CHAPTER-2

REVIEW OF LITERATURE

The body's capacity to digest toxins can be enhanced through detox diets, and among these diets, detox drinks are among the most well-liked. Detox beverages come in a wide range of flavors and offer a variety of advantages. Weight loss, skin radiance, and body waste clearance are just a few advantages. After consuming detox beverages, the majority of users report feeling healthier and lighter. However, there isn't much proof from lab testing that detox beverages are efficient at getting rid of a few toxins from our bodies. Before deciding which kind of detox drink to consume, patients and therapists should be aware of their medical issues. Then it would be more appropriate to consume any detox drink (Van Rooyen et al., 2021).

A balanced diet that guarantees the health and vitality of the body includes beverages made from fruits and vegetables. They are actively involved in cellular regeneration, cleansing, and the treatment of various ailments, and nutritionists advise them to lead healthy lives. The use of fruit and vegetable beverages for the body's detoxification, regeneration, and healing is advised for two good reasons: the first is that the sap, which is obtained in the form of juice for both fruits and vegetables, is the essential component, and the second is that the juice of raw fruits and vegetables is assimilated in the body in about 10-15 minutes and is almost entirely used for feeding and regenerating tissues with little effort on the digestive system. Vegetable juices are a fantastic way to add vital nutrients to your diet. They deeply cleanse and regenerate while enhancing metabolism. As long as they are fresh, raw, free of preservatives, and correctly extracted from vegetables, they include all the essential amino acids, minerals, salts, enzymes, and vitamins. All vegetable juices contain fibers, which speed up satiety and enhance digestion. Vegetables are the healthiest food option because they contain the least amount of sugar and have fewer calories than fruit juices. The higher salt level is the only drawback (Butu & Rodino, 2019). However, the development of an herbal detox drink powder that is ready to serve is a sustainable way to preserve these vegetables for a longer period of time.

2.1 Spearmint leaves as a detoxifying ingredient

2.1.1 Chemical composition of spearmint leaves

Mint species are wide-spreading stolons that grow both under and above ground and have erect, square, branched stems. Erect, square, branching stems and wide-spreading stolons that grow above and below ground are characteristics of mint species. The oppositely oriented, oblong to lanceolate leaves have serrated margins and tend to be downy. Dark green, grey-green, purple-blue, and rarely pastel yellow are among the majority of the various colors (Taneja & Chandra, 2012). The spearmint leaves were found to contain the following amounts of moisture (76.01 ± 0.033)%, fiber (2.1 ± 0.03)%, ash (3.48 ± 0.001)%, protein (1.75 ± 0.1)%, fat (3.20 ± 0.003)%, and carbohydrates (14.46 ± 0.15)% (Patel et al., 2021). On the other hand, the acid value is 0.0614, peroxide value 1.0, iodine value 0.564, free fatty acids 0.0305, refractive index 1.4572 at 27°C, and density 0.8395 at room temperature. The proximate chemical composition of the fresh spearmint leaves is shown in Table 2.1 (Suleiman et al., 2011)-

Table 2.1 Proximate chemical composition of the fresh spearmint leaves

Parameter	Value
Moisture (%)	76.01±0.033
Ash (%)	3.48±0.001
Protein%	1.75±0.1
Fat%	2.20±0.003
Fiber%	6.2±0.03
Carbohydrates%	10.39±0.15
Na (mg/100g)	7.2
Ca (mg/100g)	13
K (mg/100g)	24
Fe (mg/100g)	2.5

2.1.2 Physico-chemical properties of Spearmint leaves

The chemical composition, antioxidant, and antimicrobial activities of the essential oil isolated from aerial parts of *Mentha spicata* L. (spearmint) were investigated by Hussain et al., 2010. 1.2% oil was discovered to be present. There are 19 distinct chemical components found in

spearmint oil. Carvone and cis-carveol were the primary ingredients. The oil under investigation showed strong antioxidant action. Disc diffusion and minimum inhibitory concentration (MIC) experiments against several strains of bacteria and yeast were used to measure the antimicrobial activity of spearmint oil. All of the examined bacteria were adversely affected, showing that spearmint oil has a sizable antimicrobial potential (Hussain et al., 2010).

The antioxidant properties of three essential oils (EOs) of *M. pulegium* (L.), *M. suaveolens* (Ehrh.) and *M. spicata* (L.) were studied by Zekri et al., 2023. The organoleptic characteristics, yields, and physical properties were determined for the selected EOs. Their chemical compositions were identified; then, their antioxidant activities were evaluated using the DPPH free radical scavenging activity and were compared with the ascorbic acid standard. The determined physicochemical parameters of dry matter and EOs demonstrated their good quality. The obtained results indicated that these EOs could be applied as natural antioxidants in the food industry (Zekri et al., 2023).

2.1.3 Herbal beverages made using spearmint leaves

The herbal blended beverage was also made with 4 herbs: mint leaves, basil leaves, fennel seed, and licorice root. Mint and basil leaves were dried using the tray method at 50° and 55°C. Antioxidant activity was measured using the DPPH free radical method and the total phenolic compound method to assess product quality. The findings indicated that drying mint and basil leaves at 55°C was preferable than drying at 50°C. A sensory investigation was done to determine the right ingredient percentage. The herbal beverage that contained 40% dried mint, 10% dried basil, 30% dried licorice root, and 20% dried fennel seed was the most well-liked (Khiewnavawongsa et al., 2018).

Juices were also made from ash gourd (*Benincasa hispida*) and mint leaves (*Mentha spicata*), which were combined in a ratio of 75:25 to create a beverage with both functional and nutritional benefits. The ash gourd-mint leaves blended juice in glass bottle physicochemical stability, microbiological stability, and sensory qualities were assessed over the course of six months at room temperature. After 6 months of storage, the juice's pH, total soluble solids, and total acidity (as citric acid) all slightly changed, while the loss of vitamin C and beta-carotene was 75 and 56%, respectively, and no noticeable difference in the juice's sensory ratings. The outcome showed that the blended juice was safe for microorganisms and acceptable for 6 months (Majumdar et al., 2010).

2.1.4 Drying of spearmint leaves

Spearmint leaf drying was examined using hot-air (HA) and infrared (IR) techniques. The maximum essential oil concentration and rehydration ratio were obtained using IR drying. Overall, it was shown that spearmint drying using IR had superior quality preservation and used less energy than drying using HA (Nozad et al., 2016).

The properties of vacuum freeze-dried herbs and the quality of the freeze-dried products revealed that chamber pressure had a significant impact on drying time and essential oil content. While longer drying times were often associated with higher chamber pressure, the main spearmint volatile components were still present in the finished product. The quality of the freeze-dried product was judged to be higher than that of a convectively dried product but lower than that of the raw material. To the drying data, four distinct mathematical models were fitted. Vacuum freeze-dried mint leaves had higher rehydration ratios than convectively dried mint leaves, according to a water absorption test (Antal et al., 2011).

Drying of spearmint leaves was studied using five different methods (convection oven drying, freeze-drying, microwave drying, and air drying with and without sun exposure) which affected its total phenolic content, hydroxycinnamic acid derivatives, and antioxidant capabilities. According to the results, freeze drying produced dried spearmint with the greatest total phenolics content and strongest antioxidant potential and the lowest in spearmint that had been microwave and convection oven-dried. This could be explained by the fact that at high temperatures, heat-sensitive phenolics decomposed or experienced biotransformation (Orphanides et al., 2013).

2.1.5 Spearmint as a functional food

The functional properties of bread incorporated with spearmint (*S*) aqueous extract ($S1 = 0\%$ (control), $S2 = 2.5\%$, $S3 = 5.0\%$, and $S4 = 7.5\%$) by measuring total phenolic content, DPPH radical scavenging activity, and ferrous-ion chelating ability were examined by Shori et al., 2021. The spearmint bread (*S2*) showed the highest organoleptic properties among spearmint bread. In conclusion, this study showed that spearmint is a promising food additive to increase the antioxidant content in bread (Shori et al., 2021).

The effects of dried herbs with excellent nutritional properties, such as basil, marjoram, and spearmint, on the physicochemical and nutritional properties, such as water absorption index, water solubility index, oil absorption capacity, water absorption capacity, solubility, iron, and

proximate analysis on rice flour with skim milk powder at different fortification levels (FLs) were studied by Dalbhagat et al., 2021. This study will aid food manufacturers in creating fortified foods with dried herbs (Dalbhagat et al., 2021).

2.2 Coriander leaves as a detoxifying ingredient

2.2.1 Chemical composition of coriander leaves

The stem of the adult Coriander (*Coriandrum sativum* L.) plant is hollow, and its basal parts can reach a diameter of up to 2 cm. The leaves alternate, and the first ones are often gathered in a rosette. The blade shape of the basal leaves is usually undivided with three lobes, while the leaves of the nodes following are to a higher degree pinnatifid. The leaves are green or light green and their underside is often shiny and waxy. During the flowering period, the leaves sometimes turn red or violet. They wither before the first fruits are ripe starting from the basal leaves (Khan et al., 2014).

The coriander fresh leaves per 100 g contain moisture (87.9 g); protein (3.3 g); carbohydrates (6.5 g); total ash (1.7 g); calcium (0.14 g); phosphorus (0.06 g); iron (0.01 g); vitamin B2 (60 mg); niacin (0.8 mg); vitamin C (135 mg); vitamin A (10,460 I.U.) The chemical composition of fresh coriander leaves is shown in Table 2.2 (Shahwar et al.,2012)-

Table 2.2 Chemical composition of fresh coriander leaves

Parameter	Value (per 100gm)
Moisture%	87.9 g
Protein %	3.3 g
Carbohydrates	6.5 g
Total ash	1.7 g
Calcium	0.14 g
Phosphorus	0.06 g
Iron	0.01 g
Vitamin B2	60 mg
Niacin	0.8 mg
Vitamin C	135 mg
Vitamin A	10,460 I.U.

2.2.2 Physiochemical activities of coriander leaves

The antioxidant activity of coriander (*Coriandrum sativum*) oil and extracts of various polarities from the leaves and seeds were examined by Wangensteen et al., 2004. Additionally, the total number of phenols was measured. The total phenolic content of the extracts and antioxidant activity were shown to be positively correlated. The ethyl acetate extract was responsible for the most antioxidant activity in both the leaves and the seeds of the coriander, which demonstrated greater antioxidant activity than the coriander's seeds. According to this study, adding coriander to meals will improve its antioxidant content and may be able to act naturally as an antioxidant to prevent harmful oxidation processes (Wangensteen et al., 2004).

The characterization of coriander essential oil was also studied by Shahwar et al., 2012. The essential oil from coriander leaves showed radical scavenging activity ($56.73 \pm 1.82\%$) at a concentration of 500 μg . This study suggested that coriander leaves can be used as a potential source of food flavoring and antioxidants (Shahwar et al., 2012).

Coriander leaves are abundant in essential oils, trace elements, and antioxidants, all of which have positive effects. Therefore, bread supplemented with coriander leaf powder is likely to be more popular with consumers than bread that isn't. The baking and preserving qualities of such bread at supplementation levels of 1.0, 3.0, 5.0, and 7.0% (w/w) on wheat flour, as well as the antioxidant and sensory analyses. The results demonstrate that adding coriander leaf powder improved the crumb's moisture content while just slightly increasing the stiffness of the crumb. With the added bread, significant improvements in sensory qualities were seen. The antioxidant content in the enriched loaves increased significantly (Das et al., 2012).

2.2.3 Herbal beverage made using coriander leaves

Development of a ready-to-serve (RTS) herbal drink using coriander, ginger, and long pepper was studied by Bimsara et al., 2023. A sensory evaluation was carried out to select the most preferred sweetener. A freeze-dried herbal drink was used to determine the antioxidant properties using in vitro bioassays. The developed herbal drink contains phenolic compounds including flavonoids and possesses abilities to scavenge DPPH radicals, reduce ferric ions, and chelate ferrous ions. These findings indicated the antioxidant potential of the developed herbal drink (Bimsara et al., 2023).

An attempt has been made to develop a cucumber-based blended herbal beverage using sugarcane juice, citric acid, mint, and coriander extract along with salt at varying

concentrations using the response surface methodology by Heena et al., 2017. The physicochemical and sensory analysis revealed that the best blend was obtained with a sugarcane juice concentration of 30.14%, a salt concentration of 1.5%, citric acid, mint, and a coriander extract concentration of 1%. It is apparent from the study that cucumber juice can be successfully blended with sugarcane juice to enhance its sensory properties, as well as its phytochemical potential, which will open a new door in the beverage industry (Heena et al., 2017).

2.2.4 Drying of coriander leaves

Different pretreatments and methods on the basis of quality and rehydration characteristics on coriander leaves were studied. The best pretreatment was found to be dipping for 15 min in a solution of 0.1% Magnesium chloride, 0.1% Sodium bicarbonate, and 2.0% KMS in water at room temperature and the best method was drying in the mini multi-rack solar dryer (Kaur et al., 2006).

Drying characteristics and quality attributes of coriander leaves at selected drying air temperatures were also studied by Ahmad et al., 2001. Water blanching at 80°C for three minutes resulted in greater chlorophyll retention in the product. Drying took place in the falling rate period and the drying rate of unblanched samples was significantly faster than the blanched leaves. Temperature dependence of the rate constant was adequately described by the Arrhenius equation and the activation energy values for unblanched and blanched leaves were 26.5 and 24.6 kJ mol⁻¹ respectively. Chlorophyll content and rehydration capacity were found to be maximum when the blanched leaves were dried at 45°C (Ahmad et al., 2001).

The biochemical changes associated with the storage of coriander (*Coriandrum sativum* L.) green foliage leaf juice for up to 48 hours. The tender, stem, leaves, and flowers of this crop have a pleasant aromatic odor and are the richest source of vitamins A and C. Studies on biochemical changes during storage revealed that the dry matter (DM), Ph, nitrogen (N) content and chlorophyll content in the juice gradually decreased and increase in the amount of lactic acid is observed when the juice was stored up to 48 hours (Salve, 2019).

2.2.5 Coriander leaves as a functional food

The use and inclusion of fenugreek (*Trigonella foenum-graecum*), curry (*Murraya koenigii* Linn. Sprengal), and coriander (*Coriandrum sativum*) leaves were examined by Chakraborty et al., 2016. To create leaf blend powder, three different types of dry leaf powder were

combined in an exact ratio. In six distinct formulations, the leaf blend was co-extruded in a 1:1 ratio with rice and maize flour to create extruded snacks. Leaf blend was also combined with wheat flour to create pasta. The functional, proximate, sensory, and antioxidant aspects of the extruded snacks and pasta were examined. It was discovered that when the leaf blend inclusion increased, the extrudates' expansion ratio decreased. With increasing amounts of blended leaves, the leaf-based extrudates demonstrated high water retention capacity. The snacks' antioxidant activity demonstrated increased properties. The processed goods had higher levels of polyphenolic content and radical-scavenging capacity, was discovered. Pasta cooking tests yielded positive outcomes. The most acceptable pasta product was found by sensory analysis to have 2gm of leaf blend per 100gm of composite flour mix (Chakraborty et al., 2016).

2.3 Ginger as a detoxifying ingredient

2.3.1 Chemical composition of ginger

Ginger (*Zingiber officinale*) is a member of a plant family that includes cardamom and turmeric. Gingerols, which seem to be the principal component of ginger, are among the ketones that give the spice its characteristic scent. The primary part of ginger that is consumed is the rhizome, which is the horizontal stem from which the roots grow.

Different analytical techniques have helped to identify at least 115 different components in both fresh and dried ginger variants. The main ingredient in fresh ginger is gingerol. Since ancient times, ginger has been used to cure a variety of conditions, including colds, nausea, arthritis, migraines, and high blood pressure (Bode & Dong 2011). Ginger has been demonstrated to be useful for postoperative nausea and vomiting as well as nausea brought on by pregnancy (Rajathi et al., 2017).

The minerals in ginger include salt, potassium, calcium, phosphorus, and iron (Otulona et al., 2010), while thiamine, riboflavin, niacin, and vitamin C are the main vitamins found in ginger (Zachariah, 2008). At least 14 bioactive substances have been also isolated from ginger (Koh et al., 2009). Fresh ginger's primary nutrients are water (80.9%), carbs (12.3%), fiber (2.4%), proteins (2.3%), minerals (1.2%), and fats (0.9%). The chemical composition of ginger is shown in Table 4.3 (Shakya, 2015)-

Table 2.3 Chemical composition of fresh ginger

Parameter	Value (%)
Water	80.9
Carbohydrates	12.3
Fiber	2.4
Proteins	2.3
Minerals	1.2
Fats	0.9

2.3.2 Drying of ginger

The drying of whole and sliced ginger rhizomes in lengths of 5, 10, 15, 20, 30, 40, and 50 mm, from an initial moisture content of 81.3% to a final moisture content of less than 10% using a variety of drying techniques, including cabinet tray drying, solar tunnel drying, and sun drying were done. As the slice length shrunk, it was discovered that the amount of essential oil and oleoresin significantly dropped. The drying trials revealed that entire ginger rhizomes dried outside in the sun or within a solar tunnel dryer maintained the highest levels of dry ginger's essential oil and oleoresin content (Jayashree et al., 2014).

The effects that processing has on the total phenolic and flavonoid content of ginger (*Zingiber officinale*) and its antioxidant potential were studied by Offei-oknye et al., 2015. In addition to oven drying, sun drying, and freeze drying as processing techniques, fresh ginger was employed as a control. In comparison to other treatment groups, ginger that had been freeze-dried showed a much better capacity to scavenge free radicals. However, sundried ginger exhibited the highest ferric-reducing antioxidant efficacy. The samples of processed ginger that were freeze-dried had the highest flavonoid concentration (Offei-Oknye et al., 2015).

2.2.3 Ginger as a functional food

Ginger has been used in the creation of edible films and coatings as an essential oil, powder, extract, or additive (Beristain-Bauza et al., 2019). Ginger powder (1-2%) was added to rabbit burgers and monitored the results over the course of seven days at 4°C. The findings showed that adding ginger powder to raw or cooked hamburgers resulted in a high concentration of polyunsaturated fatty acids and a low concentration of saturated fatty acids. Furthermore, a

stronger antioxidant capacity was seen when ginger concentration was increased (Mancini et al., 2017).

2.4 Lemon as a detoxifying ingredient

2.4.1 Chemical composition of lemon

Lemon (*Citrus limon* L.) is an important medicinal plant of the family Rutaceae. The oblong citrus fruit known as lemon has smooth, permeable skin. Lemons are grown on thorny, little trees that are 10 to 20 feet tall (Mohanapriya et al., 2013). It is farmed mostly for its alkaloids, which have anticancer properties, and it has been reported that crude extracts of Lemon leaves, stem, root, and flower have antibacterial ability against clinically important bacterial strains (Kawaii et al., 2000).

Research has been done on significant effects such as desorption, anti-fungal, anti-oxidant, anti-inflammatory, anti-cancer, anti-bacterial, and anti-ulcer of lemon. Lemon has significant natural ingredients with high concentrations of ascorbic acid, minerals, citric acid, essential oils, and flavonoids. Due to the presence of alkaloid constituents in various lemon leaf, stem, root, and flower portions, lemon has anticancer and antibacterial activities (Rafique et al., 2020).

A 100-gram serving of raw lemons without the peel contains the following nutrients: Energy 121 kJ (29 kcal), Carbohydrates 9.32 g, Sugars 2.50 g, Dietary fiber 2.8 g, Fat 0.30 g, Protein 1.10 g, Pantothenic acid (4%), Vitamin B6 (6%), Folate (3%), Vitamin C (88%), Calcium (3%), Iron (5%), and Magnesium (2%), Zinc (1%), Potassium (3%), and Phosphorus (2%). The chemical composition of fresh lemon without peel is shown in Table 4.4 (Mohanapriya et al., 2013)-

Table 2.4 Chemical composition of fresh lemon without peel

Parameter	Value (per 100gm)
Energy	121kJ (29kcal)
Carbohydrates	9.32 g
Sugars	2.50 g
Dietary fiber	2.8 g
Fat	0.30 g
Protein	1.10 g

Pantothenic acid%	4
Vitamin B6%	6
Folate%	3
Vitamin C%	88
Calcium %	3
Iron%	5
Magnesium%	2
Zinc	1
Potassium%	3
Phosphorus%	2

2.4.2 Drying of lemon

The impact of dry lemon pomace polyphenols and antioxidant capacity on freeze-drying, hot air drying, and vacuum drying at 70, 90, and 110 °C were examined by Papoutsis et al., 2017. In comparison to lemon pomace dried by freeze-drying, lemon pomace dried by hot air or under vacuum had a higher total phenolic content and antioxidant capacity, which also rose as the temperature rose. The pomace dried under vacuum at 70 and 90 °C had the highest total flavonoid concentration. The most neo hesperidin was found in lemon pomace that had been freeze-dried, while the most rutin and p-coumaric acid were found in pomace that had been dried under vacuum at 70 °C. The pomace dried by hot air at 110 °C had the highest gallic acid concentration. According to the results, the drying method should be carefully chosen depending on the bioactive components that are intended to be extracted (Papoutsis et al., 2017).

Lemon slices were dried and color changes were examined as a result of microwave-convective heating. Six mathematical models that were published in the literature were used to fit the drying data. The model that effectively captured the dynamics of drying lemon segments was discovered. The dried lemon slices' color shift was examined and taken into account as a factor in the product's drying quality. With increasing microwave power, the values of lightness/darkness, yellowness/blueness, and hue angle increased while the value of redness/greenness declined (Darvishi et al., 2014).

2.4.3 Lemon as a functional food

The hypolipidemic effects of citrus lemon juice in rabbits following a high cholesterol diet for four weeks to see how some of the elements in citrus improve health and offer protection against chronic disease. Citrus lemon juice showed a substantial decrease in serum triglycerides, low-density lipoprotein, and cholesterol levels while increasing high-density lipoprotein. These findings imply that the antioxidant properties of citrus lemon juice may account for its hypocholesterolemic benefits (Khan et al., 2010).

CHAPTER-3

MATERIAL

3.1 Collection of Raw Materials

Spearmint leaves, coriander leaves, lemon, and ginger were procured from a local vegetable vendor at Tedhipulia, Lucknow, Uttar Pradesh.

Cumin powder and salt were procured from a supermarket near Integral University, Lucknow, Uttar Pradesh.

3.2 Chemicals, glassware/tools used

Chemicals, glassware/tools were procured from the Integral University laboratory, which are listed below-

Table 3.1 List of chemicals

S. No.	Chemicals	Chemical Formula	Company
1.	Sodium Hydroxide	NaOH	Fisher Scientific (Qualigens)
2.	Phenolphthalein	C ₂₀ H ₁₄ O ₄	Qualigens, Nice and Merck
3.	2,2-diphenyl-1-picrylhydrazyl (DPPH)	C ₁₈ H ₁₂ N ₅ O ₆	Sisco Research Laboratory
4.	Folin–Ciocalteu reagent	C ₁₀ H ₅ NaO ₅ S	HiMedia Laboratories
5.	Sodium carbonate	Na ₂ CO ₃	Thermo Fisher Scientific (Qualigens)
6.	Potassium acetate	CH ₃ CO ₂ K	Thermo Fisher Scientific (Qualigens)
7.	Aluminium Chloride	AlCl ₃	Drashti Chemicals

8.	Ethanol	C_2H_6O	Changshu Sheng Chemical	Song Fine
9.	Methanol	CH_3OH	Thermo Scientific (Qualigens)	Fisher

Table 3.2 List of Glassware/ Tools

S. No.	Glassware/ Tools	Specification	Quantity	Company
1.	Petri plates	100 mm × 15 mm	15	Borosil
2.	Conical flask	50 ml 100 ml 250 ml 500 ml	2 4 3 2	Borosil
3.	Beaker	50 ml 100 ml 250 ml 500 ml	2 4 3 5	Borosil
4.	Measuring cylinder	10 ml 50 ml 100 ml 250 ml	3 2 3 1	Borosil
5.	Test tubes	10ml 15 ml 20 ml 25 ml	10 15 5 5	Borosil
6.	Volumetric Burette	50 ml 100 ml	1 1	Borosil
7.	Micropipette	200 μ l 1000 μ l	1 1	Tarsons
8.	Crucible	30 ml	1	Thiasil

		50 ml	2	
		100 ml	1	
9.	Pasteur pipette	5 ml	2	Igneus Life Science
		3 ml	1	
10.	Spatula	20 cm	2	Indofab Engineering
11.	Glass cuvette	10 mm	4	RP scientific store
11.	Glass bottles	100 ml	10	Borosil
12.	Stainless steel utensils	Cook and serve big bowl	2	Hawkin
		Cook and serve medium bowl	2	
		Tablespoon	2	

3.3 Instruments used

The instruments used were available in the Integral University, Lucknow which are as follows-

Hand-held refractometer- A hand-held refractometer (model no. A22-962) of ERMA company was used to measure the Total Soluble Solid (TSS) of the developed herbal detox drinks.



Figure 3.1 Hand-held Refractometer

pH meter- A pH meter (model no. Type-361) of Systronics company was used to measure the pH of the developed herbal detox drinks.

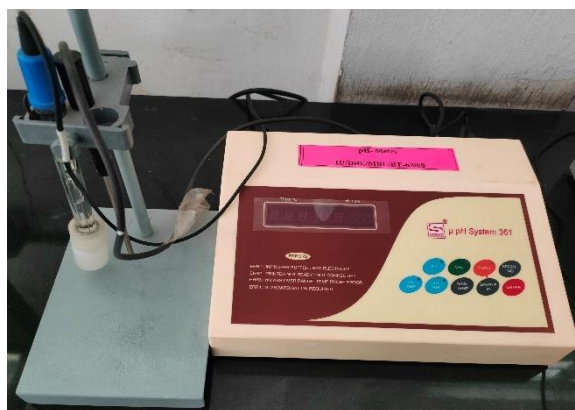


Figure 3.2 pH meter

Weighing balance- A weighing balance (model no. BL P3B/6002) of Kerro company was used to weigh all the raw materials for the development of herbal detox drinks.

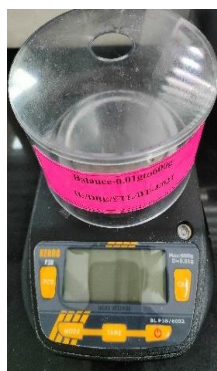


Figure 3.3 Weighing balance

Electronic balance- An electronic balance (model no. BL P7/2204) of Kerro company was used to weigh all the chemicals and herbal detox drink powder for the physicochemical analysis.

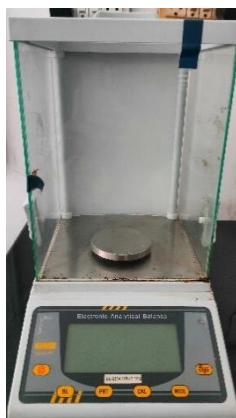


Figure 3.4 Electronic balance

Mixer grinder- A mixer grinder (model no. GX 15) of Bajaj company was used to extract juices from mint, coriander, and ginger separately which were further used to develop different blends of herbal detox drinks.



Figure 3.5 Mixer grinder

Citrus fruit juicer- A citrus fruit juicer (model no. 651E) of Sokany company was used to extract juice from lemons which were further used in the development of different blends of herbal detox drinks.



Figure 3.6 Citrus fruit juicer

Magnetic Stirrer- A magnetic stirrer (model no. 2MLH) of Remi company was used to stir the herbal detox drinks and to preparation of the chemical solution required for the physicochemical analysis.



Figure 3.7 Magnetic Stirrer

Vortex- A vortex (model no. CM-101 plus) of Remi company was used to stir the various solutions for physicochemical analysis for the herbal detox drinks and the prepared herbal detox drink powder.



Figure 3.8 Vortex

Spectrophotometer- A spectrophotometer (model no. LMSP-V325) of Labman company was used to measure the absorbance of the sample at different wavelengths for physicochemical analysis of the herbal detox drink and the prepared herbal detox drink powder

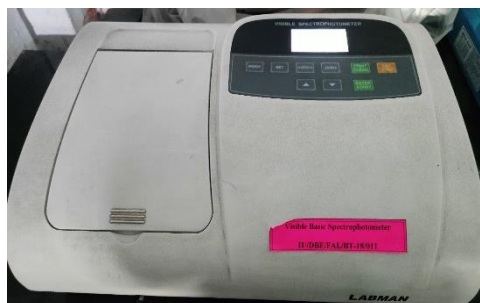


Figure 3.9 Spectrophotometer

Autoclave- An autoclave (model no. 1049) of Science Tech (India) company was used to sterilize media prepared and glassware required for anti-microbial analysis.



Figure 3.10 Autoclave

Vertical laminar airflow- A vertical laminar airflow (model no. R1925) of Science Tech (India) company was used to perform an anti-microbial analysis of the developed detox drink powder.



Figure 3.11 Vertical laminar airflow

Incubator- An incubator (model no. CI-10 plus) of Remi company was used to incubate the prepared petri plates for anti-microbial analysis of developed herbal detox drink powder.



Figure 3.12 Incubator

Muffle Furnace- A muffle furnace (model no. BPI-18) of BP Industries company was used to determine the ash content of the developed herbal detox drink powder.



Figure 3.13 Muffle Furnace

Hot air oven- A hot air oven (model no. BP-9) of BP Industries company was used to determine the moisture content of the developed detox drink powder.



Figure 3.14 Hot air oven

Lyophilizer- A lyophilizer (model no. FD 5-2.5 E) of Gold-Sim company was used to develop the herbal detox drink powder from the selected composition of herbal detox drink.

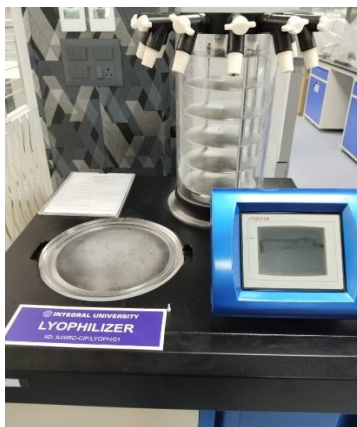


Figure 3.15 Lyophilizer

Table 3.3 List of instruments

S. No.	Instruments	Model number	Company
1.	Hand-held Refractometer	A22-962	ERMA
2.	pH meter	Type-361	Systronics
3.	Weighing Balance	BL P3B/6002	Kerro
4.	Electronic Balance	BL P7/2204	Kerro
5.	Mixer Grinder	GX 15	Bajaj
6.	Citrus fruit juicer	651E	Sokany
7.	Magnetic Stirrer	2MLH	Remi
8.	Vortex	CM-101 plus	Remi
9.	Spectrophotometer	LMSP-V325	Labman
10.	Autoclave	1049	Science Tech (India)
11.	Vertical Laminar airflow	R1925	Science Tech (India)
12.	Incubator	CI-10 plus	Remi
13.	Muffle Furnace	BPI-18	B P Industries
14.	Hot air Oven	BPI-9	B P Industries
15.	Lyophilizer	FD 5-2.5E	Gold-Sim

3.3 Media used

3.3.1 Plate Count Agar - PCA agar (Plate Count Agar) is a medium recommended for the standardized enumeration of aerobic bacteria in water, dairy products and foods, cosmetics, or pharmaceuticals. PCA medium is non-selective and relatively rich in nutrients, tryptone, vitamin factors from yeast extract, and glucose used as an energy source to promote the growth of most bacteria.

Table 3.4 Composition of Plate Count Agar (PCA)

S. No.	Constituents	Quantity (g/L)
1.	Tryptone	5.0
2.	Yeast extract	2.5
3.	Dextrose (Glucose)	1.0
4.	Agar	15.0
5.	Sodium Chloride	8.0

METHODOLOGY

3.4 Processing of Raw Materials

3.4.1 Preparation of Mint and Coriander Juice

Mint and coriander leaves were selected on the basis of their freshness and color (dark green for mint and light green for coriander). Then it was separated from the stalk, sorted, and thoroughly washed with running water. The juice was then extracted mechanically from both mint and coriander leaves separately by using a mixer grinder. Mint and coriander juices were then filtered using sterilized muslin cloth separately. Filtered juices were then stored in a sterilized bottle and kept in refrigeration until it was used for blending.

3.4.2 Preparation of Ginger and Lemon Juice

Ginger and lemon were selected on the basis of their freshness, color, and texture.

Raw gingers were peeled and washed with running water properly. The juice was then extracted mechanically by a mixer grinder. Ginger juice was then filtered using a sterilized muslin cloth. Filtered juices were then stored in a sterilized bottle and kept in refrigeration until it was used for blending.

Lemons were washed with running water properly. Then the juice was extracted by a mechanical citrus juicer and then filtered using a sterilized muslin cloth. Lemon juice was stored in a sterilized container and kept in the refrigerator until it was used for blending.

3.5 Preparation of herbal detox drinks

Nine different herbal detox drink formulations were made by altering the Coriander and Mint juice ratio. Other compositions such as Lemon Juice, Ginger Juice, Salt: and Cumin Solution (10%) have been kept constant based on the results obtained from preliminary trials. The developed different blends were filled into sterilized glass bottles and stored in the refrigerator till further analysis.

Table 3.5 Composition of Blended Herbal Detox Drink

Samples	Coriander juice (ml)	Mint juice (ml)	Lemon juice (ml)	Ginger juice (ml)	Salt and Cumin powder (1:1) 10%
C1	0	80	5	5	10
S1	10	70	5	5	10
S2	20	60	5	5	10
S3	30	50	5	5	10
S4	40	40	5	5	10
S5	50	30	5	5	10
S6	60	20	5	5	10
S7	70	10	5	5	10
C2	80	0	5	5	10

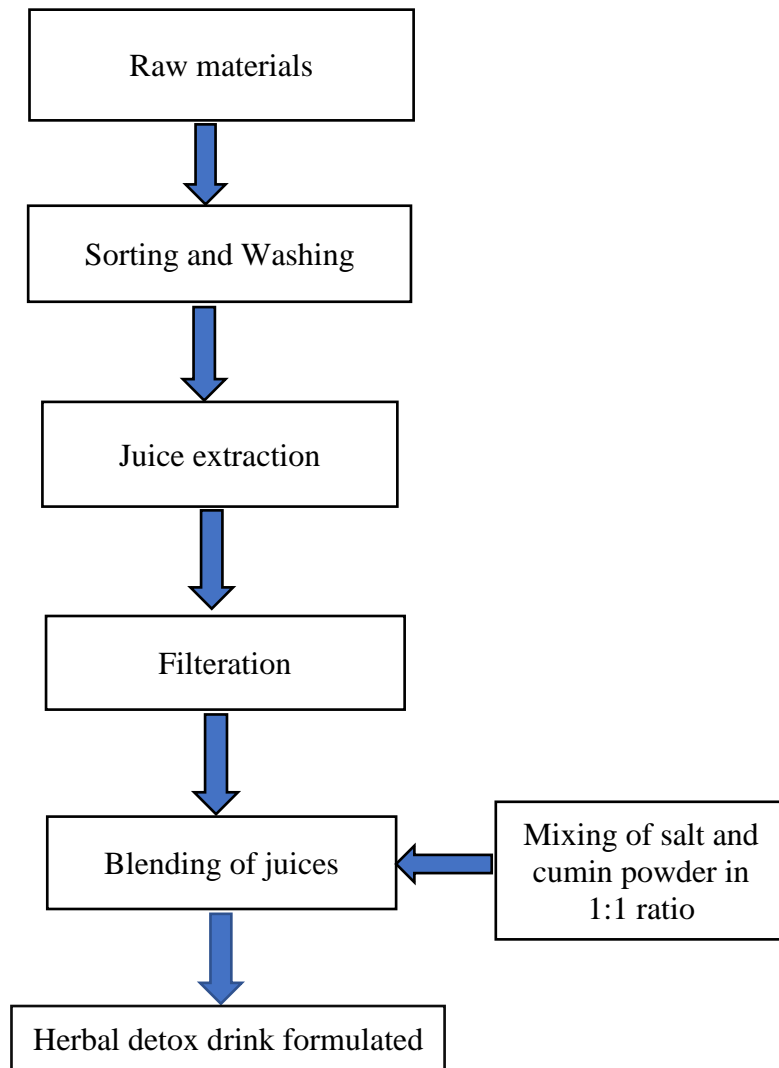


Figure 3.16 Preparation of Herbal Detox Drink

3.6 Physico-chemical Analysis of Herbal Detox Drinks

Total soluble solids (TSS), titratable acidity, and pH of all the different blended formulations were determined using standard methods (Horwitz, 1975). Antioxidant activity was determined by the 2, 2-diphenyl-2-picryl hydrazine (DPPH) inhibition method (Thakur et al., 2019).

3.6.1 Total Soluble Solids (TSS)

Total soluble solids were analyzed by using a handheld refractometer. A drop of the filtrate was put on a refractometer prism and TSS was recorded as °Brix.

3.6.2 Titratable Acidity

2 ml of well-mixed juice was diluted to 48 ml with boiled water. Titration was done with 0.1N NaOH, 0.3 ml phenolphthalein indicator was added to the sample solution and titration was done using a volumetric burette up to the end point of light brown color.

$$\text{Acidity(\%)} = \frac{N \times T.V. \times \text{Mol. wt.}}{W} \times 100$$

Where, N = Normality of titrant, usually NaOH,

T.V. = Titre Value of titrant,

Mol. wt. = molecular weight of predominant acid

W = mass of sample (g)

3.6.3 pH

pH was taken by using a pH meter with a buffer of pH 7.

3.6.4 Antioxidant activity

The free radical scavenging activity of all the formulations of herbal detox drinks was measured by: the evaluation of the free radical-scavenging effect on the 2,2-diphenyl 2-picrylhydrazyl radical. 0.1 ml of samples were mixed with 0.9 ml of 0.4 mM DPPH ethanol solution. The mixture was thoroughly mixed and kept in the dark for 30 minutes. The absorbance was measured later, at 517 nm, against a blank of ethanol. The radical scavenging capacity can be expressed as a percentage effect (E%) and calculated using the following equation (Thakur et al., 2019):

$$E \% = \frac{A(c) - A(s)}{A(c)} \times 100$$

Where, A(c) = Absorbance of the control

A(s) = Absorbance of the sample

3.7 Sensory Analysis

The coded samples of all the different blended formulations were served to a panel of 10 semi-trained members at the Department of Food Technology, Integral University Lucknow, India,

to evaluate the sensory characteristics on a nine-point hedonic scale. A training session was conducted to familiarize the panelists with the hedonic scale before sensory analysis. They were then asked to evaluate the samples for various quality attributes, i.e., color, odor, flavor, aftertaste, and overall impression, and to award a score (from 1 = dislike extremely to 9 = like extremely) depending on their respective impact. All the evaluations were carried out at room temperature (i.e., 25°C). The panelists used plain water to rinse their mouths in between the tasting sessions. The mean of all the attributes was used to define the overall acceptability of the herbal detox drink formulations (Joshi et al., 2017).

Table 3.6 Hedonic Scale

Rating	Score
Like Extremely	9
Like very much	8
Like Moderately	7
Like Slightly	6
Neither like nor dislike	5
Dislike Slightly	4
Dislike Moderately	3
Dislike very much	2
Dislike extremely	1

On the basis of the result of sensory analysis, blended formulation i.e., S3 with coriander to mint juice ratio 30:50 was selected as the best formulation for the development of Herbal detox drink powder.

3.10 Lyophilization of the selected sample

Selected herbal detox drink i.e., S3 was prepared in adequate amounts and kept in the deep freezer at -80°C for 2 hours, and then the sample was dried for 48 hours in a freeze dryer. The process was carried out in three phases i.e., freezing, primary drying, and secondary drying. Following lyophilization herbal detox drink was transformed into powder form which was further kept in an air-tight container as it was hygroscopic in nature. The herbal detox drink powder was stored for further experimental studies.

3.11 Herbal Detox Drink Powder preparation

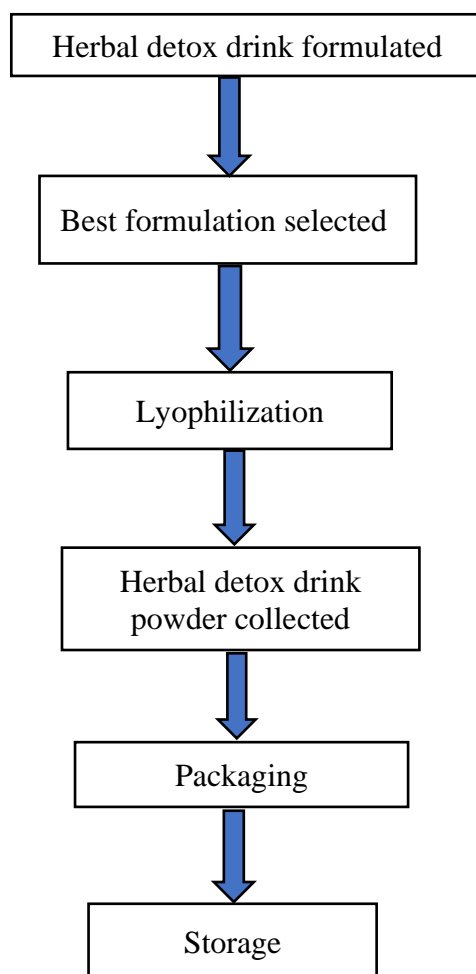


Figure 3.17 Preparation of Herbal Detox Drink Powder

3.12 Quality Analysis of Herbal Detox Drink Powder – Herbal detox drink powder analysis was done every tenth day up to the 40th day.

3.12.1 Physiochemical Analysis

Moisture content and ash content of the developed herbal detox drink powder were determined by using standard methods (AOAC, 2005). Antioxidant activity was determined by the 2, 2-diphenyl-1-picryl hydrazine (DPPH) inhibition method, total phenolic content was determined by using the Folin–Ciocalteu method, and Colorimetric aluminum chloride method was used for total flavonoid content determination.

3.12.1.1 Moisture Content – Moisture content was estimated by using 1g of sample. Initially, the weight of the empty petri dish was determined using an electronic balance. In flat

bottom petri plates, 1g of sample was weighed. The petri dish with its sample was placed in a hot air oven that was thermo statistically controlled at 105°C and heated for 2 hours and then readings were calculated. Finally, the petri dish with the sample was removed from the oven and placed in a desiccator to cool before being weighed again.

The resultant loss in weight was calculated as a percentage of moisture content on a dry basis.

$$\text{Moisture}(\%) = \frac{W1 - W2}{W} \times 100$$

Where, W = Weight of sample

W1 = Weight of sample + weight of petri dish

W2 = Weight of dried sample + weight of petri dish

3.12.1.2 Ash Content – Ash content was estimated by using 1g of sample. The empty weight of the crucible was measured. In the crucible 1g of sample was weighed. For 4 hours, the crucible having a sample in it was placed in a muffle furnace at 550°C. Then crucible was placed in the desiccator for a few minutes to cool down.

The resultant loss in weight was calculated as a percentage of ash content on a dry basis.

$$\text{Ash}(\%) = \frac{W2 - W}{W1 - W} \times 100$$

W = Weight of sample

W1 = Weight of sample + weight of petri dish

W2 = Weight of dried sample + weight of petri dish

3.12.1.3 Antioxidant activity of powder- Free radical scavenging activity of samples was measured by: the evaluation of the free radical - scavenging effect on the 2,2-diphenyl 2-picrylhydrazyl radical. 0.1 ml of samples were mixed with 0.9 ml of 0.4 mM DPPH ethanol solution. The mixture was thoroughly mixed and kept in the dark for 30 minutes. The absorbance was measured later, at 517 nm, against a blank of ethanol (Thakur et al., 2019).

The radical scavenging capacity can be expressed as a percentage effect (E%) and calculated using the following equation:

$$E\% = \frac{A(c) - A(s)}{A(c)} \times 100$$

Where, A(c) = Absorbance of control

A(s) = Absorbance of sample

3.12.1.4 Total Phenolic Content (TPC) - The total phenolics were determined by using Folin–Ciocalteu method and Gallic acid was used as a standard equivalent (in mg/g). Approximately, 5 ml of Folin–Ciocalteu reagent was mixed with 1 ml diluted herbal detox drink powder. After 3 or 5 min, 4 ml 7.5% sodium carbonate was added to the mixture and stood for 30 min at room temperature. The absorbance was measured by a spectrophotometer at 765 nm (Ghasemi et al., 2009).

3.12.1.5 Total Flavanoid Content (TFC)- The total flavonoids were estimated by the Aluminium chloride colorimetric technique. 1 ml of diluted herbal detox drink powder was mixed with 0.2 ml of 10% Aluminium chloride, 0.2 ml of potassium acetate (5%) was added, and then 5.6 ml of distilled water was added. The solution was left for 30 minutes at room temperature and the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was plotted by different concentrations of quercetin equivalents (in mg/g) (Ghasemi et al., 2009).

3.12.2 Anti-microbial effect

3.12.2.1 Plate Count Agar - PCA agar (Plate Count Agar) is a medium recommended for the standardized enumeration of aerobic bacteria in water, dairy products, foods, cosmetics, or pharmaceuticals. PCA medium is non-selective and relatively rich in nutrients, tryptone, vitamin factors from yeast extract, and glucose used as an energy source to promote the growth of most bacteria.

3.12.2.2 Preparation of Plate Count Agar (PCA) media

1. Suspend 3 g of plate count agar powder in 100 ml of distilled water. Mix 1g of agar agar into this mixture.
2. Stirred this mixture to fully dissolve all components.
3. Autoclave the dissolved mixture along with the required petri dishes at 121°C for 15 minutes.
4. Once the plate count agar has been autoclaved, allow it to cool down for a few minutes in laminar airflow.

5. Pour plate count agar up to half of each dish and leave dishes on the sterile surface until solidification.

6. Place the lid of each petri dish and store the dishes in air laminar flow.

3.12.2.3 Spreading of strain

1. *Escherichia coli* were obtained from stock cultures at the Department of Food Science and Technology, Integral University, Lucknow, Uttar Pradesh.

2. Bacteria were spread on plate count agar (PCA) and left for 10-15 minutes.

3.12.2.4 Procedure

1. Make well into each petri dish in which *Escherichia coli* was spread.

2. Pour the diluted detox drink powder sample into each well with a control of distilled water in each dish.

3. Incubate petri dishes at 37°C for 24 hr.

4. The colonies were observed and counted.

3.13 FTIR spectroscopy of Herbal Detox Drink Powder

The chemical characterizations of herbal detox drink powders were performed by Fourier transform infrared spectroscopy (FTIR). The recommended technique for IR spectroscopy is called Fourier transform infrared, or FTIR. IR radiation is transmitted through a sample during IR spectroscopy. The sample absorbs part of the infrared light, but some of it also passes through (transmits). The resulting spectrum serves as a molecular fingerprint of the material by depicting molecule absorption and transmission. Similarly, no two distinct molecular structures produce the same IR spectrum, and no two fingerprints ever match. Because of this, IR spectroscopy can be used for several kinds of analysis. No two compounds create the same IR spectrum because each has a different composition and, consequently, a different arrangement of atoms. Therefore, every type of substance may be positively identified (qualitatively analyzed) using IR spectroscopy (Berthomieu & Hienerwadel, 2009).

CHAPTER-4

RESULTS AND DISCUSSIONS

This chapter presents the experimental results conducted to analyze the quality of herbal detox drink powder under ambient conditions. All parameters were important in determining the quality of the detox drink powder.

4.1 Physicochemical Analysis of the Herbal Detox Drink Formulations

Among all the formulations, Total Soluble Solid (TSS) ranged from 4.00–6.00 °Brix, titratable acidity from 1.14–2.2%, pH from 3.74–4.22, antioxidant activity from 29.15–57%, and hedonic reading from 5-8.5 which are represented in table 4.1

Table 4.1 Physicochemical and Sensory analysis of blended formulation

Samples	TSS (°Brix)	Titratable Acidity (%)	pH	Antioxidant Activity (%)	Hedonic
C1	6.0	1.76	3.74	57.0018	5
S1	4.0	1.49	3.83	41.0716	6
S2	4.2	1.76	3.89	39.9448	7
S3	4.9	1.76	4.06	34.6507	8.5
S4	5.1	1.93	3.97	29.8805	7.5
S5	5.3	1.93	4.22	30.0205	6
S6	5.8	2.2	3.87	29.1599	5.5
S7	6.0	1.14	4.09	31.0551	5
C2	5.0	2.2	4.06	42.7849	5

4.1.1 Total Soluble Solid (TSS) of Herbal Detox Drink Formulations

The Total Soluble Solid (TSS) of all herbal detox drink formulations ranged from 4.00 to 6.00 °Brix. Approximately similar results were also observed by Heena et al., 2017 in the cucumber-based blended herbal beverages in which TSS ranged from 9.00–15.00 °Brix (Heena et al., 2017).

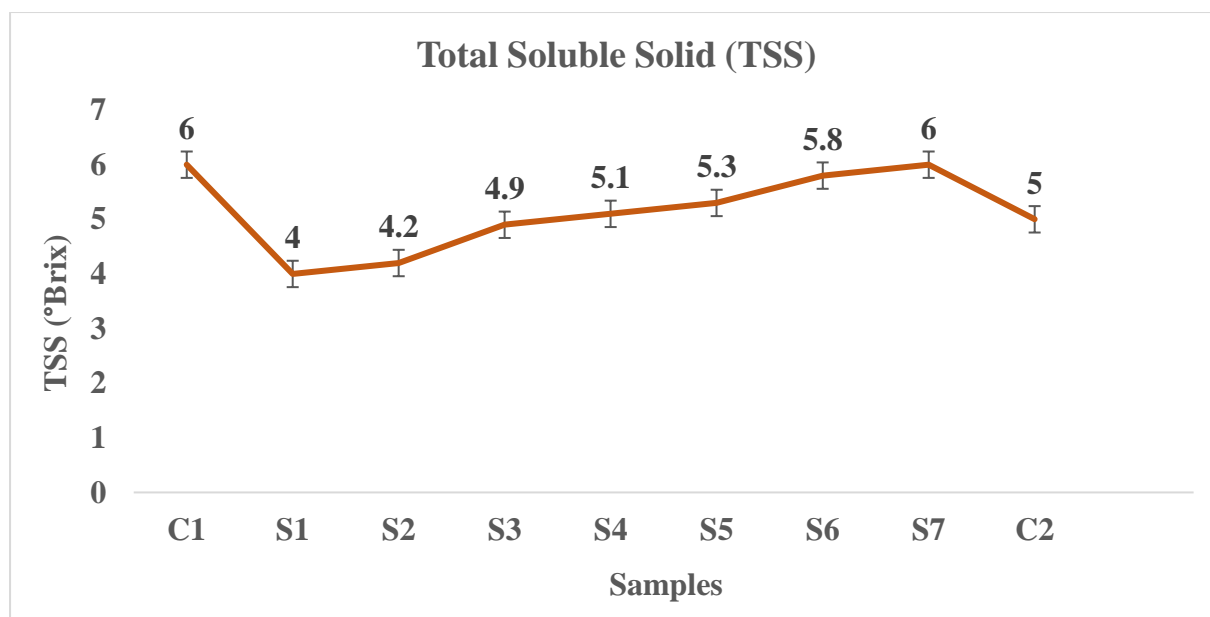


Figure 4.1 TSS of herbal detox drink formulations

4.1.2 Titratable Acidity of Herbal Detox Drink Formulations

The titratable acidity of all herbal detox drink formulations ranged from 1.14 to 2.2%. Heena et al., 2017) also observed approximately similar results in the cucumber-based blended herbal beverages in which titratable acidity ranged from 0.22 to 1.30% (Heena et al., 2017).

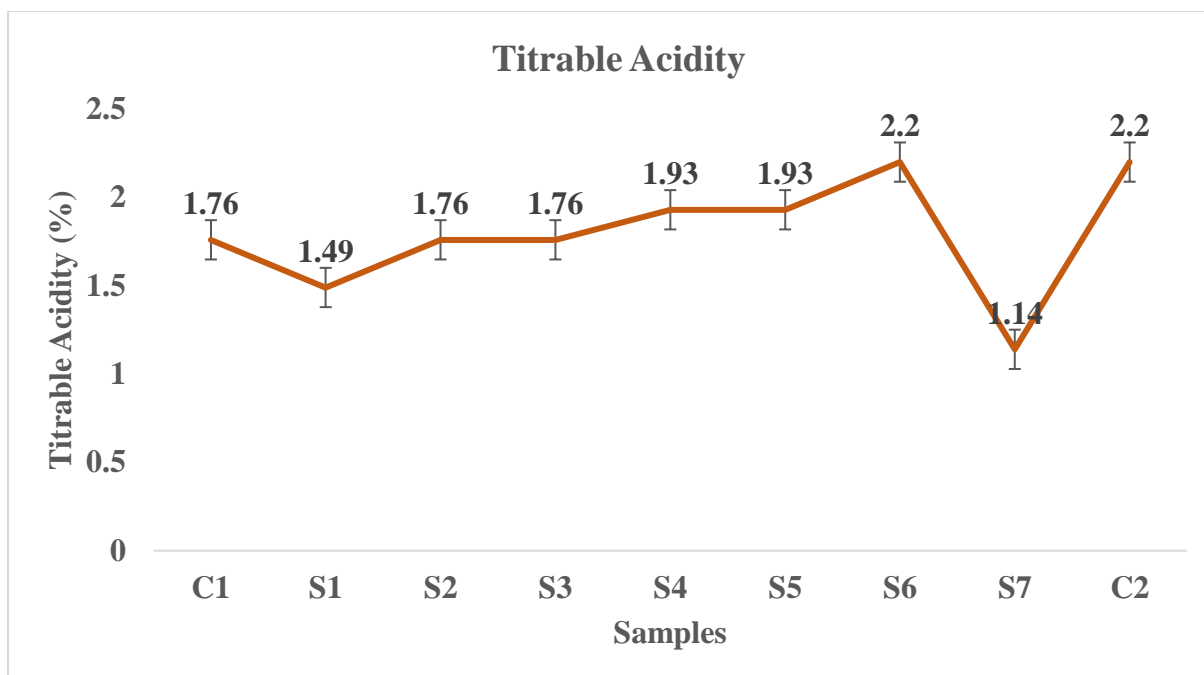


Figure 4.2 Titratable acidity of herbal detox drink formulations

4.1.3 pH of Herbal Detox Drink Formulations

The pH of all herbal detox drink formulations ranged from 3.74 to 4.22. Heena et al., 2017 also observed approximately similar results in the cucumber-based blended herbal beverages in which pH ranged from 2.96 to 5.30 (Heena et al., 2017).

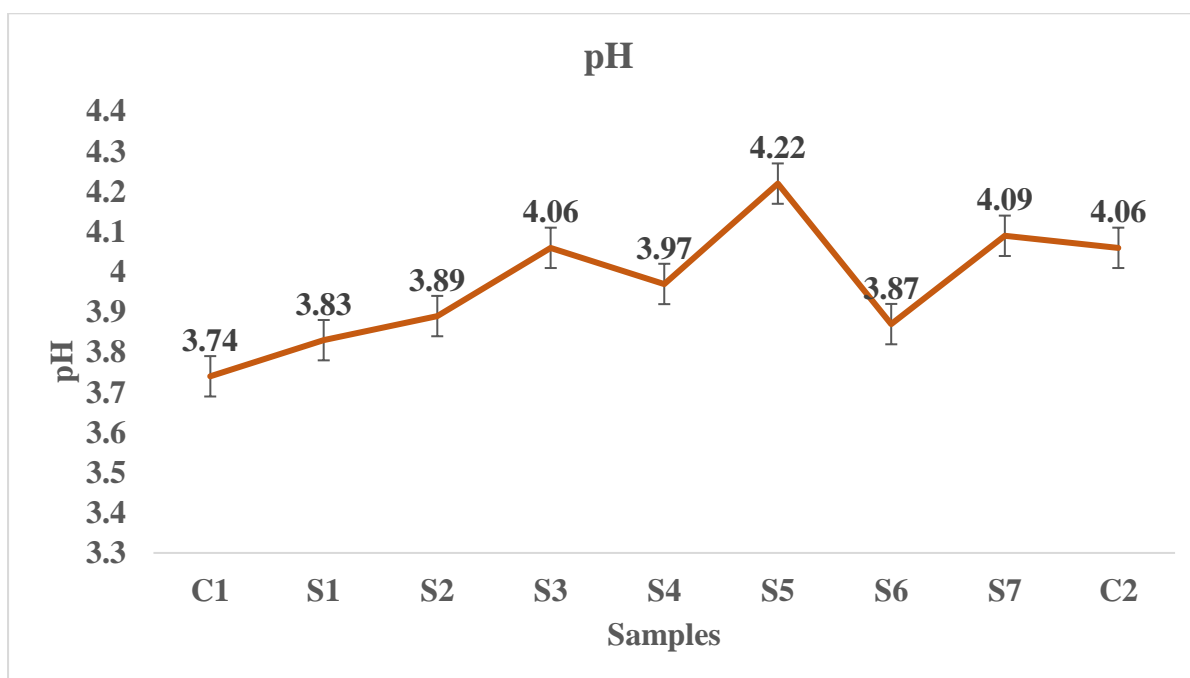


Figure 4.3 pH of herbal detox drink formulations

4.1.4 Antioxidant Activity of Herbal Detox Drink Formulations

The antioxidant activity of all herbal detox drink formulations ranged from 29.15 to 57%. Mahar et al., 2023 also observed approximately similar results in lemongrass and celery-based detoxifying drinks which ranged from 21.37 to 56.81% (Mahar et al., 2023).

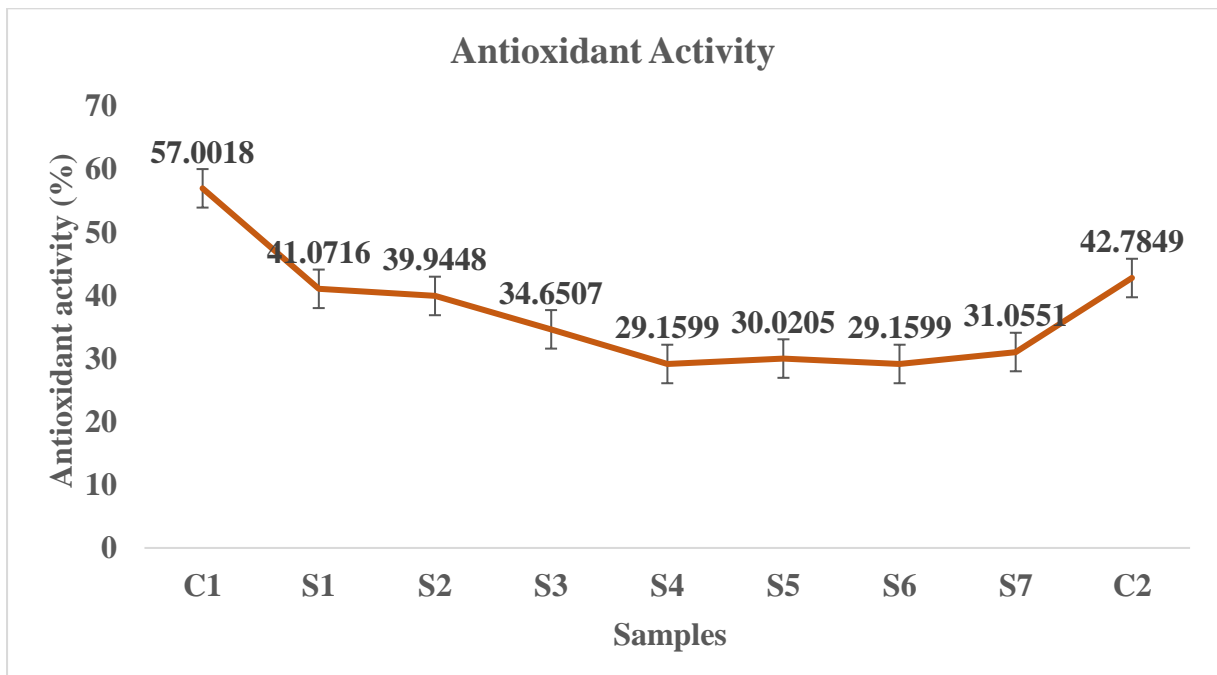


Figure 4.4 Antioxidant activity of herbal detox drink formulations

4.1.5 Sensory Analysis of Herbal Detox Drink Formulations

The hedonic scale of all herbal detox drink formulations ranged from 5 to 8.5.

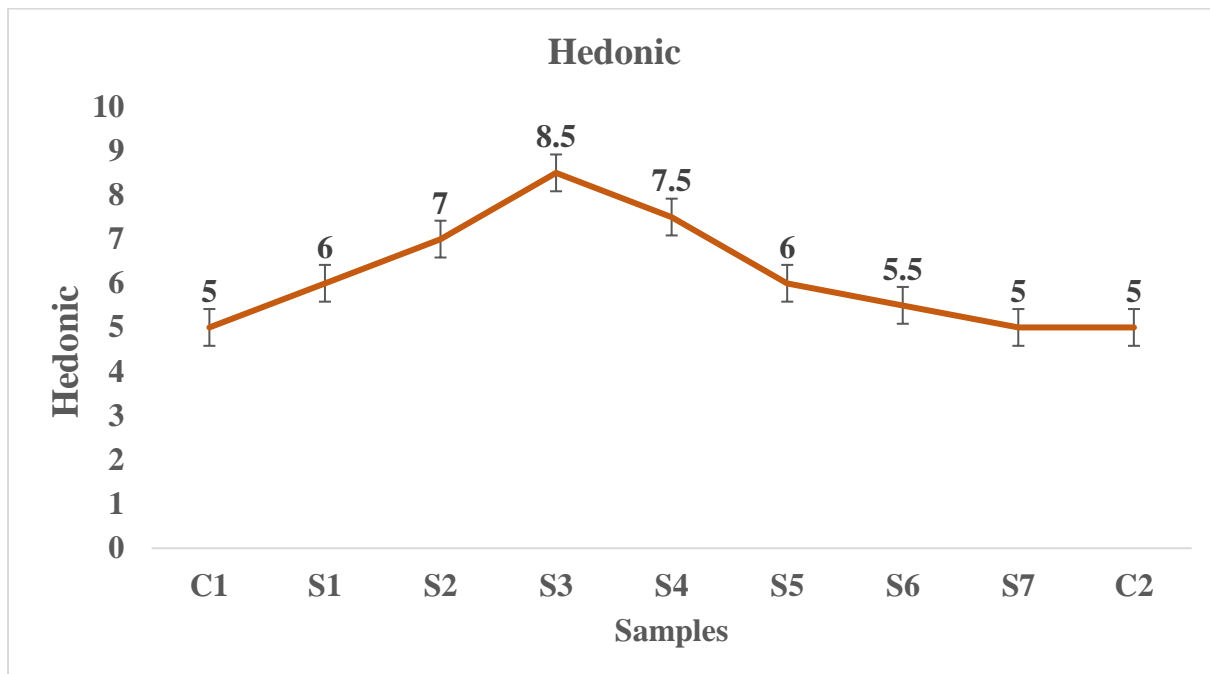


Figure 4.5 Hedonic scale of herbal detox drink formulations

All the nine formulations of herbal detox drink were evaluated by a nine-point hedonic scale sensory evaluation which revealed that the best blend among them was S3, which was made by blending mint juice, coriander juice, lemon, and ginger juice (50:30:10) along with cumin seed powder: salt (1:1) and have the highest hedonic reading- 8.5

The selected sample was further lyophilized to transform the detox drink into powder form and thus, the final quality analysis was done on that powder.

4.2 Physico-chemical analysis and shelf-life estimation of developed herbal detox drink powder

4.2.1 Moisture content

The moisture content of herbal detox drink powder was 5.14%, which was further increased during the storage period as represented in Figure 4.6. Similar results were also observed by Mahesh et al., 2018 in the herbal energy booster powder drink in which the moisture content was 5.6% (Mahesh et al., 2018).

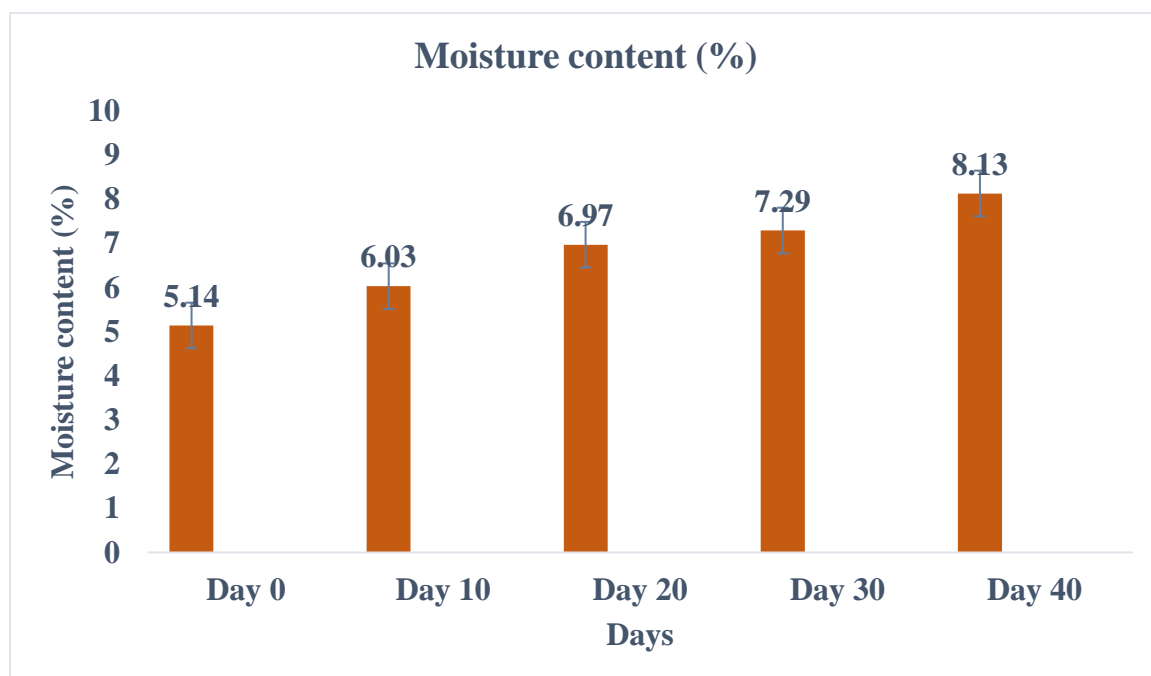


Figure 4.6 Moisture content (%) of herbal detox drink powder



Figure 4.7 Moisture content analysis of herbal detox drink powder

4.2.2 Ash content

The ash content of herbal detox drink powder was 2.89%, which was further increased during the storage period as represented in Figure 4.7. Similar results were also observed by Javaid et al., 2023, in which the ash content was 2.98% in mint and 2.8 % in coriander leaves (Javaid et al., 2023).

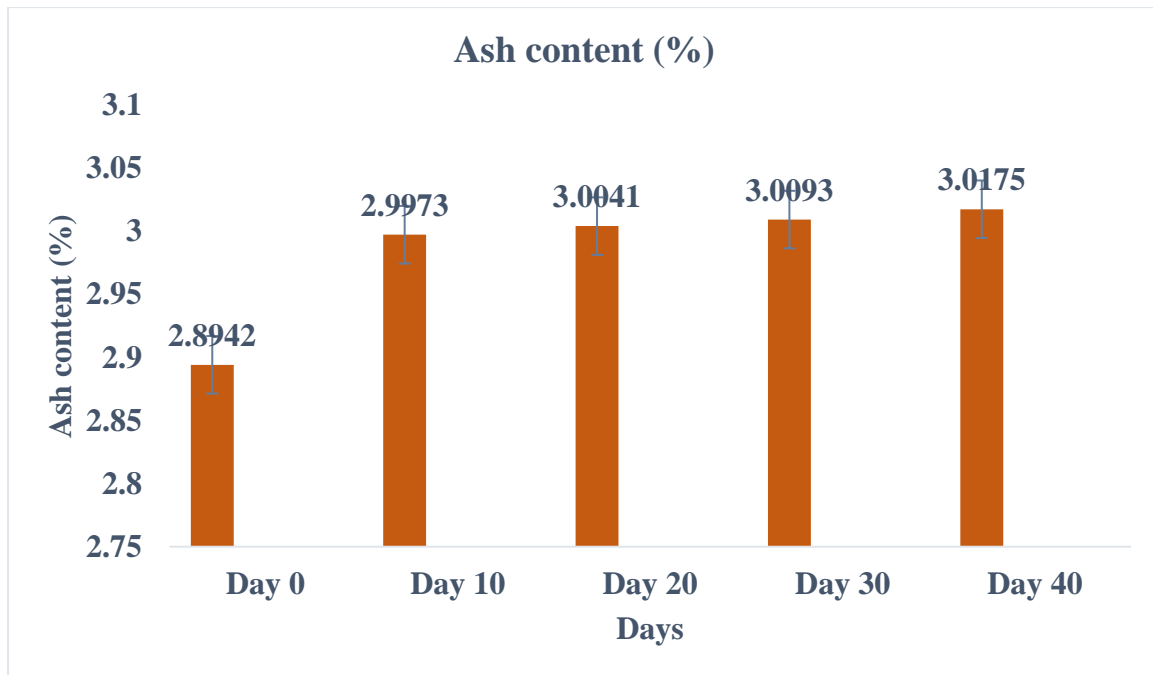


Figure 4.8 Ash content (%) of herbal detox drink powder



Figure 4.9 Ash content analysis of herbal detox drink powder

4.2.3 Antioxidant activity

The antioxidant activity of herbal detox drink powder was 40.76%, which was further decreased during the storage period and showed considerable changes as represented in Figure 4.8. Mahar et al., 2023 also observed similar results in lemongrass-based detoxifying drinks which were approximately 40.76% (Mahar et al., 2023).

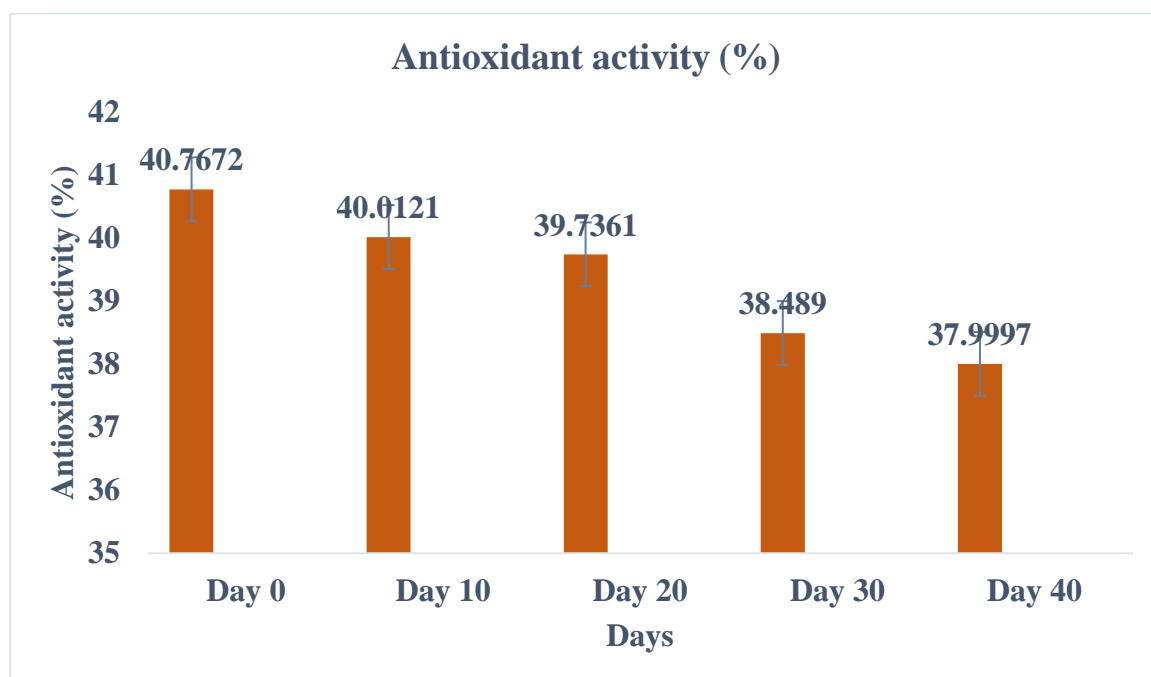


Figure 4.10 The Antioxidant Activity (%) of herbal detox drink powder

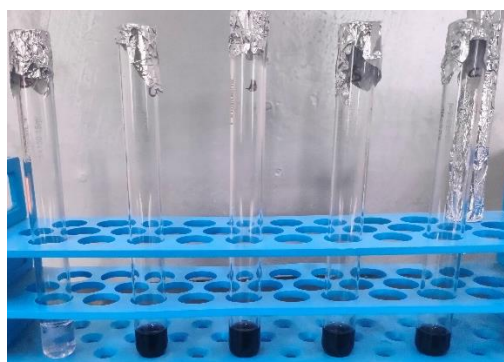


Figure 4.11 Antioxidant activity analysis of herbal detox drink powder

4.2.4 Total Phenolic Content (TPC)

The total phenolic content of herbal detox drink powder was 76.7 mg GAE/g which was further decreased during the storage period and showed minimum variation as represented in Figure 4.9. Mahar et al., 2023 also observed that the total phenolic content of lemongrass-based detoxifying drinks was approximately 72.08 mg GAE/100ml (Mahar et al., 2023).

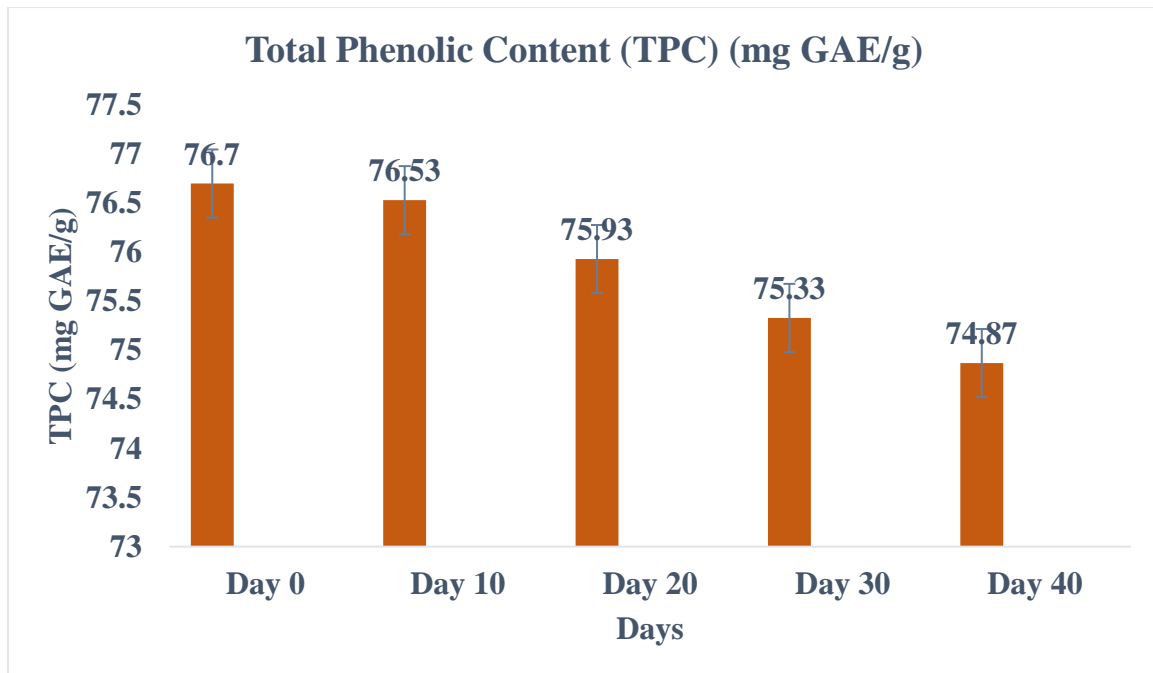


Figure 4.12 Total Phenolic Content (TPC) of herbal detox drink powder

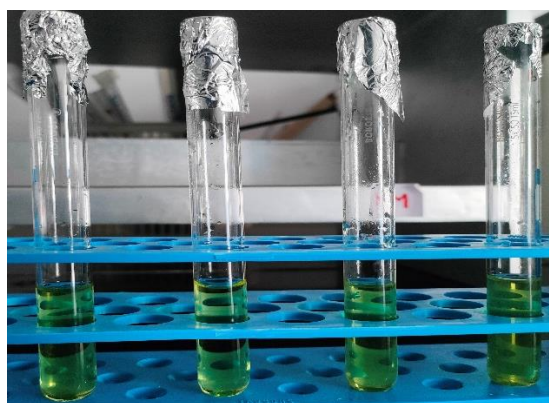


Figure 4.13 Total phenolic content analysis of herbal detox drink powder

4.2.5 Total Flavonoid Content (TFC)

The total flavonoid content of the herbal detox drink powder was 12.5 mg QE/g which was further decreased during the storage period and showed minimum variation as represented in Figure 4.9. Mahar et al., 2023 also observed that the total flavonoid content of lemongrass-based detoxifying drinks was approximately 10.03 mg QE/100ml (Mahar et al., 2023).

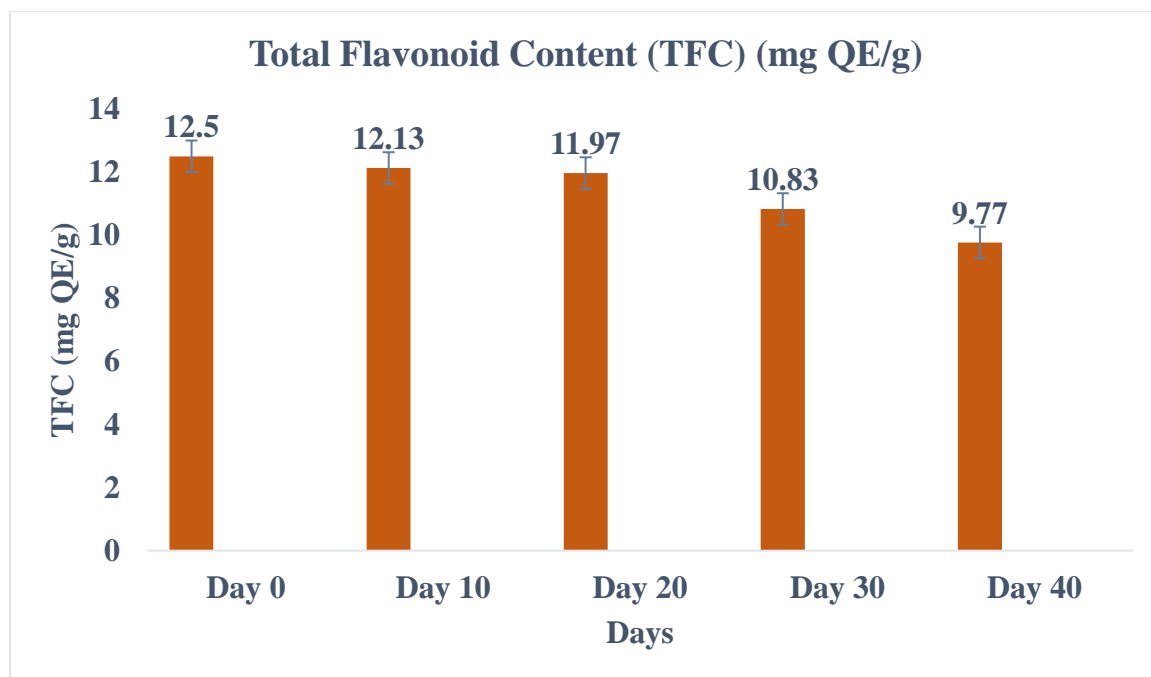


Figure 4.14 Total Flavonoid Content (TFC) of herbal detox drink powder

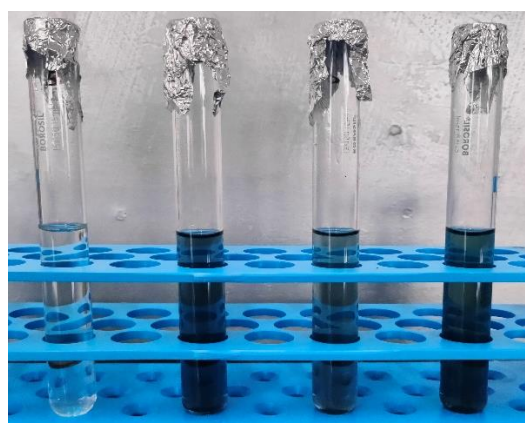


Figure 4.15 Total flavonoid content analysis of herbal detox drink powder

4.3 Antimicrobial effect

The antimicrobial activity of detox drink powder was examined based on the diameters of the clear zone of inhibition surrounding the Petri plates against *Escherichia coli*. Samples were taken at 2 mg/ml concentration. Plate count agar (PCA) media were used.

After a few days, it was observed that the inhibition zone diameter against *E. coli* was 11.30mm as shown in Figure 4.11



Figure 4.16 Antimicrobial activity of herbal detox drink powder

4.4 FTIR Spectroscopy of Herbal Detox Drink Powders

FTIR analysis was performed to detect the functional groups available on the surface of herbal detox drink powders. It has been observed from the analysis of the IR spectra that different functional groups are present in the herbal detox drink powder such as hydrogen-bonded alcohol, phenols, alkanes, alkenes, alcohol, ethers, carboxylic acid, and ester. The spectrum shows a major peak at 3400.37 cm^{-1} and the other peaks are at 2924.38 , 2854.22 , 1743.16 , 1625.27 , 1383.43 , and 1026.63 cm^{-1} . It was observed that at the bands, 3400.37 cm^{-1} corresponds to the hydrogen-bonded alcohol and phenols. The band at 2924.38 and 2854.22 cm^{-1} both corresponds to the C-H stretching of alkanes. The band at 1743.16 cm^{-1} corresponds to C=C stretching. The band at 1625.27 cm^{-1} corresponds to O-H stretching. The band at 1383.43 cm^{-1} corresponds to C-C (CH₃) stretching. The band at 1026.63 cm^{-1} corresponds to N hydrides ether and carboxylic group. The FT-IR spectrum of Herbal detox drink powder confirms the presence of functional groups for phenolics (OH) and flavonoids (CO), which are

widely reported for their antioxidant potential. Flavonoids and phenolic acids have antibacterial, antifungal, antiviral, hepatoprotective, immunomodulating, and anti-inflammatory properties.

Table 4.2 FTIR result of herbal detox drink powder

S. No.	Wave number (cm ⁻¹)	Functional groups
1.	3400.37	Hydrogen-bonded alcohol and phenol
2.	2924.38, 2854.22	C-H stretching of alkanes
4.	1743.16	C=C stretching
5.	1625.27	O-H stretching
6.	1383.43	C-C (CH ₃) stretching
7.	1026.63	N hydrides ether and carboxylic group

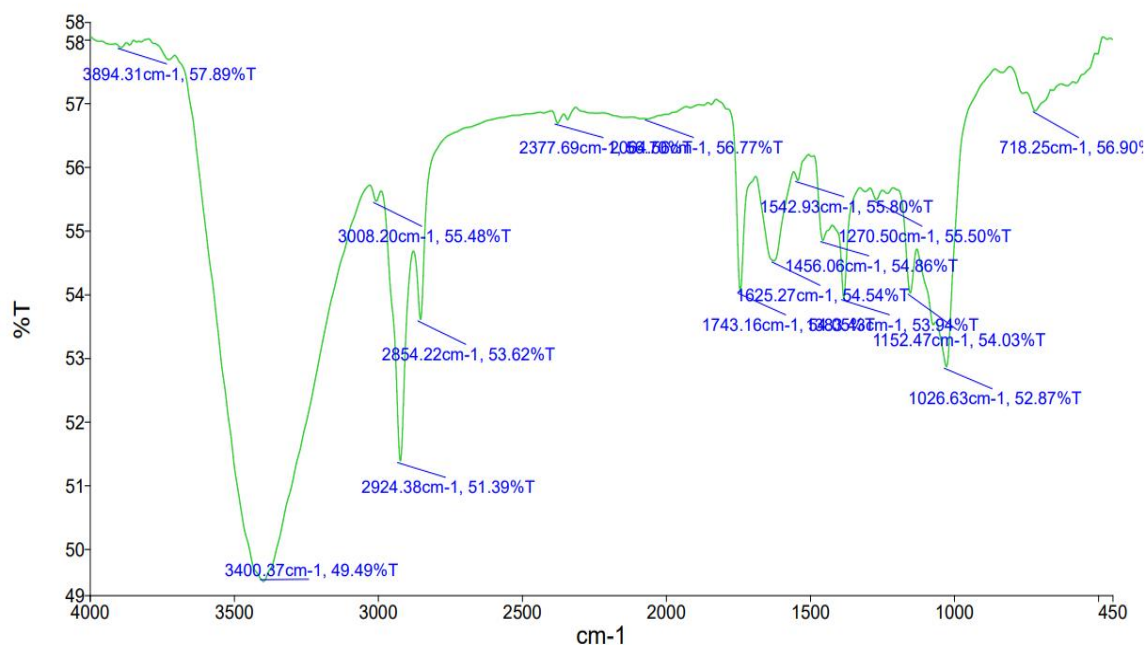


Figure 4.17 FTIR graph of herbal detox drink powder

SUMMARY AND CONCLUSION

Herbal detox drinks are marketed as natural remedies to cleanse the body, eliminate toxins, and promote overall health. The body has its own natural detoxification processes, primarily through the liver, kidneys, lungs, and skin. However, in today's world, with increased exposure to environmental toxins and unhealthy lifestyles, many people turn to various detox methods like liquid diet and juices. Lyophilization is a very promising technique to preserve these detoxifying drink forms without damaging their nutritional properties. The herbal detox drink powder prepared by lyophilization from natural herbs like mint and coriander leaves is a natural way to preserve these perishable vegetables.

The preliminary trials and sensory analysis of the different blended formulations reveal the selection of the best blend for the preparation of the herbal detox drink. The antioxidant activity analysis of these blends reveals that the developed herbal detox drink has antioxidant properties that play a crucial role in maintaining health by neutralizing free radicals, thereby reducing their harmful effects. The selected blend from different formulations was converted into powdered form by lyophilization to develop herbal detox drink powder. The prepared herbal detox drink powder also undergoes certain physicochemical analyses which reveal that it also contains antioxidant properties, phenolic content, and flavonoid content.

Ultimately, this research aims to provide a balanced and experimental-based assessment of ready-to-serve herbal detox drink powders, and their shelf-life estimation reveals that developed herbal detox drink powder is safe for consumption and can be used for more than one month. It also highlights that this developed herbal detox drink powder be considered a viable and safe option for those seeking to enhance their health and well-being.

Future prospects of herbal detox drink powder-

In the future, herbal detox drink powders may be formulated based on an individual's specific health needs, preferences, and dietary restrictions. Herbal detox products will also likely feature cleaner ingredient lists with minimal additives, preservatives, and artificial flavors or colors. There will likely be a greater emphasis on sustainable and ethical sourcing of herbal ingredients. Herbal detox products will also become integrated with traditional medicine systems, combining indigenous knowledge with modern science.

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