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Unveiling The Phytochemical Richness, Antioxidant Potential, And Shelf-Life Stability of Multi-Herbal Powder Incorporated Tea

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ABSTRACT

This study evaluated the phytochemical composition, antioxidant potential, and shelf-life stability of multi-herbal powder incorporated tea (MHT) formulations (MHT1 to MHT4) in comparison to a control. Quantitative phytochemical analysis revealed substantial enhancements in key bioactive compounds, with alkaloid content increasing from 12±0.01 mg/100g in the control to 25±0.1 mg/100g in MHT4, saponin levels rising from 8±0.02 mg/100g to 21±0.3 mg/100g, and tannin content from 10±0.03 mg/100g to 23±0.2 mg/100g. These increases reflect the diverse phytochemical profiles contributed by the herbal blends, known for their antioxidant, anti-inflammatory, and antimicrobial properties. Antioxidant assays further confirmed this enhancement, with total phenolic content rising from 56.63±1.56 mg/100g to 80.27±0.61 mg/100g and flavonoid content from 19.07±1.46 mg/100g to 35.17±0.91 mg/100g, indicating stronger radical scavenging capacity. MHT4 consistently demonstrated the highest antioxidant activity, as indicated by DPPH (IC_{50}: 79.24±0.21 µg/ml), ABTS (98.24±0.21 µg/ml), and FRAP (87.13±1.01 µg/ml) assays. Shelf-life studies revealed that MHT2 exhibited the lowest microbial counts at the 30th day (2.01 x 10^6 CFU/g TPC, 1.75 x 10^5 CFU/g fungal count), suggesting better long-term stability. These findings indicate that MHT formulations, particularly MHT4, offer substantial health benefits as functional beverages, with rich phytochemical profiles, strong antioxidant activity, and promising shelf stability. However, further optimization is recommended to enhance microbial resistance and extend commercial viability. Future research should focus on clinical validation and sensory evaluations to fully assess the therapeutic potential and consumer acceptability of these formulations

Keywords: *Herbal Tea, Phytochemical, Antioxidant, Shelf Life, Total Plate Count*

1. INTRODUCTION

Tea has a rich history, having been used for thousands of years in China. Non-camellia tea (herbal tea) can be traced back to the Tang Dynasty (Zhu, 2018). Herbal teas vary widely in their composition; they are made from natural products, mainly a variety of herbs, and have a variety of benefits for human health. Plant materials used in herbal teas include fresh or dried roots, stems, leaves, fruits, flowers, seeds, bark, or whole plants from one or more herbal tea plant species (Liu et al., 2013). Herbal tea is not tea in the traditional sense (Camellia), but is prepared by brewing or boiling the plant materials. However, some herbal teas, such as leaf tea, flower tea, and fruit tea, produced by green tea technology, can be prepared by directly soaking in cold water. In the past few years, the worldwide consumption of tea has increased, mostly due to its recently confirmed positive health benefits (Cooper et al., 2005). It has now become an indispensable aspect of modern life (Sun et al., 2019).

The chemical composition of tea is complex and includes polyphenols, alkaloids (caffeine, theophylline and theobromine), amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, minerals, trace elements and other unidentified compounds. Among these, polyphenols constitute the most interesting group and are the main bioactive molecules in tea

(Cabrera et al., 2003). The major polyphenolic compounds in tea are the flavan-3-ols called catechins which include: (-)-epicatechin (EC), (-)-Epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epigallocatechingallate (EGCG), (-)-Gallocatechins (GC) and (-)-gallocatechin gallate (GCG). Catechins are present in large amounts in green tea (Peterson et al., 2005). Based on their chemical structure, catechins that contain three hydroxyl groups in the B ring (positions 3', 4' and 5') are called gallocatechins while gallic acid substitution in position 3 of the ring is characteristic of catechin gallate (Pellilo et al., 2002). Catechins account for 6 - 16% of the dry green tea leaves with EGCG constituting 10 - 50% of catechins and being the most potent due to its degree of gallation and hydroxylation (Stewert et al., 2004). TFs and TRs are another group of polyphenolic compounds found in both black and oolong teas (Obanda et al., 2001).

The tea beverage has continued to be considered a medicine since the ancient times because of its polyphenols. Research on the effects of tea on human health has been fuelled by the growing need to provide naturally healthy diets that include plant-derived polyphenols. In line with this, there is need to elucidate how known functional components in foods could expand the role of diet in disease prevention and treatment (Mandel et al., 2006). There is already growing evidence that tea polyphenols reduce the risk of heart diseases and cancer in humans (Vanessa and Williamson, 2004). In some studies, tea has been associated with anti-allergic action (Yamamoto et al., 2004) and antimicrobial properties (Paola et al., 2005). Further studies have demonstrated that the co-administration of drugs with catechins (EC and EGCG) inhibits glucoronidation and sulfonation of orally administered drugs thereby increasing the bioavailability of such drugs (Hang et al., 2003). Moreover, some epidemiological studies have associated consumption of tea with a lower risk of several types of cancer including those of the stomach, oral cavity, oesophagus and lungs (Cabrera et al., 2003; Hakim and Chow, 2004). Therefore, tea appears to be an effective chemo-preventive agent for toxic chemicals and carcinogens.

The ability to scavenge for free radicals by tea polyphenols due to possession of a phenolic hydroxyl group attached to the flavan-3-ol structure has been associated with teas' therapeutic action against free radical mediated diseases thereby attracting tremendous research interest (Amie et al., 2003). Free radicals are known to contribute to numerous disorders in humans including cancer, artheroscerolosis, arthritis, ischemia, Central Nervous System (CNS) injury, gastritis, dementia, renal disorders and Acquired Immune Deficiency Syndrome (AIDS) (Pourmorad et al., 2006; Rao et al., 2006). Free radicals are constantly generated due to environmental pollutants, radiation, chemicals, toxins, physical stress and the oxidation process of drugs and food. Many plant phenollics have been reputed to have antioxidant properties that are even much stronger than vitamins E and C. In addition, currently available synthetic antioxidant like butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT) and gallic acid esters have been suspected to cause or prompt negative health effects and hence the need to substitute them with naturally occurring antioxidants (Amie et al., 2003; Aqil et al., 2006). In light of these literatures, the objective of this study was to evaluate the phytochemical composition, antioxidant potential, and shelf-life stability of multi-herbal powder incorporated tea (MHT) formulations. Specifically, the study aimed to analyze the bioactive compounds in MHT formulations, assess their antioxidant activity through various assays, and determine their microbial stability over a 30-day period to identify the formulation with the best shelf-life.

2. MATERIALS AND METHODS

Extraction

The herbal powder was subjected to sequential extraction using a soxhlet apparatus. Initially, defatting was conducted using petroleum ether (60–80°C), succeeded by extraction with 70% ethanol. Whatman No. 1 filter paper was employed for filtration of the extracts. This filtrate was concentrated to a minimal volume using a rotary evaporator at 40°C under reduced pressure (70-100 mBar). Subsequent to each extraction phase, the marc was air-dried at room temperature before to advancing to the next solvent. The yield of hydroalcoholic extract was calculated. The hydroalcoholic extract of herbal powder was further analyzed for phytochemical constituents and evaluated for antioxidant and to optimize its production (Ansari *et al.*, 2021)

Phytochemical Screening Secondary Metabolites

Quantitative and qualitative of phytochemical screening for alkaloids, flavonoids, glycosides, phenols, cardiac glycosides, saponins, sterols, tannins and anthraquinones were determined using various established methods.

Qualitative Determination of Chemical Constituents

"Test for alkaloids:

A few drops of dilute iodine solution were added into 3 ml test solution added. Blue colour appeared; and disappeared on boiling and reappeared on cooling" (Khandelwal 2008).

"Test for flavonoids:

2-3 ml. of extract and few drops of sodium hydroxide solution were added into a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicates the presence of flavonoids" (Szultka *et al.* 2013).

"Test for phenols:

0.5 ml of FeCl3 (w/v) solution was added into 2 ml of test solution, formation of an intense colour indicates the presence of phenols" (Baciocchi *et al*. 2001).

"Test for saponin:

0.5g extract was diluted with 20 ml of distilled water and was shaken in a graduated cylinder for 15 minutes. A 1 cm. layer of foam, indicates the presence of saponins" (Aziz *et al*. 2019).

"Test for tannins:

Few drops of 10% lead acetate solution were added into 5 ml of extract. Formation of yellow or red precipitate indicates the presence of tannins" (Atanassova and Christova-Bagdassarian, 2009).

Quantitative Determination Chemical Constituents

Determination of alkaloid content

"Tea sample (5 g) was weighed into 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hrs. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was removed and washed with 1% ammonium hydroxide and then filtered. The residue is the alkaloid and this was oven dried for 30 mins at 60oC and reweighed" (Adeniyi et al. 2009). The alkaloid content of the samples was determined by difference using the equation:

Percentage alkaloid = W2 – W1 X 100 W

Where, W = weight of sample W1 = weight of empty filter paper W2 weight of paper + precipitate

Determination of saponin content

"Tea sample (20 g) each were put into conical flask and 100 mL of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55oC. The mixture was filtered and the re extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90oC. The concentrate was transferred into a 250 mL separatory funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 mL n butanol was then added. The combined n butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation, the samples were dried in the oven to a constant weight" (Aziz *et al.* 2019). The saponins content was calculated thus: % Saponin = Weight of Saponin/ Weight of Sample X 100.

Determination of tannin content

Tea sample (500 mg) was weighed into a 50 mL plastic bottle. 50 mL of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipetted out into a test tube and mixed with 2 mL of 0.1 M FeCl3 in 0.1 N HCl and 0.008 M Potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min. (Antonova *et al*. 2015).

Antioxidant assay of multi herbal tea

Determination of total phenolics content (TPC)

Total phenolics was determined using Folin Ciocalteu's reagent as adapted from Velioglu et al. (1998). One hundred microliters of extract were mixed with 0.75 mL of Folin-Ciocalteu's reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 oC for 5 min; 0.75 mL of sodium bicarbonate (60 g/L) solution was added to the mixture. After 90 min at 22 oC, absorbance was measured at 725 nm. Result was expressed as gallic acid equivalent (mg GAE/g).

Determination of total flavonoid content (TFC)

Total flavonoid was measured according to Zhishen et al. (1999). One ml aliquot of extract and appropriately diluted standard solution of quercetin (20, 40, 60, 80 and 100 mg/l) was added into a 10 ml volumetric flask containing 4 ml deionized water. At zero-time, 0.3 ml of 10% $AlCl_3$ was added. At 6 minutes, 2 ml of 1M NaOH was added to the mixture. Immediately, the reaction flask was diluted to the volume with the addition of 2.4 ml of deionized water and thoroughly mixed. Absorbance of the mixture, pink in colour was determined at 510 nm versus prepared water blank. Total flavonoid of the samples was expressed on a dried weight as quercetin equivalent (mg QE/g).

DPPH (2, 2 -diphenyl-1-pycryl-hydrazyl) free radical scavenging activity

According to Mensor et al. (2001), 1 mL from 0.3 mM methanol solution of 2, 2 -diphenyl-1 pycrylhydrazyl (DPPH) was added into 2.5 mL sample or standards. The solution was mixed vigorously and left to stand at room temperature for 30 min in the dark. The mixture was measured spectrophotometrically at 518 nm. The percentage inhibition was calculated against

a control and compared to BHT standard curve (0-1000µm). The antioxidant activity (AA) was calculated as below:

AA% = 100 – [(Abs sample - Abs empty sample)/Abs control x 100 where Abs is absorbance Empty sample= 1 mL methanol + 2.5 mL extract Control sample= 1 mL 0.3 mM DPPH + 2.5 mL methanol IC_{50}, the amount of sample extracted into 1 mL solution necessary to decrease by 50% the initial DPPH concentration, was derived from the % disappearance versus concentration plot (at this point concentration means mg of sample extracted into 1.0 mL solution).

FRAP (Ferric reducing/antioxidant power) assay

This procedure was carried out according to Benzie and Strain (1996) with slight modification. The working FRAP reagent was produced by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2, 4, 6 tripyridyl-s-triazine (TPTZ) solution and 20 mM FeCl3 .6H2 O in a 10:1:1 ratio prior to use and heated to 37°C in water bath. A total of 3.0 mL FRAP reagent was added to a cuvette and blank reading was then taken at 593 nm using spectrophotometer. A total of 100 µL selected plant extracts and 300 µL distilled water was added to the cuvette, and a second reading at 593 nm was performed after 4 min. The changes in absorbance after 4 min from initial blank reading were then compared with standard curve. A standard of known Fe (II) concentrations was carried out using several concentrations from 100 to 1000 µM. A standard curve was plotted by plotting the FRAP value of each standard versus its concentration. The FRAP values for the samples were determined using this standard curve. The final result was expressed as the concentration of antioxidant having a ferric reducing ability.

$ABTS^{++}$ decolourization assay

2,2'-azinobis(3-ethylbenzthiazoline)-6 sulphonic acid or ABTS free radical decolourization assay was done according to Re *et al*. (1999) with some modification. Briefly, the pre-formed radical monocation of ABTS was generated by reacting ABTS solution (7mM) with 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was allowed to stand for 15 h in the dark at room temperature. The solution was diluted with ethanol to obtain the absorbance of 0.7 ± 0.2 units at 734 nm. The aliquot of 200 µl of each sample was added to 2000 µl of ABTS free radical cation solution. The absorbance, monitored for 5 min was measured spectrophotometrically at 734 nm using a spectrophotometer. Appropriate solvent blanks were run in each assay. The percentage inhibition was calculated against a control and compared to a Trolox standard curve (10-100 mM). The radical-scavenging activity was expressed in IC_{50}, the amount of sample extracted into 1 mL solution necessary to decrease by 50% the initial ABTS concentration.

Shelf-life evaluation

The storage stability of herbal powder incorporated tea was carried out for a period of 30 days at ambient conditions. All the samples were drawn periodically after 0, 15, and 30 days and analyzed for its TPC and fungal count. The total plate count was done by using the method of Aneja, (2003).

Statistical analysis

All experiments were carried out in triplicate and presented as mean ± standard deviation of mean (SD) using SPSS version 15.0. The data were statistically analysed by one-way ANOVA and Duncan's test. Correlations among data obtained were analysed using Pearson's coefficient. A significance difference was considered at the level of $p < 0.05$.

3. RESULTS AND DISCUSSION

Phytochemical constitutes of multi herbal powder incorporated tea

Table 1 Phytochemical constitutes of multi herbal powder incorporated tea

Phytochemical Constituent	Control	MHT1	MHT2	MHT3	MHT4
Qualitative Analysis					
Alkaloids	+	+++	++	+++	++
Flavonoids	+	+++	++	++	++
Phenol	+	+++	+++	++	++
Saponin	-	+++	++	+++	++
Tannin	+	+++	++	+++	++
Quantitative Analysis (mg/100g)					
Alkaloids	12±0.01	15±0.01	18±0.1	22±0.1	25±0.1

Saponin	8±0.02	12±0.02	15±0.2	18±0.2	21±0.3
Tannin	10±0.03	13±0.01	16±0.3	20±0.1	23±0.2

Phytochemicals are bioactive compounds naturally present in plants, known for their significant health benefits, including antioxidant, anti-inflammatory, and antimicrobial properties. The qualitative and quantitative analysis of these compounds in multi-herbal powder incorporated tea provides valuable insights into their potential therapeutic effects. Qualitative Analysis of Phytochemicals resulted that the control sample showed a moderate presence (+) of alkaloids. MHT1 and MHT3 exhibited high levels (+++), indicating a rich presence of alkaloids, while MHT2 and MHT4 had moderate levels (++). Alkaloids are known for their diverse pharmacological activities, including anti-inflammatory, analgesic, and antimicrobial effects, which can enhance the overall health benefits of the tea. Flavonoids were present in all samples, with the control having a moderate amount (+). MHT1 contained the highest concentration (+++), while the other formulations (MHT2, MHT3, and MHT4) had moderate levels (++). Flavonoids are powerful antioxidants that can scavenge free radicals, reduce oxidative stress, and provide cardiovascular protection. Phenolic compounds were present in moderate amounts (+) in the control, increasing to the highest levels (+++) in MHT1, MHT2, and MHT4. MHT3 exhibited a slightly lower concentration (++), suggesting variation in the phenolic content depending on the herbal mix. Phenols are major contributors to antioxidant activity, supporting cellular defense against oxidative damage. Saponins were absent (-) in the control but highly present (+++) in MHT1 and MHT3, with moderate levels (++) in MHT2 and MHT4. These compounds are known for their cholesterol-lowering, anti-inflammatory, and immune-boosting properties. Tannins were present at moderate levels (+) in the control, increasing to high levels (+++) in MHT1 and MHT3, while MHT2 and MHT4 had moderate levels (++). Tannins possess strong astringent, antimicrobial, and antioxidant properties, contributing to the overall health benefits of the tea.

Quantitative Analysis of Phytochemicals (mg/100g) stated that the alkaloid content ranged from 12±0.01 mg/100g in the control to 25±0.1 mg/100g in MHT4, reflecting a significant increase. MHT3 (22±0.1 mg/100g) and MHT2 (18±0.1 mg/100g) also showed notable alkaloid enrichment, indicating a substantial contribution from these herbal mixtures. The saponin content increased from 8±0.02 mg/100g in the control to 21±0.3 mg/100g in MHT4, showing a significant boost. This increase aligns with the qualitative findings, supporting the presence of these bioactive compounds in more concentrated herbal formulations. Tannin content ranged from 10±0.03 mg/100g in the control to 23±0.2 mg/100g in MHT4, indicating a substantial enrichment. This consistent increase in tannin levels supports the observed qualitative data, highlighting the impact of herbal incorporation on tannin concentration. The data indicate that multi-herbal powder incorporation significantly enhances the phytochemical profile of the tea, with notable increases in alkaloids, flavonoids, phenols, saponins, and tannins. MHT1 showed the highest qualitative presence for most phytochemicals, indicating a potentially more potent therapeutic profile.MHT4 consistently had the highest quantitative levels, suggesting a rich concentration of bioactive compounds. The varying levels of saponin and tannin in different formulations reflect the unique phytochemical profiles contributed by the specific herbal combinations.

Antioxidant activities of multi herbal powder incorporated tea

Table 2 Antioxidant activities of multi herbal powder incorporated tea

Antioxidant activities	**Control**	**MHT1**	**MHT2**	**MHT3**	**MHT4**
Total phenolic content (mg/100g)	56.63±1.56c	63.87±1.36c	71.13±1.02b	72.70±0.46b	80.27±0.61a
Total flavonoids content (mg/100g)	19.07±1.46c	20.90±0.36c	31.83±0.80b	32.33±0.50ab	35.17±0.91a
DPPH (IC_{50}) (µg/ml)	$64.26±0.01^{a}$	$67.89±0.01^{b}$	$71.29±0.01^{ac}$	$75.89±0.01^{b}$	$79.24±0.21^{ac}$
ABTS (µg/ml)	$72.46±1.01^{a}$	$78.01±1.03^{ac}$	$84.72±1.01^{ab}$	$92.21±0.99^{b}$	$98.24±0.21^{ac}$
FRAP (µg/ml)	$51.11±1.06^{ac}$	$63.42±1.06^{b}$	$67.19±1.01^{a}$	$74.12±096^{b}$	$87.13±1.01^{ab}$

$^{a-c}$ Means in a column with common superscript are not significantly different at the 0.05 probability level by Duncan's multiple range test. MHT – multi herbal powder incorporated tea

The total phenolic content (TPC) and total flavonoid content (TFC) are critical indicators of the antioxidant potential of multi-herbal tea formulations. Phenolic compounds, including flavonoids, are known for their ability to donate hydrogen atoms or electrons, stabilizing free radicals and preventing oxidative damage to cells and tissues. In the data provided, the TPC increased significantly from 56.63 ± 1.56 mg/100g in the control to 80.27 ± 0.61 mg/100g in the MHT4 formulation, reflecting a nearly 42% improvement. Similarly, the TFC increased from 19.07 ± 1.46 mg/100g in the control to

35.17 ± 0.91 mg/100g in MHT4, indicating a substantial enrichment of antioxidant components. These increases are statistically significant ($p < 0.05$), suggesting that the multi-herbal formulations contribute a diverse range of phenolic and flavonoid compounds, which collectively enhance the antioxidant potential of the tea. This is consistent with previous studies demonstrating that herbal blends often contain complementary bioactive compounds, leading to synergistic effects that boost overall antioxidant activity (Ghasemi et al., 2014; Hamad & Hartanti, 2025). The high phenolic content in these formulations likely contributes to their health-promoting properties, including reduced oxidative stress and inflammation.

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay is a widely used method for evaluating the free radical scavenging capacity of antioxidants. It measures the ability of antioxidants to reduce the stable purple DPPH radical to a yellow-colored diphenylpicrylhydrazine. Lower IC_{50} values in this assay indicate stronger antioxidant activity, as less sample is required to inhibit 50% of the DPPH radicals. In the presented data, the IC_{50} values increased from 64.26 ± 0.01 µg/ml in the control to 79.24 ± 0.21 µg/ml in MHT4, suggesting a significant improvement in free radical scavenging ability with increasing herbal concentration. This trend indicates that the antioxidant potential of the multi-herbal formulations is closely related to their phenolic and flavonoid content, as supported by prior studies that have reported a strong correlation between total phenolics and DPPH radical scavenging capacity (Sen *et al*., 2013).

The ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assay measures the ability of antioxidants to quench the ABTS radical cation, resulting in a color change that can be quantitatively measured. This assay is particularly useful for evaluating the antioxidant capacity of both hydrophilic and lipophilic substances, making it a comprehensive indicator of antioxidant potential. The ABTS values in the data set increased from 72.46 ± 1.01 µg/ml in the control to 98.24 ± 0.21 µg/ml in MHT4, reflecting a significant enhancement in radical scavenging capacity as the concentration of herbal powders increased. This pattern indicates a higher overall antioxidant potential in the more concentrated formulations, aligning with the observed increases in phenolic and flavonoid content. Studies have shown that polyphenolic compounds, such as those present in herbal teas, are highly effective in neutralizing ABTS radicals, contributing to their protective effects against oxidative stress (Minarti *et al*., 2022).

The FRAP assay assesses the ability of antioxidants to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions, generating a blue-colored complex that can be measured spectrophotometrically. This method directly reflects the reducing power of antioxidants, which is a critical mechanism by which they protect cells from oxidative damage. In the data, the FRAP values increased from 51.11 ± 1.06 µg/ml in the control to 87.13 ± 1.01 µg/ml in MHT4, indicating a substantial increase in reducing power. This improvement is consistent with the higher phenolic and flavonoid contents observed in the MHT formulations, supporting the idea that these compounds play a central role in the antioxidant mechanisms of herbal teas. The results align with previous findings that suggest a direct relationship between phenolic content and reducing power, emphasizing the importance of these compounds in enhancing the antioxidant profile of multi-herbal formulations (Oubihi *et al.*, 2020).

Antioxidant activity is vital for human health mainly because of its free-radical scavenging activity and protection against oxidative stress, which can cause diseases such as heart disease and cancer. Studies have shown that polyphenol and flavonoid compounds are effective in preventing these diseases. Salgado *et al*. (2012) reported a similar observation where the addition of antioxidant-rich pomegranate peel (Punica granatum) extract to orange and tomato juice led to an increase in antioxidant activity.

Shelf-life evaluation of multi herbal powder incorporated tea

Table 3 Shelf-life evaluation of multi herbal powder incorporated tea

Tea variations	Total Plate Count (cfu/g)			Fungal count (cfu/g)		
	Initial	15th day	30th day	Initial	15th day	30th day
Control	Nil	1.52×10^6	2.14×10^6	Nil	1.45×10^6	1.82×10^6
MHT1	Nil	1.08×10^6	2.01×10^6	Nil	1.69×10^6	1.75×10^5
MHT2	Nil	0.98×10^5	2.04×10^7	Nil	1.15×10^7	1.80×10^6
MHT3	Nil	1.10×10^7	2.16×10^7	Nil	1.35×10^7	1.65×10^6
MHT4	Nil	1.23×10^6	2.15×10^7	Nil	1.44×10^6	1.63×10^7

Nil – No multiplication; MHT – Multi Herbal powder incorporated tea

The microbial shelf-life evaluation of multi-herbal powder incorporated tea formulations (MHT1 to MHT4) and the control sample over a 30-day period reveals critical insights into their microbiological stability. The analysis included Total Plate Count (TPC) and fungal count measurements, both essential for assessing the safety and quality of these food products. On

15th Day Counts, the TPC for the control sample reached 1.52 x 10^6 cfu/g, reflecting significant microbial proliferation. MHT1 had a notably lower count (0.98 x 10^5 cfu/g), suggesting better initial microbial inhibition, possibly due to the presence of bioactive compounds with antimicrobial properties. MHT2 (1.08 x 10^6 cfu/g), MHT3 (1.10 x 10^7 cfu/g), and MHT4 (1.23 x 10^6 cfu/g) exhibited higher counts, indicating varying degrees of microbial resistance. On 30th Day Counts, all samples showed significant microbial growth, with the control reaching 2.14 x 10^6 cfu/g. MHT1 exhibited the highest count (2.04 x 10^7 cfu/g), suggesting that despite its lower initial count, it supported substantial microbial growth over time. MHT3 also recorded a very high count (2.16 x 10^7 cfu/g), indicating potential issues with long-term microbial control. MHT2 (2.01 x 10^6 cfu/g) and MHT4 (2.15 x 10^7 cfu/g) displayed slightly better but still concerning levels. The variation in TPC across the samples suggests that while some multi-herbal formulations may offer initial antimicrobial benefits, their long-term stability may be compromised. This could be due to factors like moisture content, pH, and nutrient availability in the herbal mixes, which can promote bacterial growth over time.

Fungal Count resulted that all samples were free from detectable fungal contamination (Nil) at the initial stage, reflecting good initial quality. On 15th Day Counts, the control sample exhibited 1.45 x 10^6 cfu/g, indicating early fungal contamination. MHT1 (1.15 x 10^7 cfu/g) and MHT3 (1.35 x 10^7 cfu/g) showed much higher counts, suggesting that these formulations might be more prone to fungal spoilage, potentially due to higher moisture content or nutrient availability. MHT2 (1.69 x 10^6 cfu/g) and MHT4 (1.44 x 10^6 cfu/g) had relatively lower counts, indicating better fungal resistance. On 30th Day Counts, the control reached 1.82 x 10^6 cfu/g, reflecting steady fungal growth. MHT1 decreased to 1.80 x 10^6 cfu/g, indicating some natural microbial inhibition over time, possibly due to drying or other inhibitory effects. MHT2 dropped significantly to 1.75 x 10^5 cfu/g, suggesting better long-term fungal resistance, potentially due to specific bioactive components. MHT3 (1.65 x 10^6 cfu/g) and MHT4 (1.63 x 10^7 cfu/g) continued to show high fungal counts, highlighting their vulnerability to fungal spoilage. The varying fungal counts suggest that certain multi-herbal powder formulations are more susceptible to mold and yeast contamination. The significant reduction in MHT2's fungal count by the 30th day indicates it may contain compounds with more robust antifungal properties, potentially providing a longer shelf life compared to other formulations. During storage, dried foods like tea powder experience deterioration in their physicochemical properties including moisture absorption, color changes and browning, along with a decline in sensory properties such as the development of off-flavors (Jena & Das, 2012). Temperature is a critical factor influencing changes in food quality during storage. Higher storage temperatures generally accelerate quality deterioration (Man & Jones, 2000). Knowledge of the influence of storage temperatures helps to estimate suitable storage conditions for preserving the qualities of the products (Kim *et al*., 2022; Shukla *et al*., 2020; Zhang *et al*., 2021).

4. CONCLUSION

There are a wide variety of herbal teas based on folk medicinal plants with long histories. There are local herbal tea recipes worldwide that have reliable traditional uses. The multi-herbal powder incorporated tea (MHT) formulations demonstrated substantial enhancements in phytochemical content, antioxidant activity, and shelf-life stability compared to the control. Quantitative analysis revealed significant increases in key bioactive compounds, with alkaloid levels rising from 12±0.01 mg/100g in the control to 25±0.1 mg/100g in MHT4, saponin content from 8±0.02 mg/100g to 21±0.3 mg/100g, and tannin levels from 10±0.03 mg/100g to 23±0.2 mg/100g. Antioxidant assays further confirmed this trend, with total phenolic content increasing from 56.63±1.56 mg/100g to 80.27±0.61 mg/100g and flavonoid content from 19.07±1.46 mg/100g to 35.17±0.91 mg/100g. MHT4 consistently showed the highest antioxidant capacity, as indicated by DPPH (IC_{50}: 79.24±0.21 µg/ml), ABTS (98.24±0.21 µg/ml), and FRAP (87.13±1.01 µg/ml) assays. Shelf-life studies revealed that MHT1 exhibited the lowest microbial counts at the 30th day (2.01 x 10^6 cfu/g TPC, 1.75 x 10^5 cfu/g fungal count), suggesting better long-term stability. These findings indicate that MHT formulations, particularly MHT1, have strong potential as functional beverages, offering rich phytochemical profiles and potent antioxidant properties, though further optimization is needed to enhance microbial stability.

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Conflict of interest

There were no documented conflicts of interest by the authors of the paper.

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Abbreviations:

MHT -Multi Herbal Powder incorporated tea; EC -epicatechin, EGC -Epigallocatechin; ECG -epicatechin gallate (ECG), EGCG- epigallocatechingallate, GC -Gallocatechins and GCG- gallocatechin gallate (GCG)

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