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Development and evaluation of phytochemical components in spiced herbal tea

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Abstract
Traditional medicines and formulations of edible herbs are made of green and dried spices, flowers, vegetables, leaves, nuts, barks and stems, marketed in loose form or in bags. Matured curry leaves were combined with supporting herbs such as moringa, amla and dry ginger, along with active herbs such as celery and sweet basil. Spiced herbal tea blends were processed to perform shelf life experiments at ambient conditions. The volatile compounds found in the spiced herbal tea blends were evaluated by GC-MS. During storage, moisture and pH were found to be lower in the metallized polypropylene pouch (P_3), ash values increased significantly during storage period of 180 days whereas protein, ascorbic acid, carbohydrates, iron, crude fibre, fat and folic acid values decreased considerably during storage period of 180 days. Antimicrobial effects were active against bacteria, fungi and yeast, suggesting that the regular consumption of spiced herbal tea can ensure human health. Based on the results obtained, curry leaves are suggested for herbal tea formulation as they have a strong antioxidant activity and fast absorption of iron, which is beneficial to human health.

Keywords: curry leaves, herbal tea, GC-MS, storage study, bioactive compounds

Introduction

Herbs and spices have been used as food and medicine for centuries. Herbal bioactive is a critical group of nutraceuticals that provide a mass of health-promoting therapeutic residences, in addition to proteins, minerals and assorted live additives (Craig, 1999) [5]. The word nutraceuticals was invented in 1979 with the support of Stephen De Felice, founder and chairman of the inspiration for innovation in medicine headquartered in Cranford, New Jersey. It was described as "a food or part of a food" that provides scientific or health benefits, such as disease prevention and treatment (Dureja *et al.*, 2003) [8]. Nutraceuticals, also referred to as phytonutrients, are natural bioactive substances which have health promotion, disease prevention or medicinal properties (Mohd and Kaur, 2020) [22].

Beverages have a vital role to play in our everyday lives. Beverages can be described as any fluid that is consumed by drinking. Wide spectrums of bioactive compounds are used for the processing of beverages. Many have benefited from drinks made from leaves, roots, sap, berries, tubers, and seeds (Samuelsson and Bohlin, 2017) [30]. Herbal tea, in compliance with many, seems like tea and is brewed because it's the same way as tea, but in real sense it's not believed to be tea at all. This is due to the fact that they no longer come from the *Camellia sinensis* bush, the plant from which all teas are produced.

Herbal teas are genuine mixtures of several ingredients and are specifically called 'tisanes.' The herbal teas are made from mixtures of dried leaves, seeds, grasses, nuts, barks, fruit, flowers or other botanical elements that give them their flavour and the benefits of herbal teas. Tisanes are often fed for their physical or medicinal effects, especially for their stimulating, relaxing or sedative residences. They too taste terrific, and they are easy to consume. It is important to note that various herbs might have different medicinal properties, so that we can produce our own aromatic infusions according to how we want to gain us from the cup of tea. (Killedar *et al.*, 2017) [18].

Curry leaves tea makes it easier to control harmful cholesterol. Drinking this tea is a way to alleviate tension. Drinking curry leaves tea may help to provide a treatment for vomiting, nausea and morning sickness. Drinking curry leaves tea can help to regulate the blood sugar levels in the body. Curry leaves have digestive enzymes that improve digestive health; (Perera and Dahanayake, 2015) [23]. Amla tea is commonly preferred for fats burn and weight reduction (Bhandari *et al.*, 2012) [3]. *Moringa oleifera*, abundant in phenolic compounds, with its health advantages, may be used for the practise of phytochemical-rich realistic antioxidant tea (Zhang *et al.*, 2015 and Serafini *et al.*, 2006) [38, 31]. Ginger tea prevents inflammation, relieves muscle discomfort, a strong anti-inflammatory effect, decreases blood sugar, reduces the risk of coronary heart disease, stops and beats cancer, removes menstrual cramps, improves metabolism and respiration (Shahrajabian *et al.*, 2019) [32]. Juice derived from celery petioles can be used for neuropathy, rheumatic tendency, gout, flatulence, continuous pulmonary catarrh, relatively closer to obese, and loss of appetite (Tyagi *et al.*, 2013) [36].

Herbal teas have therefore been formulated using curry leaves to enhance the flavour, aroma and medicinal properties that may overtake commercial green tea. Herbs specifically *Murraya koenigii* leaves, *Moringa oleifera* leaves, *Phyllanthus emblica* berries, *Zingiber officinale* rhizome, *Apium graveolens* leaves, *Ocimum basilicum*

leaves have been used to produce a balanced ideal and safe tea for all age categories.

Materials and Methods

Collection of spices and herbs

The fresh curry leaves (*Murraya koenigii*) were collected from the organic curry leaf field, Karamadai, Coimbatore. Organic curry leaves were obtained in the period of November 2019. Environmental conditions had average maximum/minimum temperature of 28/22°C and precipitation of 9 mm. Moringa (*Moringa oleifera*) leaves were collected from Orchard, Tamil Nadu Agricultural University, Coimbatore. Fresh (*Pyllanthus emblica*) Amla berries, Celery (*Apium graveolens*), Sweet basil (*Ocimum basilicum*), Ginger (*Zingiber officinale*) void of spoilage was collected from Kovai Pazhamudhir Nilayam, RS Puram West, Coimbatore.

Standardization of spiced herbal tea blends

The key herb used to prepare herbal tea blends was matured curry leaves in the following study. Supporting herbs (Moringa leaves/ Amla berries) and stimulating herbs (Ginger/ Celery leaves/ Sweet basil leaves) were mixed with the predominant herb. To achieve optimum preservation of bioactive compounds, the chosen spices and herbs were subjected to drying treatment. They closely investigated fresh curry leaves and removed all foreign materials and then softly rinsed them in tap water. Cleaned curry leaves, moringa leaves, amla berries, ginger slices, celery leaves, sweet basil leaves were thinly scattered on aluminium trays of cabinet drier and the curry leaves, moringa, celery and sweet basil were dried for 3 hours at a temperature of 60 °C, amla and ginger for 4 hours at a temperature of 60 °C.

Formulation of blended herbal tea powder

By preparing teas with various combinations, the curry leaf infused herbal tea was formulated. The most suitable sensory score was the mixture of 50% curry leaves, 20% dry amla berries, 10% moringa and dry ginger leaves, and 5% celery and sweet basil. A bag of dip tea was filled with the accepted spiced herbal tea powder.

Standardization of herbal tea infusions

In a non-drip tea bag, the prepared herbal tea blend (2g) was wrapped. The herbal infusions were made by dipping the tea bag for 1, 2 and 3 minutes in 150 ml of hot water (Horzic *et al.*, 2009) [13]. It was standardised and the organoleptically appropriate infusion time is 3 minutes (Fig.1).

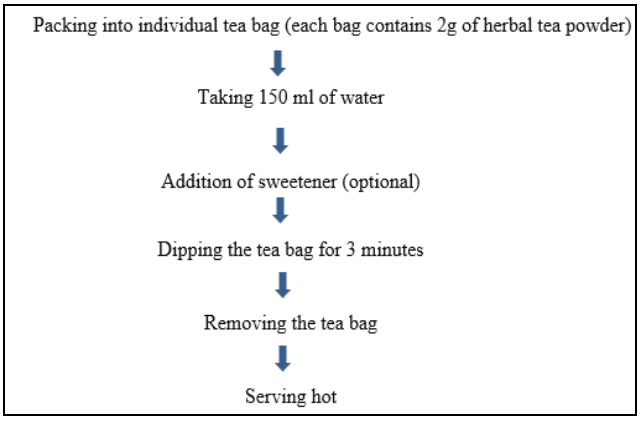

Fig 1: Standardized herbal tea blend

Chemical characteristics of developed spiced herbal blend and infusions

The chemical characteristics of developed herbal blend such as moisture (%), pH, ash values (%), crude fibre (g/100g), fat (g/100g), protein (g/100g), carbohydrates (mg/100g), iron (mg/100g), vitamin – C (mg/100g) and folic acid (µg/100g) were analyzed.

Microbial studies

The microbial load of the stored sample has been enumerate d by the concept described by (Farkas, 1984) [9]. The media used for bacteria- nutrient agar (Allen, 1953) [1], for fungi- Martin's rose Bengal agar (Martin, 1950) [20] and for yeast- yeast extract malt agar medium (Wickerham, 1951) [37]. There was a serial dilution of the samples. A dilution of 10^{-3}, 10^{-5} and 10^{-6} was used for all the investigation. One ml of the serial dilutions of the samples was taken from the sterile petridishes and appropriate media was inserted for the specific organism. The plates were incubated for about 48 hours at room temperature for bacteria, 3 days for leaves and 5 days for fungi, and the colonies were counted (Frazier *et al.*, 1967) [10].

Analysis of bioactive compounds by GC-MS in spiced herbal tea powder

Sample preparation

For GC-MS study, 10g of spiced herbal tea blend was taken and saturated into 100ml of HPLC graded Methanol. It was kept for 24 hours by continuous stirring of the sample. It was initially filtered with muslin cloth and then with Whatman No.1 filter paper. The filtered extract was then concentrated in the flash evaporator to achieve water-free extract after filtering with anhydrous sodium sulphate. Water free extract was used for the assessment of bioactive components by GC-MS (Janes *et al.*, 2009) [14].

Instruments and chromatographic conditions

GC-MS research was carried out on the Shimadzu GC-MS QP 2020 device consisting of an automated sampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument using the following conditions: Column Elite-1 fused silica capillary column (30mm×0.25mm I.D ×1 µ M df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/minute and an injection volume of 1.0 µl was employed (split less) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 minutes) with an increase of 10°C/minute to 200°C, then 5°C/minute to 280°C, ending with an isothermal temperature of 9 minutes at 280°C. Mass spectra were taken at 70 EV, with a scan interval of 0.5 seconds and fragments ranging from 45 to 450 Da. Maximum GC run time was 34 minutes (Karthika *et al.*, 2013) [17].

Storage studies

The prepared herbal tea blend was packed in non-drip tea bags. Each tea bag was packed with approximately 2g of product. Further the herbal tea blend were packed in aluminium foil pouches and metallized polypropylene pouches and labeled to conduct the shelf life studies under ambient temperature. To study the storage behavior of the prepared herbal tea blend, the changes in the chemical and

organoleptic characteristics in the infusions were analyzed once in 30 days during the storage period of three months.

Chemical analysis

Moisture

The moisture content of the sample was estimated by the hot air oven method suggested by (Ranganna, 1979) [26]. About 5 to 10 g of sample was weighed accurately and dried in a hot air oven at 70°C. The drying was continued till a constant weight was obtained. The moisture content was expressed as percentage.

$$\text{Moisture (\%)} = \frac{W_2\text{-}W_3}{W_2\text{-}W_1} \times 10$$

W_1= Weight of empty plate
W_2= Weight of empty plate + Sample before drying
W_3= Final weight of empty plate + Sample after drying

Sample preparation

The infused tea samples were filtered through a Whatman filter paper No. 41 and then tea samples were extracted with 80% ethanol by centrifugation @ 3000 rpm for 20 minutes. The supernatant thus was collected and stored at 4°C for further analysis.

pH

The pH of the sample was estimated by the method described by Jayaraman *et al.* (1976) [15]. One gram of sample was mixed well by stirring with 50 ml of distilled water using a glass rod and the pH of the suspension was determined in the pH meter.

Total ash

Accurately weighed 3 grams of air dried powder sample was obtained in a tared silica crucible and incinerated in the furnace by steadily increasing the temperature to make it dull red hot (400°C) free of carbon. Cooled and measured, repeated at constant value. The percentage of total ash was then examined with reference to the air-dried sample.

$$\text{\% Total ash values} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Crude fibre

The crude fibre of the herbal tea sample was determined as stated (Sadasivam and Manickam, 1996) [29]. The 1g tea sample powder was defatted with the use of petroleum ethers in the soxhlet apparatus for 16 hours or until the oil ether extraction process was colourless. Petroleum ether was evaporated and this defatted residue was used for measurement of crude fibre. The residue was taken in a 50 ml beaker and then digested with 25 ml of 0.255N H2SO4 supplemented by 25 ml of 0.313N NaOH solution before the solvent had evaporated. The digested sample was moved to a cloth of cheese put in a funnel embedded in a 250 ml container. The sample was washed with boiling water followed by absolute ethanol, until the washing was clear. Then the residue was shifted to the ashing dish (pre weighed dish W1). The residue had been drying at 130 ± 2°C for 2 hours. The dish was cooled and weighed in a desiccator (W2). Then it was burned at 600 ± 15°C for 30 minutes. Once again it was cooled in a desiccator and reweighed (W_3). The crude fibre was calculated as follows:

$$\text{Crude fibre (\%)} = \frac{\text{Loss in weight on ignition } (W_2\ W_1)\ (W_3\ W_1)}{\text{Weight of the sample}} \times 100$$

Fat

The fat content of the sample was measured using the soxhlet fat extraction process (Sadasivam and Manickam, 1996) [29]. 250 ml of boiling flask washed thoroughly and dried in the oven at 105°C for 30 minutes, then placed in the desiccator to cool. 2g of the dry sample was then carefully measured into the marked thimbles. Cooled boiling flask was filled with 200 ml of petroleum ether and boiled at 40-60°C. The extraction thimble was gently plugged into cotton wool, and the boiling flask containing the petroleum ether was brought to boil in the extraction thimble, and the soxhlet apparatus was allowed to reflux for six hours. The thimble was gently removed, and the petroleum ether on top of the bottle was gathered and poured into another container for re-use. When the flask was free of petroleum ether, it was extracted and boiled at 105°C for an hour. It was eventually moved from the oven to the desiccator to cool before measuring.

Total protein

The overall protein content of herbal tea infusions was determined by Lowry's procedure (Lowry *et al.*, 1951) [19]. Grind 0.5 g of the sample with an effective solvent method (water or buffer) in the pestle and mortar. Centrifuge to use the supernatant to estimate the protein. Pipet out 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard solution (BSA) in a series of test tubes. Pipet out 0.1 ml and 0.2 ml of the sample extract into two additional test tubes. Make a volume of 1 ml of water in all the tubes. The tube with 1 ml of water is used as a blank. Add 5 ml of alkaline copper solution mix well and incubate at room temperature for 10 minutes. Add 0.5 ml of FCR, stir well immediately and incubate at room temperature in the dark for 30 minutes. Read the absorbance against the blank at 660 nm. Draw a standard graph and measure the quantity of protein in the sample and give the results as a g/100g tea sample.

$$\text{The amount of protein present in the sample} = X \times \frac{25}{1} \times \frac{1000}{250}$$

Carbohydrates

The estimation of carbohydrates in an herbal tea sample was measured by the anthrone method using a calorimeter (Hedge and Hofreiter, 1962) [12]. Weigh 100 mg of the sample in a boiling tube. Hydrolyze by holding it in a hot water bath for 3 hours with 5 ml 2.5N Hcl and cool to room temperature. Neutralize with strong sodium carbonate until effervescence ends. Make a volume of 100 ml and a centrifuge. Collect the supernatant and use 0.5 and 1 ml aliquots for analysis. Prepare the standards by adding 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. '0' is used as blank. Get the volume up to 1 ml in all the tubes, including the sample tubes, by supplying the distilled water. Add 4 ml of the anthrone reagent. Heat in a boiling water bath for 8 minutes. Cool quickly and observe the colour changes from green to dark green at 630 nm. Draw the standard graph by plotting the concentration of the X-axis standard against the Y-axis absorbance. From the graph calculate the amount of

carbohydrates present in the sample tube.

$$\text{Amount of carbohydrate Present in sample (\% mg)} = \frac{\text{Sugar value from graph (mg)}}{\text{Aliquot sample used (0.5 or 1ml)}} \text{ X } \frac{\text{Total vol. of extract (ml)}}{\text{Wt. of sample (mg)}} \text{ X 100}$$

Iron

The iron content of the herbal tea samples was measured using the AAS process. In AAS, iron is normally extracted with DTPA (Diethylene Triamine Pentaacetic Acid) extracting agent. Ion quantities are then measured using a spectrophotometer for atomic absorption. Standardization was performed using crystalline ferrous ammonium sulphate containing 10 to 50 pg of ferric iron. One g of the moisture-free dry sample of the tea powder was carefully weighed into a pre-weighed, ignited and cooled porcelain crucible, charred and then ignited in a muffle furnace at 550°C for 6 hours. The resulting greyish white ash was moistened with 8 drops of concentrated Hcl and 4 drops of purified water, boiled for 2 minutes, evaporated to dryness and heated to a steam bath for 3 hours to make SiO2 insoluble. It was again moistened with 5 ml of Hcl, boiled for 2 minutes and added 5 ml of distilled water, followed by steam bath heating for 10-15 minutes. The solvent was then filtered through hardened ash less filter paper and cleaned thoroughly with distilled water and the solution then made up to 100 ml with distilled water (Cunniff, 1995) [6].

In glass test tubes, five ml of the sample solution was taken in triplicate and 5 ml of purified water was applied to it. 10 ml of purified water was used for the blanks. 0.5 ml of saturated potassium per sulphate was applied to both of these test tubes, followed by the addition of 2 ml of 3N thiocyanate potassium. The solutions were combined by inverting the tubes and within 30 minutes they were read on a spectrophotometer at 540 nm. The amount of iron present in the tea sample was calculated as:

$$\text{Amount of ascorbic acid (mg/100g sample)} = \frac{0.5\text{mg}}{\text{V1}} \text{ x } \frac{\text{V2}}{15\text{ ml}} \text{ x } \frac{100\text{ml}}{\text{Wt. of the sample}} \text{ x } 100$$

Vitamin-C

The estimation of ascorbic acid in the herbal tea sample was measured using the titration process. For the study of ascorbic acid, titrimetric methods rely either on the reducing properties of ascorbic acid or on the ketonic properties of dehydroascorbic acid. Redox titration of ascorbic acid with 2, 6, dichiorophenol indophenol in acid solution involves reduction of this dye to a colourless leucobase while the ascorbic acid is oxidized to dehydroascorbic acid (Cunniff, 1995)[6]. In a 100ml conical flask, take 5ml of the working standard solution. Add 10ml of 4% oxalic acid and titrate against the colourant (V_1 ml). The end point is the pink colour presence that lasts for a few minutes. The amount of dye absorbed is equal to that of ascorbic acid. Extract the sample (0.5-5g depending on the sample) in 4 percent oxalic acid and render up to 100 ml of known volume and centrifuge. Pipet out 5 ml of this supernatant, apply 10 ml of 4 percent oxalic acid and titrate against the dye (V_2 ml).

$$\text{Total iron (mg/100g)} = \frac{\text{Sample reading}}{\text{Standard reading}} \times \text{conc. of std. (µg)} \times 1 \times \frac{100}{1000} \times \frac{\text{moisture factor}}{5}$$

Folic acid

The folic acid in the samples was calculated by the Ranganna process (1986) [27]. Folic acid was collected from the samples using a gentle alkaline buffer oxidised with potassium permanganate and the resultant amine is diazotized. The diazotized compound is coupled with N-(1-naphthol) ethylene diamine and the colour produced is calculated at 550 nm.

A known sample quantity containing approximately 100 mg of folic acid was put in a 100 ml volumetric flask. Approximately 50 ml of K_2HPO_4 solution was added and heated at a temperature not exceeding 60°C with swirling: the sample was allowed to cool and the volume was 100 ml of K_2HPO_4. The sample until it is fully distributed. The aliquot was then filtered and centrifuged, consisting around 1mg of folic acid to 100ml of volumetric flask and diluted to volume using K_2HPO_4, solution, and was used for colour development and estimation.

One ml of sodium nitrite solution and 1 ml of Hcl solutions was added to all of the tubes and tubes were allowed to stand for 2 minutes. One ml of ammonium sulphamate solution was added and then 1 ml of N-(1-naphthyl) ethylene diamine dihydrochloride solution was added and permitted to stand for 10 minutes and then 1 g of NaCl and 10 ml of iso-butyl alcohol was added and shake for 2-3 minutes and the iso-butyl alcohol layer was removed using centrifuge. The colour of iso-butyl alcohol was read as blank at 550 nm within 25 minutes using iso-butyl alcohol.

The quantity of folic acid in the prepared sample in mg/g was calculated using expression:

$$0.4\ C = \frac{A_1\text{-}A_3}{A_2\text{+}A_3\text{-}(A_1\text{+}A_4)}$$

Where C = Concentration of working standard of folic acid in mg/g, A_1, A_2, A_3, A_4, are absorbance of tubes 1, 2, 3 and 4 respectively.

Results and Discussion

In this analysis, the flavouring compounds found in the spiced herbal tea samples were analysed using gas chromatography-mass spectrophotometry (GC-MS). The GC-MS mass spectrum interpretation was done using the National Institute of Standard and Technology (NIST) database with more than 62,000 patterns.

The spectrum of the unknown component has been compared to the spectrum of documented components stored in the NIST library.

The findings revealed that the presence of volatile compounds in the sample differed in quantity, period of retention and peak area (Fig.2). Based on the percentage of peak area, the main 25 volatile compounds present in each sample are shown in (Table 1).

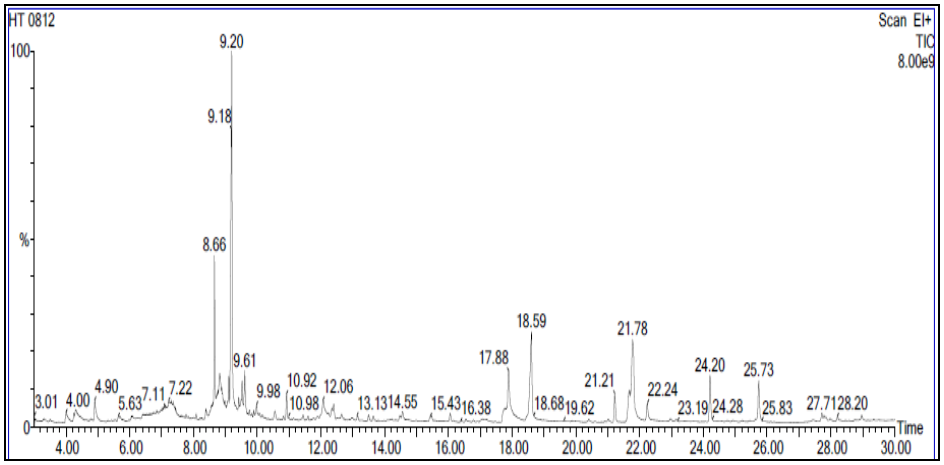

Fig 2: Chromatogram obtained from GC-MS for the methanolic extract of spiced tea

Table 1: Compounds identified in methanolic extract of spiced herbal tea powder by GC-MS

Sl.no.	Retention time	Compound name	Molecular formula	Molecular weight	Peak area (%)
1	4.009	Thymine	$C_5H_6N_2O_2$	126.11	0.830
2	4.299	1-Butanol, 3-methyl-, formate	$C_6H_{12}O_2$	116.15	1.596
3	6.425	Bestatin	$C_{16}H_{24}N_2O_4$	308.4	0.619
4	7.005	D- Pyroglutamic acid	$C_5H_7NO_3$	129.11	1.183
5	8.385	Trioxsalen	$C_{14}H_{12}O_3$	228.24	0.461
6	8.656	Caryophyllene	$C_{15}H_{24}$	204.35	4.907
7	9.111	Humulene	$C_{15}H_{24}$	204.35	0.709
8	9.976	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene	$C_{15}H_{24}$	204.35	0.638
9	10.551	Nerolidyl acetate	$C_{17}H_{28}O_2$	264.4	0.516
10	12.072	2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-	$C_{11}H_{14}O_3$	194.23	1.819
11	12.397	Neointermedeol	$C_{15}H_{26}O$	222.37	0.583
12	13.497	Senkyunolide	$C_{12}H_{16}O_2$	192.25	0.265
13	14.468	4-Phenyl-octahydro-benzo[d][1,3]oxazin-2-one	$C_{14}H_{17}NO_2$	231.29	0.341
14	14.553	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	0.681
15	16.058	Neophytadiene	$C_{20}H_{38}$	278.58	0.431
16	17.124	5,8,11-Eicosatriynoic acid	$C_{20}H_{28}O_2$	300.4	0.444
17	17.889	1-Methyl-3-(9H-thioxanthen-9-ylmethyl)piperidine	$C_{20}H_{23}NS$	309.5	6.439
18	18.594	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	7.163
19	21.211	Phytol	$C_{20}H_{40}O$	296.5	1.390
20	22.241	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	1.057
21	24.202	1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one	$C_{17}H_{24}O_3$	276.4	2.319
22	27.438	1,25-Dihydroxyvitamin D3, TMS derivative	$C_{30}H_{52}O_3Si$	488.8	0.286
23	27.778	Tetradecylphosphonate	$C_{14}H_{31}O_3P$	278.37	0.311
24	28.958	5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one	$C_{19}H_{30}O_4$	322.4	0.409
25	28.958	Gingerol	$C_{17}H_{26}O_4$	294.4	0.409

Changes in the chemical composition of the formulated spiced herbal tea during storage

The spiced herbal tea powder was packed in aluminium foil pouches (P_1), non-woven fabric tea bag (P_2) and metallized polypropylene pouches (P_3). The packed samples were stored at room temperature to study their storage behaviour *viz.*, chemical changes, microbial load and sensory characteristics. The chemical changes and organoleptic evaluation were done once in 30 days during the storage period of six months. The microbial load was analyzed before and after storage. Each chemical component of the stored sample was changed as the storage period increases irrespective of the packaging materials used (Table 2).

Table 2: Changes in the chemical composition of the formulated spiced herbal tea during storage

Parameters	Initial				Final			Mean
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	
Moisture (%)	7.50	7.50	7.50	7.5±0	8.50	8.53	7.94	8.32±0.332315
pH	6.0	6.0	6.0	6.0±0	6.7	6.8	6.6	6.70±0.100000
Ash values (%)	6.70	6.70	6.70	6.7±0	6.65	6.64	6.67	6.65±0.015275
Crude fibre (g/100g)	7.93	7.93	7.93	7.93±0	6.82	6.17	6.98	6.66±0.428991
Fat (g/100g),	6.10	6.10	6.10	6.10±0	5.20	5.03	5.60	5.28±0.292632
Protein (g/100g)	30.10	30.10	30.10	30.1±0	29.48	29.19	29.59	29.42±0.20664
Carbohydrates (g/100g)	49.30	49.30	49.30	49.3±0	48.04	47.75	48.19	47.99±0.223681
Iron (mg/100g)	55.80	55.80	55.80	55.8±0	53.02	52.15	53.12	52.76±0.53351
Vitamin – C (g/100g)	1.88	1.88	1.88	1.88±0	1.19	1.13	1.23	1.18±0.050332
Folic acid (µg/100g)	96.00	96.00	96.00	96±0	90.00	87.00	92.10	89.70±2.563201

The table indicates that the moisture content increases during the storage period of spiced herbal tea. The lowest moisture content (7.94 %) was recorded in P_3 (MPP) followed by P_1 (aluminium foil pouch) (8%) and the highest moisture content was recorded in P_2 (Non-woven fabric tea bag) (8.06%). Pua *et al.* (2008) [24] noticed that within 12 months of storage of jackfruit powder, the moisture content increased from 3.47 per cent to 3.56 per cent in aluminium laminated polyethylene and to 3.65 per cent in metallized co-extruded biaxially oriented polypropylene bags. The lowest pH (6.6) was recorded in P_3 (MPP) which was statistically on par with P_1 (aluminium foil pouch) (6.7) and the highest pH were recorded in P_2 (Non-woven fabric tea bag) (6.8). De-Heer, (2011) [7] revealed that the infusions prepared from lemongrass and moringa powder had pH values of 4.53 and 5.47 respectively. The pH of a sample affects the organoleptic character of the sample. Low pH results in sour and astringent products. The highest ash content (6.67%) was recorded in P_3 (MPP) which was statistically on par with P_1 (aluminium foil pouch) (6.65%). The lowest ash content was recorded in P_2 (Non-woven fabric tea bag) (6.64%). The higher ash content indicates a good source of minerals in P_3. Increased content of ash during storage is also reported by (Roy *et al.*, 2001) [28] in some leafy vegetables and (Singh and Sagar, 2010) [34] in dehydrated leafy vegetables. A negligible reduction in the crude fibre, fat and folic acid content of the spiced herbal tea powder were seen among the samples, irrespective of storage period and packaging material. These findings align with similar research by Arokiamary *et al.* (2003) [2], Srinivas *et al.* (2017) [35], Cai and Corke, (2000) [4] and Quek *et al.* (2007) [25]. The highest carbohydrate content was recorded in P_3 (48.19 g/100g) followed by P_1 (48.04 g/100g) and lowest was in P_2 (47.75 g/100g). Carbohydrate content was reduced by 3.45 per cent and 5.15 per cent respectively for control and value-added shrikhand during storage (Srinivas *et al.*, 2017) [35]. The highest protein content was recorded in P_3 (29.59 g/100g) followed by P_1 (29.48 g/100g) and lowest was in P_2 (29.19 g/100g). Similar findings have also been made by Singh *et al.* (2003) [33] recorded a substantial reduction in protein content up to 15 days of storage in mint, coriander, bengal gram, spinach, cauliflower leaves and carrot powder, after which protein content decreased dramatically until the end of the storage period. The highest ascorbic acid content was recorded in P_3 (1.23 g/100g) followed by P_1 (1.19 g/100g) and lowest was in P_2 (1.13 g/100g). Muhammad *et al.* (1987) [21] and Gomez *et al.* (2005) [11] also reported a similar declining trend for ascorbic acid content in different fruit beverages. Initially, the iron content in the formulated spiced herbal tea powder was 55.80 mg/100g which had decreased to 53.12 in P_3, 53.02 in P_1 and 52.15 in P_2 mg/100g. These findings align with similar research by Kadam *et al.* (2010) [16].

Microbial study

The microbial population was analyzed before and after storage of the samples selected for the study (0 and180 days). As the storage period progresses a slight increase in the microbial population was noted among the samples. In general the microbial population of the samples packed in P_3 stored in room temperature showed only a minimum count followed by the samples packed in P_1 and P_2 at the end of the storage period.

Table 3 indicates that initially the bacterial population of spiced herbal tea powder was nil which had changed from 2 to 4×10^6 $cfug^{-1}$ respectively stored in room temperature which is packed in P_1, P_2 and P_3. Initially the fungal population of the spiced herbal tea powder was nil which had changed from 2 to 3×10^4 $cfug^{-1}$ respectively stored in room temperature which is packed in P_1, P_2 and P_3. Initially there was no yeast population in the herbal tea powder. But at the end of 180 days of storage, the population $1\text{-}3\times10^4$ $cfug^{-1}$ was developed in room temperature which is packed in P_1, P_2 and P_3.

Table 3: Changes in the microbial load (cfu g^{-1}) of the formulated spiced herbal tea during storage (DWB)

Particulars	Packaging materials		
	P_1	**P_2**	**P_3**
Bacteria cfu (x 10^6 g^{-1})			
Initial	0	0	0
Final	3.0	4.0	2.0
Fungi cfu (x 10^4 g^{-1})			
Initial	0	0	0
Final	2.0	3.0	2.0
Yeast and mold cfu (x 10^4 g^{-1})			
Initial	0	0	0
Final	3.0	3.0	1.0

Organoleptic Evaluation

Evaluation of spiced herbal tea infusions was conducted organoleptically for various quality attributes like appearance and colour, flavour, consistency, taste and overall acceptability at regular intervals once in a month for 180 days. The scores of the organoleptic characteristics of the herbal tea blends during storage are tabulated in (Table 4). The score value of each attribute of each period does not show significant change during the storage period.

Table 4: Changes in Organoleptic scores during storage

Spiced herbal tea						
Quality parameters	**Initial**			**Final**		
	P1	**P2**	**P3**	**P1**	**P2**	**P3**
Colour and appearance	8.9	8.9	8.9	8.3	8.2	8.6
Flavour	8.9	8.9	8.9	8.5	8.4	8.6
Consistency	8.5	8.5	8.5	8.5	8.5	8.5
Taste	8.8	8.8	8.8	8.3	8.1	8.4
Overall acceptability	8.9	8.9	8.9	8.3	8.2	8.4

Conclusion

Functional herbal teas can be formulated using the blending of curry leaves with moringa, amla, ginger, celery and sweet basil. The spiced herbal teas produced were entirely acceptable. There was a large amount of crude fibre, fat, protein, carbohydrates, iron, vitamin C and folic acid in the spiced herbal infusions developed. Herbal teas' antimicrobial activities are effective against bacteria, fungi and yeast, suggesting that regular consumption of spiced herbal teas can ensure a person's health is maintained. Curry leaves are rich in many nutrients used to cure various conditions such as fat loss, prevention of dysentery, constipation and diarrhoea, relieves morning sickness and nausea, destroys bacteria, is safe for diabetics and eyesight, prevents inflammation and cures cuts, burns and rashes of the face. Curry leaf intake among the general population is very rare, although it is abundant in biological activities. In order to utilize the bioactive molecules of curry leaves, it can be used for beverage preparation. Hence it is recommended that herbal teas based on curry leaves can be

developed, which will be highly suitable for all age groups. As sensory attraction means more to consumer than health or dietary benefits, they will be offered new alternatives to tradition by the above-mentioned infusion.

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