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# Development and Evaluation of Bioactive Components in guava tea

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**ABSTRACT**
*Herbal teas and medicinal plant formulations are produces from green and dried herbs, flowers, fruits, leaves, seeds, barks and roots of medicinal plants and sold in a loose form or packed in bags. Matured guava leaves were blanched in hot water and blended with supporting herbs such as coriander leaf and dry ginger along with activating herbs such as cinnamon/ orange peel/ lemongrass. The herbal tea blends were packed to conduct the shelf life studies under ambient temperature. The flavouring compounds present in the herbal tea blends were analyzed by GC-MS. The compounds n-Hexadecanoic acid, Caryophyllene, 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl, (E) and 9,12,15-Octadecatrienoic acid was predominantly found in the samples. The highest extraction efficiency was obtained from 3.61 g of tea blends infused at a temperature of 94.22°C for a time 225.95 seconds to attain maximum antioxidant activity of 85.19 mg AAE/ml. A decreasing trend in total phenol content of herbal infusions was observed during storage. The maximum total phenol content was noted in T1 (113.5 mg GAE/ml) which had decreased to 105.54 mg GAE/ml at the end of storage period. The total flavonoid content was found to be decreased at the end of 180 days of storage period. Among developed herbal infusions the initial total flavonoid was in the range of 4.604 to 5.127 mg QE/ml which was declined to 3.92 to 4.51 mg QE/ml. During 180 days of storage period there was reduction in total antioxidant activity from 91.64 mg AAE/ml to 85.72 mg AAE/ml in cinnamon flavoured tea. When compared with commercial tea infusion the total antioxidant activity was maximum in the guava leaf herbal infusions.*
***Key words:*** *Guava leaf, Herbal tea, GC-MS, Phytochemical, Bioactive compounds*

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## INTRODUCTION

Humans have long benefited from the beverages that are prepared from leaves, stems, sap, fruits, tubers, and seeds [1]. Many of these beverages have become a part of everyday of modern life. Tea is the second largest consumed hot beverage in the world after water. Tea has gained popularity due to its palatability and comparatively low cost. There are several types of tea available in the market, such as flavored teas, herbal teas and specialty teas which are blended with spices/condiments or medicinal herbs, etc [2]. The tisanes of herbal teas and medicinal plant formulations are habitually consumed in many parts of the world due to their therapeutic and healing properties. In subtropical areas, herbalists and practitioners use fresh guava leaves in the form of guava leaf tea prepared from the dried

foliage of guava leaves in traditional medicine to treat various disease from diabetes and diarrhea to toothaches and sores in the mouth and swollen gums. Also in several parts of the world, folk healers recommend crushed guava leaves as an effective medicine to treat wounds and scrapes [3]. The study was formulated with the objective of developing guava leaves based tea with herbs with high antioxidant properties. The hypothesis behind this is processed guava leaf tea blended with lemongrass will have higher antioxidant property and good organoleptic properties.

## MATERIAL AND METHODS

### *Plant materials*

The fresh leaves of *Psidium guajava* plants were collected from the Orchard, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai. Intense green middle age leaves were collected during January, 2018. The environmental conditions had mean maximum/minimum temperature of 31.5/18.5°C, precipitation of 4 mm, and saturated light duration of 8.31 hours.

### Steam blanching of guava leaves

Fresh guava leaves were carefully inspected and all foreign materials removed and then gently rinsed in tap water. The guava leaves were subjected to steaming temperature of 90°C for three minutes followed by spreading the leaves on aluminium trays of the cabinet drier. The drying time and temperature was 60°C for 6 hours. The dried leaves were powdered by using blender and passed through aluminum sieve (1 mm) to get uniform particle size of herbal tea blend.

### Developing tea blends

Fresh guava leaves were carefully inspected and all foreign materials removed and then gently rinsed in tap water. The guava leaves were subjected to steaming temperature of 90°C for three minutes followed by spreading the leaves on aluminium trays of the cabinet drier. The drying time and temperature was 60°C for 6 hours. The dried leaves were powdered by using blender and passed through aluminum sieve (1 mm) to get uniform particle size of herbal tea blend.

Fresh lemongrass leaves were cleaned under running tap water and incised into small sections (5-6 cm). Then drying was done with cabinet drier at 60°C for 4 hours Coriander leaves, dry ginger and cinnamon were purchased from the local market and were used for the study. These plant materials were cleaned, dried in cabinet drier (60°C) and ground to uniform particle size (1mm) using aluminum sieve. The grounded plant materials were blended in the proportion of 75% of primary herb (guava leaves), 15% of supporting herbs (coriander leaves and dry ginger) and 10% of activating herb (lemongrass).

### Standardization of dip tea bag

The developed herbal tea blends were packed in non-drip tea bag with different quantities such as 1 g, 2 g and 3 g and then subjected to organoleptic evaluation to assess the appropriate quantity to be packed in tea bags.

### Standardization of herbal tea infusions

The prepared herbal tea blend (3 g) was packed in non-drip tea bag. The herbal infusions were prepared by infusing tea bag in 150 ml hot water for 1, 2 and 3 minutes according to method suggested by Komes *et al.*, [4]. The organoleptically acceptable time for infusion was standardized.

### Chemical characteristics of developed herbal blend

The chemical characteristics of developed herbal blend such as total phenol, total flavonoid, tannin and total antioxidant activity were analyzed. In this study Dia-g-tee which is a commercial herbal tea was taken as control and analyzed the chemical characteristics.

### Anti-microbial activity

### Test organism for evaluating antimicrobial activity

Common pathogenic food borne bacteria such as gram positive *Staphylococcus aureus* and gram negative *Escherichia coli* and *Pseudomonas aeruginosa* were used for the study. Antibacterial activities were evaluated by agar well diffusion method described by Ahmad and Beg, [5]. The cultures of bacteria were maintained in appropriate agar slants at 4°C and used as stock cultures. Bacterial strains were sub cultured in Brain Heart Infusion broth (BHI) and incubated at 37 °C for 18–24 h. The cultures were adjusted to approximately $1.5x10^8$ CFU/ml with sterile saline solution using 0.5 McFarland standards.

**Determination of antimicrobial property of tea extract**

The antibacterial activity of tea extract was determined quantitatively using the agar well diffusion method (Gurnani *et al.*, 2016)[6]. Water and methanolic extracts of tea were prepared at different concentrations. Experiments were carried out with different concentrations of tea extracts (10 %, 20 % and 30 %). The zone of inhibition on solid media was used to determine the antimicrobial effects of above mentioned tea extract concentrations against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. Bacterial dispersion ($10^6$ CFU/ml) of 10 µl was spread onto an agar plate medium. Well of 0.6 mm diameter were made with sterile borer into agar plates containing the test bacterial inoculum. 50 µl and 100 µl of the tea extracts were poured into the well of the each inoculated plate, and the diameter of the zone was measured with a scale. All tests were performed in triplicate. Methanol and sterile water was used as blank.

**Storage study**

The prepared herbal tea blend was packed in non-drip tea bags. Each tea bag was packed with approximately 3g of product. Further the tea bags were packed in glassine poly pouches. These pouches were further stored in aluminium foil packaging material 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 six months.

**Chemical analysis**

**Moisture**

The moisture content of the sample was estimated by the hot air oven method suggested by Ranganna, [7]. 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.

$$\text{Moisture (\%)} = \frac{W2-W3}{W2-W1} \times 100$$

W1= Empty weight of empty plate
W2= Weight of empty plate + Sample before drying
W3= Final weight of empty plate + Sample after drying

**pH**

The pH of the sample was estimated by the method described by Jayaraman *et al.*, [8]. 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.

**Acidity**

Acidity of the sample was estimated by the method described by Ranganna, [7]. About 5 g of the sample was weighed and dissolved in a known quantity of water and made up to 50 ml and filtered. From the filtrate an aliquot of sample was taken and titrated against 0.01 N NaOH, using phenolphthalein as indicator till the appearance of pale pink colour. The titration was repeated to obtain concordant values. The result was expressed as per cent.

**Organoleptic evaluation**

Organoleptic evaluation was conducted as per the method described by Watts *et al.*, [9]. A panel of 25 semi trained members evaluated the prepared herbal tea infusions. The tea samples were approximately 60°C to 70°C at the time of tasting. The organoleptic evaluation sessions were conducted one hour before lunch under adequate conditions of temperature, humidity and illumination. The panelists were asked to score the colour, appearance, flavour, texture, taste and over all acceptability of the herbal tea infusions on a scale of 9 to 1 point hedonic scale.

**Evaluation of bioactive compounds in the tea**

**Sample preparation**

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

**Total phenolic content (TPC)**

The total phenolic content in herbal tea infusions was determined by using the Folin-Ciocalteu method [10]. Accurately, 0.5 ml Folin-Ciocalteu reagent, 1.5 ml 7.5% sodium carbonate and 7.9 ml distilled water were introduced in a test tube containing 0.1 ml sample/standard. The solution was mixed thoroughly and allowed to stand for 2 hours in a dark place. The absorbance at 765 nm was read by UV-VIS Spectrophotometer. The TPC of herbal tea sample was expressed as mg of gallic acid equivalents (mg GAE)/ml of tea sample.

$$\text{Total phenol content} = \left[\frac{\text{Graph value}}{\text{Volume of extract}} \times \frac{\text{Total volume of extract}}{\text{Weight of sample}} \times 100\mu g\right]$$

**Total flavonoid content (TFC)**

The total flavonoids content in tea infusions was analysed according to method as described by Singh *et al.*, (2012)[11]. One ml of sample/standard was diluted with 4 ml distilled water then 0.3 ml 5% sodium nitrate solution and 0.3 ml 10% aluminium chloride were added. The mixture was kept for 5 minutes. Then, 2 ml of 1M sodium hydroxide were added to the mixture and the mixture was vortexed thoroughly. The absorbance was measured at 510 nm using UV-VIS Spectrophotometer. The results were expressed as mg of quercetin equivalents (mg QE)/ ml of tea sample.

$$\text{Total flavonoid content} = \frac{\text{Concentration of standard}}{\text{OD of standard}} \times \frac{\text{Sample OD}}{\text{Aliquot taken}} \times \frac{\text{Volume made up}}{\text{Sample taken}} \times \frac{100}{1000}$$

**Total anti-oxidant activity (DPPH method)**

The radical scavenging activity of tea infusions were determined by the 2, 2, diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The DPPH assay was performed as described in Nuengchamnong and Ingkaninan, [11] with slight modifications. DPPH is a purple coloured stable free radical that reacts with compounds that can donate a hydrogen atom. This method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolorizes the DPPH solution. The degree of discoloration indicates the scavenging potential of the antioxidant compounds.

Different aliquots (0.2 - 1 ml) of methanol extracts of each samples were pipetted out into test tubes and made up the volume in each test tube to 1ml with methanol. Then 2 ml of freshly prepared DPPH solution (0.1 mM) in methanol was added. The tubes mixed thoroughly and allowed to stand in the dark at room temperature. The absorbance decrease was determined after 30 min at 517 nm using a spectrophotometer. Methanol (1 ml) replacing the plant extract serves as a negative control, methanol replacing ascorbic acid serves as standard and methanol (2 ml) replacing the DPPH reagent serve as sample blanks. The percentage of radical scavenging activity (% RSA) or percentage inhibitions of DPPH of the methanolic extract of the samples were calculated by the following formula. The content of antioxidant was expressed as mg of ascorbic acid equivalent (mg AAE)/g.

Then graphs were plotted between the percentages of radical scavenging activity and the different concentrations of methanolic extracts of samples. IC50 value is determined as the concentration of methanol extracts of samples corresponding to 50% inhibition of DPPH free radicals. Inhibition concentration 50 % or IC50 is defined as the amount of antioxidant required to inhibit 50 % of DPPH free radicals under the experimental conditions. Antioxidant activity is inversely proportional to IC50 value.

**Tannin content**

Tannin content of the infusions was determined by Vanillin Hydrochloride Method [12]. The vanillin reagent will react with any phenol that has an unsubstituted resorcinol or phloroglucinol nucleus and forms a coloured substituted product which is measured at 500 nm. Sample was extracted with methanol by keeping it for 20-28 h, centrifuged and supernatant was collected. 5 ml vanillin hydrochloride reagent was added to 1 ml of the sample supernatant. Standard graph was drawn by using catechin as standard. After 20 mins, the absorbance was read in spectrophotometer at 500 nm. The tannin content was expressed in mg Catechin Equivalent per ml of sample

**Tannin calculation:**

$$\frac{\text{Sample absorbance-Blank absorbance} \times \text{Total volume of extract}}{\text{Slope}} \times \frac{\text{Used volume of extract}}{(W) \times (1\text{-}M/100) \times 1000} \times 100$$

**Preparation of methanolic extract**

The methanolic extract was prepared by adding 10g of developed herbal tea blend to 100ml of HPLC graded Methanol (1:10) in a conical flask. It was left for 24 hours by frequent shaking of sample in mechanical shaker. It was initially filtered with muslin cloth and then with Whatman No.1 filter paper. The filtered extract was then concentrated in flash evaporator after which it was filtered with anhydrous sodium sulphate to get water free extract. The water free extract was used for analyzing bioactive components by GC-MS [13].

***GC-MS analysis***

GC-MS analysis was carried out on Shimadzu GC-MS QP 2020 system comprising auto sampler and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument employing 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/min 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 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 34 minutes [14].

**Identification of compounds**

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

## RESULTS AND DISCUSSION

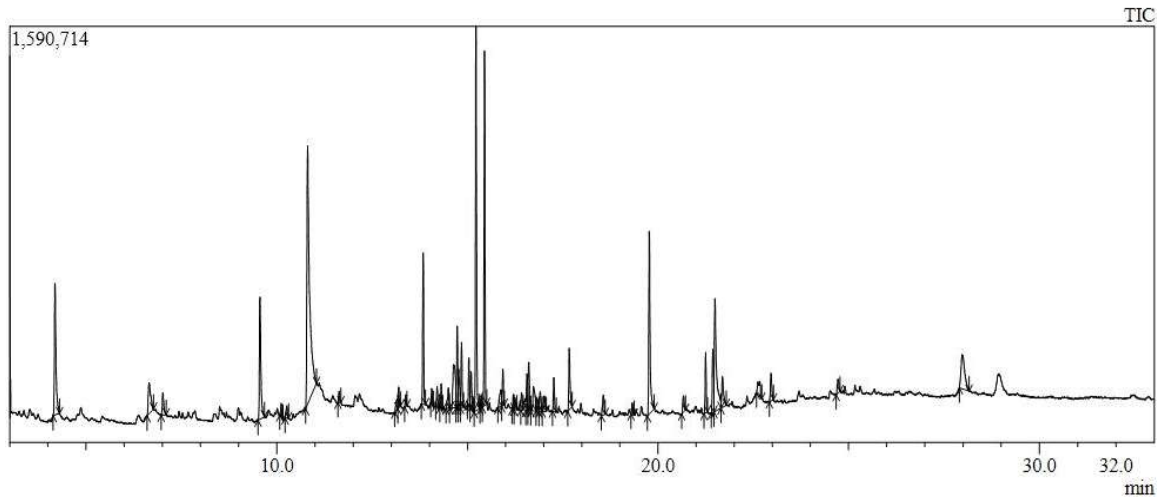


**Fig 1: Chromatogram obtained from GC-MS for the methanolic extract of $T_6$ (Steam blanched guava leaves with lemongrass combination)**

**Table 1: Compounds identified in methanolic extract of $T_6$ by GC-MS**

| S.No | Retention time | Name of the compound | Molecular formula | Molecular weight | Peak area (%) |
|---|---|---|---|---|---|
| 1. | 4.186 | Furfural 4H-Pyran-4-one, 2,3- | C5H4O2 | 96 | 4.90 |
| 2. | 9.559 | dihydro-3,5-dihydroxy-6-methyl- | C6H8O4 | 144 | 4.32 |
| 3. | 10.807 | 5-Hydroxymethylfurfural | C6H6O3 | 126 | 16.94 |
| 4. | 13.834 | Caryophyllene | C15H24 | 204 | 3.66 |
| 5. | 14.637 | .beta.-D-Glucopyranose, 1,6-anhydro- | C6H10O5 | 162 | 2.66 |
| 6. | 15.220 | Benzene, 1,2,3- trimethoxy-5-(2-propenyl)- | C12H16O3 | 208 | 9.87 |
| 7. | 15.445 | 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)- | C15H26O | 222 | 7.87 |
| 8. | 19.771 | n-Hexadecanoic acid | C16H32O2 | 256 | 5.85 |
| 9. | 21.496 | 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- | C18H30O2 | 278 | 5.74 |
| 10. | 27.976 | .gamma.-Sitosterol | C29H50O | 414 | 3.07 |

**Chemical characteristics of developed Herbal tea blends**

The results indicated that Dia-g-tee which is a commercial herbal tea was taken as control and analyzed the chemical characteristics. The chemical characteristics of herbal tea blends were highly influenced by processing methods of guava leaves.

**Table 2 Chemical characteristics and bioactive components of developed herbal tea blends**

| Chemical parameters | C | | T1 | | T2 | |
|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Initial | Final |
| Moisture (%) | 5.45 | 5.72 | 5.22 | 5.37 | 5.22 | 5.32 |
| Acidity (%) | 0.45 | 0.28 | 0.53 | 0.39 | 0.53 | 0.41 |
| pH | 5.03 | 5.44 | 5.26 | 5.88 | 5.08 | 5.76 |
| Total phenol content (mg GAE/ml) | 42.6±0.848275 | 40.6±0.848275 | 96.75±0.697952 | 95.75±0.697952 | 98.5±2.752561 | 96.5±2.872545 |
| Total flavonoid content (mg QE/ml) | 9.493±0.01179 | 8.93±0.01179 | 11.105±0.24421 | 10.055±0.24421 | 12.723±0.217859 | 12.723±0.217859 |
| Total antioxidant activity (mg AAE/ml) | 53.84±0.975579 | 50.84±0.975579 | 78.83±0.995132 | 77.39±0.995132 | 72.40±1.50088 | 72.40±1.50088 |
| Tannin (µg CE/mg) | 1.875±0.080293 | 1.256±0.080293 | 2.038±0.071557 | 1.875±0.071557 | 1.945±0.080293 | 1.265±0.080293 |

C- Control (Market sample) T1 – Unsteamed guava leaf T2 – Steamed guava leaf

The table indicates that the moisture content of the tea blends increased slightly during the storage. Sengkhamparn *et al.*, [15] stated that blanching pre-treatment softens the texture of the leaves, which makes easy to removal water from the leaves, also blanching lowers the water activity. Statistical analysis showed that there was no significant ($P \geq 0.05$) difference in the moisture content between the treatments. As the acid content of herbal infusions increased, the pH decreased during storage.

The acidity was lower in the control sample. The phenol and flavonoid content of the steamed guava leaf tea was greater than the other two samples. Phelan and Rees, (2003) reported that acidity of various commercial herbal tea ranged from 0.03 to 0.12 %. Stability is an important factor while incorporating catechins as functional ingredient [16] There was significant increase in pH of herbal tea infusions during storage. In commercial tea infusion the initial pH value was 5.03 and at the end of storage period it was 5.44. The pH values of

the present study was agreeable with the results confirmed by Christianah *et al.*, [17] who reported that the pH of commonly marketed teas in Nigeria hot water infusion was between 5.09 and 7.20. The statistical analysis infer that there is significant ($P \leq 0.05$) difference between treatments and interaction of treatment and storage period. From the above the observation, it is clear that increase in pH during storage could be attributed to the simultaneous decrease in titrable acidity.

During tea processing the total phenol content significantly increases due to oxidative degallation of phenolic esters. The higher concentration was recorded in T2 (98.5±2.752561 mg GAE/g). The antioxidant and tannin content of the T1 sample was greater than the steamed guava leaf tea blends. The TFC of commercial tea powder was 9.493 mg QE/ml which was lower than all the other treatments. TFC content was higher in steam blanched lemongrass combination.. The results are similar to the results of Wang *et al.*, [18] who showed that HPLC chromatogram of total flavanol content in steam blanched green tea leaves (1.3044) was higher when compared with roasted green tea leaves (0.9941). Saetan *et al.*, [19] studied the effect of hot water blanching process on total flavonoid content of the *Cinnamomum porrectum* herbal tea and reported that total flavonoid content was found to be higher in blanched (57.05 mg CE/g) tea leaves when compared to unblanched (45.32 mg CE/g) tea leaves.

**Table 3 Bioactive components in the tea infusions**

| **Chemical parameters** | **C** | | **T1** | | **T2** | |
|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Initial | Final |
| Total phenol content (mg GAE/ml) | 55.08 | 45.29 | 110.5 | 93.8 | 113.5 | 106.4 |
| Total flavonoid content (mg QE/ml) | 2.562 | 2.106 | 5.522 | 4.811 | 5.729 | 4.731 |
| Total antioxidant activity (mg AAE/ml) | 75.45 | 71.05 | 90.88 | 83.9 | 91.06 | 85.95 |
| Tannin (µg CE/mg) | 0.876 | 0.73 | 1.145 | 0.922 | 1.134 | 1.022 |

From the table it is inferred that total phenol content of commercial tea infusion was recorded as 55.08 mg GAE/ml, which was lower than other treatments. During 180 days of storage period there was slight decrease in TPC was observed. In the present study it is noticed that dipping of tea bags in hot water recorded the increased recovery of phenolic compounds. It might be due to water content enhances the extraction of the target compounds with better solubility in the solvent. The results of one way ANOVA reported that there was highly significant ($P \leq 0.05$) decrease between treatments and storage period in the total phenol content of the herbal infusions. Oh *et al.*, [20] investigated the antioxidant activity of various leafy herbal tea extracts and reported that TPC of green tea, rosemary tea, lemongrass tea and peppermint tea was 82.21, 30.84, 23.67 and 75.31 (mg GAE/g). The results of the above study were found to be more or less similar to the present investigation. The total antioxidant capacity is highly associated with total phenol content of herbal tea samples. Dutta *et al.*, [21] implied that total phenol content possess antioxidant property that provides protection against oxidative stress. From the table it is inferred that the initial TFC of commercial tea was 2.562 mg QE/ml, which decreased to 2.106 mg QE/ml after 180 days of storage. the highest TFC was recorded in the steam blanched guava leaf tea (5.729 mg QE/ml) and the lowest value was recorded in unprocessed guava leaf tea (4.371 mg QE/ml) sample. During storage period there was gradual decrease in TFC was observed in herbal infusions. Statistical analysis of the investigation results indicated that the significant ($P < 0.05$) reduction in TFC between the treatments and storage period.

Total antioxidant activity of herbal infusions was higher than herbal tea blends. Nicoli *et al.*, [22] has reported that this might be due to concentration of natural antioxidants was significantly reduced as a result of the thermal treatments. From the table it is inferred that the initial total antioxidant activity of commercial tea infusion was 75.45 mg AAE/ml which was decreased to 71.05 at the end of 180 days of storage period. The total antioxidant activity may get affected owing to conditions of packaging material and storage temperature. In developed herbal tea infusions the initial total antioxidant activity was found to be maximum in steam blanched treatments. The results obtained from the statistical analysis on herbal infusions indicated that treatment, days of storage and their interaction effect had highly significant ($P \leq 0.05$) impact on total antioxidant activity. The tannin content of

commercial tea infusion was 0.876 mg CE/ml, whereas it was 1.134 in steam blanched infusions.

**Changes in Organoleptic scores during storage**

| Sensory attributes | C | | $T_1$ | | $T_2$ | |
|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Initial | Final |
| Colour and Appearance | 9.0 | 8.6 | 8.0 | 7.7 | 9.0 | 8.6 |
| Flavour | 7.0 | 6.8 | 8.5 | 8.3 | 9.0 | 8.6 |
| Consistency | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| Taste | 7.0 | 6.7 | 8.6 | 8.3 | 9.1 | 8.9 |
| Overall acceptability | 7.75 | 7.53 | 8.28 | 8.08 | 8.78 | 8.53 |

Evaluation of herbal infusions was conducted organoleptically for various quality attributes like appearance and colour, flavour, consistency, taste and overall acceptability Only there was minimum decrease in the scores for the colour and appearance of herbal infusions during storage The organoleptic score for the flavour of commercial tea infusions were lower (C-7.0) among the other treatments. During storage of 6 months only there was negligible decrease in the flavour scores of herbal infusions.

**Antimicrobial activity of steam blanched guava leaf herbal infusion**

| Medium: Brain Heart Infusion (BHI) agar |
|---|
| Method: Well diffusion method |
| Type of extract – Aquous extract |

| S.No | Selected microbial strains | Zone of inhibition (mm) | |
|---|---|---|---|
| | | 50µl | 100 µl |
| 1. | *Staphylococcus aureus* | 7±0.293575 | 16±0.494931 |
| 2. | *Pseudomonas aeruginosa* | 6±0.216333 | 13±0.081957 |
| 3. | *Escherichia coli* | 5±0.116903 | 12±0.151333 |

The antimicrobial activity of steam blanched guava leaf herbal infusions against pathogenic food borne bacteria, gram positive Staphylococcus aureus and gram negative Escherichia coli and Pseudomonas aeruginosa were studied. Since these samples showed highest tannin content which is responsible for the antimicrobial activity of herbal teas. The present study was in line with the experiment done by Mailoa et al., (2013)[23] who reported that the ethanolic extract (30 %) of P. guajava leaves containing 2.351 mg/g of tannins had inhibition zones of 9 mm against the fungi Aspergillus niger and C. albicans.

## CONCLUSION

Blending of guava leaves with coriander leaf and dry ginger with cinnamon/ orange peel/ lemongrass can be used to develop functional herbal teas. The developed herbal teas were highly acceptable .The developed herbal infusions had high amount of total phenol, total flavonoid, total antioxidant activity and tannin content when compared to commercial tea infusion.Steam blanching of guava leaves could increase functional properties in terms of total polyphenol, total flavonoid, tannin and total antioxidant activity as well as sensory appeal of herbal tea. .Antimicrobial activities of herbal teas are effective against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* indicating that continuous intake of guava based teas will ensure to maintain health of a person. Guava leaves are rich in bioactive compounds which can be used in the treatment of diabetes, cardiovascular disease, obesity and atherosclerosis. The guava leaf consumption is nil among common public even though it is rich in bioactive compounds. In order to exploit. he bioactive compounds rich guava leaves which otherwise is considered as waste or unutilized, it can be used to prepare beverage.

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