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Quality and organoleptic properties of green tea blended with four different flower petals

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Abstract
The present investigation entitled "Quality and organoleptic properties of green tea blended with four different flower petals" was carried out in the Floriculture Laboratory, College of Horticulture, Rajendranagar, Hyderabad, from March 2023 to May 2023. The petals of different flowers like *Hibiscus rosa-sinensis, Rosa bourboniana, Cassia auriculata* and *Clitoria ternatea* were collected and placed in a hot air oven at 60°C for a duration of 2-3 hours. Green tea and various flower petals were blended in accordance with the designated treatment combinations. The experiment was carried out with Completely Randomized Design (CRD) with nine treatments. The data was subjected to statistical analysis for biochemical parameters, *viz.*, moisture percentage, total anthocyanin content (mg L^{-1}), antioxidant activity (% inhibition), total phenolics content (mg g^{-1} GAE) and organoleptic scoring. The results related to biochemical parameters indicated that, among the different herbal teas, the treatment T_4 (1-part green tea + 3-parts *Clitoria ternatea*) recorded the minimum moisture content (6.80%). While, T_1 (1-part green tea + 3-parts *Hibiscus rosa-sinensis*) was found to be best with total anthocyanin content (47.95 mg L^{-1}), antioxidant activity (92.39%), and total phenolics content (126.24 mg/100g). In respect to organoleptic scoring, T_1 (1-part green tea + 3-parts *Hibiscus rosa-sinensis*) registered the highest color score (7.97), whereas T_4 (1-part green tea + 3-parts *Clitoria ternatea*) recorded the highest score for flavor (7.73), taste (7.90) and overall acceptability (7.53).

Keywords: Antioxidant activity, total phenolics, total anthocyanin content

Introduction

In today's modern lifestyle people do not have enough time to pay attention to their health. Therefore, the instantaneous life style needs to be balanced by consuming a drink to keep the functional durability of the body. Herbal tea is one of the functional beverage products from plants and herbs that helps in illness treatment and also induce relaxation by its therapeutic and energizing properties (Ayu, *et al.*, 2017) [3].

Herbal teas are actually the aqueous extracts of herbal ingredients that are made from combination of seeds, flowers, grass, nuts, roots and rhizomes *etc*. These are more accurately known as 'tisanes. Obtained from the word "tisane" comes through Latin "tisana" and Greek "ptisane" give rise to archaic French and English word "tisane" that refers to any type of herbal tea.' Unlike most other forms of tea, herbal teas do not contain caffeine (Rathi *et al.*, 2019) [15]. Although the English term "TEA" is used to denote the infusion made from the leaves or seeds of plant *Camellia sinensis*. The composition and blending of ingredients are the prime consideration of reliable effects of herbal teas. Now a days, flowers are most commonly used for the fragrance and freshness they provide, in addition to the various health benefits they offer. Floral teas are blended with flower buds, petals and leaves allowing people to enjoy a beverage filled with health benefits. Some floral tea brands like "tea forte" and "ten ren teas" *etc.* are world famous just for their freshness, aroma and taste.

The synthetic antioxidants (Butylated hydroxy toluene, BHT; butylated hydroxy anisole, BHA) have always been suspected to cause or promote negative health affects at the genetic and molecular level (Barlow, 1990; Sasaki *et al*. 2002) [4, 17]. On the other hand, herbal drugs in the recent years have gained popularity because of their efficacy, cost effectiveness and negligible side effects. Accordingly, research interest has considerably increased in finding nature derived antioxidants from medicinal materials for use in foods to replace synthetic antioxidants (Pal *et al*. 2011, 2012, 2015) [10-12]. It has been advocated that there is a direct correlation between the increase in antioxidant-rich foods and the decrease in the number of human diseases.

Numerous studies were carried out on some herbs and medicinal plants resulted in the development of natural antioxidants formulations for food, cosmetic and other applications (Singh *et al.* 2002) [18].

Herbal teas have health benefits due to specific active substances known as phytochemicals. One of the crucial processes in the production of herbal tea is the drying process. Drying reduces the moisture content of materials inhibiting the growth of bacteria and fungi, as well as a decreasing enzyme activity that could otherwise damage the materials. As a result, it extends the lifetime of the product and its storage capabilities (Ayu, *et al.*, 2017) [3]. Green tea is one of the healthiest herbal teas to drink (Ravi kumar, 2014) [16] and it is made from *Camellia sinensis* leaves and buds that have not undergone the same withering and oxidation process which is used to make Oolong teas (Chinese tea) and black teas. Different flower species have their unique constituent flavour, fragrance and medicinal properties, if evaluated systematically they can be used as one of the ingredients in formulations of herbal beverages.

Materials and Methods: Experiment was carried out at Floriculture laboratory, College of Horticulture, Rajendranagar, Hyderabad, Sri Konda Laxman Telangana state Horticultural University during March-May 2023

Source of experimental material

Flower petals: The flowers of *Rosa bourboniana*, *Clitoria ternatea*, *Cassia auriculata*, *Hibiscus rosa-sinensis* were collected from college farm, College of Horticulture, Rajendranagar, Hyderabad. Petals were carefully separated from the flower petals and subsequently utilized in the experimental studies.

Green tea: Freshly prepared green tea was collected from tea estates of Darjeeling, West Bengal and utilized in the present study.

Preparation of herbal tea

Drying of petals: Fresh petals were collected and cleaned in water. The cleaned petals were then shade dried until the water had evaporated. Subsequently, the petals were placed in a hot air oven at 60 ℃ for a duration of 2-3 hours. Following the drying process, the petals were stored in airtight glass jars to facilitate their preservation for subsequent analysis.

Blending of samples: Green tea leaves and various flower petals were meticulously combined in accordance with the designated treatment combinations. This process involved skillfully blending precise proportions of green tea leaves and specific types of flower petals to create a harmonious and balanced herbal tea composition.

Table 1: Blending ratios of the tea samples

T_1	1-part green tea + 3-parts *Hibiscus rosa-sinensis*
T_2	1-part green tea + 3-parts *Rosa bourboniana*
T_3	1-part green tea + 3-parts *Cassia auriculata*
T_4	1-part green tea + 3-parts *Clitoria ternatea*
T_5	2-parts green tea + 2-parts *Hibiscus rosa-sinensis*
T_6	2-parts green tea + 2-parts *Rosa bourboniana*
T_7	2-parts green tea + 2-parts *Cassia auriculata*
T_8	2-parts green tea + 2-parts *Clitoria ternatea*
T_9	Green tea

Preparation of herbal tea

Tea infusions were prepared by boiling accurately weighed (4 g) samples in 250 ml of water at a temperature of 90°C for 5 minutes.

Total moisture content (%)

Each 100 grams of green tea blended with different flower petals was collected and dried in a hot air oven at 60°C for 2-3 hours. The dried samples were removed then placed in a desiccator to get cooled, re-weighed, placed in the oven, heated, cooled, reweighed repeatedly until a constant weight was obtained. The moisture content was then determined by differences expressed as a percentage of the initial fresh sample weight (A.O.A.C, 1990) [1].

$$\text{Total moisture content } \% = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Fresh weight}} \times 100$$

Total anthocyanin content (mg L^{-1})

Total anthocyanin content was measured by using a spectrophotometric pH differential protocol. The extracts (tea samples) were mixed thoroughly with 0.025 M potassium chloride pH 1 buffer in 1:3 or 1:8 ratio of extract to buffer and the absorbance of the mixture was measured at 520 and 700 nm against distilled water as blank. The extracts were combined similarly with sodium acetate buffer pH 4.5 and the absorbance of these solutions were measured at the same wavelengths (Lee *et al*., 2005) [9]. The anthocyanin content was calculated by using the formula mentioned below and the results were expressed as cyanidin-3- glucoside equivalent in mg/l.

Calculation

Absorbance (A) = (A520nm – A700nm) pH 1.0 – (A520nm – A700nm) pH 4.5

The total anthocyanin content of samples was calculated using the formula given below:

$$\text{Total anthocyanin content (mg L}^{-1}) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l}$$

Where, A = absorbance

MW = Molecular Weight

DF = Dilution Factor

ε = molar extinction coefficient, L x mol^{-1} x cm^{-1}

l = Path length (1 cm)

Antioxidant activity (% inhibition)

Sample extraction

Each 2g dried sample was crushed in pestle and mortar with 20 ml 80% methanol. The extract was then centrifuged at 10,000 rpm at 4 °C for 20 minutes. The supernatant was taken for determination of antioxidant activity by DPPH method.

DPPH free radical scavenging activity: The DPPH assay method is based on the reduction of DPPH, a stable free radical. The antioxidant activity of the extracts was determined using DPPH assay described by Braca *et al*. (2001) [5]. Aqueous extract 0.1 ml was added to 3.9 ml of 0.0025 M DPPH (2, 2-Diphenyl1- picrylhydrazyl) in methanol (70%). The mixture was shaken and allowed to stand for 30 min in dark at room temperature. Absorbance was read at 517 nm using UV spectrophotometer. The percent inhibition of antioxidant activity was calculated by the following formula:

Percent inhibition (%) = [(Ao-Ae)/Ao] ×100
(Ao = absorbance without extract; Ae = absorbance with extract).

Total phenolics content
Sample extraction
2 g each dried sample was crushed in pestle and mortar with 20 ml 80% methanol. The extract was then centrifuged at 10000 rpm at 4 ºC for 20 minutes. The supernatant was taken for determination of total phenolic content.

Method
The total phenol content was estimated based on Folin–Ciocalteau (FC) method (Singleton and Rossi, 1965) [19] with some slight modifications. 0.1 ml of the aliquot was diluted with 1.4 ml of Distilled water to which 0.5 ml of Folin–Ciocalteau (FC) was added and test tubes were shaken well. Then 10 ml of sodium carbonate (20%, w/v) was added. Similarly, this process was done for 0.2, 0.3, 0.4 ml aliquots and sample. These solutions were mixed well and incubated at room temperature for 60 minutes. After incubation, the absorbance was measured at 760nm.
A suitable calibration curve was prepared using gallic acid and the results are expressed in milligram per gram (mg/g) gallic acid equivalent.

$$\text{Mg gallic acid dilution equivalent per gram} = \frac{\text{O.D x Standard curve factor x volume made up}}{\text{Aliquot taken x weight of sample}}$$

Organoleptic scoring
The tea infusions were subjected to sensory evaluation for their acceptability using 9-point hedonic scale for color, flavor, taste and overall acceptability by 10 panelists.

9-point hedonic scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Statistical analysis
The experiment was replicated thrice for each treatment. The design adopted for experiment was completely randomized design concept. The variability was estimated as per procedure for analysis of variance (ANOVA) suggested by Panse and Sukhatme (1954) [13]

Results and Discussion
Total moisture content (%)
Significant difference was observed among the different treatments with respect to total moisture content. The study revealed that significantly lowest total moisture content (6.80%) was recorded in T_4 (1-part green tea + 3-parts *Clitoria ternatea*), followed by 7.26% in T_9 (green tea). Whereas, the maximum total moisture content (13.19%) was observed in T_7 (2-parts green tea + 2-parts *Cassia auriculata*), followed by 12.14% in T_3 (1-part green tea + 3-parts *Cassia auriculata*). Moisture content and water activity are most important parameters for dehydrated products for both their quality and safety. The moisture content steadily increased during the storage period, which could be due to the packaging used, which may not have provided adequate barrier against external environment (Oxygen and water vapour). Temperature and humidity might also be reasons for the increase in the moisture content in all the treatments and moreover, all the dehydrated products are hygroscopic in nature. Similar results were obtained by Costa *et al*. (2013) [21] in passion fruit (*Passiflora edulis f. flavicarpa*) powder. Similar kind of results were also reported by Amol *et al.* (2022) [2] in rose tea and hibiscus tea during the storage period of 60 days and Vyshali *et al.* (2022) [20] in green tea during the storage period of 90 days.

Total anthocyanin content (mg L^{-1})
Significant differences were observed for total anthocyanin content among the different treatments. Among all the treatments, T_1 (1-part green tea + 3-parts *Hibiscus rosa-sinensis)* significantly recorded the maximum total anthocyanin content (47.95 mg L^{-1}), followed by 14.67 mg L^{-1} in T_5 (2-parts green tea + 2-parts *Hibiscus rosa-sinensis*). Significantly, the lowest total anthocyanin content (2.10 mg L^{-1}) was observed in T_9 (green tea) followed by 3.45 mg L^{-1} in T_7 (2 parts green tea + 2- parts *Cassia auriculata*). Anthocyanin is relatively unstable and because of its high reactivity, it may be easily degraded and form colorless or undesirable brown-colored compounds during extraction processing and storage (Durst and Wrolstad, 2001) [7]. Indeed, temperature, pH, light, oxygen, metals, organic acids, sugars, enzymes, sulfur dioxide, co-pigmentation, and interactions with food components may affect both the structure and stability of anthocyanins (Idham *et al*., 2012) [8]. Ramya *et al*. (2021) [14] observed a similar decreasing trend in the total anthocyanin content of hibiscus tea, reporting that the freshly prepared hibiscus tea exhibited an anthocyanin content of 72.13 mg l^{-1}, which gradually decreased to 62.42 mg l^{-1} after 90 days of storage. Likewise, Amol *et al*. (2022) [2] found a comparable decline in the total anthocyanin content of both hibiscus tea and rose tea after 60 days of storage. Their study indicated that the anthocyanin content of hibiscus tea and rose tea on the 0^{th} day was 10.57 mg l^{-1} and 8.73 mg l^{-1}, respectively, which gradually decreased to 6.74 mg l^{-1} and 5.23 mg l^{-1} after 60 days of storage.

Antioxidant activity (% inhibition)
The differences among the different treatments were found to be significant with respect to the antioxidant activity. Among all the treatments, T_1 (1-part green tea + 3-parts *Hibiscus rosa-sinensis*) significantly recorded the maximum antioxidant activity (92.39%), followed by T_5 (2-parts green tea + 2-parts *Hibiscus rosa-sinensis*) (88.31%). While significantly minimum (66.13%) value was recorded in T_7 (2-parts green tea + 2-parts *Cassia auriculata*) followed by 73.81% in T_3 (1-part green tea + 3-parts *Cassia auriculata*). The study results indicate that there were significant differences observed among the various treatments examined in this study. Notably, green tea blended with *Hibiscus rosa-sinensis* petals exhibited the highest antioxidant content. Similar kind of results are also reported Amol *et al*. (2022) [2] and Vyshali *et al.* (2022) [20].

Total phenolics content (mg/100g GAE)

There were significant differences among the treatments for total phenolics content. Among all the treatments, T_1 (1-part green tea + 3-parts *Hibiscus rosa-sinensis*) significantly recorded the maximum total phenolics content (126.24 mg/100g), followed by T_5 (2-parts green tea + 2-parts *Hibiscus rosa-sinensis*) (96.23 mg/100g). Whereas, the significantly minimum total phenolics content (78.47 mg/100g) was noticed in T_8 (2-parts green tea + 2- parts *Clitoria ternatea*) followed by T_4 (1-part green tea + 3-parts *Clitoria ternatea*) (82.20 mg/100g). The highest total phenolic content was observed in green tea blended with hibiscus flower petals, which could be attributed to the high antioxidant activity levels in the respective herbal tea. The study findings unveil a positive correlation between antioxidant activity and total phenolics content. Similarly, a strong positive correlation between antioxidant activity and total phenolics content was also observed in commercial grape juice by Burin *et al.* (2010) [6].

Table 2: Bio-chemical parameters of herbal tea made with different flower petals and green tea

Treatments	Total moisture content (%)	Total anthocyanin content (mg L^{-1})	Antioxidant activity (% inhibition)	Total phenolics content (mg/100g GAE)
T_1 (1-part green tea + 3-parts *Hibiscus rosa-sinensis)*	9.02^f	47.95^a	92.39^a	126.24^a
T_2 (1-part green tea + 3-parts *Rosa bourboniana)*	10.03^e	11.50^c	88.14^{bc}	88.09^e
T_3 (1-part green tea + 3-parts *Cassia auriculata*)	12.14^b	5.27^f	73.81^g	90.88^d
T_4 (1-part green tea + 3-parts *Clitoria ternatea*)	6.80^i	6.29^e	79.38^f	82.20^g
T_5 (2 parts green tea + 2 parts *Hibiscus rosa-sinensis*)	11.09^d	14.67^b	88.31^b	96.23^b
T_6 (2 parts green tea + 2 -parts *Rosa bourboniana*)	11.73^c	9.64^d	84.69^d	95.90^{bc}
T_7 (2 parts green tea + 2- parts *Cassia auriculata)*	13.19^a	3.45^h	66.13^h	87.75^f
T_8 (2 parts green tea + 2- parts *Clitoria ternatea*)	8.34^g	4.17^g	87.20^c	78.47^h
T_9 (Green tea)	7.26^h	2.10^i	83.30^e	93.24^c

Organoleptic scoring

Color

Among the various treatment combinations, a significant difference was observed in the color. Among all the treatments, T_1 (1-part green tea + 3-parts *Hibiscus rosa-sinensis)* registered the highest color score (7.97) which was onpar with T_5 (2 parts green tea + 2-parts *Hibiscus rosa-sinensis)* (7.90) followed by T_8 (2-parts green tea + 2-parts *Clitoria ternatea*) (7.77). The lowest color score was registered in T_7 (2 parts green tea + 2- parts *Cassia auriculata)* (5.73).

Flavor

The differences among the different treatments were found to be significant with respect to the flavor of herbal tea. Among all the treatments, T_5 (2-parts green tea + 2-parts *Hibiscus rosa-sinensis*) recorded the highest score for flavor (7.73) followed by T_8 (2-parts green tea + 2-parts *Clitoria ternatea*) (7.20) which was onpar with T_1 (1-part green tea + 3-parts *Hibiscus rosa-sinensis)* (7.17). whereas, significantly lower score (3.93) for flavor was recorded in T_7 (2-parts green tea + 2-parts *Cassia auriculata*).

Taste

A significant difference was observed in the taste of herbal tea made from different treatment combinations. Among all the treatments, T_4 (1-part green tea + 3-parts *Clitoria ternatea*) recorded the highest score (7.90) for taste, followed by T_1 (1-part green tea + 3-parts *Hibiscus rosa-sinensis*) (7.73). Whereas, the lowest score (3.16) for taste was observed in T_7 (2-parts green tea + 2-parts *Cassia auriculata*) which was onpar with T_3 (1-parts green tea + 3-parts *Cassia auriculata*) (3.25).

Overall acceptability

A significant difference was observed in the overall acceptability of herbal tea made from different treatment combinations. Among all the treatments, T_4 (1-part green tea + 3-parts *Clitoria ternatea*) significantly recorded the highest score (8.17) for overall acceptability followed by T_5 (2 parts green tea + 2-parts *Hibiscus rosa-sinensis*) (7.83). Whereas, significantly lowest score (3.07) for overall acceptability was found in T_3 (1-part green tea + 3-parts *Cassia auriculata*). Similar results were reported by Ramya *et al.* (2021) [14] in hibiscus and green tea infusions. Among all the tea infusions, treatment T_3 (Green tea+ Hibiscus tea freshly prepared, 0-day storage) was mostly preferred by all consumers due to its bright appetizing red color with good flavor, taste and overall acceptability.

Similar results were reported by Amol *et al.* (2022) [2] in hibiscus and green tea infusions. Among all the tea infusions, T_2: P_1S_2- Rose tea (dry petals at 0 days) significantly recorded the highest score (8.30) followed by T_1: P_1S_1- Rose tea (freshly prepared, 0-day storage) (7.80) was mostly preferred by all consumers due to its bright appetizing red color with good flavor, taste and overall acceptability.

Table 3: Organoleptic scoring of herbal tea (9 - point Hedonic scale) made with different blends of flower petals and green tea

Treatments	Color	Flavor	Taste	Over all acceptability
T_1 (1-part green tea + 3-parts *Hibiscus rosa-sinensis)*	7.97^a	7.17^{bc}	7.73^b	7.53^{cd}
T_2 (1-part green tea + 3-parts *Rosa bourboniana)*	7.70^{bc}	5.80^f	6.10^f	5.77^f
T_3 (1-part green tea + 3-parts *Cassia auriculata*)	6.60^e	4.31^g	3.25^g	3.07^h
T_4 (1-part green tea + 3-parts *Clitoria ternatea*)	7.73^{bc}	7.73^a	7.90^a	8.17^a
T_5 (2 parts green tea + 2 parts *Hibiscus rosa-sinensis*)	7.90^{ab}	7.00^c	7.57^c	7.83^b
T_6 (2 parts green tea + 2 -parts *Rosa bourboniana*)	7.40^c	6.17^e	6.10^f	6.37^e
T_7 (2 parts green tea + 2- parts *Cassia auriculata)*	5.73^f	3.93^h	3.16^{gh}	3.88^g
T_8 (2 parts green tea + 2- parts *Clitoria ternatea*)	7.77^b	7.20^b	7.20^d	7.57^c
T_9 (Green tea)	7.27^d	6.50^d	6.30^e	6.70^d

Plate 1: Herbal tea prepared from different blends of flower petals and green tea

Plate 2: Different blends of flower petals and green tea

Conclusion

It is evident from the foregoing research that among various combinations studied, the treatment T_1 (1-part green tea + 3-parts *Hibiscus rosa-sinensis*) recorded the highest total anthocyanin content (47.95 mg L^{-1}), antioxidant activity (92.39%) and total phenolics content (126.24 mg/100g). In contrast, T_4 (6.80%) recorded minimum total moisture content.

In respect with organoleptic scoring, T_4 (1-part green tea + 3-parts *Clitoria ternatea*) significantly recorded the highest score for overall acceptability (8.17) followed by T_5 (7.83).

Future line of work

- Further studies may be carried out by combining herbal tea with other powders *viz*., lemongrass, Amla, ginger, mint etc.
- Further studies may be suggested on increasing the shelf life of herbal tea formulation using proper additives and packaging techniques.
- Studies on the effect of ecofriendly packaging material on herbal tea quality can be taken up.

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