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Assessment of Anti-oxidant potential and consumer acceptability of hibiscus and green tea infusions

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Abstract
Tea is a commonly preferred beverage worldwide for its refreshing quality. In the recent past, herbal tea formulations were gaining importance owing to their medicinal and anti-oxidant benefits. Among the herbal tea formulations, hibiscus tea has potential anti-oxidant qualities. The combination of hibiscus and green tea formulations have enhanced benefits both in terms of quality and health aspects. The consumer demand for such tea formulations was also rising in the past few years. Hence the present study was undertaken with the objective of preparation of hibiscus tea and to assess the anti-oxidant potential in combination with green tea formulations. Seven treatments comprising of tea formulations from shade dried red single cultivar of Hibiscus flowers were prepared in combination with green tea and stored at different intervals. The total anthocyanin content, anti-oxidant potential and colour value of the tea infusions were assessed. Among the different infusions highest anthocyanin content was recorded in hibiscus tea while the anti-oxidant potential was significantly higher in green tea followed by green tea + hibiscus tea infusions. The consumer acceptability was higher for green tea + hibiscus tea blend owing to the anthocyanin colour and flavour.

Keywords: Hibiscus and green tea infusions, antioxidant potential, colour analysis

Introduction
India is blessed with a rich heritage of traditional medical systems and is called as "Botanical Garden of the World", with an extensive wealth of biodiversity. In this modern era, the knowledge and experience of the usage of herbs were blend with advanced technologies to develop a safe and healthier herbal products (Goutam *et al.*, 2018) [6]. Green tea is one of the ancient beverages in China, being popular among researchers and public due to its various properties like antioxidant, anticarcinogenic, antihypertensive and anti-obesity (Cabrera *et al.*, 2006) [3]. Green tea consumption is associated with mild to moderate effects on four major global killers *i.e.,* cancer, stroke, type II diabetes and atherosclerosis-related cardiovascular diseases as per meta-analysis reports (Johnson *et al.*, 2012) [8]. The medicinal qualities and flavour of green tea can be enhanced by combining with any of the plants rich in anti-oxidant quality (Farooq and Sehgal, 2019) [5].
Green tea is easier to prepare and remains light, effective, inexpensive, caffeine and drug-free with rich taste and health benefits (Killedar *et al.*, 2017) [10]. *Hibiscus rosa sinensis* is one of the potential plants rich in antioxidant, antidiabetic, antimicrobial, antifertility, antiulcer, hepatoprotective and anti-inflammatory properties (Khristi and Patel, 2016) [9]. Among different varieties, red coloured single row cultivar of hibiscus flowers possesses a greater number of bioactive compounds. Hibiscus tea is prepared by dried flower petals and consumed by a large number of patients to control hypertension and hyperlipidaemia (McKay *et al.*, 2010) [16]. Hibiscus tea is in bright red colour due to the presence of anthocyanins and antioxidants which enhance the health promoting factors. The present study was carried out to investigate the antioxidant potential, total phenols, anthocyanin and sensory analysis of green tea, hibiscus tea and their combination (1:1) after a storage interval of 0 day, 45th day and 90th day of tea powder preparation and tea infusions were freshly prepared before analysis.

Methodology
This experiment was undertaken at the Department of Floriculture and Landscaping, Horticultural College and Research Institute, Coimbatore with the objective of scrutinizing the potential benefits of green tea, hibiscus tea and its combinations followed by ther consumer acceptance of tea infusions. The treatment details were mentioned below
T1: Green tea (Control)

T2: Hibiscus tea (freshly prepared, 0 day storage)
T3: Green tea + Hibiscus tea [1:1] (freshly prepared, 0 day storage)
T4: Hibiscus tea (stored for 45 days)
T5: Green tea + Hibiscus tea [1:1] (stored for 45 days)
T6: Hibiscus tea (stored for 90 days)
T7: Green tea + Hibiscus tea [1:1] (stored for 90 days)

Preparation of tea infusions
Petals of *Hibiscus rosa-sinensis* were collected and shade dried after cleaning. Tea infusions were prepared by boiling an accurately weighed (3 g) samples in 200 ml of water at a temperature of 90 ° C for 10 min. Three different tea infusions were prepared *viz*., green tea, hibiscus tea and hibiscus + green tea blend. The hibiscus green tea was prepared using 1.5 g of green tea leaves and 1.5 g of dried hibiscus flower petals. The tea infusions were freshly prepared and used for further analysis.

Antioxidant potential - ABTS radicle scavenging activity assay
ABTS radicle scavenging activity assay was performed using the method described by (Re *et al.*, 1999) [18] with slight modifications. ABTS radicle cation mixture was prepared by mixing ABTS (7 mM) with potassium per sulphate (2.45 mM) and kept in dark overnight. The working solution was then diluted with methanol to get an absorbance of 0.700 (±0.02). 3 ml aliquot of working solution was added with 100 µl of the extract at different concentrations (100 –500 µg/ml). The absorbance was measured at 734 nm after 6 min of incubation. Ascorbic acid was used as standard and the antioxidant capacity of the extract was expressed as IC_{50} *i.e.* the concentration required to inhibit 50% of the ABTS free radicle. The per cent inhibition was measured using the following formula.

$$\%\ inhibition = \frac{initial\ absorbance - final\ absorbance}{initial\ absorbance} x100$$

DPPH radicle scavenging activity assay
DPPH assay was carried out using the method of (Brand-Williams *et al.*, 1995) [2]. A stock solution of 0.1 mM DPPH in methanol was prepared and diluted to get an absorbance of about 0.980 (±0.02). 3 ml aliquot of working solution was added with 100µl of the extract at different concentrations (100 – 500 µg/ml). The reaction mixture was mixed well and then incubated in dark for 15 min. The absorbance was measured at 517 nm after incubation. Ascorbic acid was used as standard. The per cent inhibition was calculated using the following formula. The antioxidant capacity of the test samples was expressed as IC_{50}, the concentration necessary for 50% reduction of DPPH.

$$\%\ inhibition = \frac{initial\ absorbance - final\ absorbance}{initial\ absorbance} x100$$

Total phenol content
The total phenol content was estimated based on Folin - Ciocalteu (FC) method with little changes. About 0.2 ml of sample was pipetted into a test tube, where 3.3 ml of distilled water and 500 µl of Folin – Ciocalteu reagent were added. After 8 min of incubation at room temperature, 1 ml of sodium carbonate solution (20 %) was added. The mixture was incubated for 30 min at room temperature. The absorbance was read by calorimeter at 640 nm. The phenolic content of the sample was expressed in mg/g of the sample.

Monomeric anthocyanin content
Monomeric anthocyanin content was measured using a spectrophotometric pH differential protocol. The extracts were mixed thoroughly with 0.025 M potassium chloride adjusted to pH 1, in the ratio of 1:3 or 1:8 and then measured at 515 and 700 nm against distilled water as blank. The extracts were combined similarly with sodium acetate buffer of pH 4.5, and the absorbance was measured at the same wavelength (Lee *et al.*, 2005) [13]. The anthocyanin content was calculated using the formula mentioned below and the results were expressed as cyanidin-3-gluciside equivalent in mg/l.

$$\text{Total monomeric anthocyanin } (mg/l \text{ c 3 g eq.}) = \frac{(Aabs * MWt * DF * 103)}{(e * l)}$$

Where,
Abs (Absorbance) = (A520nm – A700nm) pH 1.0 – (A520nm – A700nm) pH 4.5
MWt (Molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu)
DF = Dilution factor
e = Molar extinction coefficient of cyanidin-3-glucoside (26 900 L mol $^{-}$1 cm^{-}1)
l = Path length of cuvette in cm
10^3 = Conversion Factor (g to mg)

Colour analysis
The tea infusions were subjected to colour analysis by using chromometer (3 nh spectrophotometer). Tea infusions were placed individually in specimen cell for measurements. L*, a*, b* values were displayed in the screen in which L* value indicates the lightness or darkness (lower value= darkness; higher value= lightness). The shift from negative to positive values was indicated by colour change from bluish-green to purplish-red in a* value and from blue to yellow in b* value.

Sensory analysis
The tea infusions were subjected to sensory evaluation. Each of 10 panellists were presented with tea infusions and a questionnaire to evaluate product attributes (colour, flavour, taste and overall acceptability) with a 5-point hedonic scale ranging from 1, being not acceptable to 5, highly acceptable and the preferred product was identified. The tea infusions were freshly prepared before serving.

Statistical tools
Statistical design (Completely randomized design) was performed on computer using the statistical package SPSS. Data were analysed by one-way analysis of variance (ANOVA) and the results were expressed in Mean ± SD. IC_{50} values were calculated using the statistical software, graph pad prism 5.0.

Results and Discussion
The tea infusions of green tea, hibiscus tea and its combination were evaluated on different storage days for antioxidant potential, monomeric anthocyanin content and phenolic content. IC_{50} value of each aqueous infusion was calculated in the performed antioxidant parameters. The lower the IC_{50} value, higher is the antioxidant potential of the

infusion.

Antioxidant potential of tea infusions

The reducing capacity of aqueous tea infusions to stable, non-biological radicals were investigated *via*., DPPH and ABTS assays. ABTS is an unstable, coloured, free radical and can be dissolved both in aqueous and organic phases to find the antioxidant activities of both hydrophobic and hydrophilic antioxidants of food extracts (Kim *et al.*, 2002) [11]. The highest ABTS radical scavenging activity of 201.56 was recorded in control (Green tea). It was followed by the treatments T_3, T_5, T_7, T_2 and T_4 with the values of 249.19, 292.77, 335.80, 757.88 and 798.90 respectively, and lowest scavenging activity was recorded in the treatment T_6 which contains Hibiscus tea (stored for 90 days), with the value of 828.82. Ascorbic acid used as a standard with an IC_{50} value of 81.26. An earlier report has explained that among 112 aqueous extracts of Chinese medicinal plants studied, green tea exhibited the sixth highest ABTS radical scavenging capacity (Cai *et al.*, 2004) [4].

DPPH radical scavenging efficacy of tea infusions resulted in the following order of anti-oxidant activity of the treatments T_1 [Green tea (control)] (395.68) >Treatment T_3 (733.22) >Treatment T_5 (802.25) >Treatment T_7 (875.87)> T_2 (1354.79) >Treatment T_4 (1428.74) >Treatment T_6 [Hibiscus tea (stored for 90 days)] (1507.52) as mentioned in Table.2 [IC50 of standard ascorbic acid:54.43]. Green tea leaf extract was found to be more effective in ameliorating phenyl hydrazine-mediated lipid peroxidation of erythrocytes when compared to ascorbic acid (Biswas *et al.*, 2005) [1].

Green tea as pure infusion exhibited significant ABTS and DPPH radical scavenging assays and it was succeeded by treatment T_3 which comprised of Green tea + Hibiscus tea [1:1] (freshly prepared, 0 day storage). This result is in accordance with (Farooq and Sehgal, 2019) [5] who reported that hibiscus showed the highest free radical scavenging activity than other herbal teas such as OG tea (*Ocimumgratissium*), CC tea (*Cymbopogon citratus*), CF tea (*Cymbopogon flexuosus*). Moreover, in ABTS and DPPH assays, combination of green tea+hibiscus tea infusions exhibited highest antioxidant potential than the infusions of GT+OG (*Ocimumgratissium*), GT+CC (*Cymbopogon citratus*) and GT+CF (*Cymbopogon flexuosus*). Previous research reports also confirmed the additive antioxidant interaction of green tea+hibiscus tea extracts in ABTS and DPPH assays (Fig. 1). Combining different medicinal plants showed higher antioxidant potential than using an individual plant(Yang *et al.*, 2009). The strength of combination of herbs not only increase by increase in concentration of individual herbs but also by the interaction between various herbs with different pharmacological properties (Jia *et al.*, 2004) [7]. The interaction may occur in the phytochemicals of whole extract of a single herb or between different herbs in a combination. Therefore, consuming Green tea + Hibiscus tea (freshly prepared) infusions will result in additional health benefits than the benefit of consuming green tea alone.

Table 1: ABTS and DPPH radical scavenging activity of different tea infusions

T. No.	Treatments	ABTS (IC_{50} µg/ml)	DPPH (IC_{50} µg/ml)
T_1	Green tea (control)	201.56^{g}	395.68^{g}
T_2	Hibiscus tea (freshly prepared, 0 day storage)	757.88^{c}	1354.79^{c}
T_3	Green tea + Hibiscus [1:1] (freshly prepared, 0 day storage)	249.19^{f}	733.22^{f}
T_4	Hibiscus tea (stored for 45 days)	798.90^{b}	1428.74^{b}
T_5	Green tea + Hibiscus [1:1] (stored for 45 days)	292.77^{e}	802.25^{e}
T_6	Hibiscus tea (stored for 90 days)	828.82^{a}	1507.52^{a}
T_7	Green tea + Hibiscus (90d) [1:1] (stored for 90 days)	335.80^{d}	875.87^{d}

Results expressed were the mean of three individual replicates (n = 3). Different letters expressed significant difference ($p \leq 0.05$) among the EC_{50}values of different infusion types. Lower the EC_{50}, higher is the antioxidant potential of the infusion.

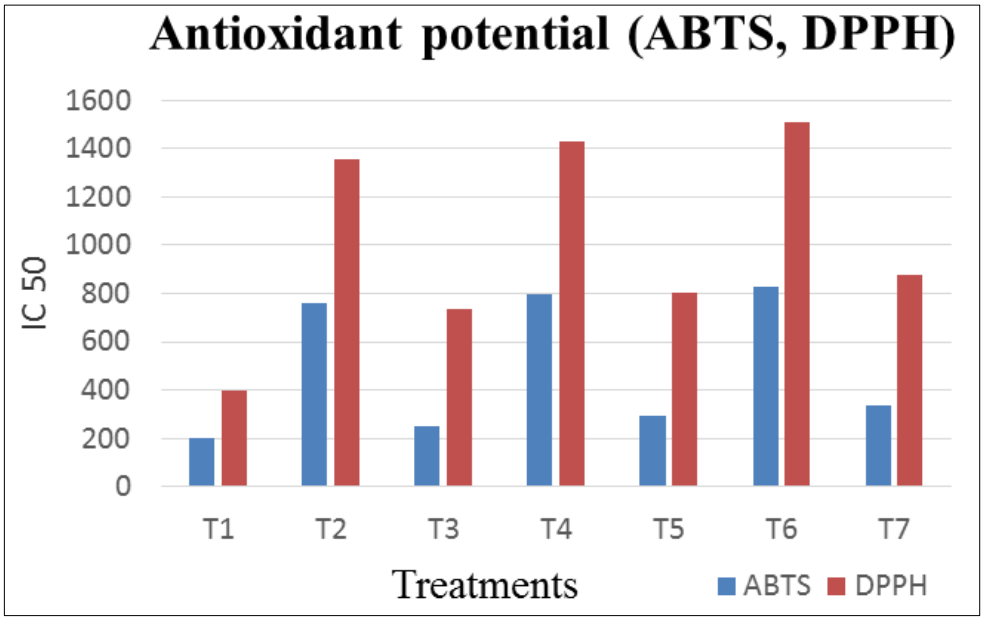

Fig 1: Comparison of antioxidant activity of different tea infusions

Estimation of total phenol content

The total phenol content was calculated on the basis of gallic acid standard curve (R^2= 0.991) and the results are given in table 2 and fig. 2. Among the seven treatments, total phenol and total anthocyanin content was significantly higher in green tea infusion (120.72 mg GAE/g). This was followed by Green tea+Hibiscus tea (freshly prepared, 0 day storage) with the value 82.57 mg GAE/g. Least phenol content was found in hibiscus tea (stored for 90 days) with the value 55.08 mg GAE/g. Freshly prepared extracts exhibited higher total phenol content when compared to stored samples for up to 90 days. There was a gradual decline in phenol content with respect to storage days.

Similar result was produced by (Farooq and Sehgal, 2019) [5] with the manifestation of highest total phenol content by green tea + hibisucs tea than green tea + cymbopogon tea. The aqueous extract of *H. rosasienensis* exhibited the highest total phenol content of 54.36 mg/g than ethanol extract of *H. rosa sinensis* (45.98 mg/g).The main class of phenolic compounds responsible for antioxidant activity of green tea is Catechins such as epigallocatechin gallate (EGCG), epigallocatechin (ECG) and epicatechin gallate (ECG) (Lee *et al.*, 2014) [14]. The individual infusion of hibiscus tea exhibited highest total phenol content than cymbopogon tea (*Cymbopogon citratus*).

The total phenol content of the tea infusions increased with decreased EC_{50} value of ABTS and DPPH, which eventually resulted in an intensified antioxidant radical scavenging activity. The combined effects of green tea and hibiscus tea extracts enhanced the total phenolic contents and thereby increased the antioxidant potential in freshly dried petals of green tea + hibiscus tea infusions.

Table 2: Estimation of total phenol content in different tea infusions

T. No.	Treatments	Total phenol content (mg GAE/g)
T_1	Green tea (control)	120.72[a]
T_2	Hibiscus tea (freshly prepared, 0 day storage)	64.79[e]
T_3	Green tea + Hibiscus [1:1] (freshly prepared, 0 day storage)	82.57[b]
T_4	Hibiscus tea (stored for 45 days)	60.35[f]
T_5	Green tea + Hibiscus [1:1] (stored for 45 days)	78.72[c]
T_6	Hibiscus tea (stored for 90 days)	55.08[g]
T_7	Green tea + Hibiscus (90d) [1:1] (stored for 90 days)	72.12[d]

Results expressed were the mean values (n = 3). Mean values followed by different letters in a row were significantly different ($p \leq 0.05$) from each other.

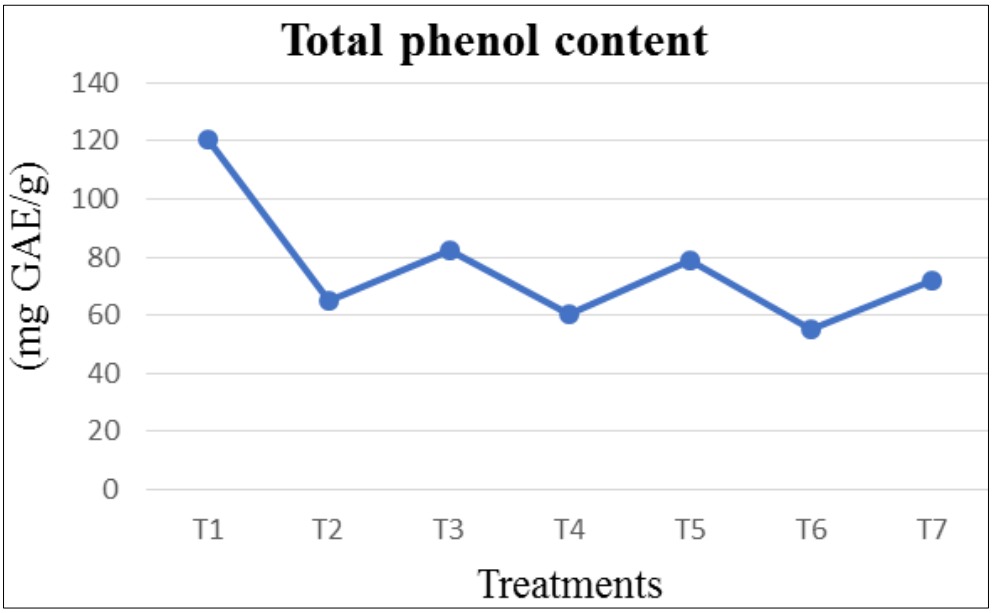

Fig 2: Total phenolic content of different tea infusions

Total anthocyanin content and colour analysis

Among the seven treatments, treatment T_2 which contains Hibiscus tea (freshly prepared, 0 day storage), exhibited the highest anthocyanin content of 72.13 mg c3g eq./l and it was followed by the treatments T_4 and T_6 with 69.11mg c3g eq./l and 62.42 mg c3g eq./l respectively. The anthocyanin content was found to decrease gradually when dried petals were stored longer. The treatment T_3 which comprises of green tea+hibiscus tea (freshly prepared, 0 day storage), reported an anthocyanin content of 37.14 mg c3g eq./l followed by treatments T_5 andT_7 with values of 33.63 mg c3g eq./l and 30.43 mg c3g eq./l respectively as mentioned in the Table 3 and Figure 3.

The petals of *Hibiscus rosa sinensis* is rich in anthocyanin pigment which provides the antioxidant activity (Mak *et al.*, 2013) [15]. In addition, they are natural bio-pigments with anti-bacterial properties as well and can be used as natural food colorants (Naz *et al.*, 2007) [17]. The total phenol content and total anthocyanin content are good indicators of *in vitro* antioxidant activity (Kong *et al.*, 2003) [12]. Strong correlation has been reported between total phenol content and total anthocyanin content as an increase in phenol content of tea infusions has in turn recorded an increased anthocyanin content.

Table 3: Evaluation of monomeric Anthocyanin content of different tea infusions

T. No.	Treatments	Monomeric anthocyanin content (c3g eq.mg/l)
T_1	Green tea (control)	1.0[g]
T_2	Hibiscus tea (freshly prepared, 0 day storage)	72.13[a]
T_3	Green tea + Hibiscus [1:1] (freshly prepared, 0 day storage)	37.14[d]
T_4	Hibiscus tea (stored for 45 days)	69.11[b]
T_5	Green tea +Hibiscus [1:1] (stored for 45 days)	33.63[e]
T_6	Hibiscus tea (stored for 90 days)	62.42[c]
T_7	Green tea + Hibiscus (90d) [1:1] (stored for 90 days)	30.43[f]

Values expressed were the mean values (n = 3). Mean values followed by different letters in a row were significantly different ($p \leq 0.05$) from each other.

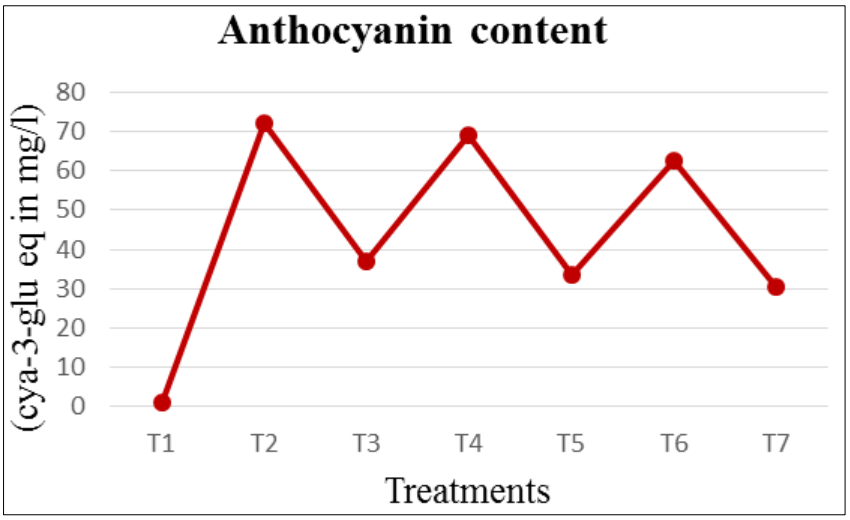

Fig 3: Monomeric anthocyanin content of different tea infusions

The total anthocyanin content is also correlated with colour values of tea infusions. L*, a*, b* values of tea infusions were calculated using 3nh chromometer. L* measures the degree of lightness and darkness of the sample (lower value=darkness; higher value= lightness). Among the different tea infusions, treatment T_2 which contains Hibiscus tea (freshly prepared, 0 day storage) resulted in the lowest L* value (18.60) which indicate more darkness compared to other infusions due to the presence of red pigment anthocyanin and the values gradually increased with the storage life of hibiscus. The highest L* value was recorded in the control which contains green tea and indicated by the lightness of the infusion. The treatments involving the combination of green tea + hibiscus tea with variation in storage days *i.e.*, treatments T_3, $T_5$and T_7 recorded a rise in L* value and drop in anthocyanin pigment. On the other hand, the shift from negative to positive values was indicated by colour change from bluish-green to purplish-red in a* value and from blue to yellow in b* value. The a* values were positive and indicated by purplish-red colour due to the presence of anthocyanin pigment in all treatments except control. On the other end, a* value was negative and indicated by bluish green colour due to the lack of anthocyanins in green tea. Similarly, b* values of all tea infusions were negative and indicated by blue colour in all treatments except control. But the control which is green tea recorded a positive value indicated by yellow colour. The colour values of all the tea infusions were furnished in Table 5.

Table 4: Colour analysis of different tea infusions as indicated by L*, a*, b* values

	Treatments	L*	a*	b*
T_1	Green tea (control)	26.84	- 0.28	0.71
T_2	Hibiscus tea (freshly prepared, 0 day storage)	18.60	0.67	-0.45
T_3	Green tea + Hibiscus [1:1] (freshly prepared, 0 day storage)	19.59	0.58	-0.38
T_4	Hibiscus tea (stored for 45 days)	18.89	0.66	-0.44
T_5	Green tea + Hibiscus [1:1] (stored for 45 days)	20.02	0.55	-0.37
T_6	Hibiscus tea (stored for 90 days)	18.95	0.66	-0.43
T_7	Green tea + Hibiscus (90d) [1:1] (stored for 90 days)	21.34	0.54	-0.35

Organoleptic scoring of tea infusions: All the tea infusions were prepared freshly and their organoleptic scorings were recorded based on the scores given by 10 panellists. The scores are presented in Table 6. Among the seven treatments conducted, the highest score of 4.5 for colour was obtained in treatment T_2 Hibiscus tea (freshly prepared, 0 day storage). The least score of 3.6 for colour was obtained by green tea. Other than colour, the preference for flavour and taste was highest for T_3 which comprised of green tea+ hibiscus tea (freshly prepared, 0 day storage) with scores of 4.2 and 4.3 respectively. Among all the tea infusions, treatment T_3, which comprised of Green tea+ Hibiscus tea (freshly prepared, 0 day storage) was mostly preferred by all consumers due to its bright appetising red colour with good flavour and taste.

Table 5: Organoleptic scoring of tea infusions

T. No.	Treatments	Colour	Flavour	Taste	Overall Acceptability
T_1	Green tea (control)	3.6	3.7	3.5	3.7
T_2	Hibiscus tea (freshly prepared, 0 day storage)	4.5	3.8	3.8	3.9
T_3	Green tea + Hibiscus [1:1] (freshly prepared, 0 day storage)	4.2	4.2	4.3	4.2
T_4	Hibiscus tea (stored for 45 days)	4.4	3.7	3.7	3.9
T_5	Green tea + Hibiscus [1:1] (stored for 45 days)	4.0	4.0	4.2	4.0
T_6	Hibiscus tea (stored for 90 days)	4.3	3.7	3.7	3.8
T_7	Green tea + Hibiscus (90d) [1:1] (stored for 90 days)	4.0	4.0	4.2	4.0

Scores were average values based on the panel of 10 judges with scoring for colour, flavour, taste and overall acceptability. Scoring details were furnished below:

Scale	Score Range	Acceptability Level
1	4.50 – 5.00	Highly acceptable
2	3.50 – 4.49	Acceptable
3	2.50 – 3.49	Moderately acceptable
4	1.50 – 2.49	Slightly acceptable
5	1.00 – 1.49	Not acceptable

Conclusion

Among all the tea infusions, green tea exhibited high radical quenching ability and total phenol content due to the presence of catechins, while hibiscus tea possesses highest anthocyanin content and an appreciable level of anti-oxidant value. The consumer acceptability was highest for green tea + hibiscus tea infusions owing to the colour, flavour and taste. Considering the above facts, aqueous infusions of hibiscus with green tea are showcased as potential health drinks with enhanced health benefits when used in combination. Hence this paper opens up the real scope of need to commercialize the combination of green tea and hibiscus tea as a health drink in addition to other beverages.

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