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Optimization of the Extraction of Antioxidant Compounds from Hibiscus Petal Tea

SG Mengade[1], SM Jogdand*[1], GB Kadam[2], MB Shete[3], AA Bhagat[3]

[1]Department of Horticulture, College of Agriculture, Pune, Maharashtra, India
[2]ICAR-IIFR, Pune, Maharashtra, India
[3]AICRP on Floriculture, ZARS, Ganeshkhind, Pune, Maharashtra, India

Abstract

Ornamental flowers are seen as a rich source of food and antioxidant compounds. Flowers with higher total antioxidant activity are *Bougainvillea glabra*, *Tagetes erecta*, *Cosmos sulphureus*, *Hibiscus rosa sinensis*. The *Hibiscus spp.* have several medicinal properties and is rich in antioxidant compounds. The hibiscus flower petals have been used in many herbal mix tea and drinks. In the present investigation to standardize the protocol for hibiscus petal tea infusions 50ºC, 60ºC and 70ºC drying temperature, 1g, 1.5g and 2 g quantity and 90 sec, 120 sec and 180 sec dipping durations were selected as independent variables. The treatment combination of 60ºC drying temperature, 2 g quantity used and 120 seconds dipping duration obtained as optimized combination of selected independent variables which gives infused tea with high amount of antioxidant activity (88.38% and 0.37 µmol TE/g) along with superior aroma, taste and good colour retention up to 90 days of storage time.

Keywords: Hibiscus tea, ABTS, DPPH, Organoleptic analysis

***Correspondence**
Author: Sunil Jogdand
Email: sunilraojogdand@gmail.com

Introduction

Hibiscus rosa sinensis is a perennial shrub widely cultivated in tropics of the world. In India different parts of plant like leaves, flowers and roots had been known for medicinal values like aphrodisiac, hemorrhagic and laxative properties. It has been used in many herbal mix and drinks. The hibiscus petal tea is rich source of antioxidants which has the potential to reduce reactive oxygen species and cholesterol. Antioxidants can stabilize free radicals before they attack cells, making them essential for maintaining optimal cellular and overall health and well-being. The antioxidant activity in foodstuffs is carried out using ET (Electron transfer) based assay and HAT (Hydrogen atom transfer) based assay. In ET-based assays, the antioxidants react with an oxidizing agent instead of peroxyl radicals. Spectrophotometric ET-based assays measure the capacity of an antioxidant in the reduction of an oxidant, which changes color when reduced. The degree of color change (either an increase or decrease of absorbance of the probe at a given wavelength) is correlated to the concentration of antioxidants in the sample. Basically, ET-based assays include ABTS assay, DPPH assay, etc. [1]

Material and Methods

Material

Fully opened hibiscus flowers were harvested from the hibiscus shrubs garden located in the campus of College of Agriculture, Pune (Maharashtra, India, N 18° 32’ 17.27”, E 73°50’ 37.21”). The freshly harvested flowers were cleaned, petals were separated from other floral parts and collected in cleaned trays, allowed for further drying in hot air oven at selected experimental conditions.

Sample collection and treatment details

Petals were dried in hot-air oven till the moisture reaches at its equilibrium moisture content (EMC). The initial moisture content of the hibiscus petals was 86 %; while after drying it reaches up to 2 % with 12 % of remained dry matter. The samples were stabilized for constant weight for three days and then utilized for tea preparation and further analysis (Figure 1).

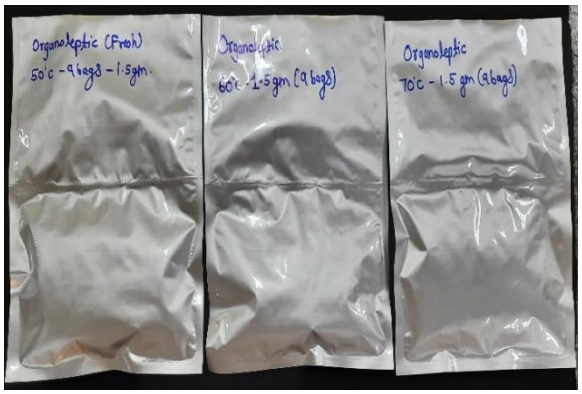
Fresh Hibiscus Flowers

Petals separated from flowers

Drying of petals in Hot air oven

Dried petals

Tea bags



Storage of tea bags

Hibiscus petal tea

Figure 1. Representative experimental setup.

The experimental plan with treatment details is presented in table:

Factors			
Factor A (Temperature for drying of petals in ºC for 24 hr)	a_1=50 ºC	a_2= 60 ºC	a_3= 70 ºC
Factor B (Quantity required for tea preparation in grams)	b_1= 1.0 g	b_2= 1.5 g	b_3= 2.0 g
Factor C (Duration in seconds)	c_1=90 sec	c_2=120 sec	c_3= 180 sec

Determination of bioactive compounds

Antioxidant activity analysis

DPPH radical scavenging Assay: The DPPH radical scavenging assay method was used to determine antioxidant capacity [2].

$$\text{Inhibition}\% = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100$$

Where, $A_{control}$=Absorbance of DPPH, A_{sample}= Absorbance of sample

ABTS radical scavenging assay: The antioxidant capacity was determined by Trolox equivalent antioxidant capacity (TEAC) method [3].

$$\text{Inhibition}\% = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100$$

where, $A_{control}$=Absorbance of ABTS, A_{sample}= Absorbance of sample

$$\text{Trolox (µmol TE/g)} = \frac{\text{Trolox (µmol/L)}}{\text{Sample (g/L)}}$$

Organoleptic Observations

Acceptance tests were conducted on seven sample infusions using ten panelists. Sample infusions were three-digit coded and served randomly to the panelists. About 30 ml of each infusion was served in transparent glass. One sample was served at a time. The nine-point hedonic scale was used for organoleptic analysis of colour, taste, aroma and overall acceptability of hibiscus petal tea.

Statistical analysis

The data was recorded and analyzed by the technique of analysis of variance and the test of significance was carried out [4].

Statistical model of factorial completely randomized design:

$$Y_{ijk} = \mu + A_i + B_j + C_k + A_iB_j + A_iC_k + B_jC_k + A_iB_jC_k + e_{ijk}$$

where, Y_{ijk} = Hibiscus petal tea from k^{th} duration (sec), j^{th} quantity (g), and i^{th} temperature (º C)

µ = overall mean
A_i = effect due to i^{th}temperature (º C)
B_j= effect due to j^{th} quantity (g)
C_k= effect due to k^{th} duration (sec)
A_iB_j= interaction effect due to j^{th} quantity (g) and i^{th}temperature (º C)
A_iC_k= interaction effect due to k^{th} duration (sec) and i^{th}temperature (º C)
B_jC_k= interaction effect due to k^{th} duration (sec) and j^{th} quantity (g)
$A_iB_jC_k$= interaction effect due to k^{th} duration (sec), j^{th} quantity (g) and i^{th}temperature (ºC)
e_{ijk}= error term

Where, e_{ijk}~N (0, σ^2)

$$\Sigma A_i = 0, \Sigma B_j = 0, \Sigma C_k = 0, \Sigma A_i B_j = 0, \Sigma A_i C_k = 0, \Sigma B_j C_k = 0, \Sigma A_i B_j C_k = 0$$

Result and discussion

The significant effect of drying temperature, petal quantity used and dipping duration were found on bioactive compounds and organoleptic observations of hibiscus petal tea with respect to the storage. The experimental data recorded during the investigation is presented in **Table 1**.

Table 1 Effect of drying temperature, petal quantity used and dipping duration on total antioxidant activity (DPPH and ABTS) of hibiscus petal tea infusions at 0, 45, and 90 days

Treatment	Antioxidant activity (DPPH)			Antioxidant activity (ABTS)		
	0 day	45 days	90 days	0 day	45 days	90 days
Drying temperature						
a_1 (50ºC)	86.60^a	84.03^a	82.50^a	0.39^a	0.32^a	0.32^a
a_2 (60ºC)	89.1^b	86.35^b	85.23^b	0.39^a	0.32^a	0.32^a
a_3 (70ºC)	89.36^b	87.29^c	85.76^b	0.41^b	0.33^b	0.32^a
SE (m) ±	0.36	0.18	0.29	0.002	0.001	0.000
CD (at 1%)	1.39	0.70	1.11	0.008	0.002	0.002
Quantity required						
b_1(1.0 g)	87.54^a	84.32^a	81.42^a	0.28^a	0.22^a	0.22^a
b_2(1.5 g)	88.59^a	86.08^b	85.82^b	0.37^b	0.30^b	0.30^b
b_3(2.0g)	88.93^a	87.26^c	86.26^c	0.53^c	0.45^c	0.45^c
SE (m) ±	0.36	0.18	0.29	0.002	0.001	0.000
CD (at 1%)	1.39	0.70	1.11	0.008	0.002	0.002
Dipping duration						
c_1(90 Sec.)	87.12^a	84.68^a	81.98^a	0.38^a	0.32^a	0.33^a
c_2 (120 Sec.)	89.09^b	86.94^c	86.66^c	0.37^a	0.32^a	0.32^a
c_3(180 Sec.)	88.85^b	86.04^b	84.85^b	0.43^b	0.33^b	0.33^b
SE (m) ±	0.36	0.18	0.29	0.002	0.001	0.000
CD (at 1%)	1.39	0.70	1.11	0.008	0.002	0.002

Note: Treatment having common super script are statistically non-significant otherwise significant.

Antioxidant activity (DPPH)

The radical scavenging activity is used to evaluate the capacity of tissue to scavenge free radicals, which mainly functions of bioactive compounds present in the tissue. The drying temperature 70ºC recorded highest antioxidant activity at 0, 45 and 90 days (89.36%, 87.29 %, 85.76%), whereas it was at par with 60ºC drying temperature at 0 and 90 days. The drying temperature 50ºC recorded significantly highest antioxidant activity at zero, 45 and 90 days (86.60 %, 84.03 %, 82.50 %) respectively (Table 1), with the increase in total phenols content DPPH radical scavenging in tea infusions increases [5]. The 2.0 g of petal quantity used recorded high antioxidant activity during storage (88.93 %) and was significantly superior over petal quantity 1.5 g and 1.0 g at 45 and 90 days whereas at zero days all quantity used were at par with each other (Table 1). Similar findings were reported by [6] in roselle tea. The antioxidant activity of hibiscus tea with dipping duration of 120 seconds (89.09 %) was at par with 180 seconds at 0 day storage, whereas it was significantly superior over the rest of treatments at 45 (86.94 %) and 90 (86.66 %) days of storage. This might be due to the optimum time exposure at 120 seconds. The [7] reported that longer brewing time produced an increase in antioxidant activity of green tea infusions this might be due to increased solubility of phenolic compounds with an increase in time.

The total antioxidant activity (DPPH) ranged from 69.90 – 90.53 % during storage. There was significant interactive effect between drying temperature, quantity used and dipping duration of petals on antioxidant activity of tea at zero days (**Table 2**, **Figure 1**). The [8] reported there is high capacity to neutralize radicals when sample was dried at 60ºC thus the treatment combination of 60ºC drying temperature, 1.5 g quantity used and 90 seconds of dipping duration recorded high antioxidant activity (90.53 %) whereas the treatment combination of 50ºC drying temperature, 1.0 g quantity used and 90 seconds of dipping duration recorded least antioxidant activity (79.75 %). During storage at 45 days, the treatment combination of 60ºC drying temperature, 2.0 g quantity used and 90 seconds

of dipping duration was superior over other interactions and maintained maximum antioxidant activity (89.44 %) while minimum antioxidant activity (78.39 %) was recorded by the treatment combination of 50ºC drying temperature, 1.0 g quantity used and 90 seconds of dipping duration, findings were in close context to *Centella asiatica* tea as observed by [9]. Similarly, for 90 days storage the treatment combination of 50ºC drying temperature, 1.5 g quantity used and 120 seconds of dipping duration was superior over other treatments and maintained high antioxidant activity (89.03 %) this might be due to synergistic effect of drying temperature, quantity used and dipping duration.

Table 2 Interaction effect of temperature, quantity, and dipping duration on antioxidant activity (DPPH) at zero, 45 and 90 days

Int. A x B x C	Mean		
(Temp. x Qty. x Duration)	Zero days	45 days	90 days
$a_1b_1c_1$	79.75^{a}	78.39^{a}	69.90^{a}
$a_1b_1c_2$	85.74^{ab}	80.40^{ab}	80.17^{c}
$a_1b_1c_3$	87.94^{c}	84.90^{de}	82.05^{cd}
$a_1b_2c_1$	82.32^{ab}	81.99^{bc}	80.22^{c}
$a_1b_2c_2$	89.37^{c}	85.79^{efgh}	89.03^{j}
$a_1b_2c_3$	87.94^{c}	85.24^{ef}	84.90^{defg}
$a_1b_3c_1$	89.82^{c}	84.79^{de}	84.90^{defg}
$a_1b_3c_2$	88.45^{c}	87.58^{ghijk}	84.62^{defg}
$a_1b_3c_3$	88.09^{c}	87.18^{fghij}	86.74^{fghij}
$a_2b_1c_1$	87.02^{c}	84.79^{de}	75.12^{b}
$a_2b_1c_2$	88.88^{c}	87.58^{ghijk}	87.08^{fghij}
$a_2b_1c_3$	89.77^{c}	86.54^{efghi}	84.09^{def}
$a_2b_2c_1$	90.53^{d}	86.79^{efghij}	86.59^{fghij}
$a_2b_2c_2$	90.23^{d}	88.89^{jk}	88.65^{ij}
$a_2b_2c_3$	89.00^{c}	86.35^{efgh}	84.90^{defg}
$a_2b_3c_1$	89.47^{c}	89.44^{k}	86.95^{fghij}
$a_2b_3c_2$	90.26^{d}	88.61^{ijk}	88.38^{hij}
$a_2b_3c_3$	89.07^{c}	86.61^{efghi}	85.31^{defgh}
$a_3b_1c_1$	88.42^{c}	82.79^{cd}	82.38^{cde}
$a_3b_1c_2$	89.84^{cd}	87.42^{ghijk}	86.91^{fghij}
$a_3b_1c_3$	90.50^{d}	86.12^{efgh}	85.07^{defgh}
$a_3b_2c_1$	89.47^{c}	85.99^{efgh}	85.37^{defghi}
$a_3b_2c_2$	89.70^{c}	87.70^{hijk}	87.18^{fghij}
$a_3b_2c_3$	88.75^{c}	85.96^{efgh}	85.51^{efghi}
$a_3b_3c_1$	87.30^{c}	87.15^{fghij}	86.41^{fghij}
$a_3b_3c_2$	89.31^{c}	88.49^{ijk}	87.93^{ghij}
$a_3b_3c_3$	88.60^{c}	85.50^{efg}	85.07^{defgh}
SE (m) ±	1.09	0.55	0.70
CD (at 1%)	4.18	2.11	2.68

Antioxidant activity (ABTS)

The antioxidant capacity is used to evaluate antioxidant potential of tissues. Among various methods used for analyzing antioxidant activity, the most popular method is ABTS assay. Different drying temperatures showed variations in total antioxidant activity of tea during storage as shown in Table 1. Drying temperature of 70ºC was found significantly superior over the rest of treatments at zero and 45 days of storage with maximum antioxidant activity (0.41 µmol TE/g, 0.33 µmol TE/g) respectively, whereas it was found at par with 50ºC and 60ºC at 90 days storage, with increase in extraction of polyphenols at high temperature, led to increase in antioxidant activity. The 2.0 g quantity used recorded maximum antioxidant activity (0.53 µmol TE/g) and it was significantly superior over 1.5 g and 1.0 g during storage up to 90 days. Antioxidant activity in roselle tea increased with increase in quantity used [6]. The higher brewing temperature and longer brewing time increase the antioxidant activity of green tea infusions as reported by [7]. The [10] found positive correlation between increased extraction time and temperature on antioxidant activity. The 180 seconds of dipping duration recorded maximum antioxidant activity (0.43 µmol TE/g) and was significantly superior over 120 seconds and 90 seconds during storage.

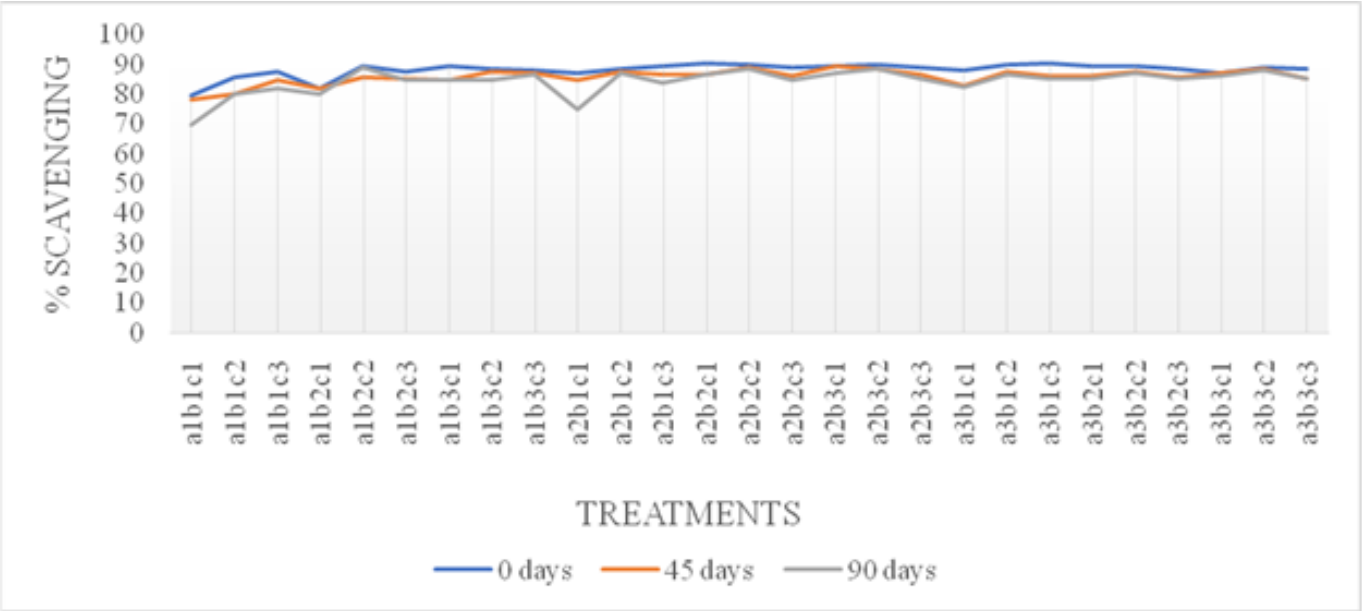


Figure 1 Interactive effect of temperature, quantity used and dipping duration on total antioxidant activity (DPPH) at zero, 45 and 90 days

The results in **Table 3**, **Figure 2** indicate that treatment combination of 70ºC drying temperature, 2.0 g quantity used and 180 seconds dipping duration was superior over other interactions and recorded maximum antioxidant activity (0.46 µmol TE/g) which was statistically at par with 50ºC and 60ºC drying temperature, 2.0 g quantity used and 180 seconds dipping duration(0.45 µmol TE/g), 70ºC drying temperature, 2.0 g quantity used and 90 and 120 seconds dipping duration (0.44 µmol TE/g), 60ºC drying temperature, 2.0 g quantity used and 120 seconds dipping duration (0.43 µmol TE/g). The synergistic effect between drying temperature, quantity used and dipping duration might have contributed to high antioxidant activity. Similar close findings were observed in Hibiscus sabdariffa by [11]. The interaction effect of drying temperature, quantity used and dipping duration on total antioxidant activity (ABTS) of tea at 45 and 90 days was found significant. The treatment combination of 50ºC, 60ºC, 70ºC drying temperature, 2.0 g quantity used and 180, 120, 90 seconds dipping duration (0.37 µmol TE/g and 0.36 µmol TE/g) were statistically at par with each other. The [12] found that large number of the polyphenols were released within 5 min of immersion of tea bags with respect to chamomile tea which contributed to high antioxidant activity.

Organoleptic analysis

The colour scores were found in decreasing trend with respect to the increase in storage time (**Table 4**). The highest sensory score for colour (8.2) of tea infusions was recorded by panel to the treatment combination of 70ºC drying temperature, 2.0 g quantity used and 180 seconds dipping duration. The [13] and [14] reported that the color intensity was strongly correlated with the infusing period because samples infused at a lower temperature but for a longer period showed a higher colour in both roselle and green tea. The taste of hibiscus petal tea infusions during storage showed the decreasing trend from 0 to 90 of days storage period (Table 4). The treatment combination of 60ºC drying temperature, 2.0 g quantity used and 120 seconds dipping duration (T_{17}) recorded highest sensory score for taste of tea infusions (8). The [14] in green tea reported that, the samples dried at higher temperatures led to dehydration of leaves producing strong bitter taste. The treatment combination of 60ºC drying temperature, 2.0 g quantity used and 120 seconds dipping duration recorded highest sensory score for aroma of tea infusions (7.4). Aroma of tea infusions declined with increase in storage duration from 0 to 90 days storage. This might be due to the drying temperature, quantity used and dipping duration produced a synergistic effect thus resulting in high aroma as observed by [13] in herb tea from *Moringa oleifera*, *Hibiscus sabdariffa* and *Cymbopogon citratus*. The overall acceptability of tea infusions was significantly influenced by colour, taste and aroma. The organoleptic analysis of tea infusions for overall acceptability recorded the highest score to the treatment combination (T_{17}) of 60ºC drying temperature, 2 g quantity and 120 sec dipping duration which also contain high total amount of antioxidants and found superior over rest of the treatment.

Table 3 Interaction effect of temperature, quantity, and dipping duration on antioxidant activity (ABTS) at zero, 45 and 90 days

Int. A x B x C (Temp. x Qty. x Duration)	Mean		
	0 days	45 days	90 days
$a_1b_1c_1$	0.35[a]	0.29[a]	0.29[a]
$a_1b_1c_2$	0.35[a]	0.29[a]	0.29[a]
$a_1b_1c_3$	0.37[a]	0.29[a]	0.29[a]
$a_1b_2c_1$	0.38[a]	0.31[b]	0.29[a]
$a_1b_2c_2$	0.38[a]	0.31[b]	0.29[a]
$a_1b_2c_3$	0.40[b]	0.32[b]	0.29[a]
$a_1b_3c_1$	0.43[c]	0.36[c]	0.37[c]
$a_1b_3c_2$	0.43[c]	0.36[c]	0.36[c]
$a_1b_3c_3$	0.45[d]	0.37[c]	0.37[c]
$a_2b_1c_1$	0.35[a]	0.29[a]	0.29[a]
$a_2b_1c_2$	0.35[a]	0.29[a]	0.29[a]
$a_2b_1c_3$	0.37[a]	0.29[a]	0.29[a]
$a_2b_2c_1$	0.38[a]	0.31[b]	0.32[c]
$a_2b_2c_2$	0.38[ab]	0.31[b]	0.31[b]
$a_2b_2c_3$	0.40[bc]	0.32[b]	0.32[b]
$a_2b_3c_1$	0.43[c]	0.36[c]	0.37[c]
$a_2b_3c_2$	0.43[cd]	0.36[c]	0.37[c]
$a_2b_3c_3$	0.45[d]	0.37[c]	0.37[c]
$a_3b_1c_1$	0.36[a]	0.29[a]	0.29[a]
$a_3b_1c_2$	0.36[a]	0.29[a]	0.29[a]
$a_3b_1c_3$	0.37[a]	0.29[a]	0.29[a]
$a_3b_2c_1$	0.39[b]	0.32[b]	0.32[b]
$a_3b_2c_2$	0.39[b]	0.32[b]	0.31[b]
$a_3b_2c_3$	0.40[b]	0.32[b]	0.32[b]
$a_3b_3c_1$	0.44[d]	0.37[c]	0.37[c]
$a_3b_3c_2$	0.44[d]	0.37[c]	0.36[c]
$a_3b_3c_3$	0.46[d]	0.37[c]	0.37[c]
SE (m) ±	0.007	0.001	0.001
CD (at 1%)	0.027	0.008	0.005

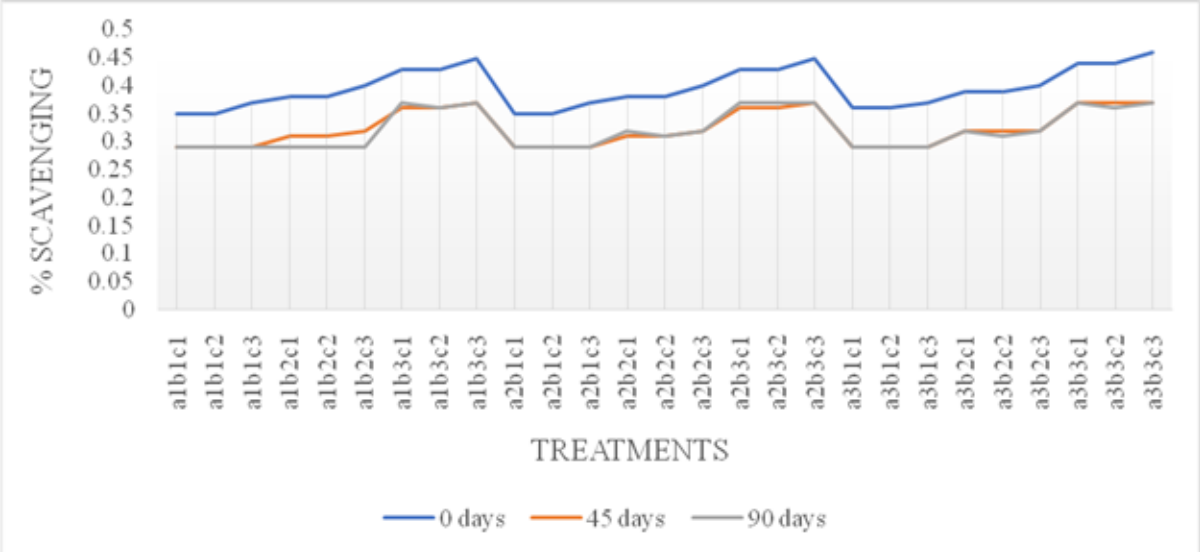


Figure 2 Interactive effect of temperature, quantity used and dipping duration on total antioxidant activity (ABTS) at zero, 45 and 90 days

Table 4 Organoleptic analysis of tea infusions for colour, taste, aroma and overall acceptability

Treatment	Colour			Taste			Aroma			Overall acceptability		
	0 Days	45 Days	90 Days	0 Days	45 Days	90 Days	0 Days	45 Days	90 Days	0 Days	45 Days	90 Days
T_5 ($a_1b_2c_2$)	7	6.7	6.5	7	6.8	6.6	6.5	6.2	6	6.9	6.8	6.5
T_8($a_1b_3c_2$)	7.6	7.1	7	6.9	6.3	6.1	7.1	6.9	6.9	6.6	6.3	6.0
T_{16}($a_2b_3c_1$)	7.5	7.2	7.1	6.7	6.1	6	7.1	6.8	6.8	6.7	6.1	6.0
T_{17}($a_2b_3c_1$)	7.4	7.2	7.1	8	7.8	7.8	7.4	7	7	7.6	7.4	7.0
T_{12}($a_2b_1c_3$)	7.9	7.4	7.2	6.6	6.1	6	7.2	7.1	7	6.7	6.5	6.3
T_{22}($a_3b_2c_1$)	7	6.8	6.8	6.7	6.3	6.1	7.2	7	7	6.2	6.1	6.0
T_{27}($a_3b_3c_3$)	8.2	8	8	6.4	6.2	6.1	7.2	7	7	6.5	6.3	6.1

Conclusion

The hibiscus petal tea infusion conditions affect the biochemical and organoleptic properties of tea, thus optimum infusion conditions are very essential for making a healthy and tasty tea, therefore the treatment combination of 60ºC drying temperature, 2.0 g quantity used and 120 seconds of dipping duration was most preferred for better taste, flavour, and colour, with more stability for total antioxidant activity.

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