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# Standardization of recipe for the preparation of drink from rhododendron (*Rhododendron arboreum* Sm.) flower extract

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**Abstract**

Rhododendron (*Rhododendron arboreum* Sm.) belonging to family Ericaceae is an evergreen shrub or small tree which bears bright red edible flowers. The flowers exhibit many medicinal properties and are rich source of carbohydrates, amino acids, sugars, pectin, anthocyanins and vitamin C. So, investigations were conducted to exploit its wide quality parameters for the development of drink on commercial scale for its proper utilization. Eight combinations of flower extract and TSS for the preparation of rhododendron drink were tried. With the increase in extract content of different recipes, there was a significant effect on physicochemical characteristics of rhododendron drink. The recipe with 14 per cent extract, 12°B TSS and 0.30 per cent acidity was found best on the basis of its sensory parameters and some physicochemical characteristics. The mean values for various sensory characteristics like colour, body, taste, aroma and overall acceptability of the standardized recipe of drink were observed as 8.37, 8.08, 8.50, 7.24 and 8.40, respectively.

## 1. Introduction

Rhododendron (*Rhododendron arboreum* Sm.) is one of the wild plants of Himachal Pradesh which is commonly known as “burans” and known for its highly valued wild edible flowers (Kashyap *et al.*, 2017). The term rhododendron has been derived from two Greek words “rhodo” and “dendron” which means rose and tree, respectively. The various species of rhododendron are concentrated in the temperate regions of Northern hemisphere especially in Sino-Himalayas including China, Japan, Myanmar, Thailand, Malaysia, Indonesia, Philippines, New Guinea, Afghanistan, Pakistan, India, Nepal, southern Europe and northern America (Heywood *et al.*, 2007; Singh *et al.*, 2009). In Himachal Pradesh, three species, namely; *Rhododendron arboreum* Sm., *Rhododendron campanulatum* D. and *Rhododendron anthopogen* D. are distributed in the forests of Bilaspur, Sirmour, Chamba, Kangra, Kullu, Shimla, Kinnaur and Solan districts (Pradhan and Lachungpa, 1990; Chauhan, 1999). Out of all species available, the most important and stately known is *Rhododendron arboreum* Sm. with deep scarlet to red, pink and white flowers (Thakur *et al.*, 2020a). The deep red to scarlet red flowers of *Rhododendron arboreum* Sm. are sweetish sour in taste which have been found to be rich source of carbohydrates, amino acids, sugars, pectin, anthocyanins and vitamin C (Solanki *et al.*, 2013; Kashyap *et al.*, 2017). Its flowers possess pharmacological and biological properties and traditionally the flowers are used for curing diarrhea, blood dysentery, high altitude sickness, headache, mental retardation, nasal bleeding, fever and stomach ache (Popesco and Kopp, 2013; Thakur *et al.*, 2020b). Its flowers being rich source of vitamin C have been found to have high antioxidant and free radical scavenging activities (Shrestha and Budhathoki, 2012). The unique antioxidant activity and various antioxidant compounds contribute towards its medicinal value, human health benefits and helps in the prevention of various neurodegenerative disorders (Kalaycioglu and Erim, 2017; Thakur *et al.*, 2019). So, being a rich source of antioxidants specially colour pigments like anthocyanins as well as sugars, this fruit can be exploited for the development of some beverages specially drink. Thus, the present studies were undertaken to standardize the recipe for preparation of drink from rhododendron (*Rhododendron arboreum* Sm.) flower extract.

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## 2. Materials and Methods

### 2.1 Raw material and extraction of extract

The flowers of *Rhododendron arboreum* Sm. procured from Rajgarh area of Sirmour district of Himachal Pradesh in the month of April were brought to the Department of Food Science and Technology, UHF, Nauni, Solan (HP), where they were used to carry out the research work. The flower identification and authentication was carried out by Department of Forest Products, Dr. YSPUHF, Nauni, Solan, India vide-UHF herbarium number-13915, YSPUHF, Solan, India. The flowers were further used for the preparation of flower extract by using flower petals and cooked in 15 per cent hot water for 6 min followed by 0.08 per cent pectinase treatment for 60 min at 50°C. This flower extract was further used for the development of rhododendron drink.

## 2.2 Development of rhododendron drink

Flower drink was prepared by mixing different proportions of rhododendron flower extract in different combinations of sugar syrup as given in Table 1. The flow sheet for the preparation of rhododendron drink has been given in Figure 1. To get the desirable concentration of acid (0.30 %) in flower drink, citric acid was added in different treatment combinations. Sodium benzoate (120 ppm) was added in all the treatments as a preservative.

**Table 1: Treatment detail of rhododendron flower drink**

| Treatment | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | $T_7$ | $T_8$ |
|---|---|---|---|---|---|---|---|---|
| Flower extract (%) | 10 | 12 | 14 | 16 | 10 | 12 | 14 | 16 |
| TSS (°B) | 12 | 12 | 12 | 12 | 15 | 15 | 15 | 15 |

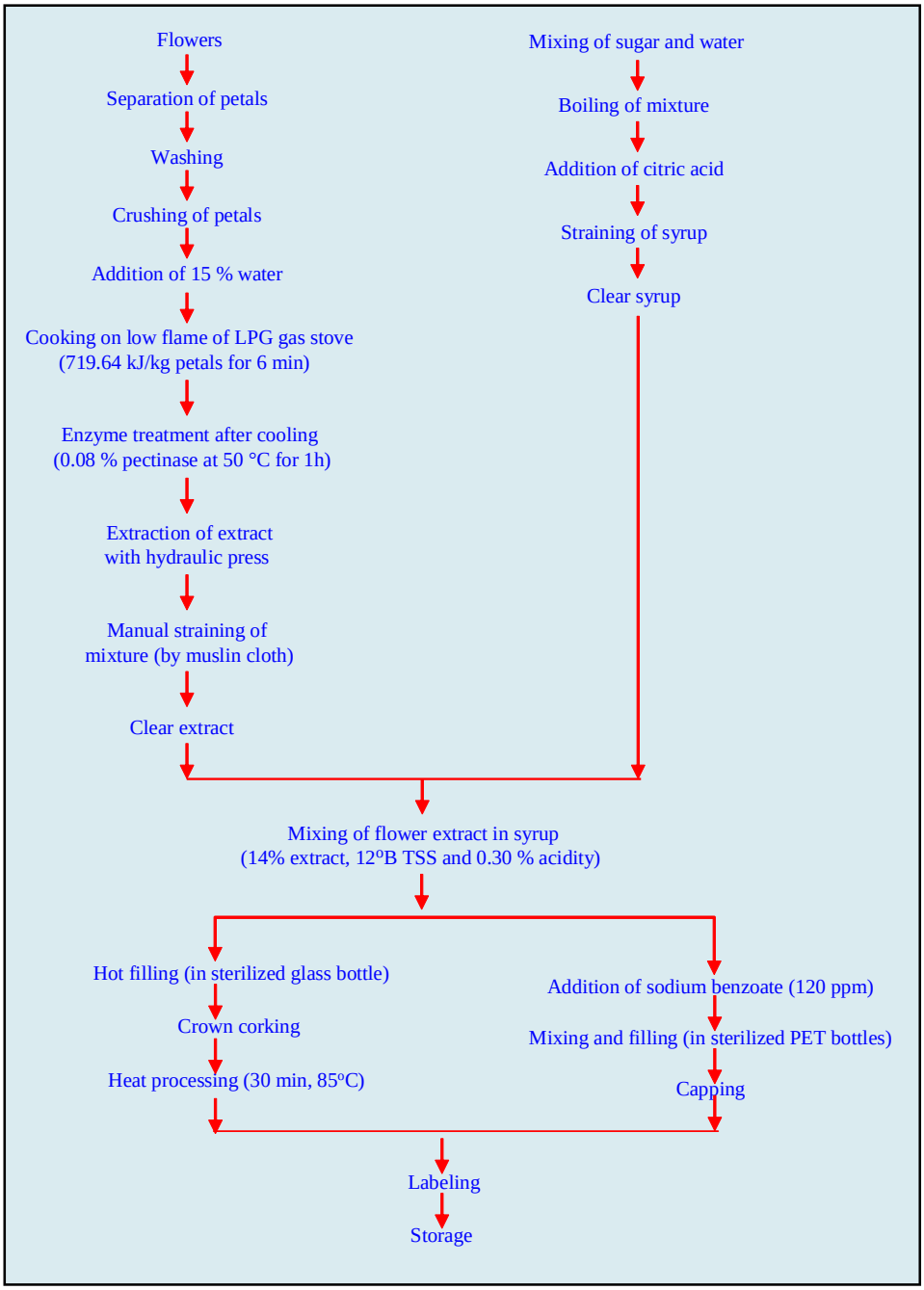


**Figure 1: Unit operations for the preparation of rhododendron drink.**

## 2.3 Physicochemical analysis

### 2.3.1 Colour

The colour of drink in terms of different units (red and yellow) was observed with a Tintometer (Lovibond Tintometer Model E).

### 2.3.2 Apparent viscosity

The apparent viscosity of the flower extract and various beverages was determined by using Ostwald viscometer and was expressed in terms of time (flow rate in minutes) taken for samples to pass through the tube.

### 2.3.3 Total soluble solids (TSS)

TSS of drink measured by hand refractrometers ranging from 0 to 32 and expressed as $^{o}$Brix ($^{o}$B).

### 2.3.4 Titratable acidity

Titratable acidity was estimated by titrating a known volume of sample against standard NaOH using phenolphthalein as an indicator. The titratable acidity was expressed as per cent citric acid (Ranganna, 2009) as per given formula:

Titratable acidity % (as citric acid) =

$$\frac{\text{Titre value} \times \text{Normality of alkali} \times \text{Volume made} \times \text{Equivalent weight of dominating acid}}{\text{Weight of sample taken for estimation} \times \text{Volume of aliquot} \times 1000} \times 100$$

### 2.3.5 pH

The pH of drink was determined by using a digital pH meter (CRISON Instrument, Ltd. Spain). Before estimating the pH of sample, pH meter was standardized with standard buffers of 4, 7 and 9.

### 2.3.6 Sugars

Sugars were estimated by Lane and Eynon method given by Ranganna (2009). A known volume of sample was neutralized with NaOH and to it 2 ml of lead acetate was added and kept as such for ten min. Excess of lead acetate was removed by adding 2 ml of potassium oxalate in 250 ml volumetric flask. After diluting it up to the mark, the solution was filtered and clear filtrate was taken to estimate reducing sugars by titrating against a known quantity of Fehling's A and Fehling's B solutions using methylene blue as an indicator. Reducing sugars were estimated as per cent and calculated as given below:

$$\text{Reducing sugars \%} = \frac{\text{*Factor} \times \text{Dilution}}{\text{Titre value} \times \text{Weight / Volume of sample}} \times 100$$

*Factor = 0.05

Total sugars were estimated by adding 5 g of citric acid to 50 ml of calibrated sample (prepared for reducing sugars) and heated for 10 min. For complete inversion of sugar samples, NaOH was added and final volume 250 ml was made. The total sugars were estimated as per cent and calculated as:

$$\text{Total sugars \%} = \frac{\text{*Factor} \times \text{Dilution}}{\text{Titre value} \times \text{Weight / Volume of sample}} \times 100$$

*Factor = 0.05

### 2.3.7 Ascorbic acid

Ascorbic acid content was determined by using 2-6 dichlorophenol indophenol visual titration method (Ranganna, 2009). Samples were prepared with 3 per cent meta-phosphoric acid and aliquot was titrated with dye to pink colour end point. The results were expressed as mg/100 ml of sample and calculated as per formula:

$$\text{Ascorbic acid (mg/100 ml)} = \frac{\text{Titre value} \times \text{*Dye factor} \times \text{Volume made}}{\text{Aliquot taken} \times \text{Weight / Volume of sample}}$$

*Dye factor = 0.5/Titre value

Titre value for dye factor was determined by titrating standard L-ascorbic acid prepared in 3 per cent metaphosphoric acid with 2-6 dichlorophenol indophenol dye to a pink colour end point.

### 2.3.8 Anthocyanins

Total anthocyanins present in all samples were determined by spectrophotometric method given by Ranganna (2009). The procedure involves extraction of anthocyanins with 85 per cent ethanolic HCl and measuring its optical density at 535 nm. Anthocyanins were calculated and expressed as mg/100 ml using the formula given below:

Total OD mg/ 100 ml (x) =

$$\frac{\text{OD of sample} \times \text{Volume made up of extracts} \times \text{Total volume at 535 nm for colour measurement}}{\text{Volume of extract used} \times \text{Volume / weight of sample taken}}$$

$$\text{Total anthocyanins (mg/100 ml)} = \frac{X}{\text{*98.20}}$$

* 98.20 = Extraction coefficient

### 2.3.9 Total phenols

Total phenols content was determined by Folin-Ciocalteu procedure given by Singleton and Rossi (1965) in which absorbance was measured at 765 nm in a colourimeter against water blank. One gram of sample was taken and ground with 10 ml of 80 per cent ethanol in pestle and mortar and centrifuged for 20 min at 10000 rpm and filtered. Filtrate was evaporated in oven up to the dryness and dried extract was dissolved in 5 ml distilled water. 2 ml aliquot was taken in separate test tube and volume was made upto 3 ml. Then, 0.5 ml Folin-Ciocalteu reagent was added. Phenols with phosphomolybdic acid in Folin-Ciocalteu reagent and in alkaline medium produce a highly dark blue coloured complex (molybdenum blue). After 3 min, 2 ml of $Na_2CO_3$ (20 %) was added and mixed. Test tubes were placed in boiling water bath for one minute and then cooled. The concentration was determined as per the standard procedure from the standard curve. A standard calibration curve of gallic acid using its different concentrations was prepared and concentration of total phenols in the given sample was calculated as mg GAE/100 ml of sample.

### 2.3.10 Antioxidant activity

Antioxidant activity (free radical scavenging activity) was measured as per the method of Brand-Williams *et al.* (1995). DPPH (2, 2-

diphenyl-1-picrylhydrazyl) was used as a source of free radical. A quantity of 3.9 ml of 6 × $10^{-5}$ mol/l DPPH in methanol was put into a cuvette with 0.1 ml of sample extract and the decrease in absorbance was measured at 515 nm for 30 min or until the absorbance become steady. Methanol was used as a blank. The remaining DPPH concentration was calculated using the following equation:

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance of blank (DPPH)} - \text{Absorbance of sample}}{\text{Absorbance of blank (DPPH)}} \times 100$$

### 2.4 Sensory evaluation

The sensory evaluation of different recipes of flower drink was carried out by hedonic rating test (Amerine *et al.*, 1965). The samples were evaluated for different sensory characteristics like colour, body, taste, aroma and overall acceptability. Semi-trained sensory panel (10 numbers at a time) comprised of faculty members and postgraduate students of Department of Food Science and Technology, UHF, Solan (HP) were selected randomly. This random selection was made so as to accommodate different sections and age groups to evaluate the various sensory parameters (Thakur *et al.*, 2018a).

### 2.5 Statistical analysis

Data on physicochemical characteristics of flower drink was analyzed by completely randomized design (CRD) whereas, data pertaining to the sensory evaluation were analyzed by using randomized block design (RBD). The experiment for recipe standardization was replicated three times.

## 3. Results

### 3.1 Standardization of recipe for the development of rhododendron drink

The data pertaining to physicochemical and sensory characteristics of rhododendron drink prepared by different recipes has been presented in Tables 2 and 3.

**Table 2: Physicochemical characteristics of different recipes of rhododendron drink**

| Treatments | Colour | | Apparent viscosity (Min) | TSS (°B) | Titratable acidity (%) | pH | Sugars (%) | | Ascorbic acid (mg/100 ml) | Anthocyanins (mg/100 ml) | Total phenols (mg GAE/ 100 ml) | Antioxidant activity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | Y | | | | | Total sugars | Reducing sugars | | | | |
| $T_1$ | 3.65 | 0.85 | 3.19 | 12 | 0.30 | 4.04 | 9.50 | 6.70 | 0.45 | 7.31 | 10.17 | 6.54 |
| $T_2$ | 3.89 | 0.68 | 4.26 | 12 | 0.30 | 4.05 | 9.76 | 6.91 | 0.85 | 8.98 | 12.21 | 8.09 |
| $T_3$ | 3.96 | 0.55 | 5.11 | 12 | 0.30 | 4.09 | 9.98 | 7.05 | 1.43 | 9.86 | 14.24 | 9.66 |
| $T_4$ | 4.01 | 0.41 | 5.27 | 12 | 0.30 | 4.16 | 10.17 | 7.21 | 1.95 | 11.44 | 16.28 | 11.21 |
| $T_5$ | 3.91 | 0.81 | 5.28 | 15 | 0.30 | 4.05 | 12.86 | 8.92 | 0.46 | 7.35 | 10.19 | 6.55 |
| $T_6$ | 4.21 | 0.65 | 5.01 | 15 | 0.30 | 4.08 | 12.90 | 8.93 | 0.88 | 9.03 | 12.22 | 8.10 |
| $T_7$ | 4.44 | 0.50 | 5.39 | 15 | 0.30 | 4.12 | 12.95 | 8.95 | 1.44 | 9.97 | 14.25 | 9.67 |
| $T_8$ | 4.59 | 0.37 | 5.51 | 15 | 0.30 | 4.17 | 13.01 | 8.96 | 1.99 | 11.52 | 16.30 | 11.24 |
| CD $_{0.05}$ | 0.13 | 0.10 | 0.24 | - | - | 0.06 | 0.15 | 0.10 | 0.27 | 0.22 | 0.37 | 0.42 |
| S.E. | 0.11 | 0.06 | 0.28 | - | - | 0.02 | 0.59 | 0.38 | 0.22 | 0.57 | 0.86 | 0.66 |

No. of replications =3; S.E.= Standard error

#### 3.1.1 Physicochemical characteristics

Data presented in Table 2 indicate that visual red and yellow TCU of drink ranged between 3.65 to 4.59 and 0.37 to 0.85, respectively among different recipes. The maximum (4.59) red TCU were recorded in $T_8$ and lowest (3.65) in $T_1$. The highest (0.85) yellow TCU were recorded in $T_1$ and lowest (0.37) in $T_8$ which were statistically at par with $T_4$. The apparent viscosity of drink ranged between 3.19 to 5.51 min. The lowest (3.19 min) apparent viscosity was recorded in $T_1$ and highest (5.51 min) in $T_8$ which was at par with $T_4$, $T_5$ and $T_7$.

During preparation of the product, TSS of first four recipes were maintained at 12°B and rest at 15°B, whereas, the titratable acidity of all the recipes was maintained as 0.30 per cent. The pH of the drink ranged between 4.04 to 4.17, highest (4.17) in $T_8$ which was at par with $T_7$ and $T_4$ and lowest (4.04) in $T_1$ which was at par with $T_2$, $T_3$, $T_5$ and $T_6$. The total and reducing sugars content of the drink ranged between 9.50 to 13.01 and 6.70 to 8.96 per cent, respectively. The highest (13.01%) total sugars content was recorded in $T_8$ which was statistically at par with $T_5$, $T_6$ and $T_7$, and the lowest (9.50 %) in $T_1$. The maximum (8.96 %) reducing sugars content was recorded in $T_8$ which was statistically at par with $T_5$, $T_6$ and $T_7$ and minimum (6.70 %) in $T_1$. The ascorbic acid content of drink varied from 0.45 to 1.99 mg/100 ml in the product and highest (1.99 mg/100 ml) was recorded in $T_8$ which is at par with $T_4$ and lowest ascorbic acid content (0.45 mg/100 ml) was observed in $T_1$ which was statistically at par with $T_5$. The anthocyanins content of different recipes of rhododendron drink ranged between 7.31 to 11.52 mg/100 ml. The highest (11.52 mg/100 ml) value of anthocyanins recorded in $T_8$ which was statistically at par with $T_4$ and lowest (7.31 mg/100 ml)

in $T_1$ which was statistically at par with $T_5$. Total phenols content of different recipes of this beverage ranged between 10.17 to 16.30 mg GAE/100 ml. It was recorded highest (16.30 mg/100 ml) in $T_8$ which was statistically at par with $T_4$ and lowest (10.17 mg/100 ml) in $T_1$ which was statistically at par with $T_5$. The antioxidant activity of drink ranged between 6.54 to 11.24 per cent and highest (11.24 %) was recorded in $T_8$ which was at par with $T_4$ and lowest (6.54 %) in $T_1$ which was at par with $T_5$.

From the above results, it is found that with the increase in extract content of different recipes a significant effect on physicochemical characteristics of rhododendron drink was observed.

**Table 3: Sensory characteristics (scores) of different recipes of rhododendron drink**

| Treatments | Colour | Body | Taste | Aroma | Overall acceptability |
|---|---|---|---|---|---|
| $T_1$ | 8.00 | 7.73 | 7.17 | 7.07 | 7.06 |
| $T_2$ | 8.07 | 7.83 | 7.33 | 7.18 | 7.20 |
| $T_3$ | 8.37 | 8.08 | 8.50 | 7.24 | 8.40 |
| $T_4$ | 8.27 | 7.97 | 7.73 | 7.22 | 8.00 |
| $T_5$ | 8.01 | 7.83 | 7.87 | 7.02 | 7.17 |
| $T_6$ | 8.11 | 7.93 | 8.03 | 7.13 | 7.60 |
| $T_7$ | 8.28 | 8.05 | 7.20 | 7.21 | 7.23 |
| $T_8$ | 8.31 | 8.07 | 7.00 | 7.22 | 7.07 |
| $CD_{0.05}$ | 0.03 | 0.02 | 0.24 | 0.01 | 0.18 |
| S.E. | 0.05 | 0.05 | 0.18 | 0.03 | 0.17 |

No. of replications =3; S.E.= Standard error

**Table 4: Physicochemical and sensory characteristics of standardized recipe of rhododendron drink**

| Characteristics | | Mean values ± SE |
|---|---|---|
| **Physicochemical** | | |
| Colour (TCU) | Red | 3.96 ± 0.07 |
| | Yellow | 0.55 ± 0.07 |
| Apparent viscosity (Flow rate in min) | | 5.11 ± 0.12 |
| TSS (°B) | | 12.00 |
| Reducing sugars (%) | | 7.05 ± 0.07 |
| Total sugars (%) | | 9.98 ± 0.07 |
| Titratable acidity (%) | | 0.30 |
| pH | | 4.09 ± 0.02 |
| Ascorbic acid (mg/100 ml) | | 1.43 ± 0.10 |
| Anthocyanins (mg/100 ml) | | 9.86 ± 0.10 |
| Total phenols (mg GAE/100 ml) | | 14.24 ± 0.12 |
| Antioxidant activity (%) | | 9.66 ± 0.40 |
| **Sensory (Scores)** | | |
| Colour | | 8.37 ± 0.08 |
| Body | | 8.08 ± 0.33 |
| Taste | | 8.50 ± 0.50 |
| Aroma | | 7.24 ± 0.33 |
| Overall acceptability | | 8.40 ± 0.40 |

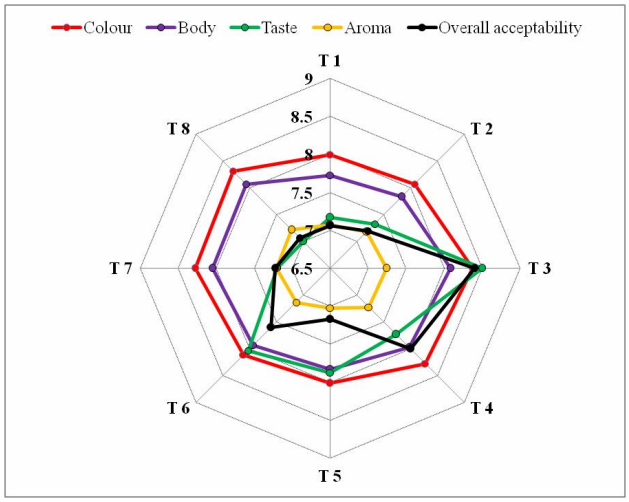


**Figure 2: Sensory characteristics (scores) of different recipes of rhododendron drink.**

### 3.1.2 Sensory characteristics

Data on sensory characteristics of different recipes of rhododendron drink given in Table 3 and Figure 2 indicate that the highest (8.37) colour score was obtained in recipe $T_3$ and lowest (8.00) in $T_1$. The recipe $T_3$ obtained maximum body score as 8.08 and minimum (7.73) in $T_1$. The highest score (8.50) of taste was also awarded to $T_3$ while $T_8$ got the lowest (7.00) which was statistically at par with $T_1$ and $T_7$. The maximum (7.24) score of aroma was obtained in recipe $T_3$ and minimum (7.02) in $T_5$. The highest score (8.40) of overall acceptability was awarded to recipe $T_3$ and the lowest (7.06) to $T_1$ which was closely followed by $T_2$, $T_5$, $T_7$ and $T_8$.

From the above results, it was concluded that the recipe with 14 per cent extract, 12°B TSS and 0.30 per cent acidity $T_3$ was found to be best on the basis of sensory and some physicochemical characteristics of the drink.

### 3.2 Physicochemical and sensory characteristics of standardized recipes of rhododendron drink

A perusal of data given in Table 4 shows that red and yellow TCU of standardized recipe of drink were observed as 3.96 ± 0.07 and 0.55 ± 0.07, respectively. Apparent viscosity, TSS, reducing sugars and total sugars of this product were recorded as 5.11 ± 0.12 min, 12.00°B, 7.05 ± 0.07 and 9.98 ± 0.07 per cent, respectively. Titratable acidity and pH of drink were observed as 0.30 and 4.09 ± 0.02, respectively. The product also contained ascorbic acid (1.43 ± 0.10 mg/100 ml), anthocyanins (9.86 ± 0.10 mg/100 ml), total phenols (14.24 ± 0.12 mg GAE/100 ml) and antioxidant activity (9.66 ± 0.40 %). The mean values of sensory characteristics like colour, body, taste, aroma and overall acceptability of standardized recipe of drink were obtained as 8.37 ± 0.08, 8.08 ± 0.33, 8.50 ± 0.50, 7.24 ± 0.33 and 8.40 ± 0.40, respectively.

## 4. Discussion

The recipe $T_4$ and $T_8$ recorded higher values of anthocyanins, total phenols, ascorbic acid, total sugars, reducing sugars, apparent viscosity and antioxidant activity which clearly due to the use of higher amount of flower extract as compared to other recipes. The

changes in extract content in different recipes had also affected the colour units of the drink. Data given in Table 3 show that there was a significant effect of extract acid-syrup blend on sensory scores of different recipes of rhododendron drink. The higher colour and body scores of recipes $T_8$ and $T_3$ might be due to better combination of extract-syrup blend as compared to other recipes. The changes in extract content in different recipes have also affected the taste and aroma score of the drink. The recipe $T_3$ obtained the highest taste score which was clearly due to the higher extract content used as well as better sugar-acid-extract blend in this recipe. The higher overall acceptability scores of recipe $T_3$ might also be due to the better combination of extract-acid-syrup blend coupled with attractive colour and body of the product. Similar results have been reported by Thakur *et al.* (2017) in box myrtle drink, Hamid *et al.* (2017) in mulberry drink, Thakur *et al.* (2018b) in wild aonla drink, Sharma *et al.* (2019) in apple-whey based herbal functional ready-to-serve beverage, Chauhan *et al.* (2019) in wild prickly pear beverage, Bhatt *et al.* (2020) in wild jamun beverage and Thakur *et al.* (2020c) in wild prickly pear fruit drink.

## 5. Conclusion

From the above results, it was observed that this standardized recipe of rhododendron drink (14 per cent extract, 12°B TSS and 0.30 per cent acidity) contained relatively higher ascorbic acid, total phenols, anthocyanins and antioxidant activity along with best sugar-acid blend, which was because of higher content of flower extract used in this recipe. This recipe also obtained maximum scores for sensory parameters like colour, body, taste, aroma and overall acceptability, which might be due to higher extract content, best combination of extract and syrup, best sugar-acid blend in the product and finally all these factors might have led the judges to award the highest scores to this recipe.

## Conflict of interest

The authors declare that there are no conflicts of interest relevant to this article.

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