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# EXTRACTION AND FUNCTIONAL APPLICATION OF ANTHOCYANINS AS FOOD COLORANTS VIA MICROWAVE-ASSISTED METHOD

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**ABSTRACT**

An experiment was conducted at the Department of Floriculture and Landscape Architecture, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, to identify the most effective anthocyanin extraction method from *Hibiscus rosa-sinensis* petals and to evaluate the physicochemical and sensory qualities of value-added edible products derived from Hibiscus extract. The initial experiment involved nine treatments combining three extraction techniques—Conventional, Microwave-Assisted, and Ultrasound-Assisted extraction—using three solvents: distilled water, 2% citric acid, and 85% ethanol. Among the treatments, $T_7$ (Ultrasound-Assisted extraction with distilled water) recorded the highest anthocyanin concentration (179.32 mg/L), phenol content (72.12 mg/g), flavonoids (36.51 mg/g) and antioxidant activity (74.21%). For other parameters, $T_4$ (Microwave-Assisted extraction with water) showed the highest pH (7.3), $T_8$ (Ultrasound-Assisted with 2% citric acid) recorded the highest titrable acidity (13.45%), and $T_3$ (Conventional with 85% ethanol) registered the highest total soluble solids (23.5 °Brix). In the second phase, edible products such as Hibiscus candy, jelly, ready-to-serve (RTS) beverage and lemon-blended Hibiscus tea were developed. Hibiscus candy showed the highest TSS (22.7 °Brix) and pH (4.2), while lemon-blended Hibiscus tea had the highest titrable acidity (0.43%). Among all products, Hibiscus RTS drink received the highest ratings in sensory evaluation, indicating superior consumer acceptability. The study demonstrates the potential of ultrasound-assisted extraction and the suitability of Hibiscus for functional edible product development.

***Key words :*** Anthocyanin extraction, *Hibiscus rosa-sinensis*, Microwave-Assisted Extraction, Natural food colorant, Value-added products.

## Introduction

*Hibiscus rosa-sinensis* popularly called as "Queen of the Tropics" belongs to the family *Malvaceae* and has distinctive, large and vivid blossoms. This ornamental plant, particularly known as "Sembaruthi" are grown for both aesthetic and therapeutic benefits. Almost all parts of the Hibiscus plants are rich in medicinal values. The majority of the red pigments found in flowers (Nakamura *et al.*, 1990) are anthocyanins, which are frequently utilized as colouring agents. Anthocyanins are safe and water soluble and can be used as natural water soluble colourants makes them as desirable food additive. Anthocyanins possess the ability to act as antioxidants, which is proven to be essential in preventing diseases including diabetes, cancer and cardiovascular disease. Anthocyanin-rich combinations of bioflavonoids may protect against DNA breakage, suppress estrogenic activity, inhibit enzymes, increase cytokin production, have anti-inflammatory effects, reduce capillary permeability, fragility and reinforce membranes (Lila, 2004). *Hibiscus rosa-sinensis* was found to have cyanidin-3-sophoroside as its primary anthocyanin.

The natural food colours can be used relatively safe in food and produce more appealing colours for food products and estimated daily intake in the United States has been determined to be 12.5 mg (Wu *et al.*, 2006).

Therefore, be used as a functional food item in our diet. Besides the beneficial nature and health benefits, anthocyanin can act as a natural substitute as food colourant. When considering the high anthocyanin content of *Hibiscus rosa-sinensis* petals and its use as edible biocolour in the food this experiment was aimed with the objective of exploring the best anthocyanin extraction method from Hibiscus (*H. rosa-sinensis*) petals and to prepare and assess the physiochemical and sensory analysis of edible value-added products using Hibiscus extract.

## Materials and Methods

An experiment was conducted at Department of Floriculture and Landscape Architecture, HC & RI, TNAU, Coimbatore under completely randomized design with 9 treatments and 3 replication (Table 1) for Extraction of anthocyanin from Hibiscus petals through different extraction methods *viz.,* Conventional extraction, Microwave assisted extraction and Ultrasound assisted extraction with different solvent such as distilled water, 2% citric acid and 85% ethanol. Fresh Hibiscus flowers of Red Single cultivars were collected and the calyx, stamen and pistil were separated from the petals. The petals were then washed with distilled water and shade dried for 3 days, until the flowers are completely dried. The dried petals are ground into powder and stored in an airtight amber container for further analysis.

**Table 1 :** Treatment details.

| | |
|---|---|
| $T_1$ | Conventional Extraction + Distilled Water |
| $T_2$ | Conventional Extraction + 2% Citric acid |
| $T_3$ | Conventional Extraction + 85% Ethanol |
| $T_4$ | Microwave Assisted Extraction + Distilled Water |
| $T_5$ | Microwave Assisted Extraction + 2% Citric acid |
| $T_6$ | Microwave Assisted Extraction + 85% Ethanol |
| $T_7$ | Ultrasound Assisted Extraction + Distilled water |
| $T_8$ | Ultrasound Assisted Extraction + 2% Citric acid |
| $T_9$ | Ultrasound Assisted Extraction + 85% Ethanol |

### Extraction of Anthocyanin

**Conventional extraction (CE)** method suggested by Du and Francis (1973) with minor modifications, was used. A 50 g of dried petals were taken in a beaker and 500 ml of solvent (distilled water, 2% citric acid, 85% ethanol) was added and mixed properly. This mixture was placed in a hot water bath at $60^0$C for 60 minutes. After the required time, the mixture was removed and allowed to cool down. Using a muslin cloth, the mixture was squeezed to remove the powdered particles. Using a rotary evaporator at 60°C and 40 rpm for 30 mins, 400 ml of the extracted liquid were concentrated to 90 ml. This concentrated liquid was then used for further analytic observations.

**Microwave assisted extraction (ME) (**Ethos X Microwave Extraction – 12 Sample system) was used in the anthocyanin extraction process. The exposure time(s), microwave power (W) and sample/solvent ratio(g/ml) standardized by Ashitha *et al.* (2020) is used in this study. The one gram of powdered sample was added to 25 ml of the solvent (1:25 ratio of distilled water, 2% citric acid, and 85% ethanol). This combination was fed with 400 W of microwave radiation into the microwave extractor cells in the Microwave assisted extraction system. The exposure period for water and 2% citric acid is 180 seconds, while for 85% ethanol it is 120 seconds. The mixture was then squeezed through a muslin cloth to remove the powdered particles. This concentrated liquid was used to make further analytical observations.

**Ultrasound assisted extraction (UAE)** with ultrasonic lab extractor was used in the anthocyanin extraction process. In the Ultrasound assisted extraction, one gram of powdered sample was combined in 1:15 ratio, with 15 ml of the solvent (distilled water, 2% citric acid, 85% ethanol) at a frequency of 40 kHz for 30 min. The mixture was squeezed through muslin cloth to remove the powdered particles.

Further analytical observations were made using this concentrated liquid. In all the treatments pigment extract were assessed for Titrable Acidity (%), pH, Total soluble solids (°degree Brix), Total Phenol Content (mg/g), Total Flavanoid content (mg/g), Total monomeric anthocyanin, Antioxidant activity (%)

### Utilization as food colorant

**Hibiscus candy :** Hibiscus candy was prepared by adding 200 g of sugar with 50 ml of water to a pan and boiled. To this mixture, 10 ml of hibiscus extract, 0.25 g of food grade citric acid and 1 g of dry ginger powder was added. This was boiled until the mixture achieves the form of a caramelization. This caramelized mixture was poured in a mould and allowed to cool. The candies prepared were packed in an airtight package for further storage and consumption.

**Hibiscus jelly :** In a pan, 75 g of sugar was added to 100 ml of water and was allowed to boil. To this boiling mixture, 15 g of gelatin and 1g of citric acid was added and mixed. This mixture was then added to the boiling sugar syrup and continuous stirring was done upto $115^0$C. This mixture was transferred into another container and allowed to cool. Desired amount of hibiscus extract was added until the favourable colour is achieved. It was then poured into moulds, stored in refrigerator conditions and left undisturbed for 2 hours.

**Lemon blended Hibiscus tea :** In a container, 50 ml of water, 3 ml of hibiscus extract, 10 g of sugar was added and boiled for 3 to 4 minutes. Turn off the heat and squeeze 5 ml of lemon extract to it. This tea was then poured in a container and served.

**Hibiscus RTS :** To 500 ml of water, 120 g of sugar was added and 0.25 g of citric acid were added to it. This mixture was allowed to boil. A 12 ml of the hibiscus extract was added to the boiling mixture. Turn off the heat and allow it to cool down. This mixture is poured in bottles. The bottle is then allowed for pasteurization at 80$^0$C for 30 minutes and cooled.

Further, for the edible product's analytical observations like Total Soluble Solids (TSS p brix), pH, Acidity (%), Sensory analysis was assessed.

### Statistical analysis

Statistical analysis of the acquired data was performed using the AGRES software programme and the MS Excel® spreadsheet. The critical difference was calculated at the 5% (0.05) level of probability.

## Results and Discussion

### Extraction of anthocyanin

**Anthocyanin content :** Anthocyanins are flavonoids, which are polyphenolic pigments that are water soluble in nature. Among the nine treatments, $T_7$ Ultrasound Assisted Extraction with water as solvent had the highest Anthocyanin concentration (179.32 mg/l), while $T_1$ conventional extraction using water as the solvent had the lowest Anthocyanin content (45.71 mg/l). In comparison to conventional extraction and microwave-assisted extraction, the results (Table 2) revealed that Ultrasound-assisted extraction recorded higher Anthocyanin content. While water as a solvent recorded highest anthocyanin content and extraction rates in the Ultrasound assisted extraction method, ethanol as a solvent recorded higher anthocyanin extraction rates in Conventional extraction and Microwave assisted extraction methods. This was due to the greater extraction rate of solvents with high surface tension, low vapour pressure and high viscosity in Ultrasound-assisted extraction (Vardanega *et al.*, 2014). Studies have shown that water has higher vapour pressure and surface tension than ethanol. Similar findings were seen when *Hibiscus sabdariffa*'s anthocyanins were extracted with ultrasound assisted extraction (Aryanti, 2019). Further research has found that in *Hibiscus sabdariffa* and Maqui berries, Ultrasound assisted extraction has higher anthocyanin extraction rate than Conventional extraction and Microwave assisted extraction (Paraíso *et al.*, 2019; Vázquez-Espinosa *et al.*, 2019). Research studies revealed that in the extraction of Roselle flowers, Microwave assisted extraction outperforms Conventional extraction (Purbowati and Maksum, 2019).

**pH :** As per U.S. Geological survey (2019), pH is to observe the relative amount of free hydrogen and hydroxyl ions in water. Among the nine treatments, the pH value ranged from 4.1 to 7.3 (Table 2). A higher pH was found in extracts using water as a solvent and the lowest pH ranged in extracts with 2% citric acid as solvent. The pH range varies due to the acidity of the solvent used in extraction. Comparative results were observed in the studies of Roselle flower (*Hibiscus sabdariffa*) (Manjula *et al.*, 2022).

**Titrable acidity :** Titrable acidity measures the total concentration of acidity in a food product. Titrable acidity

**Table 2 :** Effect of extraction methods on different physiochemical parameters – Anthocyanin, pH, Titrable acidity, TSS.

| Extraction methods | Treatments | Anthocyanin (mg/l) | pH | Tritable acidity (%) | TSS ($^0$Brix) |
|---|---|---|---|---|---|
| Conventional extraction | $T_1$ | 45.71 | 7.2 | 8.19 | 5.1 |
| | $T_2$ | 62.32 | 4.4 | 13.30 | 8.5 |
| | $T_3$ | 100.88 | 6.1 | 10.24 | 23.5 |
| Microwaveassisted extraction | $T_4$ | 96.54 | 7.3 | 8.08 | 5.5 |
| | $T_5$ | 130.12 | 4.3 | 12.99 | 8.5 |
| | $T_6$ | 155.45 | 6.4 | 9.96 | 21.5 |
| Ultrasoundassisted extraction | $T_7$ | 179.32 | 7.1 | 8.32 | 6.1 |
| | $T_8$ | 170.22 | 4.1 | 13.45 | 8.8 |
| | $T_9$ | 162.14 | 6.3 | 10.03 | 22.1 |
| | **SEd** | 2.56 | 0.17 | 0.19 | 0.29 |
| | **CD at 5%** | 5.38 | 0.36 | 0.45 | 0.61 |

shows variation for different extraction treatment (Table 2). The highest titrable acidity was found in $T_8$ Ultrasound assisted extraction using 2% citric acid as solvent (13.45%). Lowest percentage of titrable acidity was found in $T_4$ Microwave assisted extraction using water as solvent (8.08%). Comparatively higher percentage of titrable acidity was found in extracts using citric acid solvents rather than ethanol and water solvent. Similar results are observed in the study of physiochemical properties of natural pigments for *Hibiscus sabadariffa* (Abou-Arab *et al.*, 2011). Citric acid may have been added as a solvent, which may have contributed to the greater acidity percentage. It is evident from the results above that the acidity range varied depending, on the solvent used rather than the extraction method.

**Total soluble solids (TSS) :** Total soluble solids are the amount of sugar and soluble minerals dissolved in the given sample. There was significant difference in the TSS value for different extraction methods (Table 2). Among the nine treatments the TSS value ranged from $5.1^0$Brix to $23.5^0$Brix. Similar increase in the brix was recorded from the studies of Roselle flower (*Hibiscus sabdariffa*) (Abou-Arab *et al.*, 2011). A higher TSS value was found in ethanol solvent extract because the extraction rate of ethanol not only extracts higher amounts of anthocyanin, but also higher sugars, organic acids and proteins (Manjula *et al.*, 2022).

**Total phenols :** The total phenolic content significantly differed for different anthocyanin extraction treatments. The total phenolic content in this study ranged from 37.21 mg/g to 72.12 mg/g (Table 3). Comparitively, similar results were obtained in the Ultrasound-Assisted Extraction of Polyphenol Compounds from Brewer's Spent Grain (Alonso-Riaño *et al.*, 2020). The total phenol content extraction was influenced by the kind of solvent used and consequently by the solvent polarity. As a solvent, pure water produced higher extraction kinetics and greater extraction rates than pure ethanol or highly concentrated ethanol-acid combinations. Water may produce a more polar medium that makes phenolic compound extraction easier. Water and combinations with low levels of ethanol might enter cells, but excessive levels of ethanol could denaturize proteins, prevent the dissolution of polyphenols and then impact the extraction (Carciochi *et al.*, 2018). It was reported that pure water has a greater dielectric constant than combinations of ethanol and water, which enhances the solubility of phenolic compounds (Socaci *et al.*, 2018).

**Total flavonoid :** Total flavonoid content showed significant variation for different anthocyanin extraction treatment (Table 3). The flavonoid content in this study ranged from 11.12 mg/g to 36.51 mg/g. The higher flavonoid content was found in T7 Ultrasound assisted extraction using water and the lower flavonoid content was recorded in $T_1$ Conventional extraction using distilled water as solvent. Similar results were observed in the studies of Koubaa *et al.* (2015) that the total flavonoid content in aqueous solvent used in Ultrasound assisted extraction was higher than water and ethanol solvent used in Conventional extraction. In further study, it was reported that the total flavonoid content was higher in Ultrasound assisted extraction, as it makes the solvent more soluble and reduces their viscosity, which makes it easier for the solvent to get between the pores in the extraction medium. When compared to other methods of extraction, the use of microwaves considerably reduced the quantity of flavonoids. This might indicate that such substances are less stable when the microwaves are present (Hanula *et al.*, 2020).

**Total antioxidant :** Total antioxidant activity was recorded highest in $T_7$ Ultrasound assisted extraction using water as solvent (74.21%). The lowest antioxidant activity was found in $T_1$ Conventional extraction using water as a solvent (43.98%) (Table 3). The studies on Gac fruit peel and

**Table 3 :** Effect of extraction methods on different physiochemical parameters- Total phenol, Total flavonoid, Total antioxidant.

| Extraction methods | Treatments | Total phenols (mg/g) | Total flavonoids (mg/g) | Total antioxidants (%) |
|---|---|---|---|---|
| Conventional extraction | $T_1$ | 37.21 | 11.12 | 43.98 |
| | $T_2$ | 43.44 | 15.31 | 49.04 |
| | $T_3$ | 58.22 | 28.21 | 61.21 |
| Microwave assisted extraction | $T_4$ | 41.24 | 15.22 | 48.31 |
| | $T_5$ | 46.55 | 19.32 | 53.32 |
| | $T_6$ | 63.44 | 30.04 | 69.11 |
| Ultrasound assisted extraction | $T_7$ | 72.12 | 36.51 | 74.21 |
| | $T_8$ | 68.21 | 34.21 | 72.42 |
| | $T_9$ | 64.11 | 31.11 | 70.23 |
| | **SEd** | 1.36 | 0.73 | 1.27 |
| | **CD at 5%** | 2.87 | 1.54 | 2.68 |

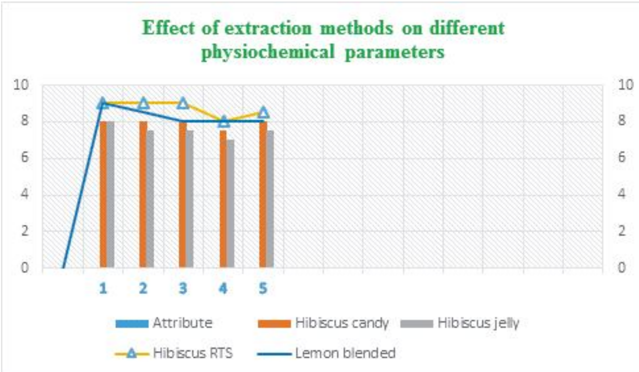


**Fig. 1 :** Graphical representation of effect of extraction methods on different physiochemical parameters - Anthocyanin, pH, Titrable acidity, TSS.

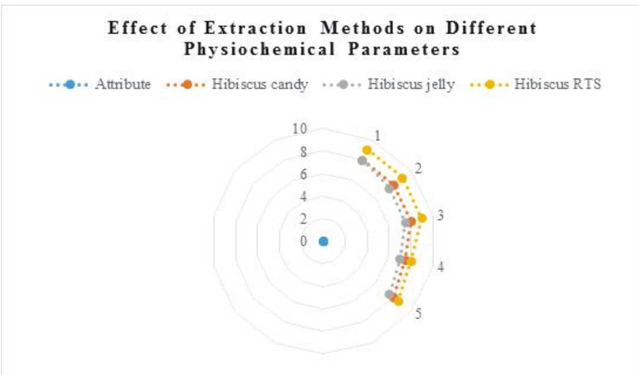


**Fig. 2 :** Graphical representation of effect of extraction methods on different physiochemical parameters- Total phenol, Total flavonoid, Total antioxidant.

their effects on antioxidant capacity of the extracts, showed similar results. Compared to Microwave assisted extraction and Conventional extraction, Ultrasound assisted extraction produced a higher yield of antioxidant capacity. The higher quantity of bioactive metabolites that were diffused into the solvent over a longer time may be the cause for increased antioxidant activity yield. It is possible that Ultrasound assisted extraction had better extraction efficiency compared to Microwave assisted extraction due to thermal deterioration of bioactive metabolites. Further in Ultrasound assisted extraction, antioxidant capacity was much higher than the Conventional method (Chuyen *et al.*, 2018).

**Preparation of edible value-added products using hibiscus extract as biocolorant**

*B. Hibiscus rosa sinensis* is a versatile plant and it has been suggested that its calyx might be used to make jelly, jam, juice, wine, syrup, pudding, cake, ice cream and flavouring. Due to its beneficial properties, it is used in medicines, cosmeceuticals, and nutraceuticals. The flower petals is the most desirable edible component of the *Hibiscus rosa sinensis* plant due to its distinctive and brilliant red colour. Although the plant can be used to make drinks, its petals are the most widely used part of the plant. Dried petals can be boiled in water to make a hot beverage or can be soaked in water to make a colourful cold beverage. Due to its high concentration of vitamin C, Anthocyanins and other antioxidants, the juice from the petals are promoted as a beverage that improves health (Akash *et al.*, 2020).

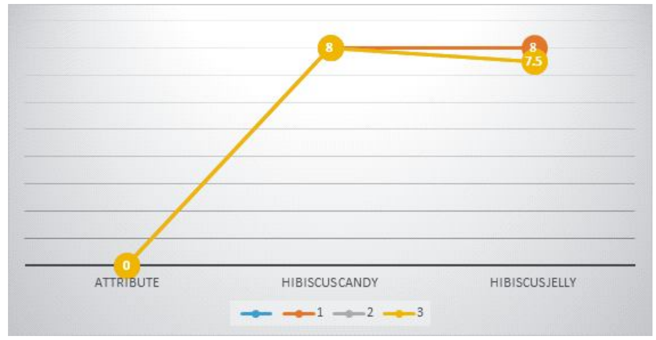


**Fig. 3 :** Graphical representation of the physiochemical parameters- pH, Total soluble solids, Titrable acidity of the prepared products from Hibiscus extracts.

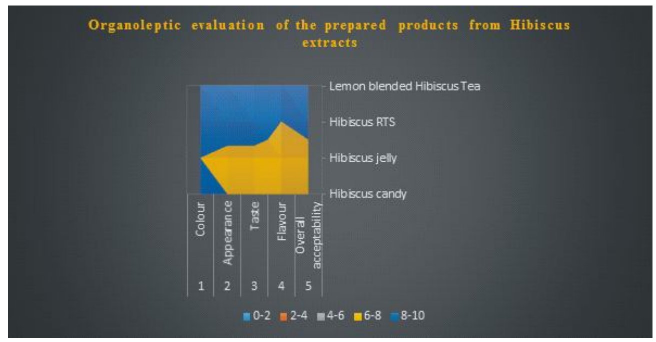


**Fig. 4 :** Graphical representation of Organoleptic evaluation of the prepared products from Hibiscus extracts.

**Total Soluble Solids (TSS) :** TSS value represent the level of sweetness and has an impact on the flavour of the food. TSS is primarily composed of total sugar content, with a minimal number of soluble proteins, amino acids and other organic components (Bexiga *et al.*, 2017). The results from Table 4 showed that, highest TSS value was recorded in Hibiscus candy and the lowest TSS value was registered in Lemon blended Hibiscus Tea. The sugar level of each product could be the cause of this variance in TSS. The investigation on changes in the nutritional and organoleptic quality of flavoured sweets made from Aonla (*Emblica officinalis* G.) produced similar results during storage (Priyanka *et al.*, 2012) and the study on impact of the addition of *Lactobacillus casei* and oligofructose on the quality parameters of orange juice and Hibiscus tea mixed beverage (Miranda *et al.*, 2019).

**pH :** In the food industry, pH is testing is done to ensure food quality, manage taste and other attributes, control microbial activity, and increase food shelf life.The

**Table 4 :** The physiochemical parameters- pH, Total soluble solids, titrable acidity of the prepared products from Hibiscus extracts.

| Products | pH | TSS ($^0$B) | Tirable acidity (%) |
|---|---|---|---|
| Hibiscus candy | 4.2 | 22.7 | 0.41 |
| Hibiscus jelly | 3.4 | 19.5 | 0.12 |
| Hibiscus RTS | 3.3 | 12.3 | 0.39 |
| Lemon blended Hibiscus tea | 3.1 | 8.5 | 0.43 |

**Table 5 :** Organoleptic evaluation of the prepared products from Hibiscus extracts.

| S. no. | Attribute | Hibiscus candy | Hibiscus jelly | Hibiscus RTS | Lemon blended Hibiscus Tea |
|---|---|---|---|---|---|
| 1 | Colour | 8 | 8 | 9 | 9 |
| 2 | Appearance | 8 | 7.5 | 9 | 8.5 |
| 3 | Taste | 8 | 7.5 | 9 | 8 |
| 4 | Flavour | 7.5 | 7 | 8 | 8 |
| 5 | Overall acceptability | 8 | 7.5 | 8.5 | 8 |

texture, flavour, nutritional content, and safety of foods are all significantly influenced by their pH. The pH is used to measure hydrogen ion concentration in the food products. Most commonly, the pH levels in food range from 2.5 to 7. The results of the study (Table 4) showed Lemon blended hibiscus tea had the lowest pH, while Hibiscus candy has the highest. This was as a result of the lemon's addition to the hibiscus tea tea's high acid level (which contains citric acid). Thus, it was evident that the acidity of food products increased with decreasing pH levels. Studies on the combined beverages of Aonla and Hibiscus tea produced similar findings (Priyanka *et al.*, 2012; Miranda *et al.*, 2019)

**Titrable acidity :** Titrable acidity, often known as total acidity, quantifies the overall acidity of a product. The titratable acidity of a food sample is a measurement of the quantity of acid or acids present. The results in this study (Table 4) showed that Hibiscus jelly and lemon blended Hibiscus tea have the greatest Titrable acidities, respectively. The higher and lower ranges of acquired acidity are caused by the acid-content found in food goods. Results from the study of Hibiscus tea mixed beverages (Miranda *et al.*, 2019), red beet (*Beta vulgaris* L.) and roselle (*Hibiscus sabdarriffa* L.) jelly production(Sara and Awad Amira, 2019), Aonla candy preparation (Priyanka *et al*., 2012) and therapeutic RTS beverage developed from aonla basil and ginger (Fegade Chandrakant and Masih, 2021) are similar.The quantity and kind of ingredients, additives and preservatives employed during product development all have an impact on the acidity of a food product (Tyl and Sadler, 2017).

**Sensory analysis :** The score for each product based on colour, appearance, taste, flavour and overall acceptability were assessed and tabulated (Table 5). The results showed that Hibiscus RTS scored the highest among the preference of the panelist. Almost similar results were obtained for Lemon blended Hibiscus Tea and Hibiscus candy. Positive feedback was given by the panelist based on the organoleptic evaluation of the products.

## Conclusion

The present study successfully identified an effective method for extracting anthocyanins from *Hibiscus rosa-sinensis* petals and explored its application as a natural food colorant in value-added edible products. Among the nine treatments tested, Ultrasound-Assisted Extraction using distilled water ($T_7$) yielded the highest anthocyanin content (179.32 mg/L), phenolic content (72.12 mg/g), flavonoid content (36.51 mg/g), and antioxidant activity (74.21%). The physicochemical parameters such as pH, titrable acidity, and total soluble solids varied significantly across the extraction methods and solvents, emphasizing the influence of solvent polarity and method efficiency. Edible products such as Hibiscus candy, jelly, RTS beverage, and lemon-blended Hibiscus tea were developed using the extracted pigment. Hibiscus candy recorded the highest TSS and pH, while lemon-blended Hibiscus tea had the highest titrable acidity. Sensory evaluation revealed that Hibiscus RTS beverage was most preferred for its color, flavor, and overall acceptability. This study demonstrates that Ultrasound-Assisted Extraction using water is a green, efficient, and non-toxic method for extracting anthocyanins from *H. rosa-sinensis*, offering a viable alternative to synthetic food colorants. Furthermore, the incorporation of Hibiscus extract enhances the functional and nutritional value of food products, making it a promising natural colorant and antioxidant source for the food industry.

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All the authors made substantial contributions to the conception of the work. Visalakshi M and Sri Raghavi R

wrote the original draft, visualization, data curation and conceptualization. Ganga M performed writing review, editing, Supervision, and Investigation. Thamaraiselvi S.P contributed to supervision and validation. Karthikeyan. S, Ganesh S and Vasanth S did data curation, supervision, and investigation. All the authors have read and approved the manuscript for publication.

### Competing interests

There are no conflicts of interest regarding the content of this article.

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