The Pharma Innovation Journal 2022; 11(8): 303-309

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(8): 303-309

www.thepharmajournal.com
Received: 16-05-2022
Accepted: 30-06-2022

Sri Raghavi R
PG Scholar, Department of Floriculture and Landscape Architecture, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Visalakshi M
Assistant Professor, Department of Horticulture, Office of Dean Horticulture, Horticulture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Karthikeyan S
Assistant Professor, Department of Horticulture, Office of Dean Horticulture, Horticulture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Amutha Selvi G
Assistant Professor, Department of Food Process Engineering, Agriculture Engineering College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Thamaraiselvi SP
Assistant Professor and Head I/C, Horticulture Research Station, Ooty. Tamil Nadu, India

Gurusamy K
Assistant Professor, Department of Biochemistry, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author:
Sri Raghavi R
PG Scholar, Department of Floriculture and Landscape Architecture, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Standardisation of anthocyanin extraction techniques from hibiscus (*Hibiscus rosa-sinensis*) petals for biocolour utilisation

Sri Raghavi R, Visalakshi M, Karthikeyan S, Amutha Selvi G, Thamaraiselvi SP and Gurusamy K

DOI: https://doi.org/10.22271/tpi.2022.v11.i8d.14647

Abstract
In recent years, the use of natural anthocyanin as a biocolour in business has grown to peak and there is an increasing desire to enhance the extraction procedures. In this study, various extraction techniques (Conventional extraction, Microwave assisted extraction and Ultrasound assisted extraction) for extracting anthocyanin from *Hibiscus rosa-sinensis* petals using water, ethanol, and citric acid as solvents have been investigated. Total Anthocyanin, Total Antioxidant, Total Phenolic Content, Total Flavonoid, Total Soluble Solids, Titrable Acidity and pH are estimated in this study. The results showed that Ultrasound assisted extraction recorded higher anthocyanin rate (179.32mg/l) when compared to Microwave assisted extraction (155.45mg/l) and Conventional extraction (100.88mg/l).This study also showed that extraction efficiency is more significant in aqueous solvent in Ultrasound assisted extraction rather than other solvents.

Keywords: *Hibiscus rosa-sinensis,* Conventional extraction, Microwave assisted extraction, Ultrasound assisted extraction

Introduction

Colour is one among the foremost vital quality attributes touching the consumer's acceptance of products like food & cosmetics since it offers the primary impression of the product's quality. The world demand for dyes is about 10,000 tonnes and is predicted to grow apace in close to future. The use of synthetic dyes which is commonly called the artificial dyes causes harm for the consumers like skin rashes, stomach infections, allergies etc. Thus people tend to invite the idea of natural colours or biocolour. Biocolour are dyes or pigments that gives colour when blended with drugs, cosmetics or foods, which has its biological origin from flowers, leaves, barks or roots. In some cases natural colours are obtained from insects and microbes. Moreover natural dyes with high antioxidant capacity results in health benefits in human, which is an added advantage.

Hibiscus rosa-sinensis colloquially called as Shoe flower or China rose, which is an ornamental shrub with glossy leaves and red flowers, belongs to the family Malvaceae. The flowers are of five petals with orange-tipped red anthers. In Hibiscus various colours such as red, pink, white, yellow are seen in different cultivars and hybrids. Hibiscus has high aesthetic value and all parts of this plant are rich in medicinal properties. It has essentially more amount of bioactive components like flavonoids, terpenoids, saponins, glycosides etc., which cures many diseases and acts as herbal alternative (Obi *et al*, 1998) [18]. Apart from aesthetic values, the major use of Hibiscus flower is its anthocyanin content which can be used as a natural colour in foods, cosmetics, drugs, dyes *etc*. The most essential anthocyanin in red pedalled *Hibiscus* is cyanidin-3-sophoroside (Lowry, 1976) [14].

Anthocyanins are polyphenolic pigments which are safe, non-toxic and natural water soluble compounds that can easily be incorporated in food products (Pazmino-Duran *et al*, 2001). Anthocyanins have high health benefits such as antidiabetic, antimicrobial, anti-obesity and prevention of cardiovascular diseases (Hock Eng Khoo *et al*, 2017) [9]. Thus, in this study, the standardisation of Anthocyanin extraction techniques from Hibiscus petals for biocolour utilisation is done. The main aim of the study is that there should be optimum extraction and the technique should be easily adaptable for commercial use. By comparing different extraction treatments, the optimum technique and solvent has been standardized.

Materials and Methods

Materials collected

The raw materials hibiscus petals were collected from the Department of Floriculture and Landscape Architecture, Botanical Garden, Tamil Nadu Agricultural University, Coimbatore during the year 2021-2022. The petals were removed from the collected flowers, washed with distilled water and were shade dried for three days. The dried petals were ground into powder. The powdered shade dried petals were further used for extraction.

Extraction: Different extraction methods along with different solvent were carried out in the anthocyanin extraction process. Solvents like water, ethanol and citric acid were used. Extraction methods include Conventional Extraction, Microwave Assisted Extraction and Ultrasound-Assisted Extraction. The treatment include a total of nine treatments with three replications. The experiment was conducted in Completely Randomized Statistical Design. The data obtained were analysed statistically.

Treatment details

T1	Conventional Extraction + Distilled Water
T2	Conventional Extraction + 2% Citric acid
T3	Conventional Extraction + 85% Ethanol
T4	Microwave Assisted Extraction + Distilled Water
T5	Microwave Assisted Extraction + 2% Citric acid
T6	Microwave Assisted Extraction + 85% Ethanol
T7	Ultrasound Assisted Extraction + Distilled water
T8	Ultrasound Assisted Extraction + 2% Citric acid
T9	Ultrasound Assisted Extraction + 85% Ethanol

Conventional extraction (CE)

In conventional extraction, Du and Francis, 1973 method was followed with few modifications. 50 g of shade dried powder of Hibiscus petals were taken in a conical flask. 500 ml of solvent (Distilled Water, 2% Citric acid, 85% Ethanol) were added and the mixture is kept in the hot water bath at 60ºC for 60 minutes. The extract along with the petals is squeezed and filtered using muslin cloth and further filtered with Whatman filter paper if needed. The extracted liquid of 400 ml was reduced into concentrated extract using rotary evaporator (SPAN Automation, SARE29G) at 60ºC, 40 rpm for half an hour till the volume is reduced to 90 ml. This concentrated extract is used for further analysis.

Microwave assisted extraction (MAE)

For Microwave assisted extraction, Ashitha *et al.,* 2020 [3] study where standardization of the exposure time (s), microwave power (W), sample/solvent ratio (g/ml) was adopted in this experiment. 1g of sample powder was taken in a beaker mixed with 25 ml solvent (Distilled Water, 2% Citric acid, 85% Ethanol) in 1:25 ratio. This mixture was fed into the microwave extractor cells (ETHOS X, Microwave assisted extraction system) with 400 W microwave power. The exposure time for water and 2% citric acid was 180s and 120s for 85% ethanol.

Ultrasound assisted extraction (UAE)

In Ultrasound assisted extraction, 1 g of powdered sample was mixed with 15 ml solvent (Distilled Water, 2% Citric acid, 85% Ethanol) in 1:15 ratio. The mixture was fed into the Ultrasound assisted extraction system at a frequency of 40 kHz for 30 min (Aryanti *et al.*, 2019) [2].

By the above techniques, the extraction was completed and observations were done by further analysis. The parameters included Titrable acidity, pH, Total phenols, Total flavonoids, Total soluble solids, Anthocyanin content and Antioxidant analysis and were observed for each treatment. Total Titrable acidity was calculated by visual titration technique given by (Ranganna, 1986) [20]. The observation for *p*H was done by using digital pH meter and the observation were taken (AOAC, 2000) [4]. Singleton and Rossi (1965) [21] method was followed to determine the Total phenolic content. Total flavonoids were observed according to the study of Liu *et al.*, 2008 [13]. Total soluble solids were expressed in ºBrix using hand Refract meter. Following the study of Lee *et al*, 2005, total anthocyanin content was determined and antioxidant were observed using DPPH radicle scavenging activity assay method following the study described by Hani *et al*, 2015 [7] with a slight modification.

Result and Discussion

Anthocyanin content

The anthocyanin content from dried petals of *Hibiscus rosa-sinensis* by different extraction techniques showed variation. Among the nine treatments, highest anthocyanin content was recorded in T7 Ultrasound assisted extraction using water as solvent (179.32mg/l). Followed by T8 Ultrasound assisted extraction using 2% citric acid as solvent (170.22mg/l). The lowest anthocyanin content was recorded in T1 Conventional extraction using water as a solvent (45.71mg/l). The results (Table 1) showed that, comparatively Ultrasound assisted extraction recorded higher anthocyanin content than Microwave assisted extraction and Conventional extraction methods. In Conventional extraction and Microwave assisted extraction methods, ethanol as solvent recorded higher anthocyanin extraction rate, whereas in Ultrasound assisted extraction method water as a solvent recorded higher anthocyanin content. This is because, in Ultrasound Assisted extraction, solvents with high surface tension, low vapour pressure and high viscosity have higher extraction rate Vardanega *et al.*, 2014 [22]. Studies showed that vapour pressure and surface tension are higher in water than ethanol. Similar results were observed in the anthocyanin extraction of *Hibiscus Sabdariffa* by Ultrasound assisted extraction (Aryanti *et al.*, 2018). According to further report, Ultrasound assisted extraction had higher anthocyanin extraction rate than Conventional extraction and Microwave assisted extraction in *Hibiscus Sabdariffa* and Maqui berries (Carolina Moser Paraiso *et al.*, 2019 [5], Mercedes Vazquez-Espinosa *et al.*, 2019) [17]. The research studies reported that Microwave assisted extraction resulted in higher extraction than Conventional extraction in Roselle flower (Ike Sitoresmi M Purbowati *et al.*, 2019) [10].

Titrable acidity

Titrable acidity measures the total concentration of acidity in a food product. Titrable acidity showed variation for different extraction treatment. The highest Titrable acidity was found in T8 Ultrasound assisted extraction using 2% citric acid as solvent (13.45%) followed by T2 Conventional extraction using 2% citric acid as solvent (13.30%). Lowest percentage of Titrable acidity was found in T4 Microwave assisted extraction using water as solvent (8.08%) (Table 1). Comparatively higher percentage of Titrable acidity was found in extracts using citric acid solvents rather than water and ethanol solvent. Similar results were observed in the study of physiochemical properties of natural pigments for *Hibiscus sabadariffa* (Abou-Arab, 2011) [1]. From the above results it is clear that acidity range differed based on the solvent used rather than the extraction technique.

*p*H

As per U.S. Geological survey (2019), pH is to observe the relative amount of free hydrogen and hydroxyl ions in water. Different treatments of anthocyanin extraction showed different pH value based on the solvent used. The highest pH value was recorded in T4 Microwave assisted extraction using water as solvent (7.3) followed by T1 Conventional extraction using water as solvent (7.2). The lowest pH range was found in T8 Ultrasound assisted extraction using 2% citric acid as solvent (4.1) which was acidic (Table 1). A higher pH was found in extracts using water as a solvent and the lowest pH ranges in extracts with 2% citric acid as solvent. Comparative results were observed in the studies of Roselle flower (*Hibiscus sabdariffa)*, (Manjula *et al.,* 2021).

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. A higher TSS value was found in T3 Conventional extraction using 85% ethanol as solvent (23.5ºB) followed by T9 Ultrasound assisted extraction using 85% ethanol as solvent (22.1ºB). A lower TSS value was found in T1 Conventional extraction using water as solvent (5.1ºB) (Table 1). Similar results were recorded from the studies of (Abou-Ara *et al.,* 2011). A higher TSS value is found in ethanol solvent extract because the extraction rate of ethanol is higher which not only extracts higher anthocyanin, but also higher solids, organic acids, proteins (Manjula *et al.,* 2021).

Total Phenols

The total phenolic content shows specifically different variation for different anthocyanin extraction treatments. Higher phenolic content was found in T7 Ultrasound assisted extraction using water as solvent (72.12mg/g) followed by T8 Ultrasound assisted extraction using 2% citric acid as solvent (68.21 mg/g) whereas lower phenolic content was observed in T1 Conventional extraction using water as solvent (37.21mg/g), (Table 2). Comparatively similar results were obtained in the study of optimization of Ultrasound assisted extraction of functional ingredients from Bertoni leaves (Jana Sic Zlabur *et al.,* 2015). This study showed that aqueous solvent in Ultrasound assisted extraction had higher phenolic extraction than the other methods.

Total Flavonoid

Total flavonoid content shows significant variation for different anthocyanin extraction treatment. The higher flavonoid content was found in T7 Ultrasound assisted extraction using water as solvent (36.51 mg/g) which was followed by T8 Ultrasound assisted extraction using 2% citric acid as solvent (34.21 mg/g). The lower flavonoid content was observed in T1 Conventional extraction using distilled water as solvent (11.12 mg/g). Similar results were observed in the studies of (Jana Sic Zlabur *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 there are microwaves present (Monika Hanula *et al.,* 2020) [16].

Total Antioxidant

Total antioxidant activity was recorded highest in T7 Ultrasound assisted extraction using water as solvent (74.21%) followed by T8 Ultrasound assisted extraction using 2% citric acid as solvent (72.42%). The lowest antioxidant activity was found in T1 Conventional extraction using water as a solvent (43.98%) (Table 2). The studies on Gac fruit peel and 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 period 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 (Hoang V. Chuyen *et al.* 2017).

Table 1: Effect of extraction methods on different parameters – Anthocyanin, pH, Titrable acidity, TSS

Treatments	Anthocyanin (mg/l)	*p*H	Titrable Acidity (%)	TSS (ºB)
T1	45.71	7.2	8.19	5.1
T2	62.32	4.4	13.30	8.5
T3	100.88	6.1	10.24	23.5
T4	96.54	7.3	8.08	5.5
T5	130.12	4.3	12.99	8.5
T6	155.45	6.4	9.96	21.5
T7	179.32	7.1	8.32	6.1
T8	170.22	4.1	13.45	8.8
T9	162.14	6.3	10.03	22.1
S.Ed	2.56	0.17	0.19	0.29
CD at 5%	5.38	0.36	0.45	0.61

Table 2: Effect of extraction methods on different parameters - Total phenol, Total Flavonoid, Total Antioxidant

Treatments	Total Phenols (mg/g)	Total Flavonoids (mg/g)	Total Antioxidants (%)
T1	37.21	11.12	43.98
T2	43.44	15.31	49.04
T3	58.22	28.21	61.21
T4	41.24	15.22	48.31
T5	46.55	19.32	53.32
T6	63.44	30.04	69.11
T7	72.12	36.51	74.21
T8	68.21	34.21	72.42
T9	64.11	31.11	70.23
S.Ed	1.36	0.73	1.27
CD at 5%	2.87	1.54	2.68

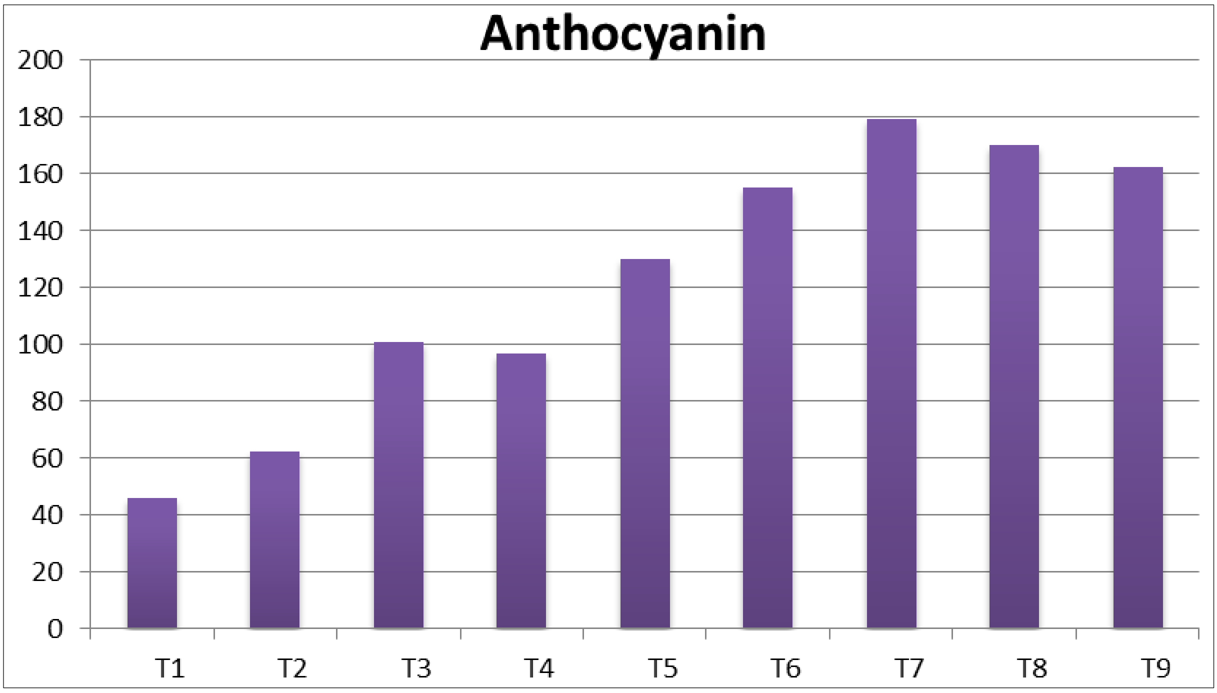

Fig 1: Effect of extraction methods on anthocyanin

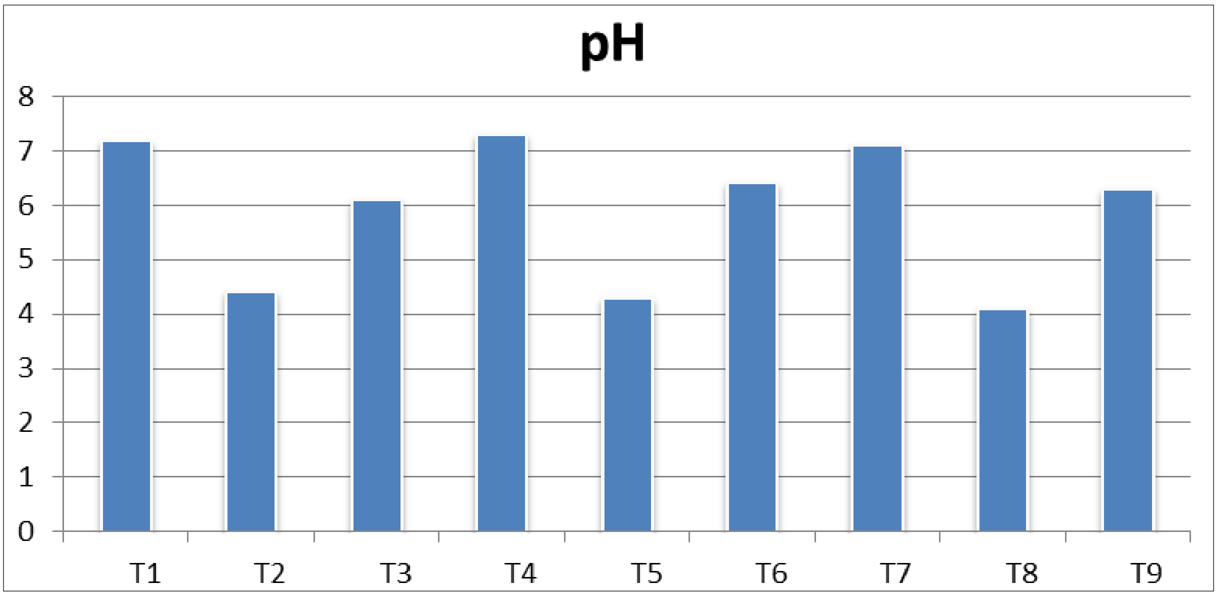

Fig 2: Effect of extraction methods on *p*H

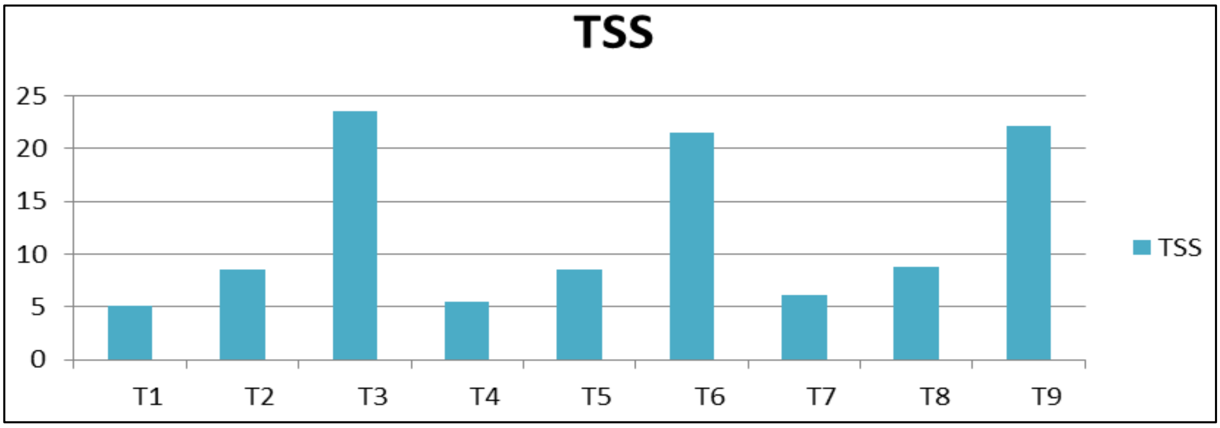

Fig 3: Effect of extraction methods on TSS

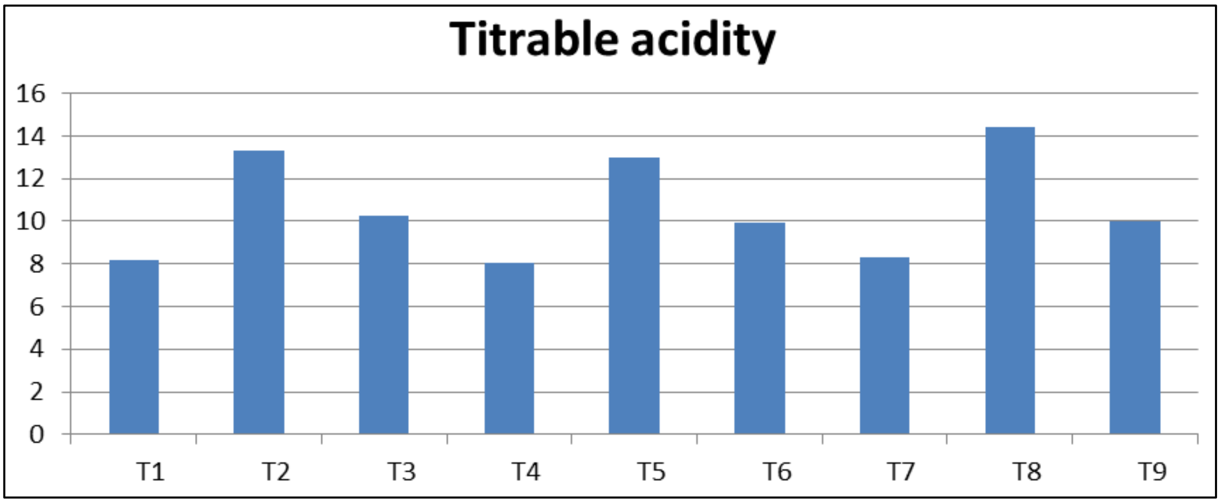

Fig 4: Effect of extraction methods on Titrable acidity

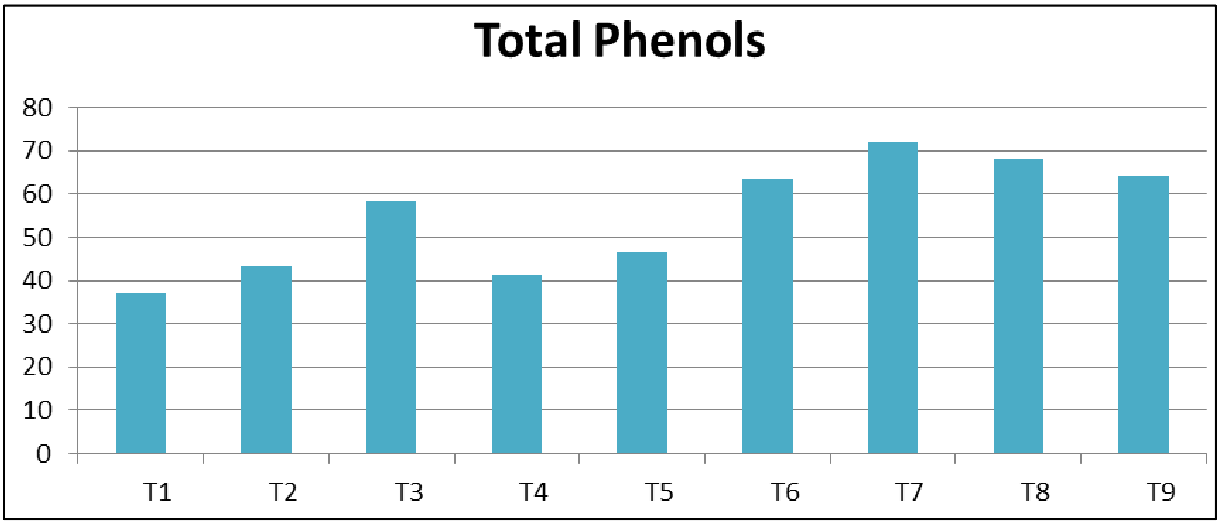

Fig 5: Effect of extraction methods on total phenol

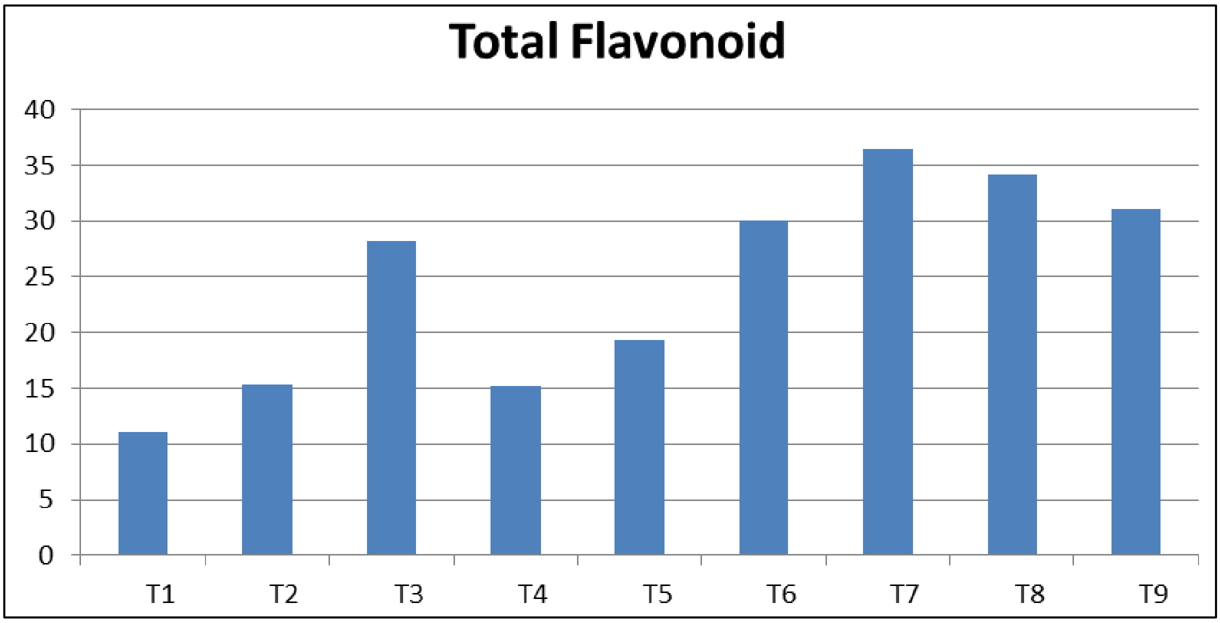

Fig 6: Effect of extraction methods on total flavonoid

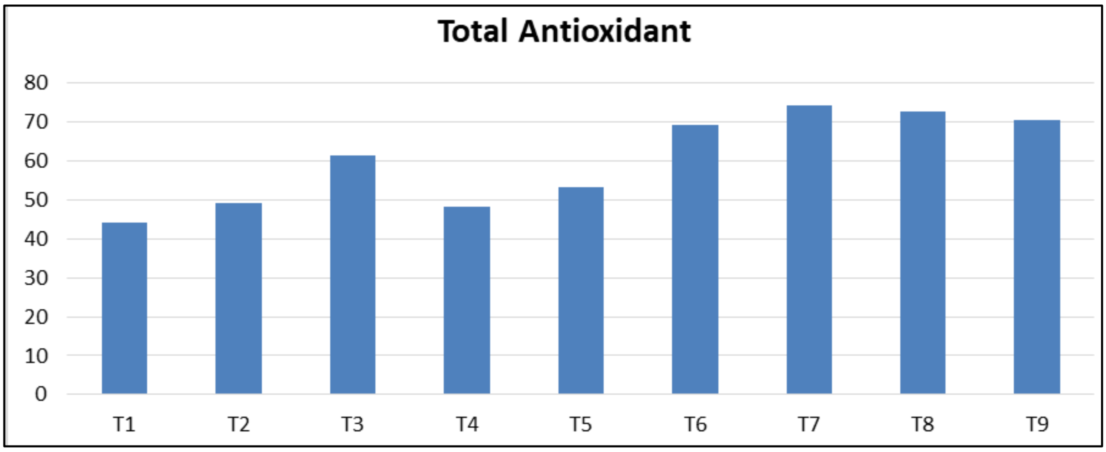

Fig 7: Effect of extraction methods on total antioxidant

Conclusion

In this article, three extraction techniques for standardisation of anthocyanin were compared (Conventional extraction, Microwave assisted extraction and Ultrasound assisted extraction). The evaluation of anthocyanin, total phenolic content, total flavonoid, Titrable acidity, pH, TSS, and antioxidant activity served as the basis for choosing the best extraction technique. Ultrasound assisted extraction turned out to be the best technique for extracting anthocyanin in comparison to conventional extraction and Microwave assisted extraction. As a further study, it was shown that using water as a solvent enhanced the efficiency of extraction in Ultrasound assisted extraction. However, additional research should be done to assess the stability of the standardised anthocyanin extract shown in this research.

References

1. Abou-Arab, Azza A, Ferial M. Abu-Salem and Esmat A Abou-Arab. Physico-chemical properties of natural pigments (anthocyanin) extracted from Roselle calyces (*Hibiscus sabdariffa*). Journal of American Science. 2011;7(7):445-456.
2. Aryanti, Nita. Conventional and ultrasound-assisted extraction of anthocyanin from red and purple roselle (*Hibiscus Sabdariffa* L.) Calyces and characterisation of its anthocyanin powder, 2019.
3. Ashitha GN, Prince MV, Sanjay P. Microwave assisted extraction of anthocyanin from *Hibiscus rosa-sinensis*. Journal of Pharmacognosy and Phytochemistry. 2020;9(2):1418-1424.
4. AOAC. Official methods of analysis of the association of official analytical chemists 17th Ed. Association of Official Analytical Chemists, USA, 2000.
5. Carolina Moser Paraíso, Suelen Siqueira Dos Santos, Vanesa G Correa, Thiago Magon, Rosane M Peralta, Jesuí V Visentainer, Grasiele Scaramal Madrona. Ultrasound assisted extraction of hibiscus (*Hibiscus sabdariffa* L.) Bioactive compounds for application as potential functional ingredient. Journal of food science and Technology. 2019;56(10):4667-4677.
6. Du CT, Francis FJ. Anthocyanins of Roselle (*Hibiscus Sabdariffa* L.). Journal of Food Science. 1973;38(5):810-812.
7. Hani, Norziah M, Siti Rashima Romli, Mehraj Ahmad. Influences of red pitaya fruit puree and gelling agents on the physico-mechanical properties and quality changes of gummy confections. International Journal of Food Science & Technology. 2015;50(2):331-339.
8. Hoang V Chuyen, Minh H Nguyen, Paul D Roach, John B Golding, Sophie E Parks. Microwave-assisted extraction and ultrasound-assisted extraction for recovering carotenoids from Gac peel and their effects on antioxidant capacity of the extracts. Food Science & Nutrition. 2018.;6(1):189-196.
9. Hock Eng Khoo, Azrina Azlan, Sou Teng Tang, See Meng Lim. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food & Nutrition Research. 2017;61(1):1361779.
10. Ike Sitoresmi M Purbowati, Ali Maksum. The antioxidant activity of Roselle (*Hibiscus sabdariffa* Linii) phenolic compounds in different variations microwave-assisted extraction time and power. In IOP Conference Series: Earth and environmental science, IOP Publishing, 2019;406(1):012005.,
11. Jana Šic Žlabur, Koubaa, Mohamed, Elena Rosello-Soto, Anet Rezek Jambrak, Mladen Brncic, *et al.* Current and new insights in the sustainable and green recovery of nutritionally valuable compounds from Stevia rebaudiana Bertoni. Journal of Agricultural and Food Chemistry. 2015;63(31):6835-6846.
12. Lee J, Durst RW, Wrolstad RE. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. Journal of AOAC international. 2005;88(5):1269-1278.
13. Liu X, Zhao M, Wang J, Yang B, Jiang Y. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. Journal of Food Composition and Analysis. 2008;21(3):219-228.
14. Lowry JB. Floral anthocyanins of some Malesian Hibiscus species. Phytochemistry. 1976;15(9):1395-1396.
15. Manjula GS, Krishna HC, Mushrif Sadanand K, Manjunatha Reddy TB. Standardization of anthocyanin extraction from Roselle (*Hibiscus sabdariffa* L.) Calyces for edible colour, 2022.
16. Monika Hanula, Jarosław Wyrwisz, Małgorzata Moczkowska, Olaf K Horbańczuk, Ewelina Pogorzelska-Nowicka, Agnieszka Wierzbicka. Optimization of microwave and ultrasound extraction methods of AÇAI

berries in terms of highest content of phenolic compounds and antioxidant activity. Applied Sciences. 2020;10(23):8325.
17. Mercedes Vázquez-Espinosa, Ana V González de Peredo, Marta Ferreiro-González, Ceferino Carrera, Miguel Palma, Gerardo F Barbero, *et al*. Assessment of ultrasound assisted extraction as an alternative method for the extraction of anthocyanins and total phenolic compounds from Maqui berries (*Aristotelia chilensis* (Mol.) Stuntz). Agronomy. 2019;9(3):148.
18. Obi, Frederick Otunuya, Ighofimoni Afonughe Usenu, Julie Osaretin Osayande. Prevention of carbon tetrachloride-induced hepatotoxicity in the rat by H. Rosa Sinensis anthocyanin extract administered in ethanol. Toxicology. 1998;131(2-3):93-98.
19. Pazmiño-Durán E, Alexandra M, Mónica Giusti, Ronald E Wrolstad, M Beatriz A Glória. Anthocyanins from Oxalis triangularis as potential food colorants. Food Chemistry. 2001;75(2):211-216.
20. Ranganna S. Handbook of analysis and quality control for fruit and vegetable products second Edn. Tata McGraw-Hill Pub Co New Delhi, India, 1986, 1138.
21. Singleton VL, Rossi JA. Acolorimetry of total phenolics with Phosphomolybdic-phosphotungstic acid reagents. American J Enol. Viticult. 1965;16:144-158.
22. Vardanega, Renata, Diego T Santos, Angela M, Meireles A. Intensification of bioactive compounds extraction from medicinal plants using ultrasonic irradiation. Pharmacognosy Reviews. 2014;8(16):88.