The Pharma Innovation Journal 2022; SP-11(3): 233-236

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(3): 233-236

www.thepharmajournal.com
Received: 13-01-2022
Accepted: 16-02-2022

Raghu A
College of Food and Dairy Technology, Koduveli, Chennai, India

Rita Narayanan
Department of Food Processing Technology, College of Food and Dairy Technology, Koduveli, Chennai, India

A Mangala Gowri
Centralized Instrumentation Laboratory, Madras Veterinary College, Vepery, Chennai, India

V Nithyalakshmi
Department of Food Process Engineering, College of Food and Dairy Technology, Koduveli, Chennai, India

Dorathy Pushparani
Department of Food Chemistry and Food Processing, Loyola College, Chennai, India

Corresponding Author
Raghu A
College of Food and Dairy Technology, Koduveli, Chennai, India

Extraction of clean-label food colourant from hibiscus

Raghu A, Rita Narayanan, A Mangala Gowri, V Nithyalakshmi and Dorathy Pushparani

Abstract
As people become more conscious of the toxicity of synthetic colours, there is a greater demand for pigments derived from natural sources. Increase in consumer demand for natural products with no chemical additives and certified dyes has necessitated the need to exploit food colourants of natural origin. Anthocyanins are gaining popularity due to potential health advantages. Present investigations were carried out to produce anthocyanin pigment as natural food colourant from *Hibiscus rosa-sinensis*. Solid-liquid extraction method was carried out with two different types of solvents *viz*. ethanol: distilled water (1:1 v/v) and distilled water, acidified with food grade citric acid in the range from 0.5% to 3% in order to recover maximum anthocyanin from the fresh flowers of *Hibiscus rosa-sinensis*. Acidified distilled water extract with maximum TPC, TMA and AA value were chosen as a standardized extract and values were found to be 5180.50±0.02 mg GAE/100g, 191.73±0.01 mg C3G/100g, 87.92±0.06%. This Natural food colourant finds applications in food products like dairy and beverage industry by replacing the existing synthetic colourants.

Keywords: anthocyanin, natural colourant, *H. rosa-sinensis*, citric acid

1. Introduction
Traditionally, several plants and their products have been used in foods as a mode of natural preservative, flavoring agent as well as a remedy to treat some of the common ailments in human (Voon *et al.,* 2012) [12]. Plants are rich source of bioactive compounds which were responsible for the prevention and treatment of chronic health conditions such as hypertension, cardiovascular diseases, inflammation and cancer. Extracts of these bioactive components can also be used as natural colorants for food because they are believed to be safe, and non-toxic to human (Boo *et al.,* 2012) [4] Among the bioactive components, Anthocyanins were identified as a potential one responsible for beneficial properties such as antioxidant and antimicrobial activities (Pires *et al.,* 2019) [10]. The synthetic colouring additives in the food industry can be replaced by anthocyanins which correspond to reddish–purple colour and present in most of the flowers and fruits (Jabeur *et al.* 2017) [7].
Hibiscus rosa-sinensis from the family Malvaceae is abundantly flowering, perennial, woody ornamental shrub widely found in the tropical regions. Studies on *H. rosa-sinensis* have revealed to possess high amount of bioactive components and is recommended as an herbal alternative to cure many diseases (Obi *et al.,* 1998) [5]. Major anthocyanins present in old strains of *Hibiscus rosa sinensis L.* is found to be cyaniding-3-β-sophoroside (Nakamura *et al*, 1990) [9] and the dark red flowers of *H. rosa-sinensis* are important source of cyanidin-3-glucoside. In Ayurveda and ancient literatures, *H. rosa-sinensis* has been used in various conditions like hypertension, pyrexia, liver disorders, antifertility activity, epilepsy, leprosy, bronchial catarrh and diabetes.
The present study was focused on the extraction of crude anthocyanins from *Hibiscus rosa sinensis* using two different solvents ie. distilled water and Ethanol: distilled water (50:50) with 0.5 to 3% acidifying agent and comparing the extraction by analyzing total polyphenols, total monomeric anthocyanin and antioxidant values. These results will demonstrate the antioxidant activity and quantification of bioactive components present in hibiscus flower after extraction and will be helpful for small-scale food business operators by reducing the usage of artificial colouring agents thereby a new marketability trend will get created. these extracts can be used as a potential natural food colourant in Food, Dairy and Beverage industries.

2. Materials and Methods

2.1 Materials

2.1.1 Plant materials

Fresh *Hibiscus rosa-sinensis* flowers were obtained from the local market, Chennai. The fresh flowers were examined for quality and bright red flowers without any damage were washed with distilled water and shade dried just to reduce surface water content and stored in nylon bags at 4°C until further analysis.

2.1.2 Chemicals

Double distilled water, Ethanol (AR 99%), Food grade citric acid, Sodium carbonate, Folin-Ciocalteu reagent (FCR), Potassium chloride, Sodium acetate, DPPH - 2, 2-diphenyl-1-picrylhydrazyl (Hi media), The reagents required for analysis were freshly prepared by adopting standard procedures.

2.1.3 Solvent preparation

Solvent 1: Ethanol-50% is prepared by adding 500ml of distilled water with 500ml of Ethanol (AR 99%) in a standard measuring flask. After preparation of solvent 1 acidification is done by adding various amounts of Food grade citric acid from 0.5% to 3%.

Solvent 2: Distilled Water Acidified with Food grade citric acid from 0.5% to 3%

2.2 Methods

2.2.1 Determination of pH

The pH of extracted samples was measured by using a bench-top pH meter (Model Susima MP1 Plus).

2.2.2 Determination of Total soluble solids (TAA)

Total soluble solids (TSS) (ºBrix) of extracted samples were determined using a hand refractometer according to AOAC (2000) at room temperature (25±1 ºC) expressed as ºBrix (0 - 90).

2.2.3 Determination of total polyphenol content (TPC)

Total polyphenols present in the exaction were determined by Folin-Ciocalteu reagent, following Bergmeier *et al.* (2014) [3]. Extract aliquots (50-100 µl) were transferred into the test tubes and the volumes were completed to 5 mL with distilled water. After adding 0.20 ml Folin-Ciocalteu reagent and 0.5 ml saturated aqueous sodium carbonate solution the tubes were vortexed and absorbance of the mixtures was recorded after 20 min., at 765 nm, by using a UV-Visible spectrophotometer (UV Mini – 1240). The amount of total polyphenols was calculated as gallic acid equivalents from the calibration curve with gallic acid standard solution. Results were expressed as mg of total phenolic content (gallic acid equivalent) per gram of fresh flower (mg GAE 100 g^{-1}dry flower)

2.2.4 Determination of total monomeric anthocyanin (TMA)

Total monomeric anthocyanin content of the extract was determined by pH differential method following AOAC Official Method 2005.02. pH 1.0 buffer and pH 4.5 buffer were made from potassium chloride (0.025M) and sodium acetate (0.4M). 1 part of the test solution is mixed with 4 parts of each buffer solution in a 50ml volumetric flask (total diluted solution should be ≤10ml) and the absorbance was measured within 20-50mins of preparation. The diluted test portions at pH 1.0 and pH 4.5 both were measured at 520nm and 700nm against distilled water as blank solution using UV-Visible spectrophotometer (UV Mini – 1240).

Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L) = $\frac{A \times MW \times DF \times 10\text{^}3}{\varepsilon \times l}$

Where,

A = ($A_{520nm} - A_{700nm}$) pH 1.0 - ($A_{520nm} - A_{700nm}$) pH 4.5

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu)

DF = dilution factor

l = pathlength in cm

Ɛ = 26 900 molar extinction coefficient, in L × mol–1 × cm–1, for cyd-3-glu.

10^3 = factor for conversion from g to mg

2.2.5 Determination of Antioxidant activity (AA)

The free radical scavenging activity of the flower extract was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method modified from Alhakmani *et al.* (2013) [3]. Briefly, the mixture contained 1 mL of 0.1mmol DPPH radical solution prepared in methanol and 0.1 mL of extract solution is mixed with DPPH solution. The solution was rapidly mixed and incubated in dark at 37 °C for 20 min. The absorbance of each solution was measured at 517 nm against 0.1mmol DPPH solution in methanol as blank using UV/Vis spectrophotometer.

% Free radical scavenging activity = $\frac{Ac - As}{Ac} \times 100$

Where, A_c – Absorbance of Control sample, A_s – Absorbance of sample.

2.2.6 Statistical analysis

VETSTAT software was used for statistical analysis and the results were given as mean± standard deviation (SD).

3. Results and Discussion

3.1 Effect of solvents on the Physico chemical properties of flower of *Hibiscus rosa-sinensis* extract

Physicochemical properties such as pH and TSS of hibiscus extracts were determined and the results are given in table 1. It is observed that the pH gradually decreased for hibiscus extract. This can be attributed to the increase in the citric acid concentration in solvent extraction method. Similar results were obtained by Arab *et al.* (2011) [1] who reported that pH of hibiscus extract increased with increased concentration of citric acid.

The TSS of the hibiscus extract increased gradually with increase in citric acid concentration. The acidified solvents degraded the cell membrane and dissolved the soluble solids into the extract thereby, increasing the TSS content. The results in the present study ranged from 3.10 to 5.43 and are in concurrence to the study reported by Arab *et al.* (2011) [1] who reported that TSS for *Hibiscus roselle* plant extract with 2% citric acid solution was 12 °Brix and 5 °Brix in aqueous solvent.

3.2 Effect of solvents on the extraction of TPC and TMA and AA from the flowers of *Hibiscus rosa-sinensis*

Extraction efficacy of ethanol with acidified water containing citric acid and distilled containing varying concentration of citric acid were compared for TPC and TMA and AA in the

crude extract of hibiscus extract. As shown in table 2, TPC and AA of hibiscus using ethanol: water acidified solvent had higher affinity of extraction than acidified water extraction.

However, it is noticed that the TMA in acidified water extract was significantly higher in aqueous acidified extract when compared to ethanol: water acidified extract. This result coincided with the findings of Vankar *et al.* (2010) [11] who also observed that TMA of acidified aqueous extract was higher than that of ethanolic extracts.

The TPC and TMA of hibiscus crude extract had highly significant yield due to the acidified solvents which degraded the cell membrane and dissolved phenolic components and stabilized them simultaneously. This may due to the formation of flaviyum cationic salts in lower acidic medium which serves as an ideal medium to extract and stabilize most of the TPC and TMA content in the extracts. But contents were not too acidic to cause partial hydrolysis of the acyl moieties in acylated anthocyanins as suggested by Andersen and Markham, (2006). Hence the TPC and TMA content increased with increase in citric acid concentration which is concordant with the observations of Chandrasekhar *et al.* (2012) [6].

However, at 3% citric acid concentration, both solvents showed a decrease in extraction yield of TPC and TMA content. This decrease might be due to the hydrolysis of the acyl moieties in acylated anthocyanins at higher concentration of acid in solvent medium (Arab *et al.*, 2011) [1].

The AA of the extract range was significantly higher in aqueous acidified solvent which is concomitant to the work of Kruawan *et al.* (2006) [8] who also observed that DPPH radical scavenging effect of Hibiscus with hot water extract was about 93.12%.

Table 1: Treatment combination for Acidified solvent extraction

Treatments	Ethanol (V/V)	Distilled Water (V/V)	Citric Acid (Food Grade)
T1	50%	50%	0.5%
T2	50%	50%	1%
T3	50%	50%	1.5%
T4	50%	50%	2%
T5	50%	50%	2.5%
T6	50%	50%	3%
T7	Nil	100%	0.5%
T8	Nil	100%	1%
T9	Nil	100%	1.5%
T10	Nil	100%	2%
T11	Nil	100%	2.5%
T12	Nil	100%	3%

Table 2: Effects of solvents on physico-chemical properties of *Hibiscus rosa-sinensis* extract

Treatments	pH	TSS(°Brix)
T1	2.43^{f}±0.00	3.26^{a}±0.02
T2	2.34^{e}±0.00	3.54^{b}±0.04
T3	2.22^{d}±0.00	3.99^{c}±0.04
T4	2.10^{c}±0.00	4.42^{d}±0.03
T5	1.97^{b}±0.00	5.13^{f}±0.04
T6	1.86^{a}±0.00	5.43^{g} ±0.02
T7	2.41^{f}±0.01	3.10^{a}±0.06
T8	2.33^{e}±0.00	3.38^{b}±0.05
T9	2.23^{d}±0.00	3.83^{c}±0.03
T10	2.11^{c}±0.00	4.27^{d}±0.03
T11	1.99^{b}±0.00	4.73^{e}±0.03
T12	1.88^{a}±0.00	5.23^{f}±0.08
F-value	1579.16**	333.04**

@Average of six trials

** statistically highly significant ($P \leq 0.01$)

Means bearing various superscripts in the same column differs highly significantly ($P \leq 0.01$)

Table 3: Effect of solvents on extraction of TPC, TMA, AA from the flowers of *Hibiscus rosa-sinensis*

Treatments	Total Polyphenol content(mg GAE/100g)	Total Monomeric Anthocyanin (mgC3G/100g)	Antioxidant Activity (%)
T1	4996.36^{b} ±0.01	159.17^{a}±0.16	83.33^{d}±0.03
T2	5069.78^{d}±0.02	167.70^{d}±0.02	85.44^{e}±0.02
T3	5102.26^{f}±0.02	172.04^{e}±0.02	89.57^{h}±0.02
T4	5153.37^{h}±0.01	188.82^{g}±0.01	92.42^{k}±0.01
T5	5254.74^{l}±0.01	190.49^{j}±0.01	91.80^{j}±0.01
T6	5185.78^{k}±0.02	189.27^{h}±0.01	90.15^{i}±0.02
T7	4986.25^{a}±0.03	160.49^{b}±0.02	73.58^{a}±0.05
T8	5004.36^{c}±0.01	165.67^{c}±0.01	78.76^{b}±0.06
T9	5074.78^{e}±0.02	173.27^{f}±0.01	82.40^{c}±0.03
T10	5122.26^{g}±0.02	188.58^{g}±0.01	85.76^{f}±0.03
T11	5180.50^{j}±0.02	191.73^{k}±0.01	87.92^{g}±0.06
T12	5176.46^{i}±0.02	189.77^{i}±0.15	85.56^{e}±0.03
F-value	19886975.17**	38280.25**	24885.12**

@Average of six trials

** statistically highly significant ($P \leq 0.01$)

Means bearing various superscripts in the same column differs highly significantly ($P \leq 0.01$)

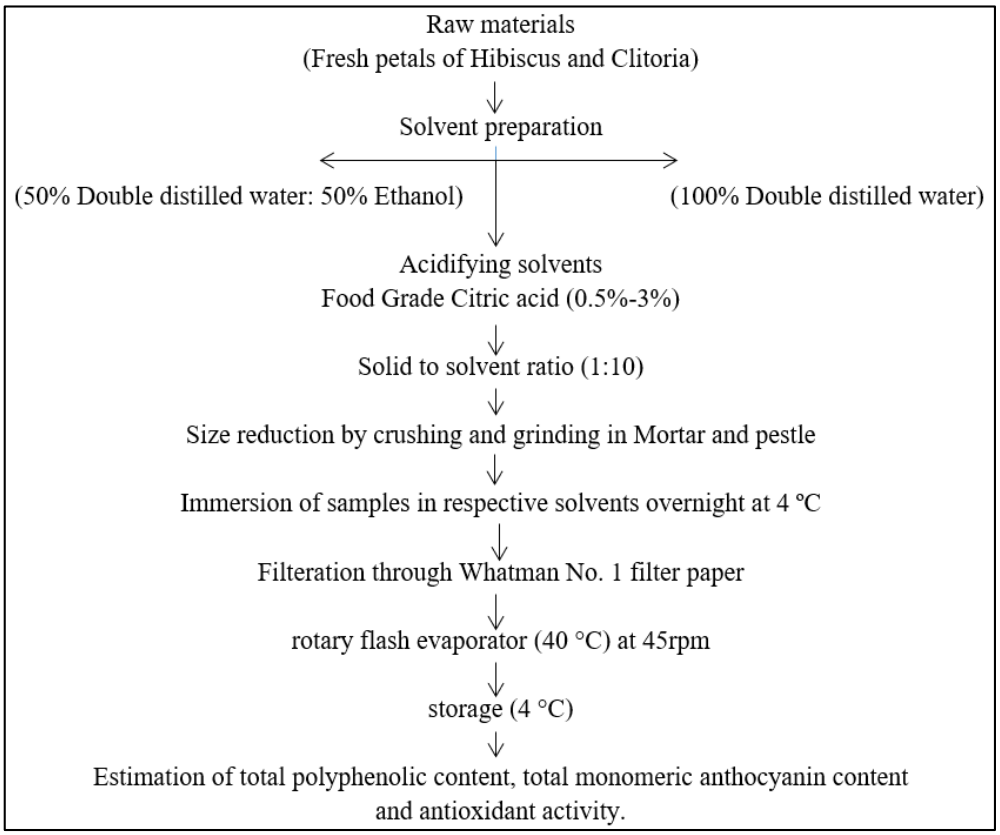

Fig 1: Flow diagram for Extraction of crude anthocyanin recovery

4. Conclusion

Acidification in extraction method increases the effective extraction and maintains the pH and colour of the extracts. This method of extraction is easy and possible for Small scale food business operator to produce natural colourant with antioxidant property hence eliminating the usage of artificial and natural identical colours in fast moving food products like milk based beverages and other beverage. Further studies may be done to extend the storage life of natural anthocyanin extracted.

5. Acknowledgement

The authors of this article would like to acknowledge College of Food and Dairy Technology, Tamil Nadu Veterinary and Animal Sciences University, Chennai for their help and motivation throughout the research work. We also like to thank all the warm hearted persons who had their part in this research work.

6. References

1. Abou-Arab AA, Abu-Salem FM, Abou-Arab EA. Physico-chemical properties of natural pigments (anthocyanin) extracted from Roselle calyces (*Hibiscus subdariffa*). Journal of American science. 2011;7(7):445-456.
2. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, *in–vitro* antioxidant and anti–inflammatory activity of flowers of Moringa oleifera. Asian Pacific journal of tropical biomedicine. 2013;3(8):623-627.
3. Bergmeier D, Berres PHD, Filippi D, Bilibio D, Bettiol VR, Priamo WL. Extraction of total polyphenols from hibiscus (*Hibiscus sabdariffa* L.) and waxweed/'sete-sangrias' (*Cuphea carthagenensis*) and evaluation of their antioxidant potential. Acta Scientiarum. Technology. 2014;36(3):545-551.
4. Boo HO, Hwang SJ, Bae CS, Park SH, Heo BG, Gorinstein S. Extraction and characterization of some natural plant pigments. Industrial Crops and Products. 2012;40:129-135.
5. Obi FO, Usenu IA, Osayande JO. Prevention of carbon tetrachloride-induced hepatotoxicity in the rat by H. rosasinensis anthocyanin extract administered in ethanol. Toxicology. 1998;131(2-3):93-98.
6. Chandrasekhar J, Madhusudhan MC, Raghavarao KSMS. Extraction of anthocyanins from red cabbage and purification using adsorption. Food and bioproducts processing. 2012;90(4):615-623.
7. Jabeur I, Pereira E, Barros L, Calhelha RC, Soković M, Oliveira MBP, *et al*. *Hibiscus sabdariffa* L. as a source of nutrients, bioactive compounds and colouring agents. Food Research International. 2017;100:717-723.
8. Kruawan K, Kangsadalampai K. Antioxidant activity, phenolic compound contents and antimutagenic activity of some water extract of herbs. Thai J Pharm Sci. 2006;30(1):28-35.
9. Nakamura Y, Hidaka M, Masaki H, Seto H, Uozumi T. Major anthocyanin of the flowers of Hibiscus (*Hibiscus rosa-sinensis* L.). Agricultural and biological chemistry. 1990;54(12):3345-3346.
10. Pires TC, Barros L, Santos-Buelga C, Ferreira IC. Edible flowers: Emerging components in the diet. Trends in Food Science & Technology. 2019;93:244-258.
11. Vankar Padma S, Dhara Bajpai. "Preparation of gold nanoparticles from Mirabilis jalapa flowers." 2010.
12. Voon HC, Bhat R, Rusul G. Flower extracts and their essential oils as potential antimicrobial agents for food uses and pharmaceutical applications. Comprehensive Reviews in Food Science and Food Safety. 2012;11(1):34-55.