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RESEARCH ARTICLE

Evaluation and selection of hibiscus (*Hibiscus rosa-sinensis* L.) genotypes for enhanced pigment content

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SUMMARY

An experiment was conducted at Department of Floriculture and Landscaping, Tamil Nadu Agricultural University, Coimbatore, India with the objectives to evaluate different hibiscus accessions for high pigment content. In this experiment, 14 hibiscus genotypes were collected from different places of Tamil Nadu and Kerala and these genotypes were evaluated continuously from June, 2014 to Sep, 2019. Among the different accessions, Acc.6 (CHR 6) was identified with highest anthocyanin yield from flower petals. The anthocyanin extract from the flowers can be used as a food colourant.

Key Words : Hibiscus, Genotype, Evaluation, Anthocyanin pigment, Food colourant

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Hibiscus rosa-sinensis is one such plant and belongs to the family Malvaceae. Anthocyanins are natural colorants which have extensive range of colours and occur widely in nature. Anthocyanins are the most important dye ranging from orange, pink, red, violet to blue in the flowers and fruits of the vascular plants. They are harmless and water soluble which makes them interesting for their use as natural water soluble colorants. Another significant property of anthocyanins is their antioxidant activity, which is known to play a vital role in the prevention of neuronal and cardiovascular illnesses, cancer and diabetes.

It is a species of tropical Hibiscus in the Hibisceae tribe. It is considered native to East Asia (Vyas, 2012). Although the plant is not related to the true roses, the term 'Rosa sinensis' literally means 'rose of China' in Latin. It was first named by Carolus Linnaeus (Oguntoye, 2014). It is abundant in the sub-tropical and tropical regions and is cultivated extensively as an ornamental plant. This plant bears large flowers on the bushy hedges. The flowers are dark red in colour and are not usually fragrant (Kumar, 2012). They are grown in different

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regions of the Asian continent and are colloquially known as Chinese hibiscus, China rose, Hawaiian hibiscus and shoeblack plant (Vyas, 2012). The plant exhibits the genetic characteristic of polyploidy. Here, the plant bears more than two complete sets of chromosomes. The side effect of this genetic characteristic is a condition in which the phenotype of the offspring may be quite different from the parent, or any other ancestor, allowing possible random expression of all or any of the previous generations (Munirajappa, 1980 and Rani, 2012). This plant has a wide range of applications. Parts of the flower are used to make a popular drink in Egypt and are also used to formulate medicines. Various parts of the plant are also used in the preparation of jams, spices, soups and sauces (Baranova, 2011). Hibiscus oil is extracted from the hibiscus plant and is considered as an essential oil. It has a wide variety of practical applications, ranging from aromatherapy to skin and hair care. There are many other benefits of using hibiscus oil on the skin. It acts as an excellent moisturizer for dry skin and it also helps to heal lesions caused by skin infections such as psoriasis or eczema (Aldouri, 2000). This oil is also found to preserve the flexibility and elastic nature of the skin and reduces the effect of aging when used on a regular basis. It also has anti inflammatory (Yazan, 2011) and astringent properties. One of the most popular applications of hibiscus oil is in the field of hair care. Hibiscus oil is obtained from its flowers and may be used alone or added to various hair care products such as shampoos and conditioners, to improve the overall condition of the hair. In this context, the present investigation was carried out to identify the *Hibiscus rosa-sinensis* genotype with high anthocyanin contents for commercial exploitation.

MATERIAL AND METHODS

The experiment was conducted at the Botanical Garden, Department of Floriculture and Landscape Architecture, Coimbatore, Tamil Nadu located at 11 0 02' N latitude and 76 0 57' E longitude at an altitude of 426.76 m above MSL during 2014 to Sep, 2019. Totally, 14 accessions of *Hibiscus rosa-sinensis* plants were sourced from different parts of Tamil Nadu and Kerala. The plants were propagated using semi hard wood cuttings. Six months old plants were used for planting. The list of accessions and place of collection are listed in the following Table A. Experimental design adopted for this study was RBD and replicated thrice and in each replication five plants were planted. Three uniformly sized plants per replications were tagged for recording observations. Anthocyanin extraction was carried out by using different solvents and that with high extraction efficiency was observed.

Table A: Accessions of *Hibiscus rosa-sinensis* collected

Sr. No.	Place of collection	Accession name	Flower colour
1.	Trichy	THR 1	Red -single
2.	Coimbatore	CHR 2	Red - single -white variegated leaf
3.	Bangalore	BHR 3	Red - single -red variegated leaf
4.	Coimbatore	CHR 4	Red – single- wider petals
5.	Thrissur	TrHR 5	Red- single - small flower
6.	Coimbatore	CHR 6	Red multi petals
7.	Thrissur	TrHR 7	Red - double
8.	Madurai	MHS 8	Red – Fringed petals
9.	Thrissur	TrHR 9	Yellow – single – small flower
10.	Coimbatore	CHR 10	Yellow – single – red throat
11.	Palladam	PaHR 11	Orange-single-red throat
12.	Coimbatore	CHR 12	Orange-double
13.	Periyakulam	PkHR 13	Pink-single
14.	Periyakulam	PkHR 14	White-single

Table B : Solvents used

Treatments	Solvent used
T_1	Water
T_2	Hot water (kept in water bath at 100°C for 30 min)
T_3	Ethanol
T_4	Ethanol : water (50:50)
T_5	Acidified ethanol (HCl: ethanol, 1:99)
T_6	Methanol
T_7	Methanol : water (50:50
T_8	Acidified methanol (HCl: methanol, 1:99)

Sample preparation:

Hibiscus flowers were collected, shade dried and grinded using a blender to obtain a fine powder. 1 gram of the fine powdered flower samples were macerated using the desired solvent and then kept in a shaker for 2 hours. The samples were then filtered and the final volume was made to 20 ml using the respective solvent. The final extracts were used for further analysis.

Estimation of monomeric anthocyanin content:

The monomeric anthocyanin content of the extract was determined using the pH differential method described by Giusti and Wrolstad (2001). The appropriate dilution factor for the extract was determined by diluting

with potassium chloride buffer (pH 1.0), until the absorbance of the extract was within the linear range of the spectrophotometer.

Two dilutions of the extract were prepared, one with potassium chloride buffer (pH 1.0) and the other with sodium acetate buffer (pH 4.5), diluting each by the previously determined dilution factor. The dilutions were allowed to equilibrate for 15 min. The absorbance of each equilibrated solution was then measured at 510 nm (lmax) and 700 nm for haze correction, using UV-VIS Spectrometer. The monomeric anthocyanin content was calculated based on cyanidin-3-glucoside (Alasalvar *et al*., 2005) with the following formula and expressed in mg/litre.

Monomeric anthocyanin content = (AxMWxDFx1000) / (ex1)A

where,

A - Absorbance of the diluted sample

$= (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$

MW- Molecular weight of cyanidin-3-glucoside (449.2)

DF- Dilution factor

ε- Molar absorptivity of cyanin-3-glucoside (26900).

The solvent in which the extraction efficiency of anthocyanin was found high was used for the preparation of extract from the flower petals of different accessions of Hibiscus. This extract was used for the antioxidant and phytochemical analysis.

Estimation of total phenol content

The total phenol content was estimated based on Folin–Ciocalteu (FC) method (Singleton and Rossi, 1965) with some slight modifications. 0.2 ml of the extract was diluted with 8.5 ml of water to which 0.5 ml of Folin–Ciocalteu (FC) was added and incubated for 3 minutes. After incubation, 1 ml of sodium carbonate (20%, w/v) was added. The solution was mixed well and incubated at room temperature for 60 minutes. After incubation, the absorbance was measured at 760nm. A suitable calibration curve was prepared using gallic acid and the results are expressed in milligram per gram (mg/g) Gallic acid equivalent.

Estimation of total flavonoids:

Total flavonoids in the sample extracts was determined using the Aluminium chloride method as described by (Liu *et al.,* 2008). 0.5 ml of the extract was added to 3 ml of Sodium nitrate (5%, w/v) and 2.5 ml of distilled water which was incubated at room temperature for 3 minutes and then 0.3 ml of Aluminium chloride (10%, w/v) was added. The reaction mixture was allowed to stand for six minutes and then 2 ml of Sodium hydroxide (1M) was added. The final volume was made upto 10 ml after 60 minutes and the absorbance was measured at 415nm. A suitable calibration curve was prepared using Quercetin and the results are expressed in milligram per gram (mg/g) Quercetin equivalent.

Total monomeric anthocyanin:

Total monomeric anthocyanin was estimated using the same procedure described in the previous experiment.

RESULTS AND DISCUSSION

The extraction efficiency of the solvents were analysed by two different methods. *i.e.* whiteness index of the bleached petals and based on the quantity of anthocyanin extracted by different solvents. Whiteness index was found directly proportional to the extraction efficiency of the solvent. It was found higher in 100% methanol (46.79). The total monomeric anthocyanin extracted was also found to be the highest (184.56 mg/l C-3-G eq.) in methanol solvent extraction. Based on the analysis, absolute methanol was found to be the suitable solvent for the extraction of anthocyanin from the hibiscus petals and the data are presented in Table 1. Anthocyanin was extracted from the hibiscus flower petals and the total phenol, total flavonoids and the total monomeric anthocyanin was estimated and their values are presented in Table 2.

A significant difference was observed among the different accessions of *Hibiscus rosasinensis* for the total phenol, flavonoid and monomeric anthocyanin contents. The data obtained was presented in Table 4. The highest phenol content was observed in Acc 6 (75.30 mg/g) and was followed by Acc 1 (62.90 mg/g). The lowest phenol content was observed in Acc14 (37.7 mg/g Gallic acid equivalent). The highest total flavonoid was observed in Acc 6 (28.20 mg/g quercetin equivalent) and was followed by Acc. 13 (25.10 mg/g quercetin equivalent). The lowest phenol content was observed in Acc. 14 (5.96 mg/g quercetin equivalent). The highest total monomeric anthocyanin content was observed in Acc 6 (81.33 mg/l cyanidin equivalent) and was followed by Acc 1 (67.82 mg/l cyanidin equivalent). The lowest anthocyanin content was observed in Acc. 14 (8.56 mg/

Table 1 : Whiteness index and total anthocyanin of hibiscus flower petals treated with different solvents

Sr. No.	Solvent	l*	a*	b*	Whiteness index	Total Anthocyanin (mg/l C-3-G eq.)
	Unbleached	26.12	3.13	0.2	26.05	
1.	Water	34.71	0.77	11.74	33.66	30.39
2.	Hot water	39.05	5.45	17.19	36.44	25.58
3.	Ethanol	24.49	1.99	-5.94	24.23	45.42
4.	50 % Ethanol	32.89	1.49	10.61	32.04	110.88
5.	Acidified methanol	42.73	6	-1.79	42.39	122.40
6.	Methanol	47.07	3.9	3.8	46.79	184.56
7.	50% Methanol	28.69	0.25	2.335	28.65	160.31
8.	Diethyl ether	27.56	4.62	-5.36	27.22	85.97
S.E.±						2.28
C.D. (P=0.05)						5.64

l* - Lightness, a* - if a is $-^{ve}$ then it is towards green and if it is $+^{ve}$ then it is towards red, b*- if b is $-^{ve}$ then it is towards blue and if it is $+^{ve}$ then it is towards yellow, C-3-G : Cyanidin 3 glucoside

Table 2 : Quantification of total phenol, flavonoid and anthocyanin of different accessions of *Hibiscus rosa-sinensis*

Accession number	Total phenol (mg/g)	Total flavonoid (mg/g)	Total monomeric anthocyanin (mg/l)
1.	62.9	24.03	67.82
2.	40.5	9.33	33.72
3.	43.86	8.26	41.86
4.	52.42	13.92	36.54
5.	53.14	19.81	63.56
6.	75.3	28.20	81.33
7.	58.26	17.11	44.70
8.	45.54	8.53	28.41
9.	45.3	6.87	27.22
10.	44.74	6.62	13.90
11.	43.7	7.14	14.96
12.	44.5	3.06	12.63
13.	55.7	25.10	37.88
14.	37.7	5.96	8.56
Mean	48.98	13.07	34.11
S.E.±	1.20	0.34	2.91
C.D. (P=0.05)	2.44	0.69	5.94

l cyanidin equivalent). These results are similar to those reported by Abou Arab *et al*. (2011), Jafarian *et al*. (2014) and Abdel-Moemin (2016) with some differences that may be due to genetic variations of different hibiscus genotypes (Babalola *et al*., 2001).

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