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RESEARCH ARTICLE

Studies on Extraction of Betalain Pigments by Different Solvents and Assessing Antioxidant Activity of *Bougainvillea spectabilis* and *Celosia argentea* Flowers

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ABSTRACT

Betalain is a natural pigment with antioxidant property which is used as a food colourant. Betalain was extracted from the fresh and dried samples of *Bougainvillea spectabilis* and *Celosia cristata* flowers by using different solvents (Aqueous, 50 % methanol, 100 % methanol, 50 % ethanol, 100 % ethanol and acetone). Total betalain (TB), betacyanin (BC) and betaxanthin (BX) content of the flowers were recorded by spectrometric methods. The results revealed that pigment yield was more in Bougainvillea flowers when compared to Celosia in aqueous extraction. On comparing the fresh and dried flowers, dried flowers yielded more betalain content in dried flowers. The extracts of the flowers were screened for antioxidant potentials like ABTS, DPPH, Chelating potential, FRAP and CUPRAC. From the study it was observed that the Bougainvillea and Celosia flowers possess antioxidant potential but the highest was found in the Bougainvillea flower.

Keywords*: Total betalain, Bougainvillea spectabilis, Celosia cristata, Antioxidant potential.*

INTRODUCTION

There has been an increasing trend towards replacement of synthetic colorants by natural pigments in the last 20 years, since the natural pigments are safety and have health benefits. Although natural pigments are generally less stable and have higher cost than synthetic colorants, their development and utilization is getting more and more attention (Cai, 2005).

Betalains occur only in the plants of the order Caryophyllales (Old name: Centrospermae), such as the family Amaranthaceae which includes several important genera, i.e. Amaranthus, *Celosia cristata* and Nyctaginaceae family that include *Bougainvillea spectabilis.* Several betalains (16 red-violet betacyanins and 3 yellow betaxanthins) were reported to be isolated and identified in different portions (*viz*. stems, leaves, and inflorescences) from plants of the Amaranthaceae family. Betalains are attracting increasing attention because of their use for food coloring and their antioxidant and radical scavenging properties against certain oxidative stress-related disorders, anticancer and antiviral properties. The present investigation deals with the extraction of betalain pigments and assessing their antioxidant properties (Shiv Narayan *et al.,* 2017).

MATERIAL AND METHODS

Sample preparation and extraction

Two ornamental flowering plants belonging to the family Nyctaginaceae and Amaranthaceae *viz*., *Bougainvillea glabra* and *Celosia argentea* were used as source materials. Flowers were collected from the Botanical garden and the local flower market. One gram sample of fresh petals and for dried sample the petals were shade dried to remove excess moisture. They were ground in a blender after shade drying and 100 mL of solvent (Aqueous, 50 % methanol, 100 % methanol, 50 % ethanol, 100 % ethanol and acetone) were added and kept for incubation in a shaker overnight and maintained at a temperature of 25 ± 2°C. Then the pigments were extracted by filtering the solution in a whattman no.30 filter paper and the filtrate was stored under -20°C for further analysis.

Analysis of total betalain

The pigment was extracted by different solvents and mixed using McIlvaine buffer (pH 6.5, citrate-phosphate buffer) for dilution of the pigment. The absorption was measured at different OD values at 538, 480, and 600 nm

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for quantification of betacyanin, betaxanthin, and total betalains respectively using UV-VIS spectrophotometer. The betalain content (BC) was analyzed by the following formula,

Betalain content ($mg.mL^{-1}$) = A*DF*MW*1000/ε*l

Here,

A - Absorption value at 600 nm

DF - dilution factor

l - Path length (1 cm) of the cuvette

For quantification of betacyanins and total betalain - the molecular weights (MW) = 550 $g.mol^{-1}$ and molar extinction coefficients (ε) = 60,000 L in H_2O; λ=538 nm for betacyanin and λ=600 for total betalains.

For quantification of betaxanthins - the molecular weights (MW) = 308 $g.mol^{-1}$; and molar extinction coefficients (ε) = 48.000 L in H_2O; λ=480 nm (Rohan Stintzing *et al.,* 2005).

Lyophilization and storage

The aqueous extracted sample was concentrated for betalain pigments using lyophilizer. By freeze drying where the water at -80 °C temperature and 0.1 mPa pressure freeze dried powder of the betalain extract was obtained and stored in vials. The extracts were stored at -80 °C in deep freezer to avoid degradation of the extracts and was used for further analysis.

DPPH radicle scavenging activity assay

DPPH is a stable free radical which do not dimerize as other free radical. Occurrence of purple colour indicates the delocalization of DPPH molecule, with an absorption of around 517 nm. The betalain extract was measured for antioxidant activity by its ability to scavenge the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) Wong, (2006). Different concentrations of the extract were prepared using distilled water as 1250, 1000, 750, 500, 250 $\mu g.mL^{-1}$.

Hundred micro litre of the prepared extract was treated with 3 mL of a DPPH reagent having absorbance of 0.980 ± 0.02 in methanol initially and incubated at normal ambient temperature. After 20 min of incubation, the absorbance was measured at 517 nm using UV/Vis spectrophotometer. Ascorbic acid at different concentrations (1 $mg.mL^{-1}$ to 0.0156 $mg.mL^{-1}$) was used as standard. The percentage of inhibition was calculated by the below formula,

$$\% \text{ inhibition} = \frac{(\text{Initial absorbance} - \text{final absorbance})}{\text{Initial absorbance}} \times 100$$

The concentration required for 50 % reduction of DPPH (IC_{50}) was used to express the antioxidant capacity of the samples.

ABTS radicle scavenging activity assay

The oxidation of ABTS cation radical is determined by the loss of electron in a nitrogen atom which is physically indicated by the presence of bluish green color. ABTS can be oxidized by potassium persulphate or manganese dioxide, giving rise to the ABTS cation radical (ABTS) which is determined by the absorption at 743 nm and it is monitored using the presence of ascorbic acid which is chosen as standard antioxidant.

Different concentrations of the extract were prepared using distilled water, as 1250, 1000, 750, 500, 250 $\mu g.mL^{-1}$ and their antioxidant capacity was measured depending upon the ability to scavenge the stable ABTS molecule. 300 µL of the extract was mixed with 3 mL of 7 mM ABTS and 2.45 mM potassium persulphate reagent having absorbance of 0.7 ± 0.05 in methanol and incubated at normal ambient temperature. After 6 min of incubation, the absorbance was measured at 743 nm using UV/Vis spectrophotometer. Ascorbic acid at different concentrations (1 $mg.mL^{-1}$ to 0.0156 $mg.mL^{-1}$) was used as standard. The percentage of inhibition was calculated by the below formula,

$$\% \text{ inhibition} = \frac{(\text{Initial absorbance} - \text{final absorbance})}{\text{Initial absorbance}} \times 100$$

The concentration required for 50 % reduction of ABTS (IC_{50}) was used to express the antioxidant capacity of the samples (Sumaira Sahreen *et al.,* 2010).

Ferric reducing antioxidant potential assay

The FRAP reagent was prepared by adding 25 mL of 300 mM Acetate buffer maintained at a pH of 3.6 to which 2.5 mL of 10 mM TPTZ (2,4,6- tri(2-pyridyl)-s-triazine) was dissolved in 40 mM HCL and added with 2.5 mL of 2.mM $FeCl_3$. 100 µL of the extract was added to 1.9 mL of FRAP reagent and the reaction mixture was incubated for 30 minutes under water bath and maintained at 36 °C. The ferric ions were reduced after adding the extract indicating the antioxidant potential of the extract was measured after incubation under UV/Vis spectrophotometer at 593 nm. The Ferric reducing antioxidant potential of the flower extracts was expressed as µM Ascorbic acid equivalent (Williams *et al.,* 1995).

CUPRAC

CUPRAC reagent was prepared by mixing together 1mL of 1.0×10^{-2} M copper (II) chloride with 1 mL of 1 M ammonium acetate buffer maintained at pH 7.0, at which the reaction mixture was added with 1mL of 7.5×10^{-3} M neocuproine solution. 100 µL of the flower extract was added to 1 mL of CUPRAC reagent. The reaction mixture was then incubated at room temperature for 30 minutes and their absorbance was recorded at 450 nm using UV/Vis spectrophotometer. A standard calibration curve was developed using ascorbic acid and CUPRAC reagent. The cupric ion reducing antioxidant capacity of the flower extract was expressed as µM Ascorbic acid equivalent (Yildiz, 2008).

Chelating power

The extracts were dissolved in water to prepare various sample solutions at 1250, 1000, 750, 500, 250 $\mu g.mL^{-1}$. The analytical procedure is that, to the 200 µL of the sample prepared 100 µL of $FeCl_2.2H_20$ (2.0 mM) was added to which 900 µL of methanol is added. After 5 min of incubation under room temperature, 5.0 mM of 400 µL of ferrozine is added. Later of 10 minute the absorbance was taken at 562 nm using UV/Vis spectrophotometer

The chelating activity (%) was calculated using the following equation,

$$\% \text{ inhibition} = \frac{(\text{Initial absorbance} - \text{final absorbance})}{\text{Initial absorbance}} \times 100$$

Ascorbic acid was used as a standard. The concentration required for the 50 % reduction of the chelates (IC_{50}) was used to express the antioxidant capacity of the samples (Karawita, 2005).

All statistical analysis were carried out in triplicates and the values were expressed in Mean ± SD. IC_{50} value was calculated using the statistical software, graph pad prism 5.

RESULTS AND DISCUSSION

Extraction efficiency of flowers

From the data (Table 1) pertaining to *Bougainvillea spectabilis* in the total betalain content, betacyanin and betaxanthin exhibited significant difference among the different solvents.

Table 1. Estimation of total betalain, betacyanin and betaxanthin content in Bougainvillea flower extracts

Treatments (Solvents)	Total betalain ($mg.mL^{-1}$)		Betacyanin ($mg.mL^{-1}$)		Betaxanthin ($mg.mL^{-1}$)	
	Fresh (F_1)	Dried (F_2)	Fresh (F_1)	Dried (F_2)	Fresh (F_1)	Dried (F_2)
T_1 (Water)	11.59 ±0.32^{a}	24.05 ± 0.16^{a}	28.47± 0.36^{a}	46.66 ± 0.04^{a}	18.17± 0.35^{a}	54.70 ± 0.60^{a}
T_2 (Hot Water)	5.17± 0.10^{b}	4.22 ± 0.12^{e}	14.50 ± 0.31^{d}	16.37 ± 0.27^{d}	9.17 ± 0.14^{c}	10.90 ± 0.1^{d}
T_3 (50% Methanol)	4.66 ± 0.19^{b}	13.71 ± 0.31^{b}	23.43 ± 0.28^{bc}	40.62 ± 0.66^{b}	13.35 ± 0.18^{bc}	40.04 ± 0.10^{b}
T_4 (100% Methanol)	2.68 ± 0.07^{c}	8.84± 0.10^{c}	6.04 ± 0.02^{e}	12.47 ± 0.12^{e}	8.02 ± 0.15^{d}	18.59 ± 0.21^{c}
T_5 (50% Ethanol)	4.33± 0.19^{b}	12.06 ± 0.15^{b}	18.87 ± 0.17^{c}	37.06 ± 0.46^{c}	11.89 ± 0.06^{c}	38.52 ± 0.27^{b}
T_6 (100%Ethanol)	1.83± 0.04^{c}	2.78 ± 0.06^{e}	2.99 ± 0.05^{e}	3.58 ± 0.06^{g}	4.80 ± 0.09^{e}	1.77 ± 0.01^{e}
T_7 (Acetone)	5.28^{b}	6.62 ± 0.01^{d}	21.65 ± 0.32^{b}	8.00 ± 0.18^{f}	10.49 ± 0.03^{b}	11.26 ± 0.13^{d}
CD (0.05) (T)		0.21		0.54		0.25
(F)		0.39		1.00		0.47
TxF		0.54		1.42		0.67

The values are represented as mean ± SD with triplicate determination

Extraction of dried flowers yielded higher pigment content than the fresh flowers. Among the different solvents, aqueous extraction (T_1) with dried flowers recorded the highest total betalain content (24.05 $mg.mL^{-1}$) followed by 50 % methanol (T_3) and 50 % ethanol (T_5) with 13.71 $mg.mL^{-1}$ and 12.06 $mg.mL^{-1}$ which were on

par with each other. The betacyanin and betaxanthin content were also higher in aqueous extraction of dried flowers (46.66 mg.mL^{-1} and 54.70 mg.mL^{1}).

The total betalain content, betacyanin and betaxanthin content in *Celosia cristata* flowers exhibited significant results among different solvents (Table 2). Among the solvents, betalain extraction with aqueous method was observed to be highly significant with shade dried flowers when compared to fresh flowers. Dried celosia flowers extracted with aqueous method (T_1) resulted in higher total betalain content of 25.75 mg.mL^{-1} followed by 50 % methanol (T_3) and 50 % ethanol (T_5) which recorded 24.13 mg.mL^{-1} and 16.32 mg.mL^{-1} respectively. Regarding betacyanin and betaxanthin content similar results were obtained with the highest content from dried flowers (50.89 mg.mL^{-1} and 31.16 mg.mL^{-1}) extracted with aqueous method (T_1) followed by 50 % methanol (T_3) which yielded 25.76 mg.mL^{-1} and 24.24 mg.mL^{-1} respectively. Significant results were obtained of the two flowers in their total betalain content, betacyanin, and betaxanthin content. Higher total betalain content, betacyanin, and betaxanthin content was observed in Bougainvillea of 24.05 mg.mL^{-1}, 46.66 mg.mL^{-1} and 54.70 mg.mL^{-1} followed by Celosia having 25.75 mg.mL^{-1}, 30.62 mg.mL^{-1} and 31.16 mg.mL^{-1} of betalain, betacyanin and betaxanthin content.

Table 2. Estimation of total betalain, betacyanin and betaxanthin content in Celosia flower extracts

Treatments (Solvents)	Total betalain (mg.mL^{-1})		Betacyanin (mg.mL^{-1})		Betaxanthin (mg.mL^{-1})	
	Fresh (F_1)	Dried (F_2)	Fresh (F_1)	Dried (F_2)	Fresh (F_1)	Dried (F_2)
T_1 (Water)	9.01± 0.06^{a}	25.75 ±0.09^{a}	16.89 ± 0.29^{a}	30.62 ± 0.32^{b}	13.15 ± 0.29^{a}	31.16 ± 0.75^{c}
T_2 (Hot Water)	2.32 ± 0.03^{d}	13.31±0.32^{c}	5.01 ± 0.11^{c}	3.72 ± 0.01^{f}	6.92 ± 0.10^{c}	11.29 ± 0.25^{e}
T_3 (50% Methanol)	6.55 ± 0.10^{d}	24.13 ±0.21^{f}	7.92 ± 0.05^{c}	25.76 ± 0.47^{d}	12.06 ± 0.10^{b}	24.24 ± 0.27^{d}
T_4 (100% Methanol)	2.10 ± 0.03^{b}	3.56 ±0.04^{b}	2.93 ± 0.01^{b}	20.16 ± 0.15^{a}	5.35 ± 0.03^{a}	16.78 ± 0.10^{a}
T_5 (50% Ethanol)	4.44 ± 0.10^{d}	16.32 ±0.15^{e}	5.70 ± 0.05^{d}	12.63 ± 0.30^{c}	8.45 ± 0.11^{c}	19.90 ± 0.51^{b}
T_6 (100%Ethanol)	2.39 ± 0.03^{d}	10.68 ±0.14^{d}	2.88 ± 0.02^{d}	2.36 ± 0.04^{g}	5.40 ± 0.12^{c}	3.84 ± 0.01^{f}
T_7 (Acetone)	1.33 ± 0.02^{c}	2.29 ±0.05^{f}	5.42 ±0.01^{c}	6.36 ± 0.02^{e}	6.60 ± 0.08^{c}	4.59 ± 0.04^{f}
CD (0.05) (T)	0.23		0.17		0.22	
F	0.43		0.32		0.40	
TxF	0.61		0.45		0.57	

The values are represented as mean ± SD with triplicate determination

Antioxidant potential of flowers

Significant results were obtained with regard to antioxidant activity by DPPH radicle scavenging activity of betalain extracts from the flowers (Table 3). It was revealed that the flower extracts scavenged the DPPH free radicals depending upon their dosage level. The highest antioxidant potential was observed with Celosia flower extracts with an IC_{50} value of 26.44 mg.mL^{-1} when compared to ascorbic acid standard (54.23 mg.mL^{-1}). This was followed by Bougainvillea flower extract which inhibition was at 41.47 mg.mL^{-1} respectively.

Table 3. Antioxidant potential of *Bougainvillea spectabilis* and *Celosia cristata* by ABTS, DPPH, and chelating potential method

Crop extract	Concentration of the extract	ABTS (IC_{50})	DPPH (IC_{50})	Chelating potential (IC_{50})
Bougainvillea spectabilis	(mg.mL^{-1})	7.17 ± 0.07^{a}	41.471 ± 0.20^{b}	0.886 ± 0.007^{a}
Celosia cristata	(mg.mL^{-1})	27.54 ± 0.54^{b}	26.449 ± 0.26^{a}	1.237 ± 0.04^{b}
CD value (0.05)		0.36	2.37	0.08
SED		0.18	1.18	0.04
Ascorbic acid (std)	(µg.mL^{-1})	81.26 ± 0.43	54.23 ± 0.22	7.17 ± 0.07

The antioxidant potential of betalain extract of flowers by ABTS method, registered significant results (Table 3). The standard ascorbic acid showed IC_{50} at 81.26 µg.mL^{-1} concentration. Among all the extracts of betalain, the highest antioxidant potential was observed in Bougainvillea extracts with 50 % inhibition at 7.17 mg.mL^{-1} while Celosia extracts exhibited 50 % inhibition at 27.54 mg.mL^{-1}.

The chelates get reduced systematically at the highest concentration of the betalain extracts and results were found to be significant among the treatments (Table 3). Among all the extracts, Bougainvillea flower extract

showed higher antioxidant potential by reducing 50 % activity of chelates at 0.88 mg.mL^{-1} concentration followed by Celosia (1.237 mg.mL^{-1}). The IC_{50} value of ascorbic acid standard is for chelating potential was 7.17 µg.mL^{-1}.

Table 4. Antioxidant potential of Bougainvillea spectabilis, Celosia cristata by CUPRAC and FRAP method

Crop extract	Concentration of the extract	CUPRAC (µg equivalence to ascorbic acid standard)	FRAP (µg equivalence to ascorbic acid standard)
Bougainvillea spectabilis	(1000 µg.mL^{-1})	85.35 ± 0.68^{a}	175.78 ± 3.99^{a}
Celosia cristata	(1000 µg.mL^{-1})	38.12 ± 0.65^{b}	88.28 ± 1.83^{c}
CD value (0.05)		2.96	4.03
SED		1.48	2.01

Antioxidant potential of betalain extracts from flowers analyzed by FRAP method exhibited a significance difference (Table 4). The values were expressed in µg equivalence to standard (*i.e*) ascorbic acid. The highest values were recorded by Bougainvillea flower extracts (175.78 µg) followed by Celosia (88.28 µg) of ascorbic acid.

Significant results were obtained with regard to antioxidant potential of betalain flower extracts by CUPRAC method (Table 4). Irrespective of IC_{50} value, the antioxidant potential is given in terms of µg equivalence to standard (*i.e*) ascorbic acid by CUPRAC method. Among extracts, Bougainvillea showed higher antioxidant potential and recorded 85.35 µg followed by Celosia (38.12 µg).

CONCLUSION

From the results it is concluded that, dried flowers expressed higher betalain content when compared to fresh flowers. Among the five methods taken for the analysis of antioxidant potential of Bougainvillea spectabilis and Celosia cristata, except ABTS method all other methods reported that Bougainvillea spectabilis could exhibit higher antioxidant potential and it may also due to the presence of higher betalain, betacyanin and betaxanthin content when compared to Celosia cristata. Even though these flowers possess antioxidant potential lower than the standard ascorbic acid, it can be used as primary antioxidants.

REFERENCES

Brand-Williams W, Cuvelier M-E and C. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. *Food science and Technology*. **28**:25-30.

Florian C. Stintzing, Kirsten M. Herbach, Markus R.Mosshammer, Reinhold Carle. 2005. Color, Betalain Pattern, and Antioxidant Properties of Cactus Pear Clones. *Journal of Agricultural and Food Chemistry.* **11**:17-19

Rohan Karawita. 2005. Reactive oxygen species scavenging, metal chelation, reducing power and lipid peroxidation inhibition properties of different solvent fractions from Hizikia fusiformis. *European Food Research and Technology*. **56**: 81-91

Cai, Y.Z. 2005. Characterization and application of betalain pigments from plants of the Amaranthaceae. *Trends in Food Science & Technology*. **43**:155-162

S.P Wong. 2006. Antioxidant activities of aqueous extracts of selected plants. Food Chemistry. 99: 775-784

Yildiz, L. 2008. Combined HPLC-CUPRAC (cupric ion reducing antioxidant capacity) assay of parsley, celery leaves, and nettle. Talanta. 77: 304-313.

Sumaira Sahreen, Muhammad Rashid Khan, Rahmat Ali Khan. 2010. Evaluation of antioxidant activities of various solvent extracts of Carissa opaca fruits. Food Chemistry. 122: 1205-1211

Shiv Narayan Amit Kumar, Sunil Kumar Ritesh, Govindasamy Sharmila, Chandrasekaran Muthukumaran. 2017. Extraction optimization and characterization of water soluble red purple pigment from floral bracts of Bougainvillea glabra. Arabian Journal of Chemistry. 46:64-69