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Profiling of carotenoid pigments and their antioxidant activities in ray florets of chrysanthemum (*Chrysanthemum*× *morifolium*)

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ABSTRACT

In present study, different varieties of chrysanthemum (*Chrysanthemum* × *morifolium* Ramat) were screened for total carotenoids, phenolic content and antioxidant activities. Among the genotypes studied, variety Jubilee (32.82 mg/100g) exhibited high total carotenoids followed by varieties Haldighati (26.71 mg/100 g), Little Orange (22.25 mg/100g), Liliput (20.77 mg/100g) and Star Yellow (19.21 mg/100g). Variety Jubilee also showed highest antioxidant activities of carotenoids {CUPRAC-Cupric reducing antioxidant capacity (149.44 µmol trolox/g), FRAP- Ferrous reducing power (40.09 %), DPPH radical scavenging activity (11.24%)}. The total phenolic content varied from 1.26 mg GAE/g (Ram Lal Dada) to 31.14 mg GAE/g (Jubilee) on fresh weight basis. Further, five promising varieties having high carotenoids, were quantified for lutein and β-carotene using High Performance Liquid Chromatography (HPLC). Highest lutein content was found in variety Jubilee (19.90 µg/g) followed by varieties Haldighati (8.67 µg /g), Liliput (4.77 µg /g), Star Yellow (3.93 µg /g) and Little Orange (3.69 µg/g). Highest β-carotene content was found in Little Orange (5.51µg/g) followed by the varieties Haldighati (2.04 µg /g), Jubilee (1.77 µg /g), Liliput (0.54 µg /g) and Star Yellow (0.50 µg/g). A high correlation between carotenoids, total phenolic and antioxidant activities was observed. Variety Sadwin Yellow showed highest total carotenoid (0.48 mg/g) in leaves, whereas chlorophyll a and total chlorophyll was highest in variety Punjab Anmol (1.32 mg/g, 1.56 mg/g) and high chlorophyll b in variety Pusa Aditya (0.28 mg/g).

Key words: Antioxidant activity, Carotenoids, Chrysanthemum, Lutein, Phenolic content

Among horticultural crops, flowers are also recognized as potential sources of pigments, however, due to lack of awareness it remained as an unexploited area. Chrysanthemum (*Chrysanthemum* × *morifolium* Ramat.) is one of the most economically important flower crops cultivated all over the world and It belongs to family Asteraceae and is native to Asia and North Eastern Europe.Its flowers are considered as an important source of carotenoid pigments and have been consumed as herbal medicines, beverages and vegetables (Park *et al*. 2015). These are known for their beautiful flowers with different colours including yellow, orange, orange-red, which are mainly contributed by carotenoids. These pigments play an important role in human health because of their capacity to act as antioxidants (Groteworld 2006, Mlodzinska 2009). These pigments are recognized as safe molecules for nutraceutical purpose due to their concentrated colour, as precursor for vitamin A synthesis (Romer *et al.* 2000) and their antioxidant activity in human being (Stahl and Sies 2003).

Lutein is one of the potential natural plant based antioxidant which reduces the risk of chronic diseases, auto oxidation of cellular lipids and age related macular degeneration, provides protection against oxidant induced cell damage and enhances immune function. Therefore, the assessment of carotenoids and their antioxidant activities is of great significance. Presently, food safety is an important public concern that has spurred movement towards the use of natural source of colourant like carotenoids due to the fact that nutraceutical pigments play a major role in health improvement. As per available literature and our knowledge, there are very few published reports on the profiling of carotenoids and antioxidant activities of chrysanthemum flowers and correlation between them. Keeping these considerations in view, the present investigation was carried out on profiling of pigments, their antioxidant activities and association among themselves.

MATERIALS AND METHODS

The plant material utilized for conducting the experiment consisted of 25 genotypes of chrysanthemum namely Classic,

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Geetanjali, Little Orange, Mayur-5, Punjab Anmol, Aparajita, Sadwin Yellow, Yellow Gold, Vijay Kiran, Ajay Orange, Pusa Aditya, Mallika Yellow, Ram Lal Dada, Yellow Reflex, Haldighati, Pusa Sona, Jubilee, Jayanti, Pusa Centenary, Liliput, Star Yellow, Teri, Kundan and Texas Gold. The main features and photograph of genotypes used in the present investigation are given in Table 1, respectively. These were grown and maintained at research farm of the Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi during 2014-16. Fresh ray florets were harvested at full bloom stage for determination of carotenoid pigments, phenolic content and antioxidant activity. Fresh leaves were also harvested for estimation of chlorophyll and carotenoids present in the leaves.

The total carotenoids were extracted and estimated using method given by Ranganna (1995) with minor modifications. Sample preparation for Lutein and β-carotene was done using a modification of procedure described by Barba *et al.* (2006). Analysis of lutein and β carotene was carried out using High Performance Liquid Chromatography (HPLC). The phenolic compounds in vacuum dried petals of marigold were extracted using a modification of the procedure described by Uzelac *et al*. (2005). Total phenolic content (TPC) was estimated according to procedure given by Singleton and Rossi (1965).

The sample was extracted using the procedure as in case of phenolic compounds. Total antioxidants were estimated using FRAP (Ferric Reducing Antioxidant Potential) method as described by Benzie and Strain (1996), DPPH assay described by Braca *et al.* (2001) and CUPRAC method standardized by Apak *et al.* (2004).

Chlorophyll and carotenoid content from leaves were estimated as per the method described by Hiscox and Israelstam (1979). Chlorophyll a, chlorophyll b and total chlorophyll content were estimated using the formula given by Arnon (1949) while carotenoid content was determined using formula given by Lichtenthaler and Welburn (1983).

The data was statistically analyzed in completely randomized design (CRD) using Statistical analysis system (SAS) software. All determinations were done at least in triplicate and all were averaged. The confidence limits used in this study were based on 95% confidence ($P<0.05$).

RESULTS AND DISCUSSION

Estimation of total carotenoids, phenolic content and antioxidant activities in ray florets

The data presented in Table 2 showed that there were

Table 1 Salient features of chrysanthemum genotypes

Genotype	Flower type	Flower size	Flower colour	Flowering time	Source
Classic	Semi double	Medium	Light Orange	Mid Nov-Mid Jan	ICAR-IARI
Geetanjali	Semi double	Medium	Yellow	Mid Nov-Mid Jan	ICAR-IARI
Little Orange	Double	Medium	Orange	Mid Nov-Mid Jan	ICAR-IARI
Mayur-5	Semi double	Medium	Yellow	Mid Nov-Mid Jan	ICAR-IARI
Punjab Anmol	Single	Small	Yellow	Mid Nov-Mid Jan	ICAR-IARI
Aparajita	Semi double	Medium	Yellow	Mid Nov-Mid Jan	ICAR-IARI
Sadwin Yellow	Semi double	Medium	Light Orange	Mid Nov-Mid Jan	ICAR-IARI
Yellow Gold	Double	Large	Orange	Mid Nov-Mid Jan	ICAR-IARI
Vijay Kiran	Semi double	Medium	Yellow	Mid Nov-Mid Jan	ICAR-IARI
Ajay Orange	Double	Medium	Orange	Mid Nov-Mid Jan	ICAR-IARI
Pusa Aditya	Single	Medium	Yellow and Red mix	Mid Nov-Mid Jan	ICAR-IARI
Mallika Yellow	Semi double	Medium	Yellow	Mid Nov-Mid Jan	ICAR-IARI
Ram Lal Dada	Single	Small	Yellow	Mid Nov-Mid Jan	ICAR-IARI
Yellow Reflex	Double	Large	Yellow	Mid Nov-Mid Jan	ICAR-IARI
Haldighati	Semi double	Medium	Dark Yellow	Mid Nov-Mid Jan	ICAR-IARI
Pusa Sona	Single	Small	Dark yellow	Mid Nov-Mid Jan	ICAR-IARI
Jubilee	Semi double	Large	Orange	Mid Nov-Mid Jan	ICAR-IARI
Jayanti	Double	Medium	Yellow	Mid Nov-Mid Jan	ICAR-IARI
Basanti	Semi double	Medium	Yellow	Mid Nov-Mid Jan	ICAR-IARI
Pusa Centenary	Double	Large	Yellow	Mid Nov-Mid Jan	ICAR-IARI
Liliput	Double	Small	Yellow	Mid Nov-Mid Jan	ICAR-IARI
Star Yellow	Double	Large	Yellow	Mid Nov-Mid Jan	ICAR-IARI
Teri	Semi double	Medium	Yellow	Mid Nov-Mid Jan	ICAR-IARI
Kundan	Double	Small	Yellow	Mid Nov-Mid Jan	ICAR-IARI
Texas Gold	Single	Small	Light Orange	Mid Nov-Mid Jan	ICAR-IARI

Table 2 Estimation of total carotenoids and phenol content and their antioxidant activities in promising genotypes of chrysanthemum

Genotypes	Total carote-noids (mg/100g)	Total phenolic content (mg GAE/g)	CUPRAC (µmol trolox/g)	FRAP (µmol trolox/g)	DPPH (%)
Classic	9.67	12.53	120.47	25.18	4.03
Gheetanjali	1.94	1.76	63.21	7.96	1.16
Littal Orange	22.25	25.75	139.95	34.74	6.57
Mayur-5	4.4	3.54	85.74	13.07	2.37
Punjab Anmol	2.45	3.08	76.13	10.92	1.95
Aparijita	5.45	4.89	93.34	16.16	2.85
Sadwin Yellow	10.26	18.97	90.60	28.91	4.97
Yellow Gold	7.70	9.43	115.74	22.92	3.80
Vijay Kiran	6.79	7.91	112.15	21.71	3.59
Ajay Orange	6.46	6.27	107.25	19.37	3.28
Pusa Aditya	12.66	13.72	121.24	26.14	4.08
Mallika Yellow	3.23	2.86	78.53	11.06	2.05
Ramlaldada	1.06	1.26	61.19	6.29	1.02
Yellow Reflex	8.16	11.15	118.26	24.15	3.96
Haldighati	26.71	28.02	142.96	36.97	11.24
Pusa Sona	6.05	5.69	100.96	18.78	3.14
Jubile	32.82	31.14	149.44	40.09	12.58
Jayanti	5.81	5.05	96.55	16.79	2.93
Basanti	4.72	3.41	66.41	9.13	1.24
Pusa Centenary	14.84	3.63	90.18	14.82	2.57
Lilliput	20.77	25.45	134.27	31.27	9.94
Star Yellow	19.21	25.45	125.18	29.09	5.15
Teri	3.51	21.88	128.31	30.14	8.46
Kundan	2.19	2.22	80.47	12.48	2.15
Texas Gold	2.05	2.07	71.16	9.9	1.55
SEm±	0.01	0.06	133.27	0.01	0.01
LSD (P=0.05)	0.56	0.41	18.93	0.17	0.04

significant differences at 5% level among the genotypes for all the parameters studied.

Total carotenoids

Among the chrysanthemum genotypes studied, the total carotenoid content ranged from 1.06 to 32.82 mg/100g fresh weight of ray florets (Table 2). Variety Jubilee showed highest total carotenoid (32.82 mg/100g) followed by Haldighati (26.71 mg/100g), Little Orange (22.25 mg/100g), Liliput (20.77 mg/100g), Star Yellow (19.21). However, lowest total carotenoids were obtained by Ram Lal Dada, Geetanjali and Kundan (1.06, 1.94, 2.19 mg/100g) on the basis of fresh weight of ray florets, respectively. The variability in the different genotypes for carotenoids is because of the genetical variability in the chrysanthemum varieties. Our results are in accordance with the study conducted by Kishimoto *et al.* (2007) in *Chrysanthemum morifolium* where they reported that the ray florets of both orange- and yellow-flowered cultivars of chrysanthemum species contained yellowish carotenoids. Our study showed that orange coloured varieties accumulated more carotenoids than yellow coloured ones. Kasemsap *et al.* (1990) and Gregory *et al.* (1986) reported that the dark orange coloured flowers have high carotenoids than light orange or yellow coloured flowers of chrysanthemum (Hayashi *et al.* 1998, Park *et al.* 2015, Akshaya *et al.* 2015.). Carotenoids are responsible for the deep yellow and orange colouration in chrysanthemum flower.

The twenty five genotypes were grouped into three groups, based on carotenoid content in the ray florets, shown in Table 3. Group I included genotypes which contain less carotenoids ranging from 1-5 mg/100g fresh weight of ray florets. Group II included genotypes which contain medium carotenoid content of 6-10 mg/100g fresh weight of ray florets. Group III included genotypes which contain high carotenoid content of 12-22 mg/100g fresh weight of ray florets.

Total phenolic content (TPC)

The total phenolic content varied from 1.26 to 31.14 mg GAE/g fresh weight of ray florets among various genotypes of chrysanthemum. Variety Jubilee (31.14 mg GAE/g) exhibited highest phenolic content followed by Haldighati (28.02 mg GAE/g), Little Orange (25.75 mg GAE/g), Liliput (25.45 mg GAE/g) and Star Yellow (21.88 mg GAE/g). Ram Lal Dada showed lowest phenolic content (1.26 mg GAE/g) on fresh weight basis (Table 2). Similar findings were also observed by Feng *et al.* (2010) in *Chrysanthemum* × *morifolium* where phenolic content was in a range of 14.79 to 42.13 mg GAE/g.

Table 3 Grouping of chrysanthemum genotypes on the basis of carotenoid content of ray florets

Group-I	Group-II	Group-III
Low carotenoid content (1-5mg/100g fresh weight of petals)	Group-II Medium carotenoid content (610mg/100g fresh weight of petals)	Group-III High carotenoid content (12-22mg/100g fresh weight of petals)
Aparajita	Sadwin Yellow	Jubilee
Basanti	Classic	Star Yellow
Mayur-5	Yellow Reflex	Haldighati
Teri	Yellow Gold	Little orange
Mallika Yellow	Vijay Kiran	Liliput
Punjab Anmol	Ajay Orange	Pusa Centenary
Kundan	Pusa Sona	Pusa Aditya
Texas Gold	Jayanti	
Geetanjali		
Ram Lal Dada		

ANTIOXIDANT ACTIVITIES

CUPRAC (Cupric Reducing Antioxidant Capacity) assay

CUPRAC assay measures the copper (II) or cupric ion reducing ability of polyphenolics (Apak *et al*. 2004). The CUPRAC values of the chrysanthemum flower of different genotypes are shown in Table 2. The CUPRAC values among genotypes of chrysanthemum ranged from 52.15 µmol trolox/g fresh weight to 287.94 µmol trolox/g fresh weight. The highest reducing power was observed in Red Gold (287.94 µmol trolox/g fresh weight) followed by Lalpari (269.17 µmol trolox/g fresh weight, Red Stone (245.51 µmol trolox/g fresh weight), Red Spoon (239.39 µmol trolox/g fresh weight), Jaya (222.12 µmol trolox/g fresh weight). However, Pink Star exhibited lowest reducing power (6.22 µmol trolox/g fresh weight).

FRAP (Ferric Reducing Antioxidant Potential) assay

The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex and producing a colored ferrous tripyridyltriazine (Fe^{2}-TPTZ) (Benzie and Strain 1996). The FRAP values among genotypes of chrysanthemum ranged from 20.22 µmol trolox/g fresh weight to 145.56 µmol trolox/g fresh weight. The highest reducing power was observed in Red gold (145.56 µmol trolox/g fresh weight) followed by Lalpari (134.33 µmol trolox/g fresh weight, Red Stone (128.65 µmol trolox/g fresh weight), Red Spoon (123.87 µmol trolox/g fresh weight), Jaya (119.2 µmol trolox/g fresh weight). However, Pink Cloud exhibited lowest reducing power (16.18 µmol trolox/g fresh weight) (Table 2).

DPPH free radical scavenging activity

The DPPH assay is a preliminary test to find out the antioxidant potential of the sample. It has been widely used to test the free radical scavenging ability of various samples (Shimoji *et al*. 2002). The DPPH value among genotypes ranged from 2.04 (Pink Star) to36.44% (Red Gold).The highest DPPH radical scavenging was observed in Red Gold (36.44%) followed by Lalpari (32.09%), Red stone (30.77%) , Red Spoon (35.14%) and Jaya (28.73%), whereas Pink Star exhibited lowest DPPH radical scavenging activity (2.04%) (Table 2).

It was concluded from the results that Jubilee recorded highest antioxidant activities by these three methods. The similar results were observed by Feng *et al* (2010) and reported that *Chrysanthemum morifolium* had a CUPRAC value of 80.96mg GAE/g and FRAP value of 149.24 µmol $FeSO_4$/g. The similar results were also reported in chrysanthemum (Kaisoon *et al.* 2012), in *Tagetes erecta* (Munira 2014, Pratheesh *et al.* 2009) and in *Tagetes lucida* (Aquino *et al.* 2002).

Correlation analysis

A positive correlation between carotenoid content and total phenolic content is evident from the Table 4. A strong correlation between antioxidant capacity and total phenolic content indicated that phenolic compounds could be main contributor of antioxidant capacities of chrysanthemum flowers. Cetkovi *et al.* (2004) also supporting the results of our studies, the antioxidant activities were in correlation with the contents of total phenolic compounds and it ranged from 14.49-57.57 mg/g. The analysis exhibited a significant and strong correlation of total carotenoids with total phenolic (0.973) and a weak correlation with FRAP (0.439) and DPPH (0.333) assay as shown in Table 4. Total carotenoids, total phenolic content showed a negative correlation with CUPRAC. The phenolic content showed a positive correlation with FRAP (0.526) and DPPH (0.418), however, a negative correlation with CUPRAC. Hence, it was concluded that total carotenoid content and total phenolic content contributed significantly to antioxidant activity. However, weak correlation was observed between total phenolic content and antioxidant activities by FRAP and DPPH methods and a negative correlation with CUPRAC.

Table 4 Linear correlation coefficient (r) between total carotenoids, total phenolic content, and antioxidant assay (DPPH, FRAP and DPPH) in chrysanthemum genotypes obtained by Pearson's analysis

	Total caro-tenoids	Total phenolic content	CUP-RAC	FRAP	DPPH
Total carotenoids	1	**0.97395	-0.21337	*0.43914	*0.33343
Total phenolic content		1.00000	-0.19806	*0.52691	*0.41866
CUPRAC			1.00000	*0.30907	*0.42319
FRAP				1.00000	**0.9854
DPPH					1

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level.

Scatter plot diagrams of carotenoids and phenolic content *versus* antioxidant activities in chrysanthemum flowers is given in Fig 1. Similar findings of correlation between phenolic content and antioxidant activities were also studied in vegetable, Colorado (Zhou and Yu 2006) and in cauliflower (Dey *et al.* 2015). Gong *et al.* (2012) and Meneses *et al.* (2013) also showed the highly positive correlation of total phenolic content with antioxidant activities. The strong and positive linear correlation among different antioxidant capacities (FRAP and TEAC) were also analyzed by Deng *et al.* (2013) in vegetables.

Estimation of chlorophyll and carotenoids in leaves of chrysanthemum

The data presented in Table 5 showed that there were significant differences at 5% level among the genotypes for all the parameters studied. Among the genotypes studied, the Chlorophyll *a* ranged from 0.35 to 1.32 mg/g. The highest chlorophyll a was showed by Punjab Anmol (1.32

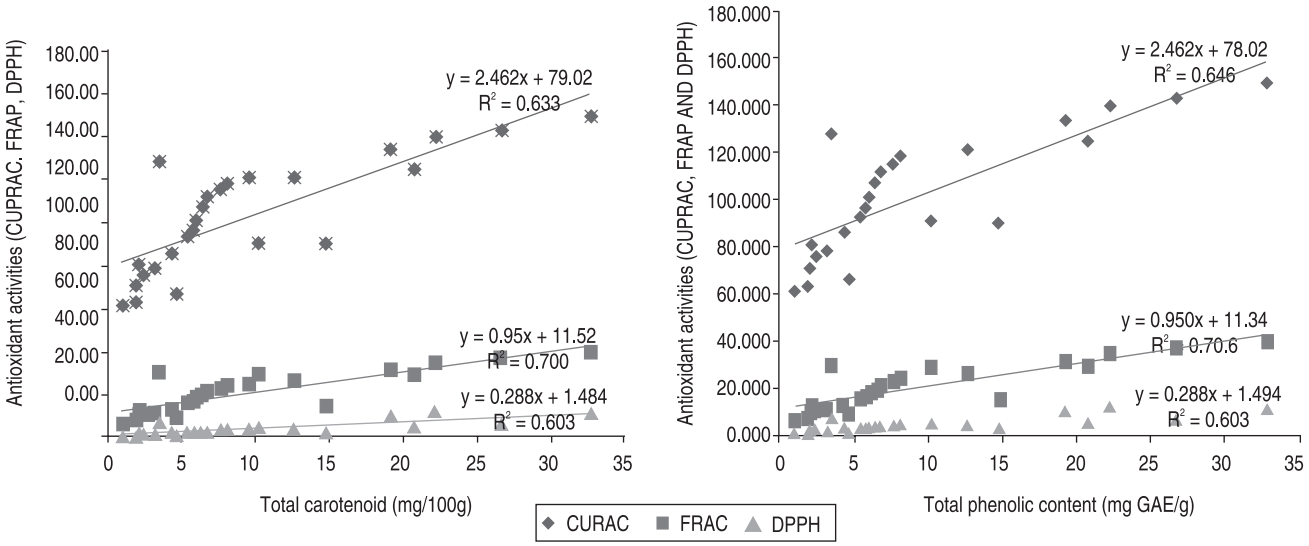

Fig 1 Scatter Plot Diagram of total carotenoids and phenolic content *v/s* antioxidant activities in chrysanthemum flowers; A) Total carotenoid v/s antioxidant activities, B) Total phenolic content v/s antioxidant activities.

Table 5 Estimation of chlorophyll and carotenoid contents in leaves of chrysanthemum genotypes

Genotype	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Total carotenoids (mg/g)
Classic	0.68	0.08	0.76	0.27
Geetanjali	0.35	0.21	0.56	0.21
Haldighati	0.89	0.15	1.04	0.41
Mayur-5	0.44	0.06	0.50	0.12
Punjab Anmol	1.31	0.28	1.59	0.38
Aparajita	0.85	0.18	1.02	0.25
Sadwin Yellow	1.06	0.28	1.35	0.48
Yellow Gold	0.92	0.11	1.05	0.27
Vijay Kiran	0.39	0.31	0.70	0.11
Ajay Orange	0.68	0.10	0.78	0.22
Pusa Aditya	1.16	0.28	1.44	0.43
Mallika Yellow	0.56	0.16	0.73	0.22
Ram Lal Dada	0.80	0.06	0.87	0.29
Yellow Reflex	0.87	0.07	0.95	0.42
Star Yellow	0.98	0.13	1.12	0.39
Pusa Sona	0.61	0.05	0.66	0.24
Jubilee	0.82	0.05	0.87	0.32
Jayanti	1.12	0.03	1.15	0.43
Basanti	0.66	0.15	0.83	0.16
Pusa Centenary	0.92	0.108	1.032	0.313
Little orange	0.82	0.037	0.863	0.262
Liliput	0.96	0.226	1.190	0.367
Teri	0.47	0.317	0.790	0.273
Kundan	0.74	0.110	0.860	0.181
Texas Gold	0.91	0.128	1.042	0.302
LSD (P=0.05)	0.01	0.02	0.02	0.01

mg/g) followed by Pusa Aditya (1.17 mg/g), Jayanti (1.12 mg/g), Star Yellow (0.98 mg/g) and Liliput (0.96 mg/g). However, the chlorophyll b ranged from 0.04 to 0.32 mg/g. The highest chlorophyll b was showed by Teri (0.32 mg/g) followed by Vijay Kiran (0.31 mg/g), Pusa Aditya (0.28 mg/g), Sadwin Yellow and Punjab Anmol (0.28 mg/g) and Geetanjali (0.21 mg/g).

Among the chrysanthemum genotypes studied, the total chlorophyll content ranged from 0.51 to 1.51 mg/g fresh weight of leaves. Punjab Anmol showed highest total chlorophyll (1.59 mg/100g) followed by Sadwin Yellow (1.45 mg/g), Pusa Aditya (1.36 mg/g), Little Orange (1.19 mg/g) and Star Yellow (1.12 mg/g). Lowest total chlorophyll was obtained in Geetanjali (0.56 mg/g) fresh weight of petals. Our results are in agreement with Ruminska *et al* (2013) where they reported 1.01-1.32 mg/g chlorophyll content in the chrysanthemum leaves. The total carotenoid content in leaves ranged from 0.11 to 0.48 mg/g. Pusa Aditya (0.48 mg/g) showed highest carotenoid content followed by Ajay Orange (0.43 mg/g), Jayanti (0.43mg/g), Yelllow Reflex (0.42mg/g), Haldighati (0.417 mg/g). Lowest total carotenoid was obtained in Vijay Kiran (0.11 mg/g).

Quantification of carotenoids in ray florets of promising genotypes using High Performance Liquid Chromatography (HPLC).

In HPLC chromatogram, the characteristic peaks show the typical absorbance of carotenoids. The per cent area of individual peak in terms of total peak area of lutein and β-carotene content is represented in Table 6 and Fig 2. Lutein and β-carotene were taken as standards to quantify the carotenoids, which were expressed in terms of µg/g. Peaks were detected at respective l_{max} of 444 and 453 nm for lutein and β-carotene. Highest lutein content of 19.90 µg/g from the peak area of 96.19% was identified in variety Jubilee, followed by 8.67 µg/g in Haldighati from the peak

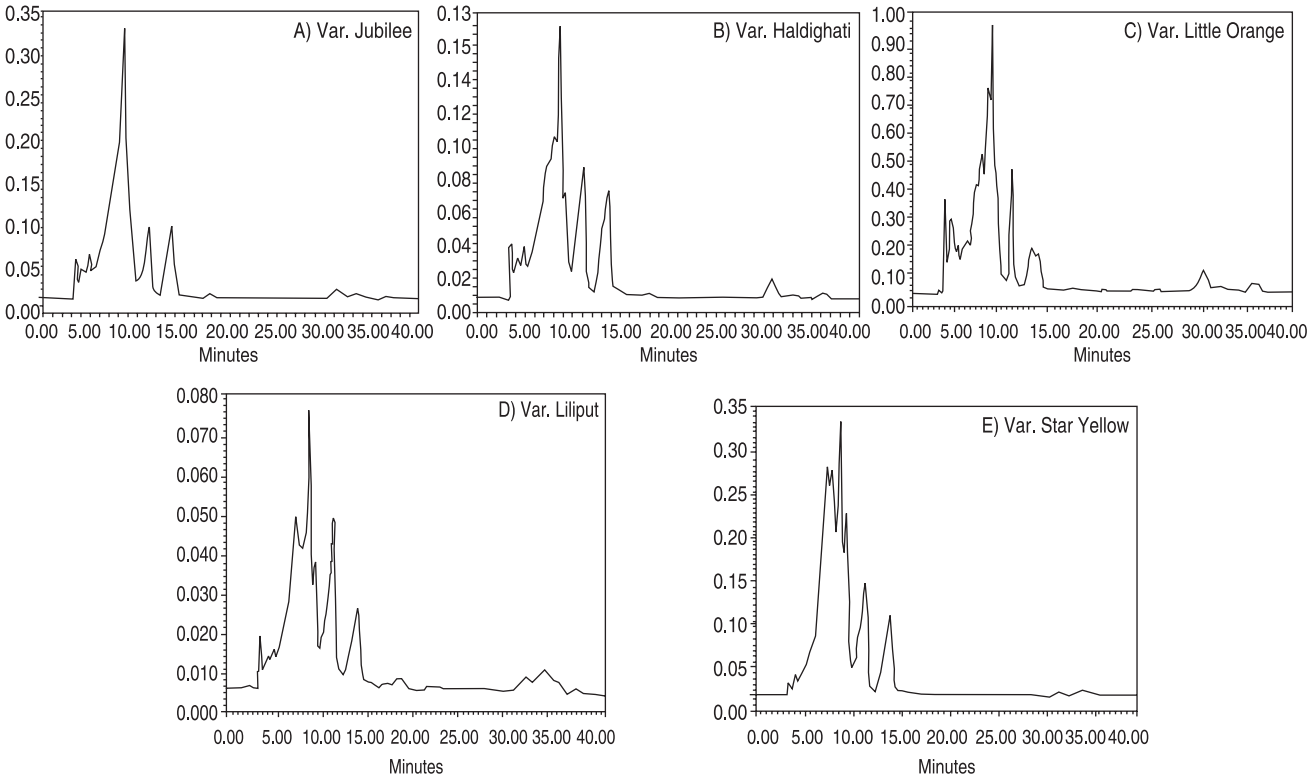

Fig 2 HPLC chromatogram of carotenoid profile of promising genotypes of *Chrysanthemum* × *morifolium* A) Var. Jubilee, B) Var. Haldighati, C) Var. Little Orange, D) Var. Liliput and E) Var. Star Yellow.

Table 6 Quantification of carotenoid pigments using High Performance Liquid Chromatography

Genotype	Retention Time (Min)	Peak area (%)	Lutein content (µg/g)	Reten tion Time (Min)	Peak area (%)	β-carotene content (µg/g)
Little Orange	8.54	60.11	3.69	36.16	39.89	5.51
Haldighati	8.72	90.51	8.67	36.36	9.49	2.04
Liliput	8.84	95.23	4.77	37.12	4.77	0.54
Jubilee	8.78	96.19	19.90	36.75	3.81	1.77
Star Yellow	8.95	98.43	3.93	37.56	1.57	0.50
LSD (P=0.05)			1.8807	LSD at 5%		2.57

area of 90.51%, 4.77 µg/g in Liliput from the peak area of 95.23%, 3.93 µg/g in Star yellow from the peak area of 98.43%, 3.69 µg/g in Little Orange from the peak area of 60.11%. Highest β- carotene content of 5.51 µg/g from the peak area of 39.89% was identified in Little Orange, followed by 2.04 µg/g from the peak area of 9.49% in Haldighati, 1.77 µg/g in Jubilee from the peak area of 3.81%, 0.54 µg/g in Liliput from the peak area of 4.77%, and 0.50 µg/g in Star Yellow from the peak area of 1.57 %. In all the five promising varieties the lutein content was identified at a retention time of eight to nine minutes and the β- carotene content is identified at a retention time of 36 to 37 min. Similar studies are reported by Kishimoto *et al.* (2004) in ray florets of chrysanthemum. Our findings were also in an agreement with the reports of Kishimoto *et al.* (2007), showing that the lutein and lutein-5, 6-epoxide are the main carotenoid components in petals of *Chrysanthemum morifolium*, *Helianthus annuus*, *Tagetes erecta* and *Tagetes patula*. Piccagalia *et al.* (1998) estimated lutein and lutein esters in African marigold and French marigold using HPLC and observed that lutein esters constituted about 80% of the chromatogram. Sivel *et al.* (2004) also showed similar findings in marigold (*Tagetes erecta*).

Chrysanthemum × *morifolium* could be considered as new source of safe natural pigments for versatile industries such as food industry, pharmaceutical industry, etc. The present study will be helpful in development of varieties and hybrids rich in carotenoids and antioxidant capacities.

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