



Determination and correlation of carotenoid pigments and their antioxidant activities in marigold (*Tagetes* sp.) flowers

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ABSTRACT

Marigold (*Tagetes* sp.) flowers are considered as an important source of carotenoid pigments namely xanthophylls (lutein, zeaxanthin) and yellow carotenoids (β -carotenes). In present study, different marigold genotypes were evaluated for colour values for abaxial and adaxial surface of petals, total carotenoids, phenolic and flavonoid content and their antioxidant activities. The colour parameters such as L, a, and b for abaxial and adaxial petal surfaces exhibited the significant differences for colour values among genotypes. Among the various genotypes studied, selection Af/w-6 had highest total carotenoids (525.68 mg/100g) on fresh weight basis followed Pusa Narangi Gainda (339.92 mg/100g) and Pusa Arpita (160 mg/100g). Total phenolic content on fresh weight basis of petals ranged from 81.93 to 136.17 mg GAE/g whereas, flavonoid content ranged from 37.11 to 65.13 mg RE/g. Highest antioxidant activity measured by DPPH radical scavenging activity and Ferrous Reducing Antioxidant Power (FRAP) was found in selections Af/w-6 (891.16 μ mol FeSO₄/g and 82.17%) followed by Af/w-4 (809.29 μ mol FeSO₄/g; 81.55%). A high correlation between carotenoids, total phenolic and flavonoid content, antioxidant activities was observed.

Key words: Antioxidant activity, Carotenoid, Flavonoid, Marigold, Phenol

The bioactive compounds play an important role in plant as well as human health. The various bioactive compounds such as polyphenols, flavonols, pigments, etc. are distinctly found in most of the plant species and serve many important ecological functions (Grotewold 2006). Plant pigments provide wide array of colours to the plant and also serve many utilities. Flower crops are one of the potential sources of pigments as they provide an array of pigments, however, due to lack of awareness it remained as an unexploited area. Among flowers, marigold (*Tagetes* sp. L.) is considered as a richest source of carotenoid pigments and it is being extracted from flowers and traded worldwide. Marigold (*Tagetes* sp.) is an important flower crop grown worldwide, native of Mexico and belongs to family Asteraceae. The genus *Tagetes* comprises of about 33 species of which, *Tagetes erecta* L. (African marigold) and *T. patula* L. (French marigold) are grown commercially for loose flowers and *T. minuta* L. for essential oil purpose. The flowers of *Tagetes erecta* and *T. patula* are also rich

source of carotenoid pigments and hence, also grown for extraction of natural carotenoid pigment which is used in poultry feed to impart intense pigmentation to egg yolk and broiler skin colour, as a natural food colourant in various industries and ingredient in cooking as the fresh petals or as dried powder.

Carotenoid pigments are found in all parts of marigold plant such as leaf, roots, flowers, seeds etc., however, marigold flower petals are considered as an important source of carotenoid pigments, especially the yellow carotenoids (β -carotenes), xanthophylls (lutein, zeaxanthin) (Ahluwalia *et al.* 2014) and polyphenols (Siriamornpun *et al.* 2012). These pigments are recognized as safe chemicals for nutraceutical purpose because of their concentrated colour, their role as precursor for vitamin A synthesis and antioxidant activity in human being. Carotenoids are one of the potential natural plant based antioxidant which reduces the risk of chronic diseases such as cancer and enhance immune function, reduces auto oxidation of cellular lipids and age related macular degeneration, provides protection against oxidant induced cell damage, etc, therefore, the assessment of colour, carotenoids, their antioxidant activities, phenol and flavonoid content of marigold is of great significance. To the best of our knowledge and available literatures, there are very few published reports on the determination of total carotenoids, phenolic and flavonoid content, antioxidant activities of marigold flowers and their correlation. Keeping these considerations in view, the present investigation

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was carried out to find out the presence of pigment, their antioxidant activities and correlation among themselves.

MATERIALS AND METHODS

The plant material utilized for conducting the experiment consisted of eight genotypes of African marigold namely Af/w-1, Af/w-2, Af/w-3, Af/w-4, Af/w-6, Afw-7, Pusa Narangi Gainda and Pusa Basanti Gainda and Pusa Arpita of French Marigold. The features and photograph of genotypes used in the present investigation are given in Table 1 and Fig 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 2013-15. Fresh marigold flowers were harvested at full bloom stage for determination of colour values of the petals, carotenoid pigments, antioxidant activity and other bioactive compounds.

Colour values of sample was measured by a Hunter Lab colour measurement instrument L, a, b colour scales. The L-value shows brightness whereas positive and negative a values determine the redness and greenness and positive and negative b values exhibit yellowness and



Fig 1 Genotypes used for determination of colour values, total carotenoids, phenolic and flavonoid content and antioxidant activities of petals of marigold

Table 1 Salient features of marigold genotypes

| Genotype | Flower type | Flower form | Flower size | Flower colour | Species | Flowering time | Source |
|------------------------|-------------|-------------|-------------|---------------|--------------------------|-------------------------|-----------|
| Af/w-1 | Semi double | Petalous | Medium | Orange | <i>Tagetes erecta</i> L. | Mid Feb.,- End March | ICAR-IARI |
| Af/w-2 | Semi double | Petalous | Medium | Light Orange | <i>Tagetes erecta</i> L. | -do- | ICAR-IARI |
| Af/w-3 | Semi double | Petalous | Medium | Yellow | <i>Tagetes erecta</i> L. | -do- | ICAR-IARI |
| Afw/-4 | Semi double | Petalous | Medium | Yellow | <i>Tagetes erecta</i> L. | -do- | ICAR-IARI |
| Af/w-6 | Semi double | Petalous | Medium | Orange | <i>Tagetes erecta</i> L. | -do- | ICAR-IARI |
| Afw-7 | Semi double | Petalous | Medium | Yellow orange | <i>Tagetes erecta</i> L. | -do- | ICAR-IARI |
| Pusa Arpita | Semi double | Petalous | Medium | Orange | <i>Tagetes patula</i> L. | Mid Dec.,- Mid Feb., | ICAR-IARI |
| Pusa Narangi Gainda | Semi double | Petalous | Medium | Orange | <i>Tagetes erecta</i> L. | Mid Feb., Mid April | ICAR-IARI |
| Pusa Basanti Ganida | Semi double | Petalous | Medium | Yellow | <i>Tagetes erecta</i> L. | Mid Feb.,- Mid March | ICAR-IARI |

blueness, respectively (Nourian and Ramaswamy 2003). The calibration of the instrument was done against a white standard. Measurements were individually taken for three samples per treatment and the average of readings was calculated. The colour value of variety Pusa Narangi Gainda of marigold was taken as standard colour value to find out colour difference ΔE among genotypes.

The colour difference (ΔE) was calculated from the L, a, b values, using the Hunter–Scotfield equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

The total carotenoids were extracted using method given by Ranganna (1995). A known weight of sample, i.e. 0.1 g fresh marigold flower petals were weighed and grounded finely with mortar and pestle in acetone till residue becomes colourless. The acetone extract was collected in a conical flask. Separating funnel was used for the separation of the carotenoid pigments. The carotenoid extract was transferred to separating funnel, then added petroleum ether with addition of 10% Na_2SO_4 . Swirl the funnel to separate the carotenoid layer. The isolated carotenoids were collected in volumetric flask. The process was repeated till remained extract shows no colour. The absorbance was read at 452 nm spectrophotometrically. Total carotenoids were estimated using following formula

$$\text{Total carotenoids (mg/} \times \text{volume make up} \times \text{dilution} \\ \text{100g fresh weight basis)} = \frac{3.87 \times \text{absorbance (452 nm)} \\ \text{factor} \times 100}{\text{weight of sample (g)} \times 1000}$$

The phenolic compounds in fresh petals of marigold were extracted using a modification of the procedure described by Uzelac *et al.* (2005). Each 0.2 g sample was extracted with 20 ml ethanol (80%). The extract was then centrifuged at 10000 rpm at 4°C for 20 minutes. The supernatant was taken for determination of total phenolic and total flavonoid content.

Total phenols were estimated according to procedure given by Singleton and Rossi (1965). A 5 g sample was

extracted with 50 ml of methanol (80%). The aliquot (1 ml) were taken in the test tubes and were added with 2.9 ml of Folin and Ciocalteu's Phenol Reagent (1N). To that, 0.5 ml of distilled water was added and all the tubes were shaken well. Then, 2 ml of sodium carbonate (20%) solution was added to all the tubes and kept for incubation at room temperature for 30 min. The colour developed was read in spectrophotometer at 750 nm wavelength. Standard curve was drawn using gallic acid as standard. Different concentrations of gallic acid were prepared and OD was read at 700 nm wavelength. The concentration of samples was calculated based on the standard curve. Total phenolic content was estimated using following formula

$$\text{Total phenolic content} \\ \text{(mg gallic acid equiva-} \\ \text{lence per gram)} = \frac{\text{OD} \times \text{standard curve factor} \times \\ \text{volume made up} \times \text{dilution}}{\text{aliquot taken} \times \text{weight of} \\ \text{sample}}$$

The colorimetric method described by Abu Bakar *et al.* (2009) was used to determine total flavonoid content. 0.3 ml of the extract was mixed with 2.1 ml of absolute alcohol in a test tube. Then, 0.3 ml of 5% NaNO_2 solution was added. After 5 min, 0.3 ml of 10% AlCl_3 solution was added to it followed by 3.4 ml of 4 N NaOH after 5 min. The mixture was vortexed and incubated at room temperature. The absorbance was read at 510 nm after 5-10 min of incubation using spectrophotometer. Standard curve was drawn using Rutin as standard. Different concentrations of rutin were prepared and O.D. was read at 510 nm. The concentration of samples was calculated based on the standard curve. Total flavonoid content was estimated using following formula.

$$\text{Total flavonoid content} \\ \text{(mg Rutin equivalence} \\ \text{per gram)} = \frac{\text{OD} \times \text{standard curve factor} \\ \times \text{volume made up} \times \text{dilution}}{\text{aliquot taken} \times \text{weight of} \\ \text{sample}}$$

The sample for assessment of antioxidant activity 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).

Preparation of working FRAP reagent: Acetate buffer 300 mM pH 3.6, TPTZ (2, 4, 6-tripyridyl-s- triazine) 10 mM in 40mM HCl and Ferric Chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) 20 mM in 10:1:1 ratio were freshly prepared on the same day. The ethanolic extract (0.1 ml) of the sample was taken in test tubes and 3 ml of working FRAP reagent was added. Then, the tubes were kept for incubation at room temperature for 4-5 min. The blue colour so developed was read in a spectrophotometer at 593 nm using the FRAP reagent as a blank and expressed as Trolox equivalents. Standard curve was drawn using FeSO_4 as standard. Different concentrations of FeSO_4 were prepared and OD was read at 593 nm. The concentration of samples was calculated based on the standard curve.

$$\text{FRAP } (\mu \text{ FeSO}_4/\text{g}) = \frac{\text{OD} \times \text{factor} \times \text{volume made up}}{\text{aliquot taken} \times \text{weight of sample}}$$

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The antioxidant activity of the extracts was determined using DPPH assay described by Braca *et al.* (2001). Aqueous extract 0.1 ml was added to 3.9 ml of 0.0025 M DPPH (2, 2-Diphenyl-1-picrylhydrazyl) in methanol (70%). The mixture was shaken and allowed to stand for 30 min. in dark at room temperature. Absorbance was read at 517 nm using spectrophotometer. The per cent inhibition of activity was calculated by the formula:

$$\text{Per cent inhibition (\%)} = \frac{[(\text{Ao}-\text{Ae})/\text{Ao}] \times 100}{1}$$

(Ao = absorbance without extract; Ae = absorbance with extract).

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).

Pearson's correlation analysis also performed to study correlation between variables.

RESULTS AND DISCUSSION

Determination of colour values

Colour is an important aspect of any products that affects the pleasure of any intake. Colour of any genotype of marigold varies from genotype to another, hence; affect the pigments and other bioactive compounds present in the flowers. Therefore, colour values of abaxial and adaxial surfaces of petals were measured using the Hunter Colour Lab and are shown in Table 2. The colour parameters such as L, a, and b for abaxial and adaxial petal surfaces exhibited the significant differences among genotypes. The L value of abaxial and adaxial petal surface of different genotypes showed the colour brightness. The var. Pusa Narangi Gainda, Pusa Arpita, Af/w-6 and Af/w-1 are brighter than remaining genotypes. The abaxial and adaxial petal surface of different genotypes exhibited positive a values leading towards more redness, except Pusa Basanti Gainda which showed -a values (abaxial: -2.34 and adaxial: -2.85). Similarly, abaxial and adaxial petal surface of various genotypes revealed positive b values meaning more yellowness.

The colorimetric parameter, i.e. total colour difference, ΔE , which is a combination of parameters L, a, and b values is extensively used to characterize the variation of colours in marigold germplasm. The results this study advocate that the changes in ΔE of abaxial and adaxial petal surface of genotypes such as Af/w-1 (8.71, 9.52), Af/w-6 (4.91, 8.77), Pusa Arpita (7.36, 7.36) and Pusa Narangi Gainda (14.88, 12.78) is lesser than other genotypes meaning that they are more close in their colour. The colour parameters were also estimated by Siriamornpun *et al.* (2012) in marigold.

The data presented in Table 3 showed that there were significant differences at 5% level among the genotypes for all the characters studied.

Table 2 Colour values of abaxial and adaxial surface of petals of different genotypes of marigold

| Genotype | Abaxial surface of petal | | | | Adaxial surface of petal | | | |
|---------------------|--------------------------|-------------|--------------|------------|--------------------------|-------------|-------------|------------|
| | L | a | b | ΔE | L | a | b | ΔE |
| Af/w-1 | 58.95 | 37.73 | 94.34 | 8.71 | 56.62 | 25.02 | 65.76 | 9.52 |
| Af/w-2 | 67.10 | 21.36 | 101.06 | 24.65 | 67.71 | 15.69 | 74.80 | 15.76 |
| Af/w-3 | 67.24 | 22.52 | 97.74 | 22.22 | 66.21 | 17.12 | 75.42 | 15.84 |
| Afw/-4 | 68.44 | 22.53 | 96.81 | 22.35 | 66.51 | 17.41 | 76.27 | 14.08 |
| Af/w-6 | 59.11 | 37.02 | 89.04 | 4.92 | 59.72 | 28.33 | 69.41 | 8.77 |
| Afw-7 | 64.07 | 36.35 | 103.06 | 18.16 | 68.22 | 21.35 | 72.91 | 11.08 |
| Pusa Arpita | 55.17 | 37.81 | 87.32 | 7.36 | 61.79 | 29.46 | 70.72 | 1.58 |
| Pusa Narangi Gainda | 59.33 | 43.84 | 100.23 | 14.88 | 60.80 | 19.23 | 65.67 | 12.98 |
| Pusa Basanti Ganida | 79.56 | -2.34 | 80.08 | 47.54 | 79.80 | -2.85 | 57.42 | 39.32 |
| Mean | 64.33 | 28.53 | 94.41 | 18.97 | 65.26 | 18.97 | 69.82 | 14.32 |
| Range | 55.17-79.56 | -2.34-43.84 | 80.08-103.06 | 4.92-47.54 | 56.62-79.80 | -2.85-29.46 | 57.42-76.27 | 1.58-39.32 |
| CD(P \leq 0.05) | 3.97 | 1.77 | 5.90 | 3.95 | 8.24 | 2.93 | 7.45 | 5.47 |

Table 3 Estimation of total carotenoids, phenol and flavonoid content and their antioxidant activities in promising genotypes of marigold

| Genotype | Total carotenoids (mg/100g) | Total phenolic content (mg GAE/g) | Total flavonoid content (mg RE/g) | FRAP ($\mu\text{mol FeSO}_4/\text{g}$) | DPPH (%) |
|---------------------|-----------------------------|-----------------------------------|-----------------------------------|--|-------------|
| Af/w-1 | 115.46 | 85.58 | 40.89 | 476.02 | 68.52 |
| Af/w-2 | 26.06 | 85.58 | 48.25 | 486.08 | 70.01 |
| Af/w-3 | 23.73 | 119.83 | 50.89 | 663.28 | 77.10 |
| Af/w-4 | 19.61 | 136.17 | 65.13 | 809.30 | 81.55 |
| Af/w-6 | 525.68 | 125.48 | 55.17 | 891.16 | 82.17 |
| Af/w-7 | 75.21 | 106.00 | 45.54 | 633.74 | 75.67 |
| Pusa Arpita | 160.22 | 84.24 | 42.56 | 466.68 | 76.47 |
| Pusa Narangi Gainda | 339.92 | 81.93 | 37.11 | 711.39 | 76.02 |
| Pusa Basanti Ganida | 33.80 | 89.20 | 42.41 | 613.80 | 74.15 |
| Mean | 146.63 | 101.55 | 47.55 | 639.04 | 75.74 |
| Range | 19.61-525.68 | 81.93-136.17 | 37.11-65.13 | 466.68-891.16 | 68.52-82.17 |
| CD(P \leq 0.05) | 13.66 | 2.93 | 3.71 | 97.28 | 2.75 |

Total carotenoids

Among the marigold genotypes studied, the total carotenoid content ranged from 19.61 mg/100g fresh weight to 525.68 mg/100g fresh weight of petals. Af/w-6 showed highest total carotenoids (525.68 mg/100 g followed by Pusa Narangi Gainda (339.92 mg/100g), Pusa Arpita (160.22 mg/100g), Af/w-1 (115.46 mg/100g). However, lower values of total carotenoids were obtained by Af/w-4, Af/w-3 and Af/w-2 (19.61 23.73 26.06 mg/100g, respectively) fresh weight of petals (Table 3.) Our results are in confirmation with Saha (2005) and Panwar (2012). Saha (2005) evaluated marigold genotypes and reported that range for carotenoid content from 366.99mg/100 g to 415.84 mg/100 g. The total carotenoid content in variety Pusa Narangi Gainda was 385.48 mg/100 g which is comparable to our results of 339.92 mg/100 g in Pusa Narangi Gainda. The results on the total carotenoid content are similar to the studies of Kasemsap *et al.* (1990) and Gregory *et al.* (1986). They reported that dark orange coloured flowers of marigold were found to have high carotenoids than light orange or yellow coloured flowers and moreover because of high total carotenoids they are highly suitable for carotenoid pigment extraction for commercial use.

Total phenolic content (TPC)

The total phenolic content varied from 81.93 to 136.17 mg GAE/g fresh weight of petals among various genotypes of *Tagetes* sp. taken into the study. Af/w-4 (136.17 mg GAE/g fresh weight) showed highest phenolic content followed by Af/w-6 (125.48 mg GAE/g fresh weight), Af/w-3 (119.83 mg GAE/g fresh weight) and Af/w-7 (106.00 mg GAE/g fresh weight) (Table 3). The IARI developed orange coloured varieties Pusa Narangi Gainda and Pusa Arpita exhibited 81.93mg GAE/g fresh weight and 84.24mg GAE/g fresh weight phenolic content respectively, which is less as compared to new selections. Similar findings were observed by Ahluwalia *et al.* (2014) as they reported phenolic content in marigold variety Pusa Basanti Gainda

as 109.4 mg GAE/g and Pusa Narangi Gainda as 102.2 mg GAE/g, which is comparable to our results on phenolic content for Pusa Basanti Gainda and Pusa Narangi Gainda as 89.20 and 81.93 mg GAE/g, respectively. Gong *et al.* (2012) also reported total phenolic content in the petals of *Tagetes erecta* and it was estimated as 70.01 mg GAE/g. Hemali and Sumitra (2014) also reported similar range in phenolic content of *Tagetes erecta* as 102.34 mg GAE/g.

Total flavonoid content (TFC)

Total flavonoid content among various genotypes of marigold was ranged from 37.11 to 65.13 mg RE/g fresh weight. The selection Af/w-4 showed highest total flavonoid content (65.13 mg RE/g fresh weight) followed by Selection Af/w-6 (55.17 mg RE/g fresh weight), Af/w-3 (50.89 mg RE/g fresh weight), Af/w-2 (48.25 mg RE/g fresh weight), Af/w-7 (45.54 mg RE/g fresh weight), Pusa Arpita (42.56 mg RE/g fresh weight) and Pusa Basanti Gainda (42.41 mg RE/g fresh weight). However, variety Pusa Narangi Gainda exhibited lowest total flavonoid content (37.11 RE mg/g fresh weight). The results are comparable with Kaisoon *et al.* (2012) who reported that flowers of *Tagetes erecta* had 68.9 mg/g of flavonoid content. In previous studies, the flavonoid content (25.12%) was also found out in flowers of marigold (*Tagetes patula* L.) by Munhoz *et al.* (2014).

Antioxidant activity

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 coloured ferrous tripyridyltriazine (Fe^{2+} -TPTZ) (Benzie and Strain, 1996). The FRAP values of the marigold flower extract of different genotypes are shown in Table 3. The FRAP values among genotypes of marigold ranged from 466.68 to 891.16 $\mu\text{mol FeSO}_4/\text{g}$ fresh weight. The highest reducing power was observed in selection Af/w-6 (891.16 $\mu\text{mol FeSO}_4/\text{g}$ fresh

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

| Traits | | Total carotenoids | Total phenolic content | Total flavonoid content | FRAP | DPPH |
|-------------------------|---|-------------------|------------------------|-------------------------|---------|---------|
| Total carotenoids | G | | 0.868** | 0.832** | 0.418** | 0.423** |
| | P | | 0.785** | 0.683** | 0.309 | 0.256 |
| Total phenolic content | G | | | 0.015 | 0.656** | 0.776** |
| | P | | | 0.001 | 0.545** | 0.613** |
| Total flavonoid content | G | | | | 0.500** | 0.647** |
| | P | | | | 0.170 | 0.259 |
| FRAP | G | | | | | 0.498** |
| | P | | | | | 0.172 |
| DPPH | G | | | | | |
| | P | | | | | |

** Significant at 1% level; G- Genotypic level; P- Phenotypic level.

weight) followed by Af/w-4 (809.30 $\mu\text{mol FeSO}_4/\text{g}$ fresh weight), Pusa Narangi Gainda (711.39 $\mu\text{mol FeSO}_4/\text{g}$ fresh weight), Af/w-3(663.28 $\mu\text{mol FeSO}_4/\text{g}$ fresh weight) and Af/w-7 (633.74 $\mu\text{mol FeSO}_4/\text{g}$ fresh weight). However, selection Pusa Arpita exhibited lowest reducing power (466.68 $\mu\text{mol FeSO}_4/\text{g}$ fresh weight).

DPPH 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). DPPH scavenging activity of flower petals of various marigold genotypes is presented in Table 3. The DPPH value among genotypes ranged from 68.52% (Af/w-1) to 82.17% (Af-6). The highest DPPH radical scavenging was observed in Af/w-6 (82.17%) followed by Af/w-4 (81.55%), Af/w-3 (77.10%), Pusa Arpita (76.47%) and Pusa Narangi Gainda (76.02%) whereas Af/w-1 exhibited lowest DPPH radical scavenging activity (68.52%). The antioxidant activities of different genotypes were similar to results obtained from FRAP assay.

The similar results were reported by Kaisoon *et al.* (2012). They studied antioxidant activities of 12 edible flowers in Thailand and reported that *Tagetes erecta* had the highest ferric reducing antioxidant power (FRAP) value (600 $\mu\text{mol FeSO}_4/\text{g}$ dry weight) and DPPH inhibition of 85.70%. Similar to our results, *Tagetes erecta* is reported to have FRAP values from 329.4–609.2 and DPPH radical scavenging of 94.3%. (Cavaiuolo *et al.* 2013). The antioxidant activity was also reported by Munira (2014) and Pratheesh *et al.* (2009) in *Tagetes erecta*; Aquino *et al.* (2002) in *Tagetes lucida*.

Correlation analysis

Correlation coefficient among the parameters was analyzed to observe the direction and magnitude of associations at genotypic and phenotypic levels (Table 4). A high correlation between carotenoids, total phenolic and flavonoid content, antioxidant activities indicated that these compounds could be main contributors of antioxidant activities of marigold flowers. Genotypic

correlation coefficients were high in magnitude than of the corresponding phenotypic correlation coefficients. The analysis exhibited a significant and positive correlation of total carotenoids with total phenolic (0.868 and 0.785), flavonoid content (0.832 and 0.683), FRAP (0.418 and 0.309) and DPPH (0.423 and 0.256) assay as shown in Table 4. Significant positive genotypic and phenotypic correlation was also observed in total phenolic content with FRAP (0.656 and 0.545) and DPPH (0.776 and 0.613) assay and significant genotypic correlation in total flavonoid content with FRAP (0.500) and DPPH (0.647) assay. However, positive but non-significant phenotypic correlation was found in flavonoid content with FRAP (0.170) and DPPH (0.259). Significant positive genotypic correlation of FRAP with DPPH (0.498) was observed whereas; they exhibited positive but non-significant phenotypic correlation (0.172). Munhoz *et al.* (2014) observed the positive correlation between flavonoid content and free radical scavenging activity of flower extract. Hence, it was concluded that flavonoid content contributes significantly to antioxidant activity. However, weak correlation was observed between total phenolic content and flavonoid content (0.015 and 0.001). 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 and flavonoid 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.

The present study on estimation and correlation of carotenoids and antioxidant activities in marigold will be helpful in development of varieties and hybrids rich in carotenoids and having high antioxidant capacities.

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