Research Article

# Biochemical Constituents Studies in African Marigold Germplasm

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**Abstract**

The present study was carried out at Department of Horticulture, CCS HAU, Hisar with the objective to find out some promising African marigold genotypes having the higher recovery of different biochemical constituents of industrial importance. In this study, twenty genotypes of African marigold were evaluated for extraction of biochemical constituents like carotenes, xanthophylls and chlorophylls. Maximum Total carotenes (77.36 mg/100g) and di-hydroxy pigments (229.18 mg/100g) were recorded in genotype 160-9-2, while maximum xanthophylls (362.03 mg/100g) were obtained in genotype 160-5 followed by 148-3-1 (357.93 mg/100g) and 160-5-1 (355.36 mg/100g) genotypes, respectively. African marigold genotype 160-8 (PN) recorded maximum mono-hydroxy pigments (121.18 mg/100g) followed by genotype 148-3-1 (116.27 mg/100g). Chlorophyll 'a' (3.68 mg/100g) and chlorophyll 'b' (6.20 mg/100g) were found higher in genotype 146-2 while genotype 148-3-3 recorded maximum Total chlorophyll (9.88 mg/100g).

**Keywords:** Carotenes, Xanthophylls, Chlorophylls, Genotypes, African marigold

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## Introduction

Marigold (*Tagetes erecta* Linn.), a member of the Asteraceae family, is native to Central and South America mainly Mexico. Its flowers are extensively used for making garlands, wreath, religious offerings and exhibitions, natural color pigments, insect and nematodes repellants, nutrient supplement for poultry feed and cut flower purpose. Its essential oil has been mainly used for the compounding of high-grade perfumes and also has anti-inflammatory and antiseptic properties. African marigold is gaining industrial importance as a potential source of carotenoids that are used as food colorants, feed additives and possess anticancer and anti-ageing effects. Carotenoids furnish flowers and fruits with different colors varying from yellow to red and are essential components for photosynthesis. These are also required by the immune system where they act as detoxifiers neutralizing free radicals before they damage DNA, lipids & proteins [1]. *Tagetes erecta* petals have more carotenoids than did the seeds or sepals. Oven drying of petals results into a greater loss of carotenoids than shade drying. Maximum carotenoid content (240.25 mg/100g) was measured in dry flowers of *Tagetes erecta* while fresh flowers of *Tagetes erecta* recorded 25.71 mg/100g carotenoid content [2]. Carotenoid fractions like lutein, carotenes, mono-hydroxy pigments, di-hydroxy pigments etc. impart orange-yellow, yellow, orange and red color, respectively for use in food, beverage and textile industries. These colors are safe for human consumption, unlike other artificial colors which are carcinogenic in nature. Marigold cultivars with orange flowers have higher xanthophylls as compared with cultivars having yellow flowers [3]. Well preserved flowers of the marigold exhibit a high yield of xanthophyll content (105.19 g/Kg) as compared to the unpreserved flowers (54.87 g/Kg) [4]. Xanthophylls inhibit auto-oxidation of cellular lipids, protect against oxidant-induced cell damage, cancer and cardiovascular disease and more importantly, protect against age-related macular degeneration (AMD) [5]. Marigold flower represents a rich source of lutein. It acts as an effective antioxidant, namely in the protection of eyes, because it neutralizes free radicals formed by the action of ultraviolet radiation on eye retina. Dried marigold flowers contain 0.1–0.2% dry matter of carotenoids, out of which 80% are lutein diesters [6]. These are also primarily being used by the poultry industries as feed additives to color egg yolks orange and poultry skin yellow [1]. Considering the potential of African marigold as an emerging avenue for diversification, employment and income, this study was carried out to find out some promising African marigold genotypes having the higher recovery of different biochemical constituents of industrial importance.

## Materials and methods

The experimental material for the present investigation consisted of twenty genotypes of African marigold (*Tagetes erecta* L.). This experimental material was taken from the previously maintained germplasm of the Department of Horticulture, CCS Haryana Agricultural University, Hisar. The fresh flowers of twenty different African marigold genotypes were harvested at full bloom stage in the morning hours when there were no dew drops present on them. The flowers were then taken to the laboratory and stored in open space at ambient conditions and analyzed for different biochemical constituents. Complete flower head was taken for this analysis experiment. Various reagents were used during the extraction of biochemical constituents from African marigold flowers. All the stock solutions of the following reagents were prepared for their further use during the chromatographic separation.

### *Reagents*

*Extractant:* A combination of Hexane-Acetone-Ethanol-Toluene in the ratio of 10:7:6:7 v/v was used.
*Absorbent:* It is the inert substance used during chromatographic separation in order to increase the contact area with the extraction solvent and help filtration. Hyflosupercel and Silica Gel G (Diatomaceous earth) were used as an absorbent and mixed in a blender for 1-2 hrs in the ratio of 1:1 w/w.
*Sodium sulphate (10%):* It is used as a moisture absorbent during the extraction process. It is prepared by dissolving 10 g of anhydrous $Na_2SO_4$ dissolved in 100 ml distilled water.
*Solvents:* Elution of different compounds in the column is performed by a gradient of solvents with different polarities.

| | |
|---|---|
| Carotenes | : Hexane and Acetone (96:4) |
| Mono-hydroxy pigments | : Hexane and Acetone (90:10) |
| Di-hydroxy pigments | : Hexane and Acetone (80:20) |
| Total xanthophylls | : Hexane, Acetone and Methanol (80:10:10) |
| Chlorophyll | : Ether and Methanol (50:50) |

### *Procedure*

Three steps were involved in identification and separation of carotenoids from marigold flowers. All these steps were carried out with full precaution and accuracy.

*Sample preparation*

Five grams of the sample was homogenized with the help of mortar and pestle. The samples were then put into 100 ml beaker and 40 ml of the extractant was pipette into it. The beaker was then covered with aluminum foil and kept in dark for 24 hours. The extractant was then collected in a conical flask. Three extractions were carried out and pooled to assure complete removal of carotenoids. Carotenoids were then filtered through glass wool and transferred to petroleum ether in order to remove water along with other solvents. The remaining traces of water were removed by addition of anhydrous $Na_2SO_4$ (10%). The process was repeated until no more color was extracted. This experimental sample was further used for chromatography.

*Chromatography*

Carotenoids were fractioned into carotenes, mono-hydroxy pigments, di-hydroxy pigments, chlorophylls etc. with the help of open column chromatography method. An absorbent cotton plug was inserted into the column with the help of a glass rod on the surface of the septa present above the stop cork. The column was packed with 12 cm layer of the mixture of absorbent under vacuum. A small amount of anhydrous $Na_2SO_4$ was added on the top of the column to absorb residual water from the sample. To flatten the surface of absorbent, the inverted cork was used for uniform absorption of the sample. The extraction sample was first added to the column. As the sample was absorbed by the absorbent, different color of the bands were formed which indicated different compounds or fraction of carotenoids.

*Recovery of biochemical constituents*

The elution of following compounds was performed by a gradient of solvents with different polarities.

*Total carotenes (mg/100g)*

Carotene solvent (Hexane and Acetone, 96:4) was added as the last solution entered the absorbent and continued until carotene band was collected in the flask. The flask was inverted several times to mix the eluted compound thoroughly before taking its absorption spectra @ 440 nm wavelength. Total carotene was then determined with the formula given in calculation.

*Mono-hydroxy pigments (mg/100g)*

As the previous solvent level approached the absorbent surface, MHP solvent (Hexane and Acetone, 90:10) was added to the column. The band of mono-hydroxy pigments (Zeaxanthin, cryptoxanthin, etc.) or di-esters was collected in a 25 ml volumetric flask and kept in dark. Absorption maxima were recorded at 474 nm wavelength and calculations were done according to the formula given under calculations.

*Di-hydroxy pigments (mg/100g)*

As the MHP solvent approached the absorbent surface, DHP solvent (Hexane and Acetone, 80:20) was added which carried the DHP band (Lutein, Zeaxanthin and their easters) through the column and was collected in a different volumetric flask. The absorption maxima were recorded at 474 nm wavelength and further calculations were done according to the formula given under calculations.

*Total xanthophylls (mg/100g)*

For Total xanthophylls, a fresh extraction sample was used. First of all, carotene was eluted by the above method followed by the elution of total xanthophylls with the solvent (Hexane, Acetone and Methanol, 80:10:10).

*Chlorophyll (mg/100g)*

The solvent used for chlorophyll extraction was Ether and Methanol (50:50). The absorption maxima was recorded on the spectrophotometer at two different wave lengths i.e. 663 nm and 645 nm for further calculations of chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll.

***Calculations***

The following carotenoid fractions were calculated according to the method given in AOAC and modified by Singh *et al.* (2008) [7]:
Total Carotenes (mg/100g):

$$\frac{OD_{440} \times 3.86 \times \text{Dilution of aliquant loaded} \times \text{Total dilution}}{\text{Aliquant loaded} \times \text{weight of sample (g)} \times 1000} \times 100$$

Mono-hydroxy and di-hydroxy pigments (mg/100g):

$$\frac{OD_{474} \times 3.86 \times \text{Dilution of aliquant loaded} \times \text{Total dilution}}{\text{Aliquant loaded} \times \text{weight of sample (g)} \times 1000} \times 100$$

Total xanthophylls (mg/100g):

$$\frac{OD_{474} \times 3.86 \times \text{Dilution of aliquant loaded} \times \text{Total dilution}}{\text{Aliquant loaded} \times \text{weight of sample (g)} \times 1000} \times 100$$

Chlorophyll ‘a’ (mg/100g):

$$\frac{[(12.7 \times A_{663}) - (2.69 \times A_{645})]}{1000 \times \text{weight of sample}} \times \text{Volume}$$

Chlorophyll ‘b’ (mg/100g):

$$\frac{[(22.9 \times A_{645}) - (4.69 \times A_{663})]}{1000 \times \text{weight of sample}} \times \text{Volume}$$

Total Chlorophyll (mg/100g):

$$\frac{[(20.2 \times A_{645}) - (8.02 \times A_{663})]}{1000 \times \text{weight of sample}} \times \text{Volume}$$

Data obtained from the present investigation was subjected to statistical analysis of variance (ANOVA) techniques by using Completely Randomized Design (CRD) to determine the significance of variance at 5% level of significance.

## Results and discussion

### *Total carotenes (mg/100g)*

Data represented in **Table 1** revealed that total carotenes ranged from 18.96 mg/100g to 77.36 mg/100g within different African marigold genotypes. The maximum total carotene content was recorded in flowers of the genotype 160-9-2 (77.36 mg/100g) followed by 160-5 (76.34 mg/100g) whereas minimum total carotene content (18.96 mg/100g) was recorded in the flowers of 160-5-2 followed by 133-1-1 (20.78 mg/100g) & 162-2-2 (21.13 mg/100g). Total carotenes content varied among different genotypes due to the different genetic makeup of these genotypes. Similar observations were made by Toiu *et al.* (2008) [2] while measuring total carotenoid content in nine marigold varieties. Similar variations in carotenoid content were also observed previously by Sestras and Boscaiu (2015) [8] while evaluating genetic variability in 23 genotypes of marigold. These findings are similar to the results obtained by Benítez-García *et al.* (2014) [9] while evaluating two varieties of African marigold.

### *Mono-hydroxy pigments (mg/100g)*

Among the different African marigold genotypes, the mono-hydroxy pigments ranged from 44.39 mg/100g to 121.18 mg/100g as represented in Table 1. The maximum mono-hydroxy pigments (121.18 mg/100g) were recorded in 160-8(PN) followed by 148-3-1 (116.27 mg/100g) whereas minimum mono-hydroxy pigments (44.39 mg/100g) were observed in 133-3-3. The variation in mono-hydroxy pigment content of different African marigold genotypes may be due to the different genetic makeup of these genotypes. Similar variations in different marigold varieties have also been observed by Singh *et al.* (2008) [7] while studying carotenes and xanthophylls fraction in six genotypes of African marigold. Such a range of variability in mono-hydroxy pigments among the marigold genotypes is mainly due to genetic nature, growing environmental conditions and cultural practices.

### *Di-hydroxy pigments (mg/100g):*

The experimental data depicted in the Table 1 reveal that di-hydroxy pigments ranged from 83.58 mg/100g to 229.18 mg/100g among the different African marigold genotypes. The maximum di-hydroxy pigments were recorded in 160-9-2 (229.18 mg/100g) whereas minimum (83.58 mg/100g) di-hydroxy pigments were recorded in the flowers of 162-2-2. This variation in di-hydroxy pigment content could be attributed to the different genetic makeup of these genotypes. Similar findings have also been reported by Singh *et al.* (2008) [7] while estimating carotenes and xanthophylls fraction in six genotypes of African marigold.

**Table 1** Evaluation of African marigold genotypes for different biochemical constituents

| Genotypes | Total carotenes (mg/100g) | Mono-hydroxy pigments (mg/100g) | Di-hydroxy pigments (mg/100g) | Total xanthophylls (mg/100g) | Chlorophyll 'a' (mg/100g) | Chlorophyll 'b' (mg/100g) | Total chlorophyll (mg/100g) |
|---|---|---|---|---|---|---|---|
| 160-9-2 | 77.36 | 89.67 | 229.18 | 285.11 | 1.43 | 1.09 | 1.74 |
| 160-8-2 | 66.85 | 72.42 | 193.89 | 243.69 | 1.61 | 1.24 | 1.54 |
| 160-8(PN) | 57.71 | 121.18 | 189.84 | 255.02 | 1.51 | 1.16 | 1.97 |
| 160-8-3 | 39.96 | 103.64 | 192.61 | 264.64 | 1.32 | 1.0 | 1.89 |
| 133-3-3 | 21.60 | 44.39 | 100.06 | 110.05 | 2.02 | 1.55 | 1.59 |
| 133-1-1 | 20.78 | 60.25 | 112.46 | 236.49 | 3.34 | 5.46 | 8.80 |
| 146-2 | 29.42 | 96.10 | 101.28 | 288.35 | 3.68 | 6.20 | 9.79 |
| 148-8-1 | 46.02 | 84.64 | 94.48 | 206.24 | 2.42 | 4.68 | 7.38 |
| 148-8-2 | 38.17 | 71.98 | 106.26 | 302.91 | 3.64 | 5.93 | 9.57 |
| 148-3-1 | 35.56 | 116.27 | 142.39 | 357.93 | 2.47 | 4.81 | 7.72 |
| 148-3-3 | 43.66 | 83.39 | 125.16 | 333.01 | 2.94 | 4.78 | 9.88 |
| 160-5-1 | 64.56 | 106.32 | 176.39 | 355.36 | 3.23 | 5.53 | 8.76 |
| 160-5-2 | 18.96 | 98.26 | 103.62 | 280.61 | 2.96 | 5.01 | 7.97 |
| 160-5-2-2 | 53.36 | 69.73 | 137.40 | 267.88 | 2.39 | 4.80 | 7.19 |
| 160-5-3 | 48.85 | 88.41 | 106.67 | 267.78 | 2.88 | 4.82 | 7.69 |
| 160-5-4 | 65.89 | 52.83 | 92.87 | 239.43 | 2.76 | 4.80 | 7.93 |
| 160-5 | 76.34 | 112.52 | 188.71 | 362.03 | 3.09 | 5.82 | 8.91 |
| 160-7 | 30.92 | 93.37 | 180.62 | 325.91 | 3.00 | 5.46 | 8.46 |
| 160-7-1P2 | 57.85 | 75.56 | 165.53 | 307.21 | 3.42 | 4.57 | 7.99 |
| 162-2-2 | 21.13 | 56.73 | 83.58 | 219.24 | 2.62 | 4.82 | 7.45 |
| CD (5%) | 6.127 | 7.597 | 6.809 | 34.139 | 0.321 | 0.331 | 0.701 |

***Total xanthophylls (mg/100g)***

Values represented in Table 1 recorded that the total xanthophylls content ranged from 110.05 mg/100g to 362.03 mg/100g among the different African marigold genotypes. The maximum xanthophyll content was observed in the genotype 160-5 (362.03 mg/100g) followed by 148-3-1 (357.93 mg/100g) whereas minimum xanthophyll (110.05mg/100g) content was observed in 133-3-3. This variation in xanthophyll content may be due to the different genetic makeup of these genotypes. These findings are in line with the results obtained by Shivakumar *et al.* (2014) [10] while characterizing fifteen genotypes of African marigold. Similar variation in xanthophyll content was also observed by Deineka *et al.* (2007) [3] and Ahmad *et al.* (2011) [11] in marigold. Similar results were also obtained by Karuppaiah *et al.* (2011) [12] while performing variability studies in thirty-four genotypes of African marigold. Similar findings to these results have been obtained by Benítez-García *et al.* (2014) [9] while studying two varieties of African marigold.

***Chlorophyll 'a' (mg/100g)***

Chlorophyll 'a' content ranged from 1.32 mg/100g to 3.42 mg/100g among the different African marigold genotypes (Table 1). The maximum chlorophyll 'a' (3.68 mg/100g) was observed in flowers of 146-2 followed by 160-7-1-P2 (3.42 mg/100g) whereas minimum chlorophyll 'a' was observed in flowers of 160-8-3 (1.32 mg/100g) followed by 160-9-2 (1.43 mg/100g) among the different genotypes. The variations in chlorophyll content may be due to the different genetic makeup of these genotypes. Similar variations in chlorophyll content were also observed previously by Sestras and Boscaiu (2015) [8] while studying genetic variability in 23 genotypes of marigold.

***Chlorophyll 'b' (mg/100g)***

Values represented in Table 1 reveal that Chlorophyll 'b' content ranged from 1.0 mg/100g to 6.20 mg/100g amidst different African marigold genotypes. The maximum chlorophyll 'b' (6.20 mg/100g) was observed in flowers of 146-2 followed by 148-8-2 (5.93 mg/100g) whereas minimum chlorophyll 'b' was recorded in flowers of 160-8-3 (1.0

mg/100g) followed by 160-9-2 (1.09 mg/100g). Similar variations in chlorophyll 'b' content were also recorded by Sestras and Boscaiu (2015) [8] while studying genetic variability in 23 genotypes of marigold.

***Total Chlorophyll (mg/100g)***

Among different African marigold genotypes, total chlorophyll ranged from 1.54 mg/100g to 9.88 mg/100g. The maximum total chlorophyll was recorded in flowers of 148-3-3 (9.88 mg/100g) followed by 146-2 (9.79 mg/100g) whereas minimum total chlorophyll was recorded in flowers of 160-8-2 (1.54 mg/100g) followed by 133-3-3 (1.59 mg/100g) as revealed by data shown in Table 1.

## Conclusion

From the present investigation, it may be concluded that the maximum carotenes (77.36 mg/100g) and di-hydroxy pigments (229.18 mg/100g) were obtained in 160-9-2 while maximum mono-hydroxy pigments (121.18 mg/100g) were reported in 160-8 (PN). The maximum total xanthophylls (362.03 mg/100g) were reported in genotype 160-5 followed by 148-3-1 and 160-5-1, respectively.

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