Research Article

Optimization of Extraction Process of Pigments from Marigold Petals

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Abstract

Extraction process of pigments from marigold flowers was carried out using dry flower petals powder of three different stage viz., (i) full bloom flower (ii) 5-7 days opened flower and (iii) senescing stage of flower with different solvent treatment viz., acetone, hexane, ethanol and mixture of acetone: hexane (1:1) and ethanol: hexane (1:1). The result indicated that extraction of carotenoids from Acetone: Hexane was most efficient followed by Acetone and Ethanol while extraction from Hexane and Ethanol: Hexane was comparatively low. Irrespective of treatment (solvent), carotenoid content of fully opened flower was higher (240.01 mg/100g) than 5-7 day opened flower (239.64 mg/100g) and senescing flower (224.81 mg/100g).The interaction effect between treatments (solvents) and stage of flower revealed maximum carotenoids in Acetone : Hexane (1:1) extracted fully open flower i.e. Acetone : Hexane x fully open flower (434.24 mg/100g) followed by Acetone : Hexane x 5-7 day opened flower (416.78 mg/100g), Acetone : Hexane x senescing flower (398.58 mg/100g) and Acetone x fully opened flower (336.54 mg/100g). Total phenol content and radical scavenging activity of Acetone: Hexane x fully opened flower (83.57 mg GAE/100g and 88.48 % respectively) and Acetone x fully opened flower (81.23 mg GAE/100g and 85.95 % respectively) was significantly higher compared to Acetone : Hexane x 5-7 day old flower (77.74 mg GAE/100g and 81.45% respectively) and Ethanol x fully open flower (76.82 mg GAE/100g and76.75 % respectively) while dry residue of extract was maximum (6.70%) in Ethanol x fully opened flower treatment.

Keywords: Marigold, pigments, solvent, carotenoids, total phenol, radical scavenging activity

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Introduction

Marigold (*Tagetes erecta* L.) is a leading loose flower of India, covering an area of 66.13 thousand hectares with 603.18 thousand metric tonnes of production in 2015-16 [1]. These flowers are mainly used for decoration purposes or as offering to God. A survey report reveals that 30-40% of the total productions of flowers are unsold and wasted everyday which are thrown in water of river Ganga or dumped which also creates water pollution as well as environmental pollution [2]. These wasted flowers can be used in various ways and we can get wealth from waste materials. It produces natural dye from its flowers (petals) consisting mainly of carotenoid-lutein and flavonoid-patuletin, which could be isolated and exploited commercially [3]. Marigold extract (lutein) is a xanthophyll that has strong antioxidant ability, and has the potentiality to be used as an additive in poultry feeds [4, 5]. Preliminary studies demonstrated the solvent extraction of petals as a source of flavonoids with antioxidant and larvicidal effect on *Aedes aegypti* mosquitoes [6]. It has the prospect of utilization as food colouring agent and nutrient supplement (food additive) in baked food, beverages, sauces, dairy products etc. [7]. Anthocyanin and betalain pigments which are water soluble are extracted from raw material with water and sometimes with aqueous methanol. For carotenoids extraction, organic solvents are good choice for initial extraction of pigment from plant material [7]. Although there are few reports of chromatographic, supercritical fluid extraction and HPLC techniques for extraction, separation, identification and quantification of constituents of oleoresin extract of marigold [8, 10], some simple innovative technique of extraction of marigold pigment by organic solvents needs to be standardized through proper method of refinement. The quality and quantity of pigment extract also depends significantly on stage of flower development. Thus considering the importance of marigold pigment, the present investigation was undertaken to optimize the extraction process of pigment from marigold petals.

Materials and Methods

The marigold flowers of *Tagetes erecta* L. (African marigold) were obtained from Horticultural Research Station (at Mondouri), Bidhan Chandra Krishi Viswavidyalaya, and the experiment was carried out in the laboratory of Department of Post Harvest Technology of Horticultural Crops during December 2016 to February 2017. Fresh

marigold flowers were harvested at three different stage of flower development (i) Full bloom flowers (ii) 5-7 days opened flowers and (iii) senescing stage of the flower. The petals were separated from the flower according to stages and dried in a cabinet dryer for about 10 hours to a level of 8 to 11 % moisture level at 58 ±1°C. Dried petal sample was grinded and the powdered sample was kept in desiccators for pigment extraction. The extraction process was carried out using powdered sample of three different stage of the flower with different solvents viz., acetone, hexane, ethanol and mixture of acetone: hexane (1:1) and ethanol: hexane (1:1). The pigment was allowed to extract for 24 hours at room temperature (20-26°C) and then carotenoid pigment was estimated. On the basis of efficiency of extraction of carotenoids pigments three solvents ie., i) Acetone ii) Ethanol iii) Acetone: Hexane were selected for the further quality character analysis of pigments.

Different treatment combinations that was used for extraction were as follows: T_1S_1= Acetone with fully opened flower, T_1S_2= Acetone with 5-7 days opened flower, T_1S_3= Acetone with Senescing flower, T_2S_1= Hexane with fully opened flower, T_2S_2= Hexane with 5-7 days opened flower, T_2S_3= Hexane with Senescing flower, T_3S_1= Ethanol with fully opened flower, T_3S_2= Ethanol with 5-7 days opened flower, T_3S_3= Ethanol with Senescing flower, T_4S_1= Acetone: hexane with fully opened flower, T_4S_2= Acetone: hexane with 5-7 days opened flower, T_4S_3= Acetone: hexane with Senescing flower, T_5S_1= Ethanol: hexane with fully opened flower, T_5S_2= Ethanol: hexane with 5-7 days opened flower, T_5S_3= Ethanol: hexane with senescing flower. The extract was characterized for quality analysis and observations of the biochemical assays which are mentioned in detail below were recorded.

Biochemical characters

Dry residue (DR)

For each extract, 10 ml were measured volumetrically, transferred to a pre-weighed small glass beaker and let to evaporate to dryness in a water bath. The dishes containing the residue were placed in a microwave, at 105°C for 10 min. the residue was calculated by

$$DR\ (\%) = (mf/mi) \times 100$$

Where, mf is the final mass of the sample in grams and mi is the initial mass, thus the results are expressed relative to 100 g of the extraction solution [11].

Determination of carotenoids pigment

The carotenoids (β-carotene) were estimated by the methods as detailed in AOAC [12] and Ranganna [13]. The carotenoid content of the sample was estimated at 436 nm using acetone, acetone - hexane and ethanol as blank for respective solvents. Carotenoid was calculated as:

$$\text{mg of β-carotene per 100 gram} = \frac{\text{µg of carotene per ml as read from the curve} \times \text{Dilution} \times 100}{\text{Weight of sample x 1000}}$$

Determination of total phenol contents and radical scavenging activity

Preparation of extract

Dry marigold petals were extracted using a modification of the procedure described by Jena [13].

Total phenol contents (TPC)

The concentration of total phenols was estimated spectrophotometrically using Folin–Ciocalteu reagent [14]. To 0.1 ml of the sample extract, 2.9 ml of deionized water, 0.5 ml of Folin–Ciocalteu reagent and 2.0 ml of 20 % Na_2CO_3 solution was added. The mixture was allowed to stand for 90 min and absorption was measured at 760 nm against a reagent blank in UV–vis spectrophotometer (spectrophotometer 166). The concentrations were determined by comparing the absorbance of the standards. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/L solutions of gallic acid, results were expressed as mg gallic acid equivalents in 100g of dry sample (mg GAE/100g DW).

$$\text{mg gallic acid equivalence per 100gram} = \frac{\text{O.D x standard curve factor x volume made up x Dilution}}{\text{Aliquot taken x weight of sample}}$$

Preparation of DPPH Solution

0.1 mM DPPH Solution prepared either using 22.2 mg in 1000 ml or 1.9 mg in 100 ml of ethanol.

DPPH free radical scavenging activity

Radical scavenging activity (RSA) using DPPH method: The estimation of the antiradical capacity of the samples was based on the reduction of DPPH. Because of its stability, DPPH is often used to evaluate the free radical-scavenging ability of the different type of samples and its determination was based on the method of [15] with minor adaptations. The sample extract (0.1 ml) was added to 3 ml of the solution of 0.001 M of DPPH in ethanol. After the incubation in darkness for 30 min, the absorbance at 517 nm. The percentage of radical scavenging activity (RSA) was calculated according to the following formula:

$$\% \text{ RSA} = \frac{\text{Abs}_c - \text{Abs}_s}{\text{Abs}_c} \times 100$$

Where Abs_c = Absorbance of control; Abs_s = Absorbance of sample

Results and Discussion

The effect of treatments (solvent), stage of flower development and interaction between treatments x stage of flower on carotenoids content were significantly different at 5% level (**Table 1**). Irrespective of the stage of flower, mean carotenoids content of Acetone + Hexane treatment was maximum (416.53 mg/100g) followed by Acetone (323.91 mg/100g), Ethanol (185.61 mg/100g), Ethanol x Hexane (142.04 mg/100g) and Hexane (105.99 mg/100g). Irrespective of treatment (solvent), mean carotenoids content of fully opened flower was maximum (240.01 mg/100g) followed by 5-7 day opened flower (239.64 mg/100g) and senescing flower (224.81 mg/100g). However, mean carotenoids of fully opened flower and 5-7 day opened flower were not significantly different. The interaction effect between treatments (solvents) and stage of flower (Treatment x stage of flower) revealed maximum carotenoids (434.24 mg/100g) in Acetone + Hexane treatment of fully open flower (Acetone + Hexane x fully opened flower) followed by Acetone + Hexane x 5-7day opened flower (416.78 mg/100g), Acetone +Hexane x senescing flower (398.58 mg/100g), Acetone x fully opened flower (336.54 mg/100g), Acetone x 5-7day opened flower (323.78 mg/100g), Acetone x sensing flower (311.41 mg/100g) and so on. Carotenoids content of fully opened flower was highest with Acetone +Hexane treatment followed by Acetone, Ethanol, Hexane, and Ethanol +Hexane respectively in that decreasing order. Thus extraction with Acetone + Hexane was best followed by Acetone and Ethanol. So further chemical analysis was carried out with these three best solvents i.e. Acetone + Hexane, Acetone and Ethanol. Hexane and Ethanol + Hexane were left out in the subsequent analysis or experiments.

Table 1 Influence of solvent and stage of flower development on carotenoids (mg /100g) content of petal extract

Carotenoids content (mg /100g)				
Stage of flower development	**Fully Opened**	**5-7 Day Opened**	**Senescing**	**Mean**
Treatment(Solvent)				
Acetone	336.54	323.78	311.41	323.91
Hexane	117.06	108.87	92.04	105.99
Ethanol	197.91	183.90	175.02	185.61
Acetone+Hexane	434.24	416.78	398.58	416.53
Ethanol+Hexane	114.30	164.85	146.98	142.04
Mean	240.01	239.64	224.81	
	SEm ±	C.D. (5%)		
Treatments (T)	0.294	0.833		
Stage (S)	0.227	0.645		
Treatments x Stage (TxS)	0.509	1.442		

The influence of solvent treatments and stage of flower on total phenol content is shown in **Table 2**. Treatment, stage of flower opening and treatment x stage of flower interaction effect was significant (at 5%). Mean phenol content of Acetone + Hexane was significantly higher (77.73 mg GAE/100g) than acetone (75.10 mg GAE/100g) and Ethanol (69.63 mg GAE/100g). Similarly, high average phenol content was recorded in fully opened flower (80.54 mg GAE/100g) which was significantly higher than 5-7day opened (71.54 mg GAE/100g) and senescing flower

(70.38 mg GAE/100g) respectively. The interaction effect of Acetone + Hexane with the fully opened flower was highest (83.57 mg GAE/100g) followed by Acetone x fully opened flower (81.23 mg GAE/100g), Acetone +Hexane (T_2) x 5-7day opened flower (S_2) (77.74 mg GAE/100g), Ethanol (T_3) x fully opened flower (S_1) (76.82 mg GAE/100g) and so on. However, T_2S_2 and T_3S_1 were at par and not significantly different.

Table 2 Influence of solvent and stage of flower development on total phenol (mg GAE/100g) of petal extract

Total phenol contents (mg GAE/100g)				
Stage of flower development	**Fully Opened**	**5-7 Day Opened**	**Senescing**	**Mean**
Treatment (Solvent)				
T_1- Acetone	81.23	73.06	71.01	75.10
T_2- Acetone+Hexane	83.57	77.74	71.87	77.73
T_3- Ethanol	76.82	63.83	68.25	69.63
Mean	80.54	71.54	70.38	
	SEm ±	C.D. (5%)		
Treatments (T)	0.201	0.578		
Stage (S)	0.201	0.578		
Treatments x Stage (TxS)	0.348	1.001		

The effect of treatments, stage of flower and interaction between treatment x stage of flower on radical scavenging activity was significant (**Table-3**). Average radical scavenging activity of T_2 (Acetone + Hexane) was significantly higher (78.67 %) than Acetone (73.43 %) followed by Ethanol (66.21 %). Similarly, of the different stage of flower development average radical scavenging activity of fully opened flower (S_1) i.e. 83.73 % was significantly higher than 5-7 day opened flower (S_2) is 73.31 % followed by senescing flower (S_3) is 61.27 %. Interaction effect of radical scavenging activity for treatment x fully opened flower interaction was observed to be in general high. The T_2 (Acetone + Hexane) x S_1 (fully opened) possessed maximum radical scavenging activity (88.48 %) followed by T_1 (Acetone) x S_1 (fully opened) i.e. 85.95 %, T_2 (Acetone + Hexane) x S_2 (5-7 day opened) is 81.45%, T_3 (Ethanol) x S_1 (fully opened) i.e. 76.75% and so on. It is interesting to note that all the interaction of the treatments x senescing flower remained significantly lower than other interactions and it varied from 55.20 % (Ethanol x senescing Flower) to 66.08 % (Acetone: Hexane x senescing Flower).

Table 3 Radical scavenging activity (%) of marigold petal extract as influenced by solvent and stage of flower development

Radical scavenging activity (%)				
Stage of flower development	**Fully Opened**	**5-7 Day Opened**	**Senescing**	**Mean**
Treatment (Solvent)				
T_1- Acetone	85.95	71.79	62.54	73.43
T_2- Acetone+Hexane	88.48	81.45	66.08	78.67
T_3- Ethanol	76.75	66.68	55.20	66.21
Mean	83.73	73.31	61.27	
	SEm ±	C.D. (5%)		
Treatments (T)	0.215	0.619		
Stage (S)	0.215	0.619		
Treatments x Stage (TxS)	0.372	1.072		

Mean dry residue content of Ethanol (T_3) is 6.21 % was recorded to be significantly higher than Acetone: Hexane (T_2) is 5.40 % followed by Acetone (T_1) i.e. 4.41 % (**Table 4**). Irrespective of treatments, mean dry residue of the fully opened flower was significantly higher than (6.12 %) than 5-7 days opened (5.31 %) and senescing flower (4.50 %). Interaction effect of treatments and stage of flower development showed maximum dried residue (6.71 %) in ethanol (T_3) x fully opened flower (S_1) followed by Acetone: Hexane (T_2) x fully opened flower (S_1) i.e. 6.30 %, Ethanol (T_3) x 5-7 days opened flower (S_2) is 6.22 %, Ethanol (T_3) x senescing flower (S_3) i.e. 5.62 %, Acetone: Hexane (T_2) x 5-7 days opened flower (S_2) i.e. 5.43 % and so on.

The result indicated that of the different treatments (solvents) used for extraction of pigments (carotenoids), Acetone: Hexane (1:1) was most efficient followed by Acetone and Ethanol while extraction from Hexane and Ethanol: Hexane were comparatively low. In general extraction from fully opened flower was higher than other stage of flower development. However carotenoids of fully opened flower and 5-7 day opened flower were at par. So three solvents i.e. Acetone, Acetone: Hexane and Ethanol was selected as treatments for subsequent quality characteristics

estimation. Total phenol content and radical scavenging activity of Acetone: Hexane x fully opened flower (T_2S_1) and Acetone x fully opened flower (T_1S_1) was high while dry residue of extract was high in general in Ethanol treatment and in particular maximum in Ethanol x fully opened flower (T_3S_1) treatment. Although [3,9] developed innovative solvent extraction of dry flowers of *Tagetes erecta* with ethanol having high extraction efficiency allowing selective extraction of flavonoids and carotenoids, [16] and [6] optimized the extraction process with acetone which is in more or less in accordance to our findings. In the present investigation however Acetone: Hexane was the best treatment followed by acetone for extraction. It is interesting to note that Acetone: Hexane has not been tried earlier for extraction of pigments from marigold petals. Moreover, [17] also confirmed the suitability of acetone and hexane among some other solvents for the extraction of pigments. The superiority of acetone extract on radical scavenging activity with low dry residue has been reported by [6] in conformity with our result. For carotenoids extraction, organic solvent like hexane and acetone are more effective because of their solubility rather than water [7]. Previous report indicated that water is not an efficient solvent for extracting phenolic compounds, which are generally more soluble in organic solvents less polar than water [18]. Several studies have reported that the extraction efficiency of phenolic compounds is increased by the use of a mixture of water and organic solvents [19] which is somewhat contradictory to our result where Acetone: Hexane and Acetone was more efficient. However, [6] found that pure acetone gave the best results, probably due to the chemical composition of French marigold confirming our findings

Table 4 Dry residue (%) of marigold petal extract as influenced by solvent and stage of flower development

Dry residue (%)				
Stage of flower development	Fully Opened	5-7 Day Opened	Senescing	Mean
Treatment (Solvent)				
T_1- Acetone	5.32	4.41	3.54	4.41
T_2- Acetone+Hexane	6.30	5.43	4.53	5.40
T_3- Ethanol	6.71	6.22	5.62	6.21
Mean	6.12	5.31	4.50	
	SEm ±	C.D. (5%)		
Treatments (T)	0.00102	0.00196		
Stage (S)	0.00098	0.00194		
Treatments x Stage (TxS)	0.00158	0.00310		

Conclusion

It can be concluded that the different treatments (solvents) used for extraction of carotenoids, Acetone: Hexane was most efficient followed by Acetone and Ethanol while extraction from Hexane and Ethanol: Hexane were comparatively low. In general extraction from fully opened flower was higher than other stage of flower development. Total phenol content and radical scavenging activity of Acetone: Hexane x fully opened flower and Acetone x fully opened flower was high while dry residue of extract was maximum in Ethanol x fully opened flower treatment.

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