BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, August 2014. Vol. **11**(2), 839-843

Studies on Valuable Pigments from Florets of Safflower (*C. tinctorius* L.,) and Their Identification by TLC Method

Ayesha Sultana and S.Y. Anwer

Department of Genetics, Osmania University. Hyderabad, India.

doi: http://dx.doi.org/10.13005/bbra/1346

(Received: 08 May 2014; accepted: 06 June 2014)

India is the largest producer of safflower in the world with highest acerage. It is one of the humanity;s oldest crop cultivated mainly for oil from the seeds and redissh dye from the flowers. Intrest in this crop has been rekindled in the last few years because of the medicinal importances of the safflower florets . In china it is being grown exclusively for its flower's as they are usedfor the treatment of many illnesses as well as in the preparation of "Green Tea". Medicinal importance of the florets are mainly attributedto the presences of two major pigments i.e carthamin and safflower yellow (carthamidin). Most of the work is confined to the Chinese and Iranian Genotypes, there is no systematic report available on the isolation and characteriztion of florets pigments for Indian Genotypes. Therefore, the present study is aimed at isolation and characterization of carthamin and carthamidin (yellow pigment) in the four Indian Genotypes.

Key words: Safflower florets, Pigments, TLC.

MATERIALS AND METHODS

Dry florets of four Genotypes(JS1-97, NARI-NH1, MANJIRA,NIRA) were collected from the field of plant Genetics Experimental farm , Department of Genetics,Osmania university ,Hyderabad.(A.P).The chemicals used in the present study were obtained from the following sources : cellulose microcrystalline Avlicel(2330), kiselgel 60F254(1005554 Merk) silica gel G(type 60) for thin layer chromatography (from merk)and spectrophotometer.

* To whom all correspondence should be addressed.

Procedure

Dye extraction

Extraction of water insoluble carthamin and yellow water soluble pigment from safflower florets were essentially carried out by (kulkerni *et al.,* 1997) but with some modifications as follows

Extraction of carthamin

Fine dry floret powder (1gr) was suspended in 20ml of 0.5% WV -1 sodium carbonate .stirred at room temperature for 30mints .The floating pieces were re moved by centrifugation at 3500rpm for 15mints and the supernant Was retained at 5±1C the resulting suspension was added to fresh 20ml 0.5% sodium Carbonate and stirred for further 30mint and centrifuged and this process was repeated for one more time .The cooled extracts were mixed together and was acidified to obtaind a pH by adding 0,5% citric acid and used for adsorption

of carthamin.Adsorption of carthamin from acid extract was performed using a modified method described by (kulkerni *et al* 1997).cellulose powder (0.5gr) was suspended in acid solution , stirred with a magnetic stirring apparatus for 30min at room temperature and centrifuge at 3500rpm for 15min. supernant was discarded. The pellet was resuspended in distilled water and centrifuged. The washing was repeated 5-6 times under the same conditions until a colourless supernant was obtained. The pellet was suspended in 10ml of acetone ,intermixed for 5min,then centrifuged for 5min at 3500rpm.The acetone layer was filtered and used for spectrophotometric measuremen.

Extraction of carthamidin (safflower yellow)

One gram of fine floret powder was suspended in 15ml distilled water and stirred for 30min. Floating pieces were removed by centrifugation and the supernant was retained at 5±1c .The resultant suspension in distilled water was stirred for further 30min and centrifuged. The supernant was then filtered to separate suspended particles of floret powder.

Spectrophotometric measurement

The spectrophotometric measurement of carthamin (acetone washing of reddish cellulose) and yellow pigment (water extract) was followed from 380-620nm for carthamin and from 385-500nm for safflower yellow.

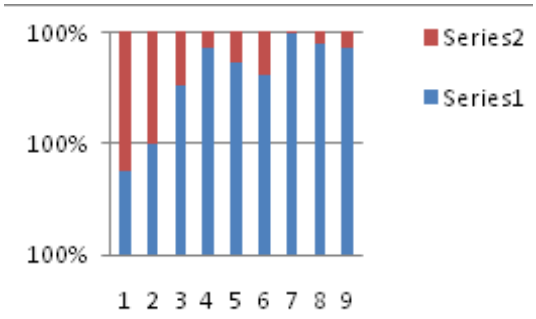
Thin layer chromatography

Thin-layer chromatographic identification was employed as reported by Rudometova *et al*., (2001) .The Rf values of yellow pigment and the red carthamin were examined on silica gel G .Two kinds of thin layer plates were used namely silica gel G and kisel gel 60 F254. The chromatographic solution consisted of distilled water: isobutanol: ethanol: formic acid. (4:7:4:t4).)

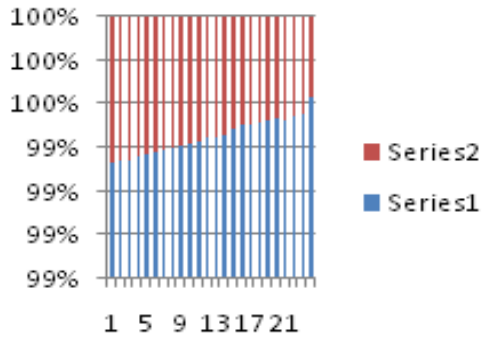
In the present study ,chromatographic and spectrometric analysis for carthmin and safflower yellow in the four Genotypes viz: NARI-NH1 and JS1-97, Manjira, NIRA are being reported in Indian safflower Genotypes . From the data presented in table the absorbance of carthamin extract is shown to have a maximum peak of light at 380-440nm (fig 1) this peak of light at 380nm

Table 1. The results of chromatographic and spectrophotometric analysis of carthmin and safflower yellow

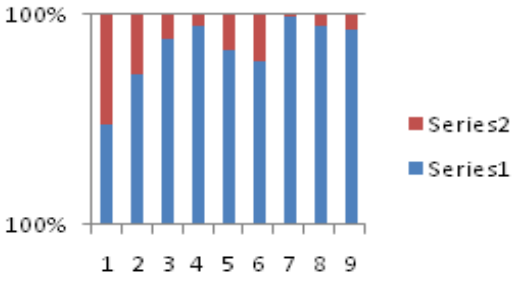
Sample	color	Maximum absorbance nm	silica gel type	RF value
Acetone washing of reddish cellulose (carthmin)	Red	380nm	Silica gel 'G' Kisel gel 60 f 254	2.0 2.2
Acetone washing of reddish cellulose(carthamin)	Red	410nm	Silica gel G Kisel gel 60f 254 (1005554)	1.6 1.9
Acetone washing of reddish cellulose (carthamin)	Red	440nm	Slica gel G Kisel gel 60 f 2254 (1005554)	2.0 2.1
Acetone washing of reddish cellulose (carthamin)	Red	380nm	Silica gel G Kisel gel 60 f 2254 (1005554)	0.9 1.2
Water-soluble yellow pigment	Yellow	385nm	Silica gel 'G' Kisel gel 60 f 254 (1005554)	2.1 2.2
Water-soluble yellow pigment	Yellow	385nm	Silica gel G Kisel gel 60f 254	2.0 2.2
Water-soluble yellow pigment	Yellow	395nm	Silica gel G Kisel gel 60f 254 (1005554)	1.3 1.2
Water-soluble yellow pigment	Yellow	385nm	Silica gel G Kisel gel 60f 254 (1005554)	1.1 1.0



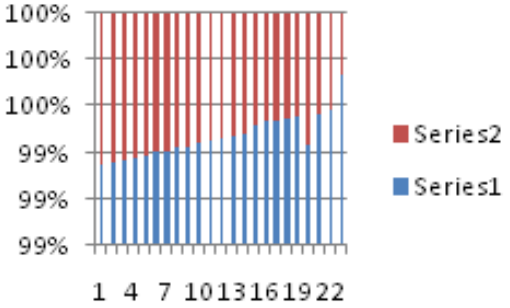
Estimation of carthamin in JS1-97 Genotype



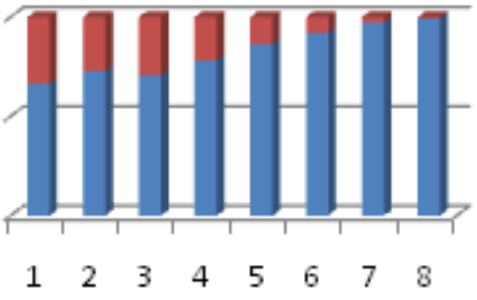
Estimation of carthamidin in JS1-97 Genotype



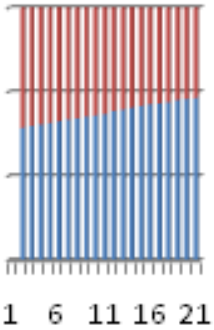
Estimation of carthamin in NARI-NH1 Genotype



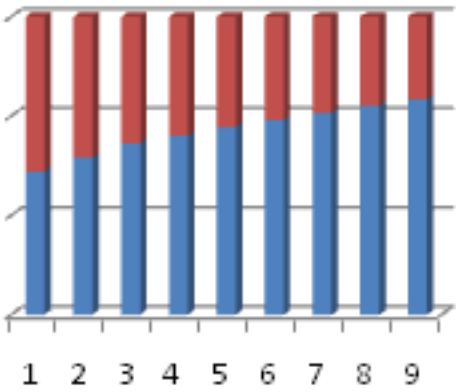
Estiamtion of carthamidin in NARI-NH1 Genotype



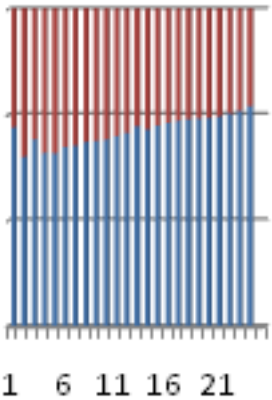
Estimation of carthamin in Manjira Genotype



Estimation of carthamidin in Manjira Genotype



Estimation of carthamin in NIRA Genotype



Estimation of carthamidin in NIRA Ge

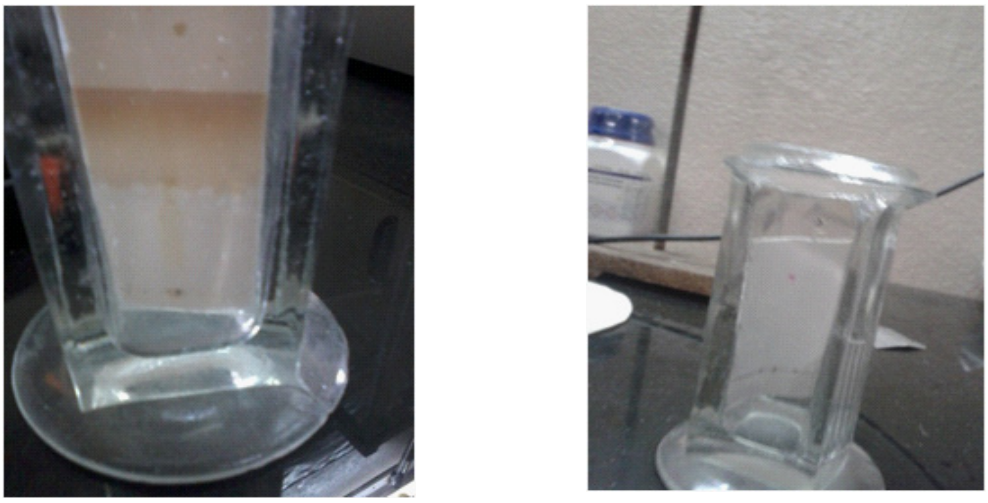


Fig. 2.

is typical for carthamin extract as shown in the earlier report (satio *et al*., 1985l) . However in case of safflower yellow the maximum peak is recorded at 385nm (fig 2) where as Kulkerni etal(5) .., 1997 has found the optical density for yellow pigment at 480nm .However Wu and Fu 1993 reported the optical density for yellow pigment at 400nm these differences in optical density for yellow pigment may be attributed to varietal differences therefore for the confirmation of differences in the results the extracts used for spectrophotometric analysis were subjected to chromatographic separation using two types of TLC plates (silica gel G ,kiesel gel 60F254) . Chromatogram of the extract is presented in fig 3 and 4 in both types of gels as for as the carthamin compound is concerned , this assended in the form a red horizontal line however when the chromatogram with safflower yellow is analyzed it is seen that the safflower yellow also assended but not in the form of a horizontal line as that of carthamin but this has taken the form of a circular yellow spot for silica gel c and tailed spot for kisel gel 60 (table1) . Analysis of the data indicates that for different silica gel different Rf values were noticed which can be attributed with the type of silica gel used. Similar results were reported by Rudometova *et al*.., 2001 .

Experimental results observed in present study demands that some additional operations are required to obtaine carthamin in required concentration and purification this can be attained by increasing the number of times the addition of Na2CO3 in florets and/or increasing the amount of sorbent cellulose powder may be it 1gm and also by increasing the number of repeated dilutions . Overall results recorded in the present study clearly indicates that a potential exist for carthamin and yellow pigment to be isolated and characterized from Indian safflower Genotype. The standardize protocol for isolation and characterization of carthamin and yellow pigment has reported in the present study may ultimately be useful for using this pigments as valuable dyes helping in food industry.

REFRENCES

1. N.Fatahi J. Carapetian and R. Heidari, Spectrophotometric measurement of valuable pigments from petals of safflower and their identification by TLC Method.
2. More S.D., Raghavaiah C.V., Hangarge D.S., Joshi B.M., Dhawan A.S., In: VI International Safflower conference (Ed.Esendal E.), Istanbul, Turkey, 2005; 180-186.
3. Rajvanshi A.K., In:VI International safflower conference (Ed. Esendal E.),Istanbul, Turkey, 2005, 80-85
4. Goda, Y., J. Suuki and T. Maitani, Structure of safflomin A and content of safflomin in commercial safflower products.Jpn., *J. Food chem*., 1997; **4**:54-58.
5. Hirokado,M ., K. Kimura, K. Suuki, Y. Sadamasu,

Y.Katsuki, K. Yasuda and M. Nishijima, 1999.
6. Detection method of madder coloure, cochineal extract,lac coloure, carthamus yellow carthamus red in processed foods by TLC.*J. Food Hyg.Soc. Jpn*., 1997; **40**: 488-493.
7. Kulkarni, D.N., S.M. Revanwar, K.D. Kulkarni and H.W. Deshpande, Extraction and uses of natural pigments from safflower florets .4th Int. safflower conf. Italy, 1997; 365-368.
8. Kuroda, C., The constituents of carthamin. *J. Soc*., 1930; 752-765.
9. Obara, H. and J.Onodera., *Structure of carthamin Chem. Lett.,* 1979; 201-204.
10. Rudometova, N.V., A.p.Pasovskij and E.A. Blohina, Method of isolation and identification of carthamin from safflower. Application's perspectives in Russian food products. 5th Int. safflower conf. Williston, N.D., U.S.A., 2001 ; 23-27.
11. Satio, K. and Y. Takahashi,Studies on the formation of carthamin in buffer solutions containing precarthamin and oxidating agents. *Acta Sic. Pol*., 1985; **54**:309-313.
12. Takahashi, Y., Miyasaka, S. Tasaka, I. Miura, S. Urano, M. Ikura, K. Hikichi, Matsumoto and M. wada, Consititution of two coloring matters in the flower petals of carthamus tinctorius L., *Tetra hedron Lett*., 1982; **23**: 5163-5166.
13. Wu, s. and J. Fu, The research and production of carthamin. Conf. proc. Safflower in china, 1993; 881-889.