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

India is the largest producer of safflower in the world with highest acreage. It is one of the humanity’s oldest crop cultivated mainly for oil from the seeds and reddish dye from the flowers. Interest 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 used for the treatment of many illnesses as well as in the preparation of “Green Tea”. Medicinal importance of the florets are mainly attributed to 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 characterization 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 Avilcel(2330), kiselgel 60F254(1005554 Merk) silica gel G(type 60) for thin layer chromatography (from merk) and spectrophotometer.

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 removed 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
Adsorption of carthamin from acid extract was performed using a modified method described by Kulkarni et al. (1997). Cellulose powder (0.5 gr) was suspended in acid solution, stirred with a magnetic stirring apparatus for 30 min at room temperature and centrifuged at 3500 rpm for 15 min. 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 10 ml of acetone, intermixed for 5 min, then centrifuged for 5 min at 3500 rpm. The acetone layer was filtered and used for spectrophotometric measurement.

**Extraction of carthamidin (safflower yellow)**

One gram of fine floret powder was suspended in 15 ml distilled water and stirred for 30 min. Floating pieces were removed by centrifugation and the supernant was retained at 5±1°C. The resultant suspension in distilled water was stirred for further 30 min 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 380-620 nm for carthamin and from 385-500 nm 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 (4:7:4:1).

In the present study, chromatographic and spectrometric analysis for carthamin 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 1 the absorbance of carthamin extract is shown to have a maximum peak of light at 380-440 nm (fig 1) this peak of light at 380 nm

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>color</th>
<th>Maximum absorbance nm</th>
<th>silica gel type</th>
<th>RF value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone washing of reddish cellulose (carthamin)</td>
<td>Red</td>
<td>380 nm</td>
<td>Silica gel ‘G’</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kisel gel 60 f 254</td>
<td>2.2</td>
</tr>
<tr>
<td>Acetone washing of reddish cellulose (carthamin)</td>
<td>Red</td>
<td>410 nm</td>
<td>Silica gel G</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kisel gel 60 f 254</td>
<td>1.9</td>
</tr>
<tr>
<td>Acetone washing of reddish cellulose (carthamin)</td>
<td>Red</td>
<td>440 nm</td>
<td>Silica gel G</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kisel gel 60 f 2254</td>
<td>2.1</td>
</tr>
<tr>
<td>Acetone washing of reddish cellulose (carthamin)</td>
<td>Red</td>
<td>380 nm</td>
<td>Silica gel G</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kisel gel 60 f 2254</td>
<td>1.2</td>
</tr>
<tr>
<td>Water-soluble yellow pigment</td>
<td>Yellow</td>
<td>385 nm</td>
<td>Silica gel ‘G’</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kisel gel 60 f 254</td>
<td>2.2</td>
</tr>
<tr>
<td>Water-soluble yellow pigment</td>
<td>Yellow</td>
<td>385 nm</td>
<td>Silica gel G</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kisel gel 60 f 254</td>
<td>2.2</td>
</tr>
<tr>
<td>Water-soluble yellow pigment</td>
<td>Yellow</td>
<td>395 nm</td>
<td>Silica gel G</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kisel gel 60 f 254</td>
<td>1.2</td>
</tr>
<tr>
<td>Water-soluble yellow pigment</td>
<td>Yellow</td>
<td>385 nm</td>
<td>Silica gel G</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kisel gel 60 f 254</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Estimation of carthamin in JS1-97 Genotype

Estimation of carthamin in NARI-NH1 Genotype

Estimation of carthamin in Manjira Genotype

Estimation of carthamin in NIRA Genotype
is typical for carthamin extract as shown in the earlier report (Satoo et al., 1985). However in case of safflower yellow the maximum peak is recorded at 385 nm (fig 2) whereas Kulkarni et al (1997) has found the optical density for yellow pigment at 400 nm. However Wu and Fu (1993) reported the optical density for yellow pigment at 400 nm. 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 asssumed in the form a red horizontal line however when the chromatogram with safflower yellow is analyzed it is seen that the safflower yellow also assensed 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 kiesel 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 obtain 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 1 gm 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.

REFERENCES
1. N. Fatahi J. Carapetian and R. Heidari, Spectrophotometric measurement of valuable pigments from petals of safflower and their identification by TLC Method.
5. Hirokado, M., K. Kimura, K. Suuki, Y. Sadamasu,


