**Comparative analysis and characterization of yellow and red pigment in safflower (*Carthamus tinctorius* L*.*)**

**ABSTRACT:**

This study explores the extraction, identification, and quantification of safflower's red (carthamin) and yellow (carthamidin) pigments, important for use in food, textiles, cosmetics, and pharmaceuticals. Carthamin forms from its yellow precursor, precarthamine, through oxidation during flower maturation. Yellow pigment was extracted with distilled water, and red with Sodium carbonate solution. Pigments were characterized spectrophotometrically, quantified using a Rutin standard curve, and identified via thin-layer chromatography (TLC) by comparing Rf values with standards flavonoids. Petals of thirrty safflower genotypes exhibiting colour variations, were collected for pigment extraction and quantification. The concentration of yellow pigment ranges between 6.9 to 28.9 mg/ml and red 2.2 to 4.3 mg/ml. Two genotypes with the highest pigment yields were identified as GMU-7923 which have 27.6 mg/ml and 3.3mg/ml and GMU-7931-1 have 28.9mg/ml and 4.3 mg/ml yellow and red content respectively. The Rf value for yellow pigment ranges from 0.32-0.80 and 0.93-0.96 for Red. GMU-7923 has Rf value 0.8 and 0.96 and GMU-7931-1 0.8 and 0.96 for yellow and red pigment respectively. Therefore, these genotypes can be exploit for it’s pigments as valuable dyes. The novelty of this study lies in exploring safflower florets as a source of natural, non-allergenic, and non-carcinogenic colorants, with the aim of identifying and extracting key pigments for use in various fields/industries.

Keywords **:** Pigment, Red, Rf value, Safflower, TLC,Yellow.

**1.INTRODUCTION:**

 Safflower ([*Carthamus*](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/carthamus) tinctorius L.) a member of the Asteraceae family, is an important [oilseed crop](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/oilseed-crops) which is cultivated over different geographical regions of Asia, including China and India (Singh, 2007).Iran is considered as one of the major origins of safflower in the old world (Golkar, 2014).In recent years, India's oilseed production has demonstrated some fluctuations, with safflower production reflecting a notable trend. According to the Economic Survey of Maharashtra 2023-24, India's safflower production during the fiscal year 2023 was estimated at approximately 57,000 metric tons (Economic Survey of Maharashtra, 2023-24). However, by the end of fiscal year 2024, India's safflower production was estimated to decrease to about 50,000 metric tons, marking a reduction from the previous year's levels ([statista.com](https://www.statista.com/statistics/937047/india-procurement-price-of-safflower/?utm_source=chatgpt.com) [apps.fas.usda.gov](https://apps.fas.usda.gov/newgainapi/api/Report/DownloadReportByFileName?fileName=Oilseeds+and+Products+Annual_New+Delhi_India_IN2024-0005.pdf&utm_source=chatgpt.com) accessed on dated 05 March, 2025).

The medicinal properties of safflower have been widely accepted. Safflower is an important industrial crop containing yellow and red colour pigments from C glucosylquinochalcone flavonoids in the petals (Kazuma *et al.,*2000). Due to their well-known roles in cardio-cerebrovascular health, safflower flavonoids are considered the main active product among the safflower metabolites. So far, over 60 kinds of flavonoids have been identified and characterized in safflower (Xian *et al.* 2022). According to the differential flavonoid components compared with other plants, safflower flavonoids can be divided into unique and common categories. The unique flavonoids are only identified in safflower, containing most quinochalcones, such as SR-derived carthamin, YRderived safflor yellow A, HSYA, safflor yellow B, and anhydrosafflor yellow B (AHSYB). Most of these unique flavonoids belong to C-glycosides. In contrast, safflower common flavonoids are also identified in many other species and mainly consist of naringenin, kaempferol, hyperoside, luteolin and quercetin, glycosylation products of which belong to O-glycosides (Wang *et al*., 2021). Flavonoids are mainly composed of the glycosides derived from shannesol and quercetin, safflower yellow A, hydroxysafflor yellow A, red pigment, apigenin, quercetin, rutin, myricetin etc. (Li *et al*. 2012). The safflower pigments includes 3-6%, red (carthamin) insoluble in water, and 24-30% water soluble yellow (carthamidin) (Machewad *et al.,* 2012). These pigments, particularly flavonoid such as safflower yellow, which contains a mixture of carthamin, have garnered significant interest due to their vibrant color properties, safety, and versatility.

 *C. tinctorius* has recently been shown to have antioxidant, anti-inflammatory and antidiabetic activities (Asgarpanah and Kazemivash 2013). Eco-friendly and biodegradable dyes derived from natural resources have emerged as an important alternative to the synthetic dyes (Jadhav and Joshi, 2015). Improvement in the chemical characteristics of ice cream is reported by the addition of carthamidin extract (Machewad *et al.* 2012) and to improve the therapeutic value of food products (Al-Snafi 2015). Safflower carthamin is widely used as stain coloring in foods such as ice-cream, jelly, soup, and as an additive in beverages and cosmetics (Singh 2007; Machewad *et al.* 2012). China has manufactured and produced carthamin as a red paint for cosmetics (Yue *et al.* 2013). The extracts of florets are used in the treatment of many illnesses such as menstrual problems, cardio vascular diseases pain, and swelling associated with trauma, heart attack and renal thrombosis, circulatory system disorders (Singh, 2007). In recent years, safflower petals have been used as herbal tea in India and China (Sultana and Anwer 2014; Al-Snafi 2015).

Considering economic importance of safflower petals, it could be worth while to explore these florets for the extraction of colorants that would be used in different food products with medicinal properties. It was therefore deemed valuable to explore the potential of safflower florets for the extraction of natural colorants, which can be utilized across a variety of industries, including food, textiles, and pharmaceuticals. In this study, a detailed examination was conducted to extract and identify the key pigments present in safflower florets. The research aimed not only to isolate these pigments but also to analyze their chemical composition, quantification, and potential applications in different sectors, offering an environmentally friendly alternative to synthetic dyes. This study contributes to a deeper understanding of the biochemical properties of safflower pigments and their possible commercial use.

**2.MATERIALS AND METHODS:**

Petals of 30 different Safflower genotypes showing variation in the flower colours from yellow to orange, brown and red were collected from field of All India Coordinated Research Project (AICRP) on Safflower, VNMKV, Parbhani and used for pigment extraction and characterization (Plate 1).





Plate 1. Petals collected from Safflower genotype

**2.1UV spectrophotometry**:

The total flavonoids in safflower exhibit distinct UV absorption peaks within a certain wavelength range. At 330 nm, there are distinctive absorption peaks. The standard flavonoid, rutin, is detected at a wavelength of 500 nm. To determine the yellow and red pigment content of safflower genotypes, a standard curve was created (Zhou *et al*., 2015).

**2.2Preparation of reference and test samples:**

The Rutin curve, a standard flavonoid, was created using Ji *et al*. (2018)'s methodology and utilized to quantitatively estimate the amount of yellow and red pigment in safflower.

**2.3Safflower Yellow Color Extraction**:

To isolate safflower yellow, 1 g of fine dry floret powder was suspended in 15 ml distilled water. The mixture was stirred and soaked twice, each for 30 minutes. After soaking, the extracts were combined and filtered through a 200 mesh nylon filter. The resulting solution was centrifuged at 2000 rpm for 10 minutes. The supernatant was filtered to remove residual particles, yielding the safflower yellow extract (Jadhav and Joshi, 2015).(Plate 4)

**2.4Safflower Red Colour Extraction:**

 The safflower residue, post-yellow pigment extraction, was thoroughly rinsed and subsequently immersed in a 1% Sodium carbonate solution at 20-25°C. The suspension was stirred and soaked twice for 30 minutes each. The extracts were combined and filtered through a 200 mesh nylon filter. The filtrate was centrifuged at 2000 rpm for 10 minutes. To the clear supernatant, a stoichiometric amount of 1% Citric acid was added, and allowed to stand for 30 minutes to facilitate sedimentation. The clarified liquid treated with 0.5% Cellulose, promoting the adsorption of the red carthamin pigment. The resulting material was rapidly dried, yielding the final red pigment powder (Jadhav and Joshi, 2015). (plate 4 and Plate 5)

**2.5Spectrophotometric Measurement**:

Spectrophotometric measurements were performed to determine the absorbance of yellow and red pigment content. The measurements for each pigment were conducted over specific wavelength ranges 385 to 500 nm safflower yellow and 380 to 600 nm for red. Spectrophotometric analysis of safflower pigments from 30 distinct genotypes revealed that the maximum absorbance for safflower yellow occurred at a wavelength of 400 nm, corresponding to its characteristic absorption profile. In contrast, the red pigment, exhibited a peak absorbance at 500 nm, reflecting its distinct spectral properties (Table no.1). These absorbance maxima are indicative of the specific molecular structures of yellow and red pigments, facilitating their quantification and comparison across the different genotypes.

**2.6Thin-Layer Chromatography**
The Rf values of yellow and red pigments were examined on Silica gel G with two standard flavonoids Rutin and Qurecetin. The mobile phase consisted of Chloroform, Methanol, and Water (4:3:1) (Rudometova *et al*. 2001). The TLC plates were visualized under UV Trans illuminator.(Plate 6)

**3.RESULT AND DISCUSSION**

Safflower plant known for its brilliantly colored petal contents. Thirty different compounds having diverse array of applications in medicinal, food and textile industries. Safflower yellow has absorption maxima at wavelength 400 nm and pH 3.55, and is water-soluble, whereas red pigment had at 500 nm and pH 8.82 and soluble in alkaline solutions, particularly Sodium carbonate (Table no.1). The characteristics of safflower yellow and red pigments described by Jadhav and Joshi (2015) include the absorption maxima, which were found to be 400 nm for the yellow pigment and 520 nm for the red pigment with pH values 3.53 and 8.73, respectively. Sultana and Anwer (2014) showed maximum peak of absorbance between 380-440 nm for carthamin extract with a specific peak at 380 nm. This peak is characteristic of carthamin extract, as reported in earlier studies by Satio *et al*. (1985). In contrast, the maximum peak for safflower yellow was observed at 385 nm. However, Kulkarni *et al*. (1997) found the optical density for safflower yellow pigment at 480 nm, while Wu and Fu (1993) reported it at 400 nm. These differences in optical density for the yellow pigment may be attributed to varietal differences. To confirm these differences, the extracts used for spectrophotometric analysis were further subjected to chromatographic separation by TLC.

**Table 1.** Characteristics of safflower yellow and red colour.

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| **Sr no.** | **Characteristics** | **Yellow colour** | **Red colour** |
| 1. | Absorption maxima | 400nm | 500nm |
| 2. | pH | 3.55 | 8.82 |
| 3. | Solubility | Water | Alkaline solution |

 Among the 30 genotypes examined in the current study, the yellow pigment ranged from 6.9 to 28.9 mg/ml and GMU-7923 exhibited the highest concentration 27.6 mg/ml of yellow pigment, suggesting significant potential of GMU-7923 for high-quality yellow pigment production. However red pigment content ranged from 2.2 to 4.3 mg/ml. GMU-7931-1 demonstrated the highest concentration of red pigment 4.3 mg/ml (Figure no.1). Jadhav and Joshi (2015) reported concentration of safflower yellow pigments ranged from 24% to 28%, while carthamin, the red pigment, exhibited a concentration range between 0.3% and 0.8%. Concentration of yellow and red pigment is notably higher than as reported in the earlier studies, indicating the genotype's potential for enhanced pigment production.



 Fig1: Concentration of yellow pigment and red pigment in safflower genotypes.

The nature of the extracted yellow (carthamidin) and red (carthamin) pigments were analyzed to assess the varietal differences among the safflower petal extracts for pigment content. The chromatographic separation was conducted using TLC, and the resulting Rf values were compared to those of standard flavonoids, specifically Rutin and Quercetin, to aid in pigment identification. The Rf value for yellow pigment ranged from 0.32 to 0.80, red pigment between 0.93 and 0.96. These findings align with the thin-layer chromatographic identification reported by Rudometova *et al*. (2001), the Rf values of safflower yellow and carthamin were observed in specific ranges on silica gel G, which corroborates the results obtained in the current study. Chavan and Nikam (2015), reported Rf values for carthamidin and carthamin as 0.81 and 0.90, respectively, which align with the findings for most of our genotypes, including the two varieties with the highest pigment content. Specifically, the Rf values for GMU-7923 were observed to be 0.80 for the yellow pigment and 0.96 for the red pigment, while GMU-7931-1 exhibited Rf values 0.80 for the yellow pigment and 0.96 for the red pigment (Figure no.2).

These values further support the correlation between pigment concentration and the observed Rf values in these genotypes. Further comparison with the findings of Sultana and Anwer (2014) revealed that Rf values for carthamin ranged from 0.9 to 2.0, while for carthamidin, 0.1 to 2.1. These differences in Rf values can be attributed to the variation in experimental conditions, including the type of solvent system used, the quality of the silica gel, and possibly the genotype specific composition of pigments in safflower. Despite these differences, the Rf values obtained in our study fall within the expected ranges for both pigments, confirming their identification as yellow (carthamidin) and red (carthamin) pigments.

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 Fig 2: Rf values of safflower genotypes.



Plate 2: TLC of extracted yellow pigment from safflower.



Plate 3: TLC of extracted red pigment from safflower.

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Plate 4: Safflower Red and Yellow Pigment

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Plate 5: Red Pigment Powder Adsorbed on Cellulose

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Plate 6: TLC of 1.Rutin and 2. Qurecetine under UV transilluminator

**4.CONCLUSION**

The carthamidin water soluble yellow pigment and carthamin alkaline soluble red pigment can be extracted from safflower petals, which is having capability as food colorant and natural yellow and red dye. In the future, this breakthrough in natural food colorant is expected to gain widespread acceptance, especially as today’s world increasingly seeks natural, non allergenic, non-carcinogenic and non-synthetic food alternative with properties such as enhanced stability across the varying pH level, temperature, and solute concentrations, as well as in the presence of polar solvent. This study demonstrates that the 30 different safflower genotypes exhibit significant potential for production of both safflower yellow and red pigments. Among the 30 genotypes studied, two genotype GMU-7923for yellow pigment and GMU-7931-1for red pigment showed highest levels of pigment content and paved its way for commercial pigment extraction and their utilization in food, dying and medicinal industries.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Authors hereby declare that NO generative artificial intelligence tools including large language models (e.g., ChatGPT, Google Gemini, Microsoft Copilot) or text-to-image generators were used in the writing, editing, data analysis, or figure preparation for this manuscript. All content is the original work of the authors.

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