Original Research Article

Quantitative estimation of Carthamin and carthamidin from the florets C. tinctorius L.., (safflower florets)

Abstract

The present investigation was undertaken to explore safflower florets for extraction and identification of colorants (yellow and red) that can be used in different food products. The safflower yellow color was extracted by suspending it in distilled water and the red colour with sodium carbonate solution. The extracted safflower yellow and red colour carthamin pigments were spectrophotometric ally characterized and further identified by using thin layer chromatography by measuring RF values. Quantitative phytochemical analysis revealed that the plant has rich amount of Carthamin and carthamidin. Comparison of the two pigments showed that carthamidin content is less when compared to carthamin.

Key Words: Safflower florets, quantitative analysis, carthamin and Carthamidin.

Introduction

During the last 40 years, at least a dozen potent drugs have been derived from flowering plants including Dioscorea species derived diosgenin from which all an ovulatory contraceptive agents have been derived; reserpine and other anti-hypertensive and tranquilizing alkaloids from Rauwolfia species; pilocarpine to treat glaucoma and dry mouth, derived from a group of South American trees (Pilocarpus species) in the Citrus family; two powerful anti-cancer agents from the Rosy Periwinkle (*Catharanthus roseus*); laxative agents from Cassia species and as a cardio tonic agent to treat heart failure from Digitalis species.

In addition to the regular metabolites that are present in each of the medicinal plants, however in addition to the natural metabolites *C. tinctures* is additionally blessed with two important pigments that are present in the florets of safflower and these two pigments have an immense medicinal properties.

Safflower, *Carthamus tinctorius* L. is a thistle herb belonging to the family Asteraceae. Safflower Plants are 30-150 cm tall with globular flower heads (capitula) and, commonly, brilliant yellow, orange or red flowers. It isone of humanity's oldest crops cultivated in India mainly for oil from the seeds and adyesfrom the flowers.Though, safflower flowers have been used in preparations of Ayurvedic medicines in India and also merit mention in European and Japanese pharmacopoeia's, the interest in this crop has been rekindled in the last few years as the medicinal use of these flowers in china, has become more widely known. China has a significant area under safflower plantation, but is grown almost exclusively for its flowers, which are harvested for use in traditional medicines. Safflower flowers are used in china for the treatment of many illnesses as well as in the preparation of "tonic tea".

Safflower is now mainly grown in India for its much-valued edible oil. Safflower produces oil rich in polyunsaturated fatty acids (linoleic acid 78 percent), which play an important role in reducing blood cholesterol.

In India, flowers of safflower are regarded as stimulant, sedative and as a promoter of menstrual discharge. In large doses, they are laxative. The main active ingredients in safflower florets are safflower yellow (carthamidin), which is water-soluble and safflower Carthamin (red pigment) which is alcohol soluble are used in some preparations. Many clinical and laboratory studies support the use of safflower medicines for Cardiovascular disease and pain and swelling associated with trauma.

To commercialize safflower flowers in India, efforts have been initiated to popularize them as a "Herbal health tea" for curing several chronic diseases. Regular users of this tea have reported its usefulness in alleviating diseases like hypertension, spondylosis, angina, arthritis, constipation and hypercholesterolemia. Analysis of flowers for nutrition qualities was conducted recently at the central food technological research institute (CFTRI). They were found to contain substantial quantities of amino-acids and minerals such as potassium, calcium, magnesium and iron.

The dried flowers of *carthamus tinctorius* (safflower) have been used in traditional Asian medicine for thousands of years. The active compounds are red and yellow pigments, which have been experimentally shown to enrich blood, to decrease fatigue (Akihisa et al., 1994). Moreover, because of restrictions on using synthetic pigments for food colorants, there has been increasing interest in the use of natural pigments. Safflower pigments have been shown to be safe for use as natural pigments. Safflower pigments have been shown to be safe for use in processed foods and soft drinks..Kanehira et al. (2003) reported that kinobeon A,isolated from safflowerexhibited stronger effect on the oxidative stresses and could be a usefillcytoprotective reagent... In 83% of patients with coronary disease, blood cholesterol levels have been reduced after 6 weeks of treatment (Guimiao and Yili, 1985). Experiments with dogs suggest injections of safflower can reduce the damage done to the heart muscle by an infarction. Heart arrhythmia and hypertension were reduced by safflower treatment three times a day for 4 weeks (Bingzhang et al. 1978; Guimiao and Yili, 1985). Injections of safflower extract at Fengfij, Yamen, Fengchi and other acupuncture points every 3 days increased blood flow in the coronary artery. Treatment of cerebral thrombosis with safflower improved and lowered blood pressure in over 90% of patients (Guimiao and Li Yili, 1985; Damao, 1987)... Safflower decoctions have been used successfully for treatment of male sterility (Yuehao, 1990) and dead sperm excess disease (Chun, 1990). Treatment with safflower resulted in pregnancy in 56 of 77 infertile women who had been infertile for 1.5-10 years (Wenyu, 1986).

Material and methods

Quantitative analysis and Spectrophotometric Measurements of Carthamin and Carthamidin from petals of Safflower Florets:

Materials:

For the analysis of the pigments ten Genotypes were screened namely they are A1, Bhīma, Jsi-97, CO-1, Manjra, a1, Pbns-12,Nari-6,Nari-28 , Sharada,SSF- 658.Dry florets were collected from the field of CPMB, Department of Genetics, and Osmania University Hyderabad.

Dye extraction: Extraction of water insoluble carthamin and yellow water solublepigment from safflower florets were essentially carried out by (Kulkarni et al., 1997) but with some modifications as follows

Extraction of carthamin

Fine dry floret powder (1gr) was suspended in 20ml of methanol, acetone, ethyl acetate and hexane 0.5% WV -1 sodium carbonate .stirred at room temperature for 30mints .The floating pieces were re moved by centrifugation at 3500rpm for 15mintutes and the supernate

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 obtained 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 (Kulkarni 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. supernate 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 supernatewas obtained. The pellet was suspended in 10ml of acetone,intermixed for 5minutes,then centrifuged for 5minutes at 3500rpm.The acetone layer was filtered and used for spectrophotometric measurement.

Extraction of carthamidin (safflower yellow):

One gram of fine floret powder was suspended in 20ml distilled water and stirred for 30min. Floating pieces were removed by centrifugation and the supernate was retained at 5±1c .The resultant suspension in distilled water was stirred for further 30min and centrifuged. The supernate was then filtered to separate suspended particles of floret powder. 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 basified toobtain a pH and used for adsorption of carthamidin.Adsorption of carthamidin from basic extract was performed using a modified method described by (Kulkarni et al 1997).cellulose powder (0.5gr) was suspended in basic solution , stirred with a magnetic stirring apparatus for 30min at room temperature and centrifuge at 3500rpm for 15minutes. Supernate was discarded. The pellet was resuspended in methanol and centrifuged. The washing was repeated 5-6 times under the same conditions until a colourless supernate was obtained. The pellet was suspended in 10ml of water, intermixed for 5minutes, then centrifuged for 5minutes at 3500rpm.The aqueous layer was filtered and used for spectrophotometric measurement.

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 for carthamin and carthamidin.The Rfvalues 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: is butanol: ethanol: formic acid. (4:7:4:t4).).

Result

Image 1 Field study

Extraction and quantification of the yellow and red pigments in the florets of *C.tinctorius* bioactive components from ten different genotypes of safflower (namely they are Manjra, Co-1, Pbns-12, SSf-658, Sharada, Nari-6, Nari-28, A1, JSI-97, Bhīma) has been done by acid and alkali methods by using different polar and non-polar solvent systems such as methanol, ethyl acetate, acetone ,hexane and aqueous. After extraction quantification of these bioactive compounds has been done .The result revealed that methanol and aqueous extracts showing more bioactive compounds when compared to others. Among the ten genotypes the maximum quantification is observed in Manjra, Pbns-12, Nari-6, SSf-658, A1, CO-1 when compared to Bhīma, Nari-28, Sharada and Jsi-97. In both methanol and aqueous extracts Manjra shows highest percentage of quantification i.e. 2.350mg/gr fallowed by Pbns-12 and Nari-6 2.21mg/gr SSf-658-1.921mg/gr A1-1.869mg/gr co-1-1.834mg/gr respectively which is shown in it at figures 1 A -E).

The results are summarized in Figures 1A

Figures 1A Quantitative analysis of Carthamin with ethyl acetate extraction

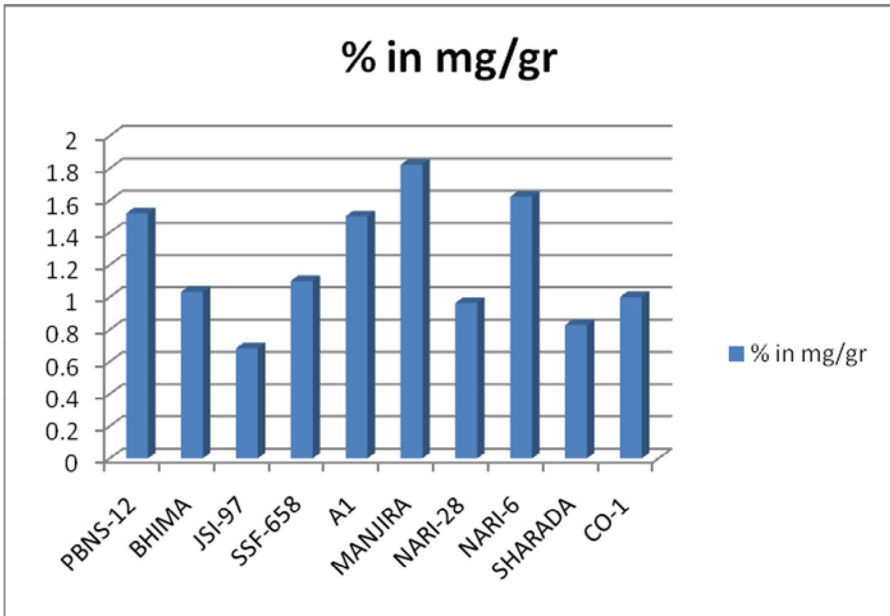


fig: 1B Quantitative analysis of carthamin with methanol extraction

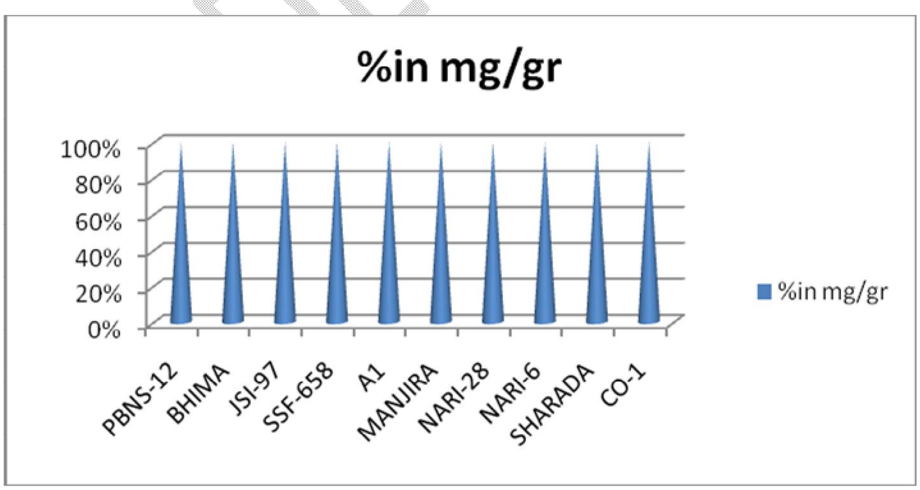


Fig 1C Quantitative analysis of carthamin with hexane extraction

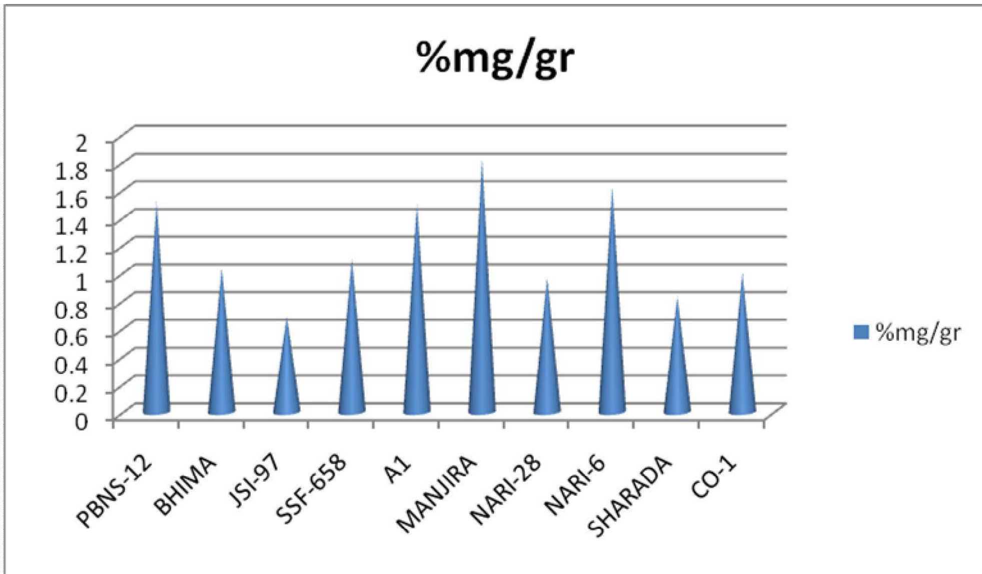


Fig :1D Quantitative analysis of carthamin with acetone extraction

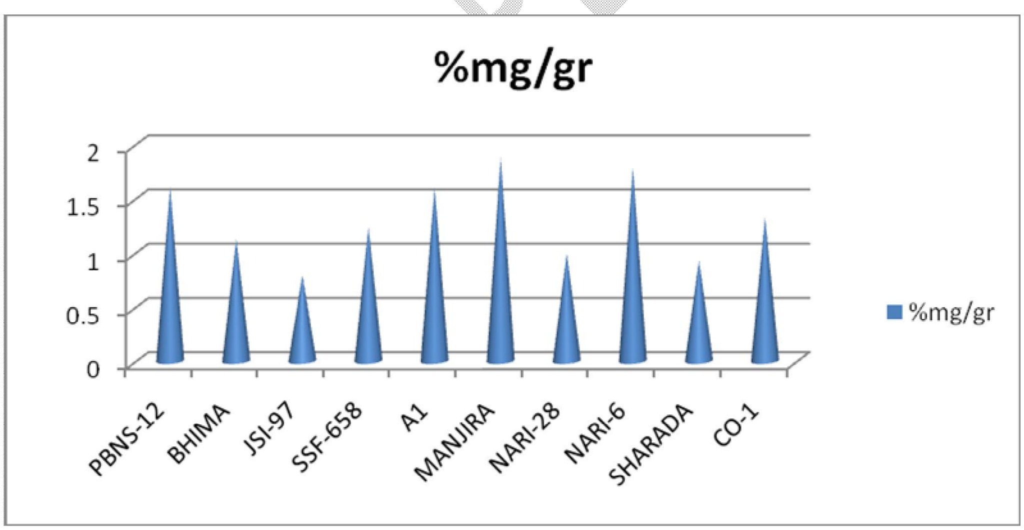
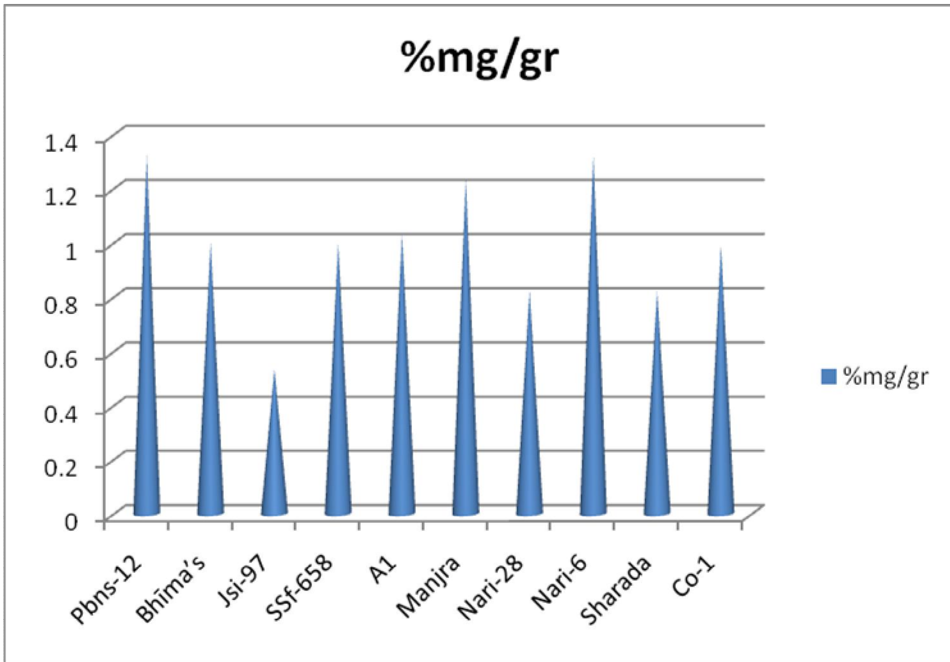


Fig : 1E.Quantitative analysis of carthamidin with aqueous extraction



Iamge 2 : Study analysis and protocol

Discussion

The quantification of Carthamin and carthamidin has been done from the petals of safflower florets by aqueous and methanolic extraction .Safflower flowers are also an excellent material for extracting the yellow and red pigments, which have multiple uses. The yellow pigment can be especially utilized in the pharmaceutical industry, while the red pigment can be used for colouring and flavouring food and textile industry. Thus, the culture of safflower can be profitable. Safflower petals have tremendous potential for value addition, as it an excellent source of different medicines and also food dyes. Health problems, particularly cancer associated with synthetic azodyes, may have further stimulated the utilization of natural chemicals such as the safflower dyes. Besides, development of safflower industry exist large market in China, it will bring with the glad tidings for 200 million of patients suffered heart and brain vascular disease, it is also good news for the middle-aged and the old.

Nowadays, the safflower products shows great potential in the market of the world with its important contribution of edible oil or industrial oil as well as its highly nutritious by-products for animal feed and its petals as colouring for food and textile. Therefore, technologies such as medicine, tea, food colours and textile colours need to be developed in the international research system and the crop can be promoted through to be increased market opportunities in the world.

Conclusion

Screening of selected medicinal plant *C.tinctorius*clearly reveals that the maximum classes of phytoconstituents are present in petals of safflower floral extracts. Hence, the above plant extract could be explored for its highest therapeutic efficacy by pharmaceutical companies in order to develop safe drugs for various ailments. Since these plants have been used in the treatment of different ailments, the medicinal roles of these plants could be related to such identified bioactive compounds. The qualitative and quantitative analyses of these phytoconstituents will be an interesting area for further study. Efforts should be geared up to exploit the biomedical applications of these screened plants due to the presence of certain class of phytoconstituents for their full utilization.

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