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Evaluation of antioxidant activity in carthamin and carthamidin from safflower florets

Dr Ayesha Sultana, Dr S.Y.Anwar
Department of Genetics and Biotechnology, Osmania University, Hyderabad. Telangana. Email:ayeshaasra68@yahoo.com

Abstract
The petals of safflower floral extracts were analysed for in-vitro antioxidant properties. The Methanolic and aqueous extract of C. tinctorius possess maximum antioxidant activity the anti- oxidative system protects the organism against ROS-induced oxidative damage. There are restrictions on the use of synthetic antioxidants such as BHT, as they are suspected to be carcinogenic. Natural antioxidants therefore have gained importance. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to form a stable diamagnetic molecule. The petals of safflower florets were found to scavenge the DPPH radicals in vitro in a concentration dependent manner. In fine, a multipronged approach adopted in this study has revealed a wide range of beneficial aspects to human health care. The results of the study are definitely pointers for further research.

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I. Introduction

The dried flowers of carthamustinctorius (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 safflower, 4 exhibited stronger effect on the oxidative stresses and could be a usefiilcytoprotective 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).

II. Material And Methods

Evaluation of antioxidant activity:
This experimental work was under taken to determine free radical depletion potential of methanolicand aqueous floral extracts of.*C.tinctorius*.

Equipment's for carrying out the antioxidant assays:
UV-visspectrophotometer: UV-1650PC Shimadzu Vortex mixer RemiCyclomixer Centrifuge m/c Incubator (37oC): Remi Scientific pH meter :Toschcon.

- 1,1-diphenyl picrylhydrazyl (DPPH) assay

- Chemicals: 1, 1-diphenyl picrylhydrazyl (DPPH), Sigma Aldrich Methanol, Merck Preparation of solutions/ reagents:
- DPPH solution (200 µM): It was prepared by dissolving 7.886 mg in 100 ml of methanol.

Methodology

The antioxidant activity of the safflower floral extracts was assessed on the basis of the radical scavenging effect using stable 1,1-diphenyl-2-picrylhydrazyl (DPPH).DPPH solution (0.004% w/v) was prepared in 95% methanol and serial dilutions were carried out with the stock solutions (20 mg/mL) of the extracts. Variousconcentrations of extracts were mixed with DPPH solution (900 µL), incubated in dark for 30 min and then absorbance was measured at 517 nm. Methanol (95%),DPPH solution and ascorbic acid (AA) were used as blank, control and reference standard respectively.

Percent DPPH inhibition calculated using the following formula:-

% DPPH inhibition = OD (control) – OD (sample) X 100 O.D (control).

Where OD is the optical density. Inhibitory concentration (IC50) is used as a measure of comparison of antioxidant activity of different extracts. It was calculated from the plot of % DPPH inhibition v/s concentration against ascorbic acid as standard (Brand-William et al., 1995; Mensor et al., 2001).

FRAP assay

The ferric ions (Fe3 +) reducing antioxidant power (FRAP) method wasused to measure the reducing capacity of safflower methanolic and aqueous extracts with a slight modification which involves the presence of extracts to reduce the ferric cyanide complex to the ferrous form. The FRAP method is based on a redox reaction in which an easily reduced oxidant (Fe3 +) is used in stoichiometric excess and antioxidants acts as reductants. Various concentrations of floral extracts (aqueous and methanolic) of*C.tinctoriusfrom* the stock solutions and the standard (ascorbic acid) were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferric cyanide (1% w/v). The mixture was incubated at 50°C for 20 min. Then 2.5 mL of trichloroacetic acid (10% w/v) was added to the reaction mixture, which was then centrifuged at 1000 g for 10 min. The upper layer of the solution (2.5 mL) was mixed with deionised water (2.5 mL) and ferric chloride (0.5 mL, 0.1% w/v). The absorbance was measured at 700 nm at the reaction time of 30 min. The reducing power of the extracts was represented as mg AAE/g of dw.

Determination of total antioxidant activity (Thiobarbituric acid method)

The total antioxidant activity of floral extracts of *c. tinctorius*were evaluated by phosphor molybdenum method. The assay is based on thereduction of Mo (VI) - Mo (V) by the antioxidant compounds and subsequent formation of a green phosphate/Mo (V) complex at acidic ph. Different extracts of 10 µL each from the stock solution were dissolved in 90 µL distilled water and 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mMammonium molybdate) in 1.5 mL tubes. The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, theabsorbance of the solution of each reaction was measured at 695 nm against blank samples. Ascorbic acid (AA) was used as standard and the total antioxidant capacity was expressed as milligrams of ascorbic acid equivalents (mg AAE/g) of dw.

III. Result

The results of the in vitro study showed that the aqueous (Carthamidin) and methanolic (carthamin)extract of safflower florets possessed significant antioxidant activity and radical scavenging ability.

Total antioxidant activity (TAA) and ferric reducing antioxidant power (FRAP)

The extracts of florets of safflower exhibited significant antioxidant activity, thus establishing the extracts as an antioxidant. The results of the antioxidant measurementsare summarized in below Table .The total antioxidant activity of carthamidin was in the range of 0.532±0.01-0.188±0.011 mg AAE/g dw in the florets extracts. The highest value 0.532±0.01 mg AAE/g dwwas observed in co-1, whereas at lowest value 0.188±0.011AAE/g dw was found in A1 shown (Table-9).

In case of carthamin the total antioxidant activity was in the range of 0.696 0.±0.512- 0.286±0.009 mg AAE/g dw observed in the floral extracts. The highest value 10.696±0.512 mg AAE/g dw was observed in Nari-6, whereas lowest value 0.286±0.009 aae/g dw observed in Manjra.The floret extracts of safflowershowed reasonably higherantioxidant activity (Table -10).

The extracts of safflower floretsexpressed electron donating activity, but their power was inferior to ascorbic acid, which is known to be a strong reducing agent. Safflower floral extracts exhibited considerably higher reducing power.The reducing ability of the carthamidin floral extracts was in range of 0.649±0.190-0.965±0.006mg AAE/g dw. The highest value was observed in Pbns-120.965±0.006 mg AAE/g dw), whereas the lowest value was recorded in Manjra0.649±0.190mg AAE/g dw (Table-9), In case of carthamin the antioxidant activity observed in the range of 0.832±0.008, 0.469±0.008mg AAE/g dw. The highest value 0.832±0.008mg/gr observed in whereasManjra lowest value 0.469 ±0.008 observed in CO-1 (Table-10).

DPPH radical scavenging activity

In this study, the extracts showed tendency to quench the DPPH free radicals, as indicated by the concentration dependent increase in percentage inhibition. Theresults revealed that both carthamin and carthamidin floral extracts had the higher DPPH radical scavenging ability. The IC 5 0 values (concentration of the extractthat was able to scavenge half of the DPPH radical) are presented in Table 11-12. Among the carthamidin extracts, SSF-658 exhibited stronger radical scavenging ability with the lowest IC 5 0 value of 42.94 ± 3.182µg GAE/Mlwhich indicates its good antioxidant potential. The other extractsshowed moderate DPPH radical scavenging effects (Table 11). On the other hand, Nari-6, A1 and Manjra showed significantly stronger activities and quenched DPPH (Table-11).

Among the carthamin extracts, A1, pbns-12 andManjra exhibited stronger radical scavenging ability with the lowest IC 5 0 value of 35.03+_5.265, 37.87+_7.361.47.40±7.33 µg GAE/ml, which indicates its good antioxidant potential. The other extracts showed moderate DPPH radical scavenging effects (Figure Table 12). On the other hand, A1, pbns-12 and Manjra showed significantly stronger activities and quenched DPPH.

Antioxidant activity by FRAP and TAAmethod
Carthamidin

Genotype	FRAP	Std mg/gr	TAA	Std mg/gr
Pbns-12	0.965 ± 0.006	0.544 ± 0.011	0.263 ± 0.010	0.119 ± 0.024
SSf-658	0.935 ± 0.009	0.484 ± 0.015	0.281 ± 0.010	0.145 ± 0.010
A1	0.723 ± 0.008	0.532 ± 0.02	0.188 ± 0.011	0.108 ± 0.008
Manjira	0.649 ± 0.190	0.542 ± 0.011	0.386 ± 0.480	0.097 ± 0.009
Nari-6	0.910 ± 0.007	0.544 ± 0.02	0.256 ± 0.01	0.162 ± 0.007
Co-1	0.862 ± 0.009	0.541 ± 0.01	0.532 ± 0.01	0.345 ± 0.007

FRAP

Carthamin

Genotype	FRAP	Std ascorbic acid mg/gr	Std ascorbic acid mg/gr	TAA
Pbns-12	0.654± 0.060	0.512± 0.008	0.432± 0.01	0.543± 0.009
Ssf-658	0.775 ± 0.1	0.457 ± 0.009	0.342± 0.008	0.543 ± 0.01
A1	0.661 ± 0.053	0.451 ± 0.009	0.233 ± 0.008	0.331 ± 0.009
Manjira	0.832 ± 0.008	0.531 ± 0.008	0.163 ± 0.009	0.286 ± 0.009
Nari-6	0.804 ± 0.008	0.701 ± 0.008	0.284 ± 0.009	0.696 ± 0.512
Co-1	0.469 ± 0.008	0.322 ± 0.009	0.162 ± 0.008	0.189 ± 0.009

Evaluation of antioxidant activity of carthamidin
DPPH

S.NO	**SAMPLE(CARTHAMIN)**	**IC50 VALUE for standard**	**IC50 Value for sample**
1	MANJIRA	107.528 ± 7.529	47.405 ±7.33
2	CO-1	133.706 ± 4.670	51.256 ± 3.218
3	SSF-658	160.604 ± 2.966	58.195 ± 5.408
4	PBNS-12	93.376 ± 4.394	37.87 ± 7.361
5	A1	102.499 ± 2.382	35.03 ± 5.265
6	NARI-6	55.105 ± 2.670	58.195 ± 5.478

IV. Discussion

Afair correlation between total terpenes content andantioxidant activity was observed in the petals of safflower florets. These observations clearly indicate aclose relationship and linkage between terpenes and antioxidant activity. Plant terpenes constitute one of themajor groups of compounds that act asprimary antioxidants or free radical scavengers. Terpenoids are probablythe most important compound that possess a broadspectrum of chemical and biologicalactivities including free radical scavenging properties. Such antioxidant properties areespecially distinct for terpenoids.Therefore, DPPHradical scavenging activity and total terpenoids contents in the extractswere determined.

V. Conclusion

In conclusion, our findings revealed that *C. tinctorius* flowers at fructification stage showed the highest carthamin content with the strongest antioxidant and significant antibacterial activities, suggesting that this stage was the best harvesting time of safflower. Moreover, this study can be considered as the report focusing on biological activities of safflower natural pigments (quinochalcone) as influenced by environmental factors. These findings underlined the potential consumption of safflower as a suitable source of natural dyes as an alternative to food synthetic colorants as well as strong antibacterial activity observed shows the presence of bioactive compounds responsible for it. Further, the research is needed for the purification of the extract which will elucidate the potential compound and its activity in the wide range of bacteria
.our result will be an encouragement for further study which will lead to the use of the active components of petals of safflower florets in drug preparation is in the near future.

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