# ASSESSMENT OF SAFFLOWER FLOWERS (*Carthamus tinctorius* L.) AS POTENTIAL FOOD ANTIOXIDANTS

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**Abstract**

Bioactive compounds have received increased attention for their use as preservatives and dyes in food by replacing synthetic antioxidants and they are also involved in prevention humans against diverse chronic diseases. Among the sources of these antioxidants, safflower (Carthamustinctorius L.), a medicinal plant cultivated in the Northeastern Sahara of Algeria for its interests as a nutritional and medicinal product. Through this research, we investigated the phenolic composition and antioxidant activity of hydroethanolic extract of safflower flowers. The extracts were prepared by macerating the flower powder in 70% ethanol. Analysis of total polyphenols was performed by the Folin-Ciocalteu reagent, flavonoids by aluminum trichloride and tannins condensed by vanillin. The antioxidant activity was evaluated by the phosphomolybdate and 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) tests. The total polyphenols content was 22.55±0.67 mg of gallic acid equivalent/g dry weight, flavonoids was 96.70±5.14 mg of rutin equivalent/g dry weight and condensed tannins was 8.50±0.49 mg of catechin equivalent/g dry weight. The extract showed a good antioxidant potential of 25.69±1.83 mg ascorbic acid equivalent/g dry weight and that it has a considerable and dose-dependent scavenging ability to reduce ABTS, with a 50% inhibition concentration of 19.64±0.07 µg/mL. This research reveals the potential of C. tinctoriusflorets for its further applications as natural sources of bioactive compounds.

**Keywords:** *Carthamustinctorius* L., total phenolics, flavonoids, condensed tannins, antioxidantactivity.

## VALORISATION DES FLEURS DE CARTHAME (*CARTHAMUS TINCTORIUS* L.) COMME ANTIOXYDANTS ALIMENTAIRES POTENTIELS

**Résumé**

Les composés bioactifs ont reçu une attention accrue pour leur utilisation comme conservateurs et colorants dans les aliments en remplaçant les antioxydants synthétiques et ils sont également impliqués dans la prévention des humains contre diverses maladies chroniques. Parmi les sources de ces antioxydants, le carthame (Carthamustinctorius L.), une plante médicinale cultivée dans le Nord-est du Sahara algérien pour ses intérêts en tant que produit nutritionnel et médicinal. A travers cette recherche, nous avons étudié la composition phénolique et l'activité antioxydante de l'extrait hydroéthanolique de fleurs de carthame. Les extraits ont été préparés par macération de la poudre de fleurs dans de l'éthanol à 70%. L'analyse des polyphénols totaux a été réalisée par le réactif de Folin-Ciocalteu, les flavonoïdes par le trichlorure d'aluminium et les tanins condensés par la vanilline. L'activité antioxydante a été évaluée par les tests du phosphomolybdate et 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammoniumsalt (ABTS). La teneur totale en polyphénols était de 22,55±0,67 mg d'équivalent acide gallique/g de poids sec, les flavonoïdes étaient de 96,70±5,14 mg d'équivalent rutine/g de poids sec et les tanins condensés étaient de 8,50±0,49 mg d'équivalent catéchine/g de poids sec. L'extrait a montré un bon potentiel antioxydant de 25,69±1,83 mg d'équivalent d'acide ascorbique/g de poids sec et qu'il a une capacité considérable et dose-dépendante de piégeage l'ABTS, avec une concentration d'inhibition à 50 % de 19,64±0,07 µg/mL. Cette recherche révèle le potentiel des fleurs de C. tinctorius pour ses applications ultérieures en tant que sources naturelles de composés bioactifs.

**Mots clés:** *Carthamustinctorius L*., polyphénols totaux, flavonoïdes, tanins condensés, activité antioxydante.



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## INTRODUCTION

The demand for natural food additives has been rising with increased toxic effects and mutagenic potential for human health of synthetic dyes [1]. Among the sources of these natural dyes, *Carthamustinctorius* L., commonly called as safflower (*English*) and Zaafor (*Arabe*), which belongs to the Asteraceae family, is an important medicinal plant originated from the eastern Mediterranean region [2] and cultivated almost exclusively for its orange-red pigment found in its flowers and the oil content in its seeds. The flowers are applied traditionally for treatment of cardiovascular, cerebrovascular and gynecological maladies [3] and utilized as a natural color additive for foods and healthy beverages, pharmaceutics and cosmetic preparations [4,5]. In Indian traditional medicine, the seed oil is used to treat sores and rheumatism and in Iran to treat liver and heart ailments [6], it is also useful in lowering the cholesterol level in the blood and in reduction of the risk of human arteriosclerosis [7].

*C. tinctorius* has been identified to consist of more than 200 substances, including flavonoids, alkaloids, lignans, serotonins, steroids, triterpenoidsaponins, alkane diols, riboflavin, quinochalcone*C*-glycosides and quinone-containing chalcones, which have been demonstrated to possess antioxidant effects [8,9]. Safflowerflowers are known as a source of redand yellow pigments whichare classified as members of the *C*-glucosylquinochalcone family of flavonoids that not detected in other natural products [9]. The major component of red pigments are carthamin, which are water-insoluble, and major components of yellow pigments are hydroxysafflor yellow A and safflor yellow B, which are freely water soluble [10]. Other minor yellow pigments so far reported are precarthamin, safflor yellow A, anhydrosafflor yellow B, safflomin A, safflomin C, tinctormine and cartormin [9,11,12,13]. These ingredients have a various biological potentials such as antioxidant and radical-scavenging properties, hydroxysafflor yellow A reduces apoptosis in pancreatic β-cells by attenuating oxidative damage and JNK/c-Jun signaling pathway [14]. In addition, these natural dyes exhibited antibacterial and antifungal proprieties thanks to the existence of quinones in their structure [15]. Therefore, the main objective of the present study was to evaluate polyphenol, flavonoid, and condensed tannin levels and antioxidant properties of hydroethanolic extracts of the flowers of *C. tinctorius*cultivated in Southern Algeria, for its potential exploitation as a functional food ingredient and natural antioxidant.

## MATERIALS AND METHODS

### Plant material

Fresh flowers of *Carthamustinctorius* L. were collected randomly in summer season from the Meggarine region (Southern Algeria). The harvested samples were dried in the shade at room temperature for three weeks and converted into a fine powder by laboratory mill. The powder was stored away from air and humidity, in hermetically sealed glass jars prior to extraction.

### Preparation of ethanolic extracts

The extraction of phenolic compounds was performed by maceration procedure. In brief, 20 g of dry safflower flower

powder were homogenized with 100 mL of 70 % aqueous ethanol at room temperature for 24 h. After filtration through a Whatman No. 1 filter paper, the extraction procedure was repeated twice more. The combined ethanolic extracts were concentrated under reduced pressure in a rotary evaporator at 40°C until dryness. The dried concentrated extract was weighed and then reconstituted in methanol at a concentration of 1 mg/mL.

The extract yield was calculated using the formula:

**Yield (%) = (weight of final dried extract / weight of initial dried matter) x 100**.

**Total polyphenolicquantification**

Total polyphenoliccontent was determined using the Folin-Ciocalteu reagent according to the method described by Lister and Wilson [16]. In test tubes, 100 µL of extract were added to 500 µL Folin–Ciocalteuaqueous solution (1:9, v/v), and then to 1000 µL distilled water. The mixture was shaken and stabilized for 3 min before adding 1.25 mL 20% sodium carbonate solution. The tubes were vortexed and incubated for 2 h at room temperature in dark, the absorbance of the resulting blue colour was measured at 765 nm with a spectrophotometer versus a prepared blank. Results were expressed as milligrams of gallic acid equivalent for each gram of dry plant extract (mg GAE/g DW).

**Flavonoids quantification**

Flavonoids quantification was estimated following the aluminiumtrichloride colorimetric method [17]. An aliquot (1 mL) of ethanolic extract was added to 1 mL of aluminum chloride methanolic solution (2%), thoroughly mixed. At the end of incubation (10 min) at room temperature, the absorbance of the reaction blend was measured by a spectrophotometer at 430 nm against blank solution. Total flavonoid contents were showen as milligrams of rutin equivalent for each gram of dry plant extract (mg RE/g DW).

**Condensed tannins quantification**

The proanthocyanidin content was using the vanillin assaywas based on the procedure reported by Julkumen-Titto [18]. To the 1 mL of sample 1,5 mL of vanillin solution in methanol (4%; w/v) and750 mL of hydrochloric acid solution (12 M) were added. The tubes were vortexed and set in a heating block set at 30 °C for 20 min. The tubes were then cooled in room temperature and the absorbance versus prepared blank was read at 550 nm. Condensed tannins values were presented in terms of milligrams catechin equivalent per gram of dry plant extract (mg CE/g DW).

**Total antioxidant capacity (Phospho-molybdenum method)**

The phosphomolybdenum method was used to evaluate the capacity of the extracts to reduce molybdenum (VI) [19]. The 0.3 mL of extract were mixed with 3 mL reagent solution which contain (0.6 M $H_2SO_4$, 28 mM $Na_3PO_4$ and 4 mM $(NH_4)_6Mo_7O_{24}.4H_2O$. The mixture was incubated in water-bath at 95°C for 90 min. After cooling in an ice bath, the absorbance of formed green phosphomolybdenum complex against a blank was determined at 695 nm using a spectrophotometer. Ascorbic acid

antioxidant equivalents were expressed on dried weight flower basis (mg ACE/g of dry weight).

### ABTS radical scavenging activity

ABTS radical cation scavenging tests were performed according to analytical method described by Re et *al*. [20]. Briefly, 5mL of ABTS solution (7 mM) was combined to 88 µl of potassium persulfate (2.45 mM), and the mixture ABTS-potassium persulfate was incubated in the dark, at room temperature for 12-16 h to produce ABTS radical. The fresh blue-greenradical solution was diluted with distilled water to give an optical density of 0.70 ± 0.02 at 734 nm.Then, 100 µL of sample extract at various concentrations were added to 2900 µL of diluted reagent solution. The reaction mixture was shaken vigorously and left reacted 5 minat room temperature in darkness. The absorbance of the resulting solution was monitored at 734 nm. Free scavenging ability was presented as the extract concentration required for 50 % inhibition of the ABTS radical (IC50) and percent inhibition was calculated using the following equation:

**% inhibition = [(Control absorbance-Extract absorbance) / (Control absorbance)] x100**.

Where control was the test solution without analyzed sample extract.

## RESULTS AND DISCUSSIONS

### Phytochemical composition

Polyphenolic metabolites are important antioxidants in both edible and inedible plants. Yield and phenolic composition of ethanolic extracts from the dried flowers of *C. tinctorius* are given in Table 1. The extraction of *C. tinctorius* flowers with 70 % aqueous ethanol gave a light brown color extract with percent yield of 29.01%, based on dried weight flowers.

**Table 1:** Extraction yield, total phenolic, flavonoid and condensed tannin contents of hydroethanolic extract from flowers of *C. tinctorius*.

| Extract yields (g/100 g DW) | Total phenolic (mg GAE/ g DW) | Flavonoids (mg RE/ g DW) | Condensed tannins (mg CE/ g DW) |
|---|---|---|---|
| **29.01** | 22.55 ± 0.67 | 96.70 ± 5.14 | 8.50 ± 0.49 |

Means of three independent measurements ± SD (standard deviation)

The results of chemical analysis revealed that the sample extracts contained total phenol, flavonoid and condensed tannin concentrations of 22.55 ± 0.67 mg GAE/g DW, 96.70 ± 5.14 mg RE/g DW and 8.50 ± 0.49 mg CE/g DW, respectively. Different from our results, Karimkhani et *al*. [21], investigated the total polyphenolic and flavonoid contents of the methanolic extracts of four different safflower cultivars (IL111, Padide, Isfahan-28 and Mahali). The values ranged from 46.2 to 62.3 mg gallic acid equivalent/g dry extract and 7.5 to 9.6 mg catechin equivalent/g dry extract, respectively. In a research conducted by Güneş et *al*. [22], the total phenolic and proantocyanidin contents of certain species

belonging to the Asteraceae family were examined. Different values were obtained depending on the species. The highest amounts of total phenolic and proantocyanidin contents in the Asteraceae family were obtained from *Echinacea purpurea* (13.34 mg GAE/g FW) and *Achilleanobilis* (83.63%) species. The lowest values were measured in *Helianthus annuus* (2.65 mg GAE/g fresh weight) and *E. purpurea* (20.85%) species, respectively, whereas the phenolic and proantocyanidin contents of *C. tinctorius* were found as 7.04 mg GAE/g fresh weight and 42.27%, respectively. While Farid et *al*. [23], reported that methanolic extracts prepared from flowers of the same specie cultivated in Egypt had the highest total phenolic and lowest flavonoid amounts, were 102.44 mg GAE/g and 13.94 mg quercetin equivalent /g, respectively. The phenolic contents differ from one study to another can be explained by differences in the nature of extraction solvent, extraction technique, agro-climatic conditions, plant cultivars, plant part, as well as plant species [24].

**Antioxidant properties**

Various *invitro* antioxidant models have been developed to assess the efficiency of natural antioxidants, either as pure compounds, compound mixture or as plant extracts. In the our present work, two different assays, phosphomolybdenum and ABTS were applied to the ethanolic extracts of safflower flowers and differ from each one in terms of basic mechanism and quantification methods.

The phosphomolybdenum method involves an electron transfer process, a potential antioxidant will reduce the molybdenum (VI) to the molybdenum (V); the latter forms a green phosphomolybdenum (V) complex in acidic medium, which increases the absorption at 695 nm [19]. The ascorbic acid antioxidant equivalent based on dried weight flowers was 25.69±1.83 mg AAE/g DW (Table 2).

**Table 2:** Total antioxidant capacity (mg ACE/ g DW) and antiradical property against ABTS radical (IC50 in µg/mL).

| Sample | Total antioxidant Capacity | ABTS scavenging activity |
|---|---|---|
| Hydroethanolic extract | 25.69 ± 1.83 | 19.64 ± 0.07 |
| Trolox | / | 03.22 ± 0.07 |

Means of three replicates ± SD (standard deviation)

The ABTS radical scavenging assay is based on the ability of putative antioxidants to reduce the free radical mono-cation $ABTS^{\bullet+}$ to the non-radical form $ABTS^{+}$ by an electron or hydrogen atom transfer mechanism [25]. The trapping capacity of ABTS radical was determined by the decrease in absorbance at 695 nm. As seen in the figure 1, the safflower flower extract showed potent scavenging activity in a dose-dependent mannerwithin the concentration range tested (06-42 µg/mL). It half maximal inhibitory concentration (IC50) value was

19.64 ± 0.07 µg/mL, which appeared approximately 6 fold lower than the value recorded for trolox standard (3.22 ± 0.07 µg/mL) (Table 2).

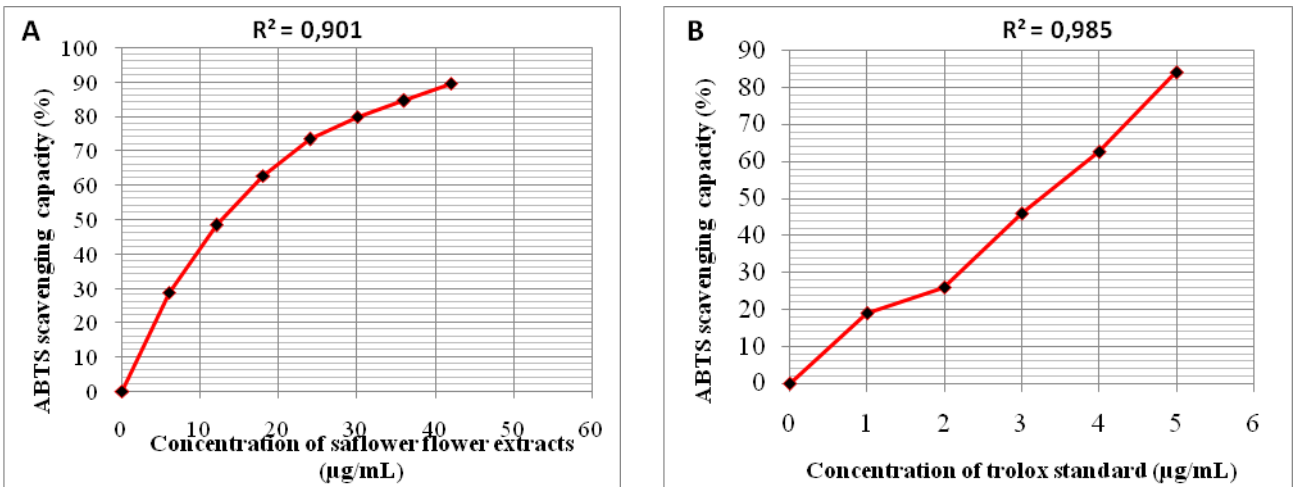


**Fig.1-** ABTS radical scavenging capacity of safflower flower extracts (**A**) and trolox standard (**B**).

Our results are higher than that of those reported by Salem et *al*. [26], who found lower ABTS trapping capacities between different development stages of *C. tinctorius* flowers. Where an increasing trend was reported from bud to full flowering stages in yellow flowers and the values ranging from (IC50= 700 µg/mL) to (IC50= 120 µg/mL), respectively, whereas constant levels were shown in orange and red flowers among three development stages of safflower, Since their antioxidant values were lower than trolox (IC50= 242.30 µg/mL).

The antioxidant activity shown by the extracts may be attributedto the presence of various phenolic components. This observation is consistent with many previous reports. Choi et al. [27], isolated twenty compounds from the methanolic extracts of *C. tinctorius*flowers. Among them, one flavonol glycosides (quercertin-3-*O*-β-D-glucopyranoside) and two flavanone glycosides ((2S)-4', 5, 6, 7-tetrahydroxyflavanone 6-*O*-β-D-glucopyranoside and (2*R*)-5, 7, 8', 4-tetrahydroxyflavanone 8-*O*-β-D-glucopyranoside), which exert a potent inhibitory effect on DPPH, with IC50 values of 56.7, 63.1 and 68.8 µM, respectively. In another study, Yoon et al. [28] revealed that three flavonol glycosides (kaempferol 3-O-sophoroside, quercetin 3-O-rutinoside, kaempferol 3-O-rutinoside) and one C-glucosulquinochalcone ( cartormin) isolated from the petals of safflower exhibited a dose-dependent activity on the ABTS radical system, with IC50 values of 4.07± 0.09, 5.26 ± 0.10, 6.08± 0.03 and 4.63± 0.10 µM, respectively. In addition, Yoon and Paik [29] isolated four *C*-glucosyl-quinochalcones (safflomin C, isosafflomin C, methylsafflomin C, and methylisosafflomin C) from the butanolic fraction of methanolic extract of safflower petals and examined their antioxidant activity. These four compounds suppressed the absorbance of the ABTS radical with the IC50 values of 2.24 ± 0.06, 2.74 ±0.01, 6.50 ± 0.02 and 6.15 ± 0.05 µM, respectively.

## CONCLUSION

Our findings revealed that *C.tinctorius* flowers is a valuable source of phenolic compounds and powerful antioxidants, and provided scientific support for potential used of safflower flowers as functional food that may assist in prevention of health against oxidative stress-related diseases.

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