

Total Phenolic Content and Radical Scavenging Activity of *Carthamus Tinctorius L.*

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Abstract

Extract from leaves of *Carthamus tinctorius L.* was prepared by using soxhlet extraction method. Total phenolic, flavonoid content and also antioxidant activity of leaves from *Carthamus tinctorius L.* were determined. To determine the antioxidant capacity, 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH•) scavenging, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS⁺) radical scavenging tests were used. Reducing power of the extract was also tested. Total phenolic and flavonoid content of the extract were determined as 77.38 ± 6.62 mg GAE/g, 25.49 ± 0.26 mg CE/g dry weight of extract respectively. DPPH radical scavenging activity was determined as 89%. ABTS radical scavenging activity was found as 58% at 1 mg/mL concentration. Reducing power was found as 0.885.

Keywords: safflower, antioxidant activity, phenolic, *Carthamus tinctorius L.*

Introduction

Free radicals and other reactive oxygen species are responsible from many diseases. To prevent these risk it is advised increased consumption of natural antioxidants abundant in foods[1]. Phenolic compounds which is found in plants exhibit antioxidant activity.

Carthamus tinctorius L. commonly known as Safflower or false Saffron, a member of Compositae family, has been used traditional medicine in Turkey. It is also used for purgative, analgesic, antipyretic, antidote to poisoning for a long time all over the world. A dye is obtained from the flower petals of *Carthamus tinctorius L.* and used for different purposes like coloring rice or bread or dyeing cloth [2]. Safflower seeds contain 13-46% oil and oil content of the seeds very similar to olive oil. It is known with high linoleic acid content. It contains linoleic acid (63-72 %), oleic acid (16-25 %) and linolenic acid(1-6%)[3]. This prevents blood cholestrol [4]. More than 200 compounds have been isolated

from *C. tinctorius*. These compounds are mostly consist of flavonoids, phenylethanoid glycosides, coumarins, fatty acids, steroids and polysaccharides [5]. Some researchers reported the antioxidant activity of seeds and flowers of *Carthamus tinctorius L.* but antioxidant activity of leaves weren't investigated yet. Therefore the aim of this study to determine phenolic and flavonoid contents of leaves of *Carthamus tinctorius L.* and also radical scavenging activities,

2. MATERIAL and METHOD

2.1. Chemicals

2,2'-azino-bis (3-ethyl benzothiazoline -6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), (+) catechin hydrate, 2,2-diphenyl-1-picryl-hydrazyl(DPPH), a-tocopherol, ascorbic acid and pyrocatechol were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid (TCA), ferric chloride were obtained from

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Merck. All other reagents used in this study were of analytical grade.

2.2. Plant materials

Carthamus tinctorius L. leaves were collected in July from Tekirdag area. Leaves were washed with distilled water and dried at room temperature.

2.3. Preparation of extract

Carthamus tinctorius L. extract were prepared by soxhlet extraction method with ethanol for 4 hours. The extract was then filtered and evaporated to dryness.

2.4. Total Phenolic Content

Total phenolic content of the extract was analyzed according to the method of Slinkard and Singleton[6] with slight modifications. The plant extract was tested in 1-4 mg concentrations. Total phenolic content of plant extract was calculated with the standard curve of gallic acid and expressed as μg gallic acid equivalent. The extract was analyzed in triplicate. Results are expressed as mean value.

2.4. Total Flavonoid Content

Total flavonoid content of the extract was determined according to the method of Zhishen[7]. Standart curve was prepared with catechin. Extract in different concentrations were analyzed in triplicate and flavonoid content expressed as $\mu\text{g}(+)\text{-catechin}$ equivalent.

2.5. DPPH radical scavenging activity

To determine the antioxidant activity of extracts DPPH radical scavenging activity was used[8]. DPPH radical solution was prepared freshly at 20 mg/L concentration in methanol. 1.5 mL of DPPH solution and 0.75 mL of extracts at different concentrations were added

into test tubes. After 30 minutes incubation in the dark, absorbances were recorded at 517 nm with spectrophotometer. Results were calculated as percentage inhibition with the following formula:

$$\text{DPPH radical scavenging activity (\%)} = (A_0 - A_1 / A_0) \times 100$$

where A_0 is the absorbance of DPPH• in methanol solution without an antioxidant, and A_1 is the absorbance of DPPH• in the presence of an antioxidant.

2.6. ABTS Radical Scavenging Activity

The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay was done according to the method of Re et al. [9]. To prepare an ABTS radical solution equal volume of ABTS(7 mM) and ammonium persulphate solutions (2.45 mM) were mixed and then incubated in the dark at 25 °C for 12–16 h and then diluted with ethanol until the absorbance at 734 nm was 0.70 ± 0.003 . Different quantities of each sample were mixed with 3 mL of ABTS radical solution and the change in absorbance was recorded at 734 nm for 6 minutes. Ascorbic acid was used as standart. The ABTS radical scavenging capacity of the sample was calculated by the following formula:

$$\text{ABTS radical scavenging activity: } ((A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}) \times 100$$

A_{blank} : Absorbance of the ABTS radical solution without an antioxidant

A_{sample} : Absorbance of the ABTS radical solution in the presence of an antioxidant

2.7. Determination of Reducing Power

Extract was tested for determination of reducing power by the method of Oyaizu[10]. Various concentrations of the extract from

safflower leaves (2.5 mL) were mixed with 2.5 mL of sodium phosphate buffer (200 mM, pH:6.6) and 2.5 mL of 1% potassium ferricyanide. The mixtures were incubated at 50 °C for 20 min. Then 2.5 mL of 10% trichloroacetic acid was added into test tubes and centrifuged. After centrifugation 2.5 mL of supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL of 1% FeCl₃. Absorbances of the mixtures were measured at 700 nm spectrophotometrically. α -Tocopherol used as standart.

3. RESULTS and DISCUSSION

3.1. Total Phenolic and Flavonoid Content

Leaves of Safflower were extracted by using soxhlet extraction method. Ethanol used as solvent. Total phenolic content of the extract was determined as 77.38 ± 6.62 mg GAE/g dry weight of extract. It is reported in the literature that total phenolic content seeds of *Carthamus tinctorius* L. as 126 ± 2.4 mg GAE/mg [11]. Kruawan et al.[12] reported that water extract from flower of *Carthamus tinctorius* L. as 139.98 ± 18.02 mg GAE/g. Our results show that extract from the leaves contains less phenolic content from seeds and flower of safflower. Total flavonoid content of the leaves were found as 25.49 ± 0.26 mg CE/g dry weight basis. The total flavonoid content seed of *Carthamus tinctorius* L. was found 62.2 ± 1.9 mg QE/g [11]. The total flavonoid content of CTE contain less flavonoid than seeds. 8 flavonoids were isolated from leaves of *Carthamus tinctorius* L.

3.2. DPPH Radical Scavenging Activity

The method based on the discolourization of deep violet colour of α, α -diphenyl- β -picrylhydrazyl radical. Therefore antioxidants act as hydrogen donor, in the presence of an antioxidant DPPH radical reduced and absorbance decreases. % DPPH radical scavenging activity of *Carthamus*

tinctorius L. extract was determined as 53%, 75%, 81%, 89% respectively at 1,2,3,4 mg/mL concentration. Inhibition percentage of the extract increased with increasing concentration. The results indicates that soxhlet extract of the leaves has significant antioxidant capacity. Trolox was used as standart. As can be seen in Fig 1. antioxidant activity of CTE is very close to trolox. % DPPH radical scavenging activity of CTE is higher than seeds of safflower (36.2 ± 0.5 % for 1 mg/mL)[11] but lower than flowers of safflower (96.65%) [12].

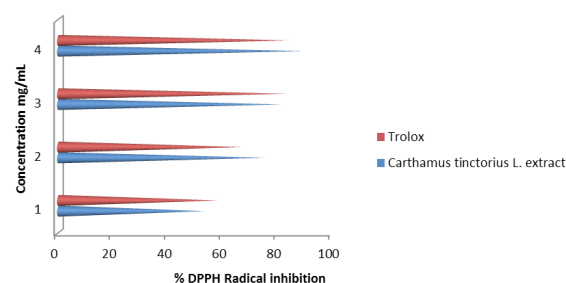


Fig 1. % DPPH Radical Scavenging Activity of *Carthamus tinctorius* L. extract

3.3. ABTS Radical Scavenging Activity

ABTS radical was prepared by mixing ABTS solution and potassium persulfate solution in 1:1 portion. Blue green ABTS radical was decolorized in the presence of H donor. Antioxidant compounds decolorize the ABTS radical because of their hydrogen donating capability. ABTS radical scavenging activity of CTE was tested for different volumes. Ascorbic acid used as reference. ABTS radical scavenging activity extract from seeds of *Carthamus tinctorius* L. 15.3 ± 4.4 % at 1 mg/mL concentration[11]. Fig 2. shows ABTS radical scavenging activity of CTE.

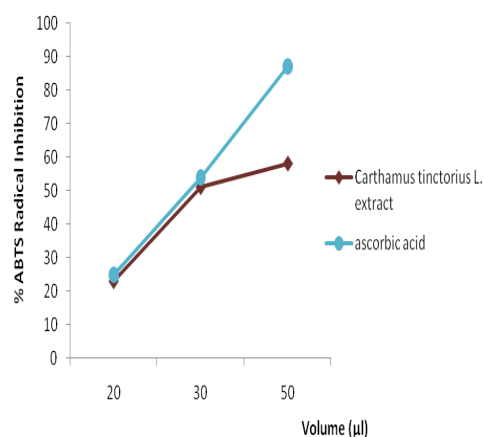


Fig 2. % ABTS Radical scavenging activity

3.4. Reducing Power

Reducing power activity is related with the antioxidant activity. In the presence of an antioxidant the yellow colors of mixture turn into blue green color [13]. As can be seen in Fig 3. CTE showed lower activity than α -tocopherol standart. Reducing power was found as 0.598, 0.688, 0.796, 0.885 respectively at 0.5, 1, 1.5, 2 mg/mL concentrations. Reducing power of plant extract are increased with the increasing concentration. These findings are very similar to the phenolic content results. It can be said that reducing power activity was originated from the phenolic content of the extracts.

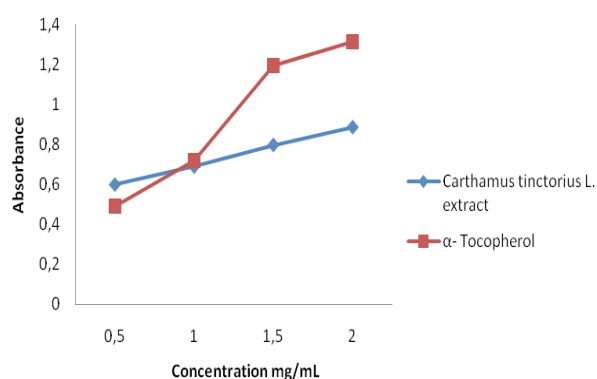


Fig 3. Reducing power of *Carthamus tinctorius L.* extract

Conclusion

According to our result leaves of *Carthamus tinctorius L.* contain important amount

phenolic compound. These results lower than other parts of the plant like seed and flower. Also plant extract has significant antioxidant activity.

REFERENCES

- [1] Altan N., Dinçel A.S., Koca C., "Diabetes Mellitus and Oxidative Stress.," *Turkish Journal of Biochemistry*, 2,41-45, 2006.
- [2] Asparganah J., Kazemivash N., "Phytochemistry, pharmacology and medicinal properties of *Carthamus tinctorius L.*" *The Chinese Journal of Integrated Traditional and Western Medicine*, 19/2,153-159,2013.
- [3] Kim S.K., Cha J.Y., Jeong S.J., Chung C.H., Choi Y.R., Cho Y.S., "Properties of the chemical composition of safflower (*Carthamus tinctorius L.*) sprout," *Korean Journal of Life Science*, 10, 68-73,2000.
- [4] Arslan B., Esendal E., Paşa C., "The economically important traits of safflower (*Carthamus tinctorius L.*) cultivars and lines cultivated in Tekirdağ, Turkey," *7th International Safflower Conference*.
- [5] Zhou F.R., Zhao M.B., Tu P.F., "Simultaneous determination of four nucleosides in *Carthamus tinctorius L.* and safflower injection using high-performance liquid chromatography.," *Journal of Chinese Pharmaceutical Sciences*. 4,1003-1057,2009.
- [6] Slinkard, K., Singleton, V.L., "Total phenols analysis: automation and

- comparison with manual methods,” *American Journal of Enology and Viticulture*, 28, 49-55, 1997.
- [7] Zhishen J., Mengcheng T., Jianming W.,” Research on antioxidant activity of flavonoids from natural materials,” *Food Chemistry Journal Elsevier.*, 64,555-559, 1999.
- [8] Jimenez-Escrig, A., Jimenez-Jimenez, I., Sanchez-Moreno, C., & Saura-Calixto, F., “Evaluation of free radical scavenging of dietary carotenoids by the stable radical 2,2-diphenyl-1-picrylhydrazyl,” *Journal of the Science of Food and Agriculture*, 80, 1686–1690, 2000.
- [9] Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., “ Antioxidants activity applying an improved ABTS radical cation decolorization assay.” *Free Radical Biology and Medicine*. 26,1231-1237,1999.
- [10] Oyaizu, M.,” Studies on products of browning reaction prepared from glucose amine,” *Japanese Journal of Nutrition*, 44, 307-315,1986.
- [11] Yu S.Y., Lee Y.J., Kim J.D., Kang S.N., Lee S.K., Jang J.Y., Lee H.K., Lim J.H., Lee O.H.,” Phenolic composition, antioxidant activity and anti-adipogenic effect of hot water extract from safflower (*Carthamus tinctorius* L.) seed,” *Nutrients*. 5,4894-4907,2013.
- [12] Kruawan K., Kangsadalampai K.,”Antioxidant activity, phenolic compound contents and antimutagenic activity of some water extract of herbs,” *The Thai Journal of Pharmaceutical Sciences*, 30, 28-35,2006.
- [13] Chung, Y.C., Chang, C.T., Chao, W.W., Lin, C.F. and Chou, S.T. , “Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1”, *Journal of Agricultural Food Chemistry*. 50, 2454-2458, 2002.