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Research Article

Total phenol and flavonoid contents, and antioxidant capacity of Silybum marianum L. Gaertner grown in Turkey

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Abstract

Because of their therapeutic properties, the aerial parts of Silybum marianum have traditionally been used in folk medicine. The purpose of this study was to determine the antioxidant capacity and total phenol and flavonoid contents of methanol extracts of Silybum marianum aerial parts. DPPH (2, 2-diphenyl-1-picrylhydrazil) free radical scavenging and reducing power assays were used to assess the antioxidant capacity of Silybum marianum. The total phenol and flavonoid contents of each extract were determined using the Folin-Ciocalteu and aluminum trichloride methods, respectively. DPPH (2, 2-diphenyl-1-picrylhydrazil) free radical scavenging assays were used to assess the antioxidant capacity of Silybum marianum extracts. All of the extracts tested showed impressive total phenolic and total flavonoid content, as well as potent antioxidant capacity. The methanolic extract was found to be containing a number of total phenols (56.95±0.78 mg of gallic acid equivalent per gram of dried material) and flavonoids (86.09±0.35mg of quercetin equivalent per gram of extract). The same extract also had a significantly higher DPPH-reducing power and scavenging capacity (Inhibition % = 91.77±0.12). These findings imply that Silybum marianum aerial parts could be used as a natural source of antioxidants in the food, pharmaceutical, and cosmetic industries.

Keywords: Antioxidant capacity, Silybum marianum, total flavonoid, total phenol.

Silybum marianum L. Gaertner'in Antioksidan Kapasitesi, Toplam Fenolik ve Flavonoid İçerikleri

Öz

Terapötik özelliklerinden dolayı Silybum marianum'un toprak üztünde kalan kısımları geleneksel olarak halk hekimliğinde kullanılmaktır. Bu çalışmanın amacı, Silybum marianum toprak üstü kısımlarının metanol ekstraktlarının antioksidan kapasitesini ve toplam fenol ve flavonoid içeriklerini belirlemektir. Silybum marianum'un antioksidan kapasitesini belirlemek için DPPH (2, 2-difenil-1-pikrilhidrazil) serbest radikal süpürme ve indirgeme kapasitesi deneyleri kullanıldı. Test edilen tüm ekstraktlar, önemli ölçüde toplam fenolik ve toplam flavonoid içeriğinin yanı sıra güçlü antioksidan kapasite göstermiştir. Metanolik ekstraktın, bir miktar fenol (kurutulmuş materyalin gramı başına 56.95±0.78 µmg gallik asit eşdeğeri) ve flavonoidler (ekstrenin gramı başına 86.09±0.35 µmg kersetin eşdeğeri) içerdiği bulundu. Ayrıca aynı ekstraktın önemli ölçüde daha yüksek DPPH indirgeme gücüne ve süpürme kapasitesine (İnhibisyon % = 91.77±0.12) sahip olduğu gözlenmiştir. Bu bulgular, Silybum marianum toprak üstünde kalan kısımlarının gıda, ilaç ve kozmetik endüstrilerinde doğal bir antioksidan kaynağı olarak kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Antioksidan kapasitesi, Silybum marianum, total flavonoid, total fenol.

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1. Introduction

Silybum marianum L.(Figure 1) are cultivated in the region from Southern Europe to Northern Africa, as its seeds have medicinal potential. The primary purpose of its cultivation is the extraction of silymarin, which is used to cure liver diseases, from its edible seeds. Silybum marianum seeds contain many flavonolignans. The main active compound in the seeds is a flavonoid known as silymarin, which is widely used to regenerate damaged liver tissues. Many research have indicated silymarin's efficacy in chemoprevention and hepatoprotection [1], [2].

Fig. 1 *Silybum marianum L.*

The antioxidant and free radical scavenging qualities of silymarin contribute to its capacity to protect the liver. Silymarin has the potential to remove free radicals from the body.It has been proven to increase superoxide dismutase activity in erythrocytes and glutathione production in hepatocytes [3]. Flavonolignans demonstrated radical scavenging properties as well as protection against lipid membrane [4] and low-density lipoprotein damage [5]. The antioxidant effect is caused by the modulation of apoptosis, cell proliferation, and differentiation pathways [6]. Many diseases, including cancer and Alzheimer's, can be caused by reactive oxygen species [7]. They are created in the human body as a result of environmental stress and normal biochemical reactions [8]. Fatty acid oxidation in cell membranes can reduce fluidity and permeability while also damaging macromolecules such as DNA, RNA, and other cellular components [9].Silymarin supports normal cell membrane fluidity by interacting with its constituents [10].

2. Material and Method

2.1. Reagents

Folin-Ciocalteu reagent, gallic acid, and quercetin standards, aluminum chloride hexahydrate, methanol, and sodium carbonate were obtained from from Sigma-Aldrich Co. (St Louis, MO, USA).

The ultrapure water used in the experimental studies with a conductivity of less than 0.05 µS cm-1 was produced using a Milli-Q System. All other chemicals were of analytical purity.

In this study, silybum marianum was grown on the grounds of Turkey's Uşak University Faculty of Agriculture and Natural Sciences. The plant's various organs were divided up and dried for 15 days in a dark environment. The dried material was crushed in a mortar to a fine powder.

2.2. Analytical Instruments

A dual beam UV-Visible spectrophotometer using 1.0 cm quartz cells and UV-Probe software was used (Shimadzu UV-1800 spectrophotometer, Japan).

2.3. Standard solutions

25 mg of reference standard was weighed precisely and transferred to a 50 mL volumetric flask, and a 15 mL methanol was added. The content of the volumetric flask s was sonicated for 5 min, and the volume was filled up to the mark with methbanol. Thus, a stock standard solution with a concentration of (500 µg mL-1) was prepared. The standard solution series (50–300 µg·mL−1, n = 6) was prepared by diluting from this stock solution with methanol. They were filtered through a membrane filter with a porosity of 0.45 µm.

2.4. Extraction

500 mg of dried, ground plant material was carefully weighed into 100 ml flasks and 50 ml of solvent (70% methanol + 30% ultrapure water) was added. Ultrasound assisted extraction (UAE) was performed for 15 min. using a Bandelin Sonorex ultrasonic bath with a frequency of 50 kHz. Erlenmeyers were placed in an ultrasonic bath for standard ultrasonic conditions. The solvent levels in the Erlenmeyers and the water level in the ultrasonic bath were kept the same. The extraction process was carried out by adjusting the temperature and time value of the ultrasonic bath. Following extraction, the mixture was filtered using white band filter paper (Whatman).

2.5. Total phenolic content

Total phenolic compounds were determined in each extract by the Folin-Ciocalteu method as previously described [11]. The extract (1 mL), 0.5 mL of Folin–Ciocalteu reagent, 2 mL of ultrapure water and 4 mL of sodium carbonate solution (75 g L-1) were mixed. After the mixture was kept in the dark at 20 °C for 40 minutes, the absorbance value was measured at a wavelength of 765 nm in a spectrophotometer device. Gallic acid methanolic solutions were used to create a calibration curve, and the results were represented as gallic acid equivalents (GAE).

2.6. Total flavonoid content

The content of flavonoids was determined colorimetrically [12]. Flavonoid content was determined from a quercetin calibration curve and results were expressed as quercetin equivalents (QE). The extract (1 mL) was mixed with 1 mL of AlCl3 solution (20 g L-1) in ethanol and diluted to 25 mL with ethanol. After the mixture was kept at 20 °C for 40 minutes, the absorbance value was measured at a wavelength of 415 nm in a spectrophotometer device. 1 drop of acetic acid was added to 1 mL of extract, then the volume was diluted to 25 ml with ethanol and blank samples were prepared. A quercetin calibration curve was also used for flavonols prepared by mixing quercetin ethanol solutions with 2 mL AlCl3 (20 g L-1) and 6 mL sodium acetate (50 g L-1). Absorbance, after 2.5 hours of incubation at 20 °C, the absorbance value of the mixture was read at 440 nm on a spectrophotometer device. The same procedure was performed with 2 mL of plant extract instead of quercetin solution. The content of flavonols was finally expressed as QE.

2.7. Total flavonoid content

The content of flavonoids was determined colorimetrically [12]. Flavonoid content was determined from a quercetin calibration curve and results were expressed as quercetin equivalents (QE). The extract (1 mL) was mixed with 1 mL of AlCl3 solution (20 g L-1) in ethanol and diluted to 25 mL with ethanol. After the mixture was kept at 20 °C for 40 minutes, the absorbance value was measured at a wavelength of 415 nm in a spectrophotometer device. 1 drop of acetic acid was added to 1 mL of extract, then the volume was diluted to 25 ml with ethanol and blank samples were prepared. A quercetin calibration curve was also used for flavonols prepared by mixing quercetin ethanol solutions with 2 mL AlCl3 (20 g L-1) and 6 mL sodium acetate (50 g L-1). Absorbance, after 2.5 hours of incubation at 20 °C, the absorbance value of the mixture was read at 440 nm on a spectrophotometer device. The same procedure was performed with 2 mL of plant extract instead of quercetin solution. The content of flavonols was finally expressed as QE

2.8. Antioxidant activity

Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazil) radical method [13]. For this, 0.0024 g of DPPH was precisely weighed and a stock solution of DPPH reagent (6 x 10 -5 M) was prepared by dissolving in 100 mL of methanol. A working solution of DPPH with a concentration (40 mg L-1) was prepared from the stock reagent solution by diluting it with methanol. 300 µL of sample extract and 5700 µL of DPPH working solution were mixed in a 10 mL test tube. The mixture was incubated for 60 minutes at room temperature in a dark environment. The absorbance of the reaction mixture against ultrapurewater was measured at 517 nm using a spectrophotometer (Shimadzu UV-1800 spectrophotometer, Japan). On the other hand, a control solution without sample extract was prepared and its absorbance against ultrapure water was measured at 517 nm in a spectrophotometer device. The antioxidant activity was calculated as:

Antioxidant activity (%) = (AC(O) 517 − AA(t) 517) / AC(O) 517 × 100

where AC(O) 517 is the absorbance of the control at t = 0 min and AA(t) 517 is the absorbance of the antioxidant at t = 1 h.

3. Results and Discussion

3.1. Total phenolic content

Calibration curve generated with gallic acid standards were presented Figure 2. TPC values of different parts of plant material are presented in Table 1 as mg gallic acid equivalent (GAE) per g dried plant.

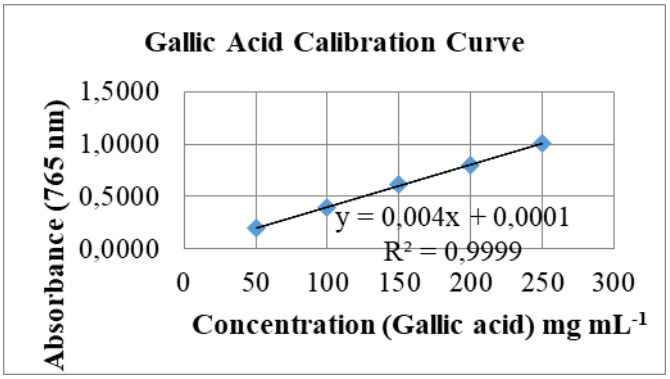

Fig. 2 Calibration curve of gallic acid standards

Sample	**TPC ± S.D.** mg. GAE/ g d.w.
Stem	56±0.77
Leaves	46±0.86
Flowers	39±1.04

Table 1. Total phenolic contents of Silybum marianum organs

3.2. Total flavonoid content (TFC)

Calibration curve generated with quercetin standards were presented Figure 3. TFC values of different parts of plant material are presented in Table 2 as mg quercetin equivalent (QE) per g dried plant.

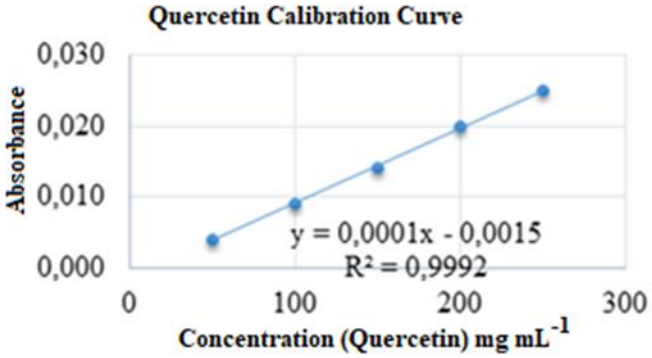

Fig. 3 Calibration curve of quercetin standards

Table 2. Total flavonoid contents of Silybum marianum organs

Sample	**TPC ± S.D.** mg. QE/ g d.w.
Stem	85±0.66
Leaves	62±0.92
Flowers	44±1.12

3.3. Antioxidant activity

Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazil) radical method. Antioxidant activity of different parts of plant material are presented in Table 3 as inhibition %.

Table 3. Antioxidant activity of Silybum marianum organs

Sample	**A.A. ± S.D.** Inhibition %
Stem	85±0.66
Leaves	62±0.92
Flowers	44±1.12

Ascorbic acid solution (500 µg mL-1) was used as the control solution in the antioxidant test. The capacity of ascorbic acid solution to inhibit DPPH radical was determined as 95%.

TFC, TPC and antioxidant activities of methanol extracts of five organs (ie main stem, leaves, flowers) of Silybum marianum L. were investigated. Total phenolic content was highest in the stem parts and then in the leaves and flowers, respectively. The total flavonoid content was similarly highest in the stem parts and then in the leaves and flowers, respectively. The organs of Silybum marianum L. can be used for flavonoid extraction. The antioxidant activity of the methanol extract from the organs of Silybum marianum L. was the highest of the stem extract, followed by the leaves. It was surprising that the phenolic, flavonoid and antioxidant activity of flowers from the stamens of Silybum marianum L. were lower than those of other organs. More studies are needed to identify other active compounds in the herb. The identity of flavonoids that may have contributed to the antioxidant activity in this herb should also be determined.

4. Conclusions and Recommendations

In conclusion, this study shows that extracts obtained from the organs of Silybum marianum L. plant have significant free radical scavenging activity on DPPH radical. The data show that methanolic extracts of Silybum marianum L. are a potential source of natural antioxidants.

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