



Comparative analysis of total phenolic and flavonoid content, antioxidant, and tyrosinase inhibitory properties of *Silybum marianum* L. from Iraq and Türkiye

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Irak ve Türkiye'de kullanılan *Silybum marianum* L.'un toplam fenolik ve flavonoid içeriği, antioksidan ve tirozinaz inhibisyon aktivitelerinin karşılaştırmalı incelenmesi

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Abstract: This study investigates the phytochemical composition and bioactive properties of *Silybum marianum* L. seeds collected from Iraq and Türkiye, two regions with a history of traditional medicinal use of this herb. Extracts were analyzed for total phenolic and flavonoid content (TPC and TFC) using colorimetric methods, and for antioxidant [2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays] and tyrosinase inhibitory activity. Results showed that Turkish extracts had significantly higher TPC and TFC (165.65 ± 6.99 mg GAE/g and 7.66 ± 0.71mg QE/g, respectively) compared to Iraqi extracts, which correlated with enhanced antioxidant and tyrosinase inhibition. The study underscores the impact of regional and environmental factors on the medicinal attributes of *S. marianum* L., offering valuable information for optimizing its application in the pharmaceutical and cosmetic industries. Turkey.

Key words: *Silybum marianum* L., antioxidant activity, phytochemical composition

Özet: Bu çalışma, bu bitkinin geleneksel tıbbi kullanım geçmişine sahip iki bölge olan Irak ve Türkiye'den toplanan *Silybum marianum* L. tohumlarının fitokimyasal bileşimini ve biyoaktif özelliklerini araştırmaktadır. Ekstreler, kolorimetrik yöntemler kullanılarak total fenolik ve flavonoid içeriği (TPC ve TFC) ve antioksidan [2,2-difenil-1-pikrilhidrazil (DPPH) ve 2,2'-azino-bis(3-etilbenzotiazolin-6-sülfonik asit) (ABTS) deneyleri] ve tirozinaz inhibitör aktivitesi için analiz edilmiştir. Sonuçlar, Türk ekstraktlarının Irak ekstraktlarına kıyasla önemli ölçüde daha yüksek TPC ve TFC'ye (sırasıyla 165.65 ± 6.99 mg GAE/g ve 7.66 ± 0.71mg QE/g) sahip olduğunu ve bunun da gelişmiş antioksidan ve tirozinaz inhibisyonu ile ilişkili olduğunu göstermiştir. Çalışma, bölgesel ve çevresel faktörlerin *S. marianum* L.'nin tıbbi özellikleri üzerindeki etkisinin altını çizmekte ve ilaç ve kozmetik endüstrilerindeki uygulamalarını optimize etmek için değerli bilgiler sunmaktadır.

Anahtar Kelimeler: *Silybum marianum* L., antioksidan aktivite, fitokimyasal bileşimi

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1. Introduction

Silybum marianum (L.) Gaertner, commonly known as milk thistle, is a herbaceous plant belonging to the *Asteraceae* family. It is native to the Mediterranean region but is now cultivated worldwide for its medicinal value (Abenavoli et al., 2010). Characterized by its thorny leaves and purple flowers, this plant thrives in well-drained soils under full sunlight. The seeds of *Silybum marianum* have long been used in traditional medicine for their hepatoprotective properties and are considered the primary source of bioactive compounds (Karkanis et al., 2018).

The phytochemical composition of *S. marianum* is diverse, with its seeds containing flavonolignans collectively known as silymarin, which is the most researched active constituent of the plant (Ahmed et al., 2020). Silymarin is a complex mixture of flavonolignans, including silybin, silychristin, silydianin, and isosilybin, with silybin being the most abundant and biologically active component (Ramasamy and Agarwal, 2008). Besides flavonolignans, the plant also contains other phytochemicals, such as flavonoids, polyphenols, fatty acids, and tocopherols,

which contribute to its antioxidant properties (Javeed et al., 2022). Pharmacologically, *Silybum marianum* exhibits a wide range of biological activities. The most notable is its hepatoprotective effect, which has been extensively studied for its role in managing liver diseases, including hepatitis, cirrhosis, and non-alcoholic fatty liver disease (Saller et al., 2001). Silymarin's hepatoprotective action is attributed to its antioxidant properties, inhibition of lipid peroxidation, and its ability to stabilize cell membranes and modulate inflammatory responses (Abenavoli et al., 2010). In addition to hepatoprotection, *S. marianum* has demonstrated anticancer, anti-inflammatory, cardioprotective, and neuroprotective effects, making it a promising candidate for various therapeutic applications (Ramasamy and Agarwal, 2008).

Studies have also revealed the plant's antioxidant potential through its high phenolic and flavonoid content. These compounds scavenge free radicals, reduce oxidative stress, and enhance enzymatic antioxidant defenses (Tedesco et al., 2004). A study emphasized *S. marianum*'s total phenol and flavonoid content as well as its antioxidant potential, demonstrating its power to scavenge free radicals and

lessen oxidative stress (Akbel and Bulduk, 2022). Studies conducted on various parts of milk thistle have shown a high amount of phenolic and total flavonoids, together with notable antioxidant properties (Sun et al., 2016). A different study assessed *Silybum marianum*'s phytochemical components and antioxidant properties, highlighting its function in boosting enzymatic antioxidant defenses (Kumar et al., 2019). Moreover, the tyrosinase inhibitory activity of *S. marianum* extracts has sparked interest in its potential applications in cosmetic formulations for skin depigmentation and anti-aging effects (Jabłonowska et al., 2021; Kim et al., 2019; Kucharska et al., 2023).

The enzyme tyrosinase, which contains copper, is essential for the manufacture of melanin, the pigment that gives skin, hair, and eyes their color. Tyrosine is oxidized by it to dopaquinone, which then goes through further processes to produce melanin. Although melanin shields the skin from UV rays, too much of it can cause age spots, hyperpigmentation, and uneven skin tone. Thus, the development of cosmetic formulations targeted at skin depigmentation and anti-aging has turned its attention to the reduction of tyrosinase activity (Kim and Uyama, 2005). Rich in naturally occurring bioactive substances like flavonoids and polyphenols, plant extracts are frequently studied for their ability to inhibit tyrosinase because they provide a more sustainable and safe substitute for synthetic inhibitors. For instance, mulberry (*Morus alba* L.), green tea (*Camellia sinensis* (L.) Kuntze), and licorice root (*Glycyrrhiza glabra* Torr) have all demonstrated strong tyrosinase inhibition and are frequently used in herbal skincare products to lessen hyperpigmentation and encourage an even complexion (Chan et al., 2008).

The widespread use of *S. marianum* in traditional and modern medicine underscores its importance as a medicinal plant. Its phytochemical richness and pharmacological versatility justify continued research into its bioactive properties and therapeutic potential. This study aims to compare the total phenol and flavonoid content, antioxidant capacity, and tyrosinase inhibitory activity of *S. marianum* collected from Iraq and Türkiye, contributing to a better understanding of regional variations in its bioactive profile. The phytochemical and pharmacological characteristics of milk thistle have been the subject of much investigation, but little comparative study has been done on the bioactive components and bioactivities of this plant according to their geographic origin. The phytochemical profiles of medicinal plants are known to be influenced by environmental factors such as soil composition, climate, and cultivation methods, which may have an impact on the plants' therapeutic efficacy (Borges et al., 2017). Although *S. marianum* is utilized extensively in both Iraq and Türkiye, no thorough investigation has yet evaluated the plant's tyrosinase inhibitory action, antioxidant capacity, and total phenol and flavonoid content across the two countries. By analyzing and contrasting the bioactive profiles of *S. marianum* from Iraq and Türkiye, this work aims to close this gap. By doing this, it will optimize the plant's use in both conventional medicine and contemporary therapeutic applications by offering insightful information on how geographical heterogeneity affects the plant's chemical makeup and biological activity. Future investigations into regional adaptations and their effects on standardization, quality assurance, and

therapeutic potential may build on the findings of this study.

2. Materials and Method

2.1. Plant Material

The seeds of *Silybum marianum* were obtained from local herbal markets in Iraq and Türkiye, regions traditionally associated with the cultivation and use of this plant. In Iraq, the seeds were sourced from markets in Kerkuk, a central hub for the trade of medicinal plants. Similarly, in Türkiye, seeds were procured from herbal markets in the Konya region (Fig. 1). To ensure the botanical authenticity and homogeneity of the seeds, a rigorous identification process was conducted. Morphological characteristics of the seeds were examined and compared against established taxonomic keys and descriptions from authoritative botanical references (Davis, 1975). The seeds are typically oval to oblong in shape, with a slightly flattened appearance. Measuring approximately 6–8 mm in length, the seeds vary in color from black to brown, often featuring a yellow ring at the apex. Their smooth, mottled surface contributes to their distinctive texture, making them easily recognizable. A key distinguishing feature is the presence of a pappus, a specialized appendage with numerous barbed bristles measuring about 2 cm in length, which enhances wind dispersal and contributes to their ecological adaptation.

2.2. Extraction Procedure

The seeds of *S. marianum* from Iraq and Türkiye were finely ground into a uniform powder using a mechanical grinder. A measured amount of the powdered seeds was soaked in 50% ethanol (v/v) at a ratio of 1:10 (w/v) to ensure optimal extraction of bioactive compounds (Jahan et al., 2016). The mixture was allowed to macerate at room temperature with occasional stirring for 24 hours. After the initial maceration, the mixture was filtered through Whatman No. 1 filter paper to separate the liquid extract from the solid residue. The filtrate was then concentrated under reduced pressure using a rotary evaporator set at 40°C to remove the ethanol, yielding a semi-solid crude extract. This extraction process was repeated twice to maximize the recovery of active constituents. The combined extracts from the repetitions were pooled, transferred to a sterile container, and stored at 4°C in a refrigerator until further analysis, including the evaluation of bioactive properties.

2.3. Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) was assessed using the Folin-Ciocalteu Singleton colorimetric method (Singleton



Figure 1. *Silybum marianum* seeds; a: from Iraq; b: from Türkiye

et al., 1999), incorporating certain modifications. In summary, 0.5 mL of extract (1 mg/mL) was combined with 2.5 mL of 10% Folin-Ciocalteu reagent and incubated for 5 minutes. Subsequently, 2 mL of 7.5% sodium carbonate solution was added, and the mixture was allowed to react in darkness at room temperature for 30 minutes. The absorbance was then measured at 765 nm using a Shimadzu UV-1800 UV-visible spectrophotometer. Gallic acid served as the standard, with results expressed in milligrams of gallic acid equivalents (mg GAE) per gram of extract.

2.4. Total Flavonoid Content (TFC)

The total flavonoid content was determined using an aluminum chloride colorimetric assay (Chang et al., 2002). Briefly, 0.5 mL of extract (1 mg/mL) was mixed with 2 mL of distilled water and 0.15 mL of 5% sodium nitrite solution. After 5 minutes, 0.15 mL of 10% aluminum chloride solution was added. The mixture was allowed to stand for 6 minutes, followed by the addition of 1 mL of 1 M sodium hydroxide solution. The mixture was then diluted to 4 mL with distilled water, and the absorbance was measured at 510 nm. Quercetin was used as the standard, and the results were expressed as milligrams of quercetin equivalents (mg QE/g extract) per gram of extract.

2.5. Antioxidant Activity

Antioxidant capacity was evaluated using the DPPH and ABTS radical scavenging assay.

2.5.1. DPPH Radical Scavenging Activity

The antioxidant activity of the extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method (Brand-Williams et al., 1995). A solution of 0.1 mM DPPH in methanol was prepared. An aliquot of 1 mL of extract (at concentrations of 10, 50, 250, 500, 750, 1000 and 2000 µg/mL) was mixed with 3 mL of DPPH solution and incubated in the dark for 30 minutes. BHT was used as a positive control. The experiments were conducted in triplicate, and the results were expressed as mean and standard error to ensure reliability and statistical accuracy. The absorbance was measured at 517 nm, and the percentage inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = (\text{A}_{\text{control}} - \text{A}_{\text{sample}}) / \text{A}_{\text{control}} \times 100$$

2.5.2. ABTS Radical Scavenging Activity Assay

The ABTS radical scavenging activity was measured based on the decolorization of the ABTS cation radical with slight modifications (Re et al., 1999). Samples were prepared following the same procedure used for the DPPH assay. The ABTS cation radical was generated by reacting 7 mM aqueous ABTS solution (15 mL) with 140 mM potassium persulfate solution (264 µL). The reaction mixture was incubated in the dark at room temperature for 16 hours before use. Prior to the assay, the ABTS working solution was diluted with methanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm and allowed to stabilize at room temperature. Trolox (concentration range from 0.1 to 1.0 mg/mL) was used as a positive control to evaluate antioxidant activity. In a 96-well microplate, 50 µL of the sample and 100 µL of the ABTS working solution were mixed. The mixture was incubated in the dark for 10 minutes, and the absorbance was measured at 734 nm against a blank. All experiments were performed in triplicate.

The percentage of ABTS radical scavenging activity was calculated using the following formula:

$$\text{Scavenging activity (\%)} = [1 - (\text{A}_1 - \text{A}_2) / \text{A}_0] \times 100$$

Where: A_0 is the absorbance of the control (without sample), A_1 is the absorbance in the presence of the sample, and A_2 is the absorbance of the sample without ABTS solution. The ability of the extracts to scavenge ABTS radicals was expressed as the IC_{50} , the concentration required to scavenge 50% of ABTS radicals. The IC_{50} values were calculated from the dose-response curves of scavenging activity (%) versus sample concentration.

2.6. Tyrosinase Inhibition Assay

The tyrosinase inhibitory activity was determined using a modified dopachrome method (Im et al., 2019). The reaction mixture, containing 40 µL of extract, 80 µL of phosphate buffer (pH 6.8), and 40 µL of mushroom tyrosinase enzyme (30 units/mL) was incubated at 37°C. The reaction was initiated by adding 40 µL of L-DOPA (1.5 mM), and the absorbance was recorded at 475 nm after 10 minutes. The Kojic acid served as the positive control. The percentage of tyrosinase inhibition was calculated as:

$$\text{Inhibition (\%)} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}} \times 100$$

2.7. Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean $\pm$ standard deviation (SD). One-way ANOVA followed by Tukey's post-hoc test was used to determine significant differences between the means ($p < 0.05$). Statistical analyses were performed using Graphpad software.

3. Results

The antioxidant activity, total phenolic and flavonoid content, and tyrosinase inhibitory activity of *S. marianum* L. extracts collected from Iraq and Türkiye were analyzed. Comparative results for each parameter are presented below.

3.1. Total Phenolic and Flavonoid Content

The calibration curve for the standards was presented as Figure 2. The equations obtained from the calibration curves for quercetin and gallic acid were as follows:

For quercetin: $y = 0.0229x + 0.0724$ with a correlation coefficient r^2 of 0.9996;

For gallic acid: $y = 0.0016x + 0.033$ with a correlation coefficient r^2 of 0.99788.

These equations were used to calculate the total phenolic and flavonoid contents in the extracts, expressed as gallic acid equivalents (GAE) and quercetin equivalents (QE), respectively. Samples from Türkiye exhibited significantly higher TPC and TFC compared to those from Iraq ($p < 0.05$). The TPC was quantified using the Folin-Ciocalteu method and expressed as mg of gallic acid equivalent per gram of extract (mg GAE/g). As shown in Figure 3, Turkish extracts exhibited a higher TPC (165.65 ± 6.99 mg GAE/g) than Iraqi extracts (158.63 ± 3.70 mg GAE/g), indicating regional differences in the phytochemical composition of *S. marianum* L. seed extracts. The TFC was determined using the aluminum chloride colorimetric method, was expressed as mg of quercetin equivalent per gram of extract (mg

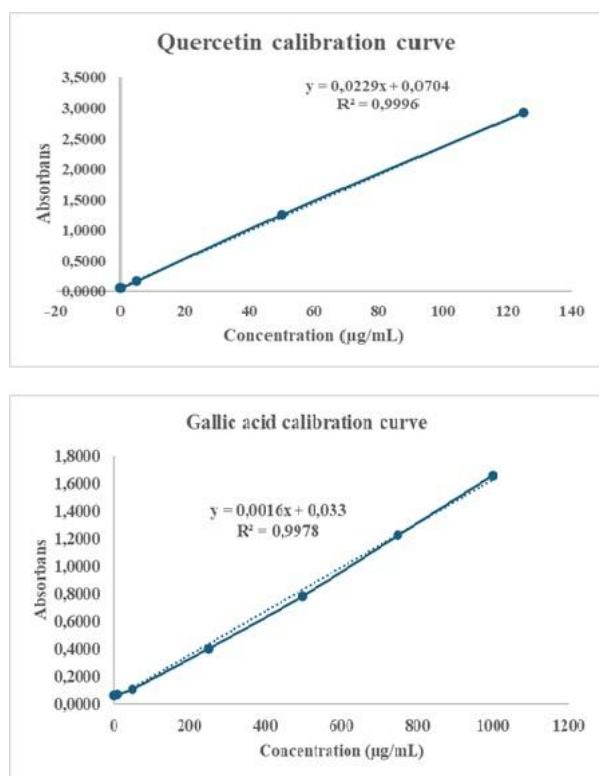


Figure 2. Standard calibration curve of Quercetin for TFC and Gallic acid for TPC

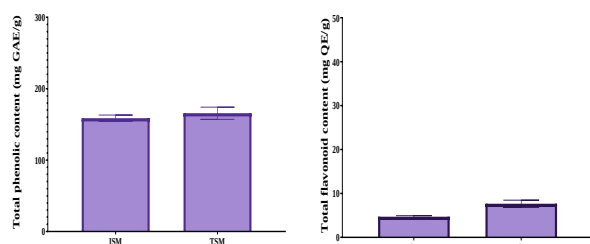


Figure 3. Total phenolic and flavonoid content of *S. marianum* L. seed extracts from Iraq and Türkiye; ISM (Iraqi *S. marianum* seeds); TSM (Turkish *S. marianum* seeds)

QE/g). Turkish extracts again surpassed Iraqi extracts, with values of 7.66 ± 0.71 mg QE/g and 4.69 ± 0.21 mg QE/g, respectively. These results are consistent with the higher antioxidant activities observed in Turkish samples.

3.2. Antioxidant Activity

The Turkish extracts demonstrated stronger antioxidant activity in both DPPH and ABTS assays, correlating with their higher phenolic and flavonoid content. Antioxidant activity was assessed using DPPH and ABTS radical scavenging assays. The IC_{50} values, indicating the concentration required to inhibit 50% of radical activity, were lower for extracts from Türkiye compared to Iraq (Figs. 4-5), suggesting stronger antioxidant capacity. The DPPH assay demonstrated a dose-dependent increase in radical scavenging activity for both extracts, with Turkish samples achieving a maximum inhibition of $56.78 \pm 2.08\%$ at 1000 $\mu\text{g/mL}$, compared to $48.07 \pm 0.87\%$ for Iraqi samples at the same concentration (Table 1). Similarly, the ABTS assay results mirrored the DPPH findings, with Turkish extracts exhibiting significantly higher scavenging

capacity. In the ABTS radical scavenging assay, the extract from Türkiye and Iraq showed nearly same activity with the inhibition of $79.22 \pm 1.60\%$ and $78.65 \pm 0.91\%$, respectively.

3.3. Tyrosinase Inhibition

Tyrosinase inhibitory activity was evaluated using the L-DOPA method, with kojic acid as the positive control. The results (Fig. 6) showed that Iraqi sample exhibited greater inhibitory activity, achieving an inhibition rate of $51.59 \pm 9.12\%$ at 2000 $\mu\text{g/mL}$ compared to $43.79 \pm 2.20\%$ for Turkish extract. IC_{50} values further confirmed the superior activity of Iraqi samples ($IC_{50} = 1789 \pm 0.81$ $\mu\text{g/mL}$) over Turkish samples ($IC_{50} = 2032 \pm 1.14$ $\mu\text{g/mL}$). The reference drug kojic acid demonstrated inhibitory activity with the IC_{50} value of 23.06 $\mu\text{g/mL}$ and showed $77.17 \pm 1.49\%$ inhibition at the same concentration.

4. Discussions

The findings of this study reveal significant differences in the total phenolic and flavonoid content, antioxidant activity, and tyrosinase inhibitory activity of *S. marianum* extracts collected from Iraq and Türkiye. These results highlight the influence of regional environmental and agricultural factors on the phytochemical composition and bioactivity of the plant.

4.1 Total Phenolic and Flavonoid Content

The significantly higher TPC and TFC observed in Turkish samples compared to Iraqi samples ($p < 0.05$) suggest that climatic conditions, soil composition, and agricultural practices play a pivotal role in determining the phytochemical profile of *S. marianum* L. Türkiye's

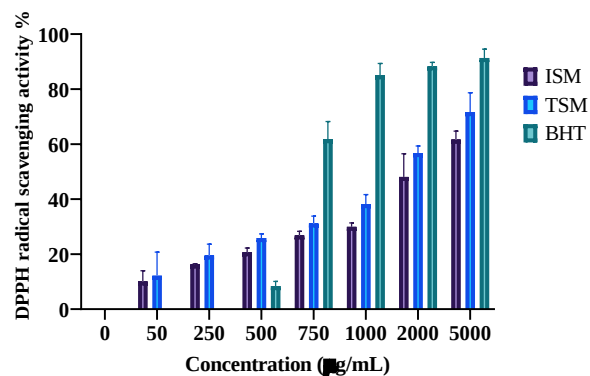


Figure 4. DPPH radical scavenging activity of *S. marianum* L. seed extracts from Iraq and Türkiye

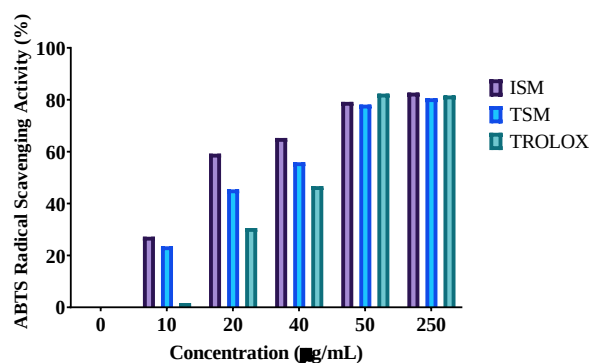
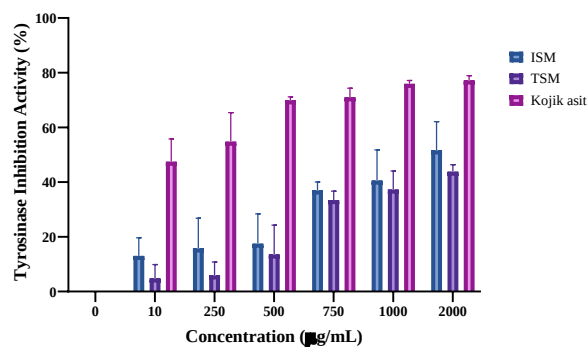


Figure 5. ABTS radical scavenging activity of *S. marianum* L. seed extracts from Iraq and Türkiye

Table 1. TPC, TFC, Antioxidant and enzym inhibitory activity of Turkish and Iraqi *S. marianum* seed extracts

	TPC mg GAE/g	TFC mg QE/g	DPPH radical scavenging activity (IC ₅₀ µg/mL)	ABTS radical scavenging activity (IC ₅₀ µg/mL)	Tyrosinase inhibitory activity (IC ₅₀ µg/mL)
TSM	165.65 ± 6.99	7.66 ± 0.71	1665 ± 0.79	25.49± 1.01	2170± 0.938
ISM	158.63 ± 3.70	4.69 ± 0.21	2652 ± 0.736	17.71 ± 0.95	1755± 0.870
BHT	-	-	705.7± 5.91	n.t.	n.t.
Trolox	-	-	n.t.	33.52 ± 2.09	n.t.
Kojic acid	-	-	n.t.	n.t.	23.27± 0.25

Note: Values expressed are means ± S.D. of three parallel measurements; n.t.: not tested. ISM (Iraqi *S. marianum* seeds); TSM (Turkish *S. marianum* seeds) BHT: Butylated hydroxytoluene

**Figure 6.** Tyrosinase inhibitory activity of Turkish and Iraqi *S. marianum* seed extracts and kojic acid

Mediterranean climate, characterized by moderate temperatures and distinct seasonal variation, may contribute to enhanced phenolic and flavonoid biosynthesis. Conversely, Iraq's arid climate with higher temperatures and lower rainfall may impose stress conditions that limit the accumulation of these bioactive compounds. Additionally, differences in soil nutrient content and cultivation practices between the two regions likely affect the secondary metabolite production in the plant. The observed variations in the extracts of *Silybum marianum* from Türkiye and Iraq in terms of TPC and TFC are consistent with findings from earlier research that emphasize the impact of environmental conditions on phytochemical profiles. For example, the production of phenolic and flavonoid chemicals in medicinal plants is known to be greatly impacted by changes in soil composition, climate, and cultivation practices. In a former study, the TPC and TFC were found as 56.95±0.78 mg GAE/g and 86.09±0.35mg QE/g in methanol extract of *S. marianum* from Türkiye (Akbel and Bulduk, 2022). Since the plant material used in this study was the above-ground part, the phenolic content was lower but the flavonoid content was higher than our results.

4.2 Antioxidant Activity

The higher antioxidant activity of Turkish extracts, as evidenced by lower IC₅₀ values in DPPH and ABTS assays, correlates with their superior phenolic and flavonoid content. Phenolic compounds and flavonoids are known for their potent antioxidant properties, functioning as radical scavengers that mitigate oxidative stress. The dose-dependent increase in antioxidant activity observed in both DPPH and ABTS assays further supports the critical role of these compounds in conferring bioactivity. The stronger antioxidant potential of Turkish samples underscores the importance of regional environmental conditions in

enhancing the medicinal value of *S. marianum* L., making them a more suitable candidate for therapeutic applications targeting oxidative stress-related diseases. The results of the DPPH and ABTS assays for *S. marianum* extracts from Türkiye and Iraq align with previous studies that emphasize the plant's antioxidant potential. The higher radical scavenging activity observed in Turkish extracts, particularly in the DPPH assay, suggests a greater concentration of bioactive compounds such as phenolics and flavonoids. This is consistent with earlier findings that environmental factors like soil composition, climate, and cultivation methods significantly influence the phytochemical profiles of medicinal plants. In a former study, *S. marianum* var. *marianum* had a maximum scavenging effect of 72.944% compared to the *S. marianum* var. *albiflorum*, (61.846%), stronger IC₅₀ was obtained by var. *marianum* with 17.027±0.064 µg/mL (Rozhgar et al., 2024).

4.3 Tyrosinase Inhibition

The greater tyrosinase inhibitory activity demonstrated by Iraqi samples, with a higher inhibition rate and lower IC₅₀ value compared to Turkish extract, highlights the potential of Iraqi *S. marianum* extract in cosmetic and dermatological applications. Tyrosinase is a key enzyme in melanin biosynthesis, and its inhibition is critical in treating hyperpigmentation disorders. The differences in tyrosinase inhibitory activity between the two regions may also be attributed to variations in secondary metabolite composition influenced by regional factors. Iraqi extracts' superior performance further supports their potential for use in developing skin-lightening and anti-aging products.

The tyrosinase inhibitory activity of *S. marianum* extracts, as indicated by the IC₅₀ values, demonstrates that Iraqi samples (IC₅₀ = 1755 ± 0.87 µg/mL) exhibit stronger inhibition compared to Turkish samples (IC₅₀ = 2170 ± 0.94 µg/mL). These findings align with previous studies that emphasize the role of environmental factors in influencing the bioactive compound profiles of medicinal plants. For instance, a study on flavonolignans from *Silybum marianum* reported IC₅₀ values ranging from 1.7 to 7.6 µM for monophenolase inhibition and 12.1 to 44.9 µM for diphenolase inhibition (Kim et al., 2019). Another investigation highlighted the tyrosinase inhibitory activity of silybin derivatives, with IC₅₀ values of 274.6 µM and 109.5 µM for specific compounds (Han et al., 2024). These studies suggest that the inhibitory potential is closely tied to the concentration and composition of flavonolignans and other bioactive compounds.

Iraq and Türkiye have different climates, soil compositions, and agricultural techniques, which are probably the cause

of the observed variances in phenolic and flavonoid content, antioxidant activity, and tyrosinase inhibitory activity. The production of bioactive chemicals may be enhanced by Türkiye's advantageous environmental conditions, such as soil fertility and farming practices. These results highlight how crucial geographical variables are to maximizing milk thistle's therapeutic and aesthetic benefits. When choosing plant sources for medical and cosmetic applications, it is important to take environmental and regional factors into account. This is demonstrated by the increased bioactivity found in Turkish *S. marianum* L. samples. Clarifying the precise environmental factors that improve phytochemical synthesis and bioactivity may be the main goal of future research. Furthermore, investigating different areas and carrying out longer-term research may offer more profound understanding of how environmental variability impacts *S. marianum* L.'s medicinal potential. These discoveries help to improve the plant's application in agriculture and industry to create high-value goods.

The significant geographical differences in the phytochemical composition and biological activity of *S. marianum* L. extracts obtained from Iraq and Türkiye are highlighted by this comparative study. In comparison to Iraqi samples, Turkish samples consistently showed higher levels of total phenolic and flavonoid content, stronger antioxidant activity, and more tyrosinase inhibitory capacity. These results highlight how the plant's therapeutic qualities are influenced by soil composition, climate, and farming methods. Turkish samples' higher bioactivity indicates their potential for improved therapeutic and

cosmetic uses, especially in treating illnesses linked to oxidative stress and hyperpigmentation disorders. These findings offer insightful information for maximizing *S. marianum* 's application in the cosmetics and pharmaceutical sectors. They also emphasize how crucial it is to choose plant sources according to environmental and regional considerations in order to optimize their therapeutic efficacy. In order to develop targeted cultivation and utilization methods, future study concentrating on a broader variety of geographic locations and environmental characteristics may help us better understand the factors impacting the phytochemical and bioactive qualities of *S. marianum*.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

Conceptualization, N.S. and N.E.; methodology, software, validation, and formal analysis, N.E.; data curation, N.S.; writing—original draft preparation, N.S.; writing-review and editing, visualization, and supervision, N.E.; funding acquisition, N.E. All authors have read and agreed to the published version of the manuscript.

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