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ORIGINAL ARTICLE / ÖZGÜN MAKALE

COMPARATIVE EVALUATION OF BIOLOGICAL EFFECT OF FOUR SUNFLOWER (*HELIANTHUS ANNUUS L.*) GENOTYPES: AGRO-FOOD BYPRODUCTS AS PROMISING NATURAL NEW FOOD ADDITIVES

DÖRT AYÇİÇEĞİ (HELIANTHUS ANNUUS L.) GENOTİPİNİN BİYOLOJİK ETKİLERİNİN KARŞILAŞTIRMALI DEĞERLENDİRMESİ: TARIMSAL GIDA YAN ÜRÜNLERİNDEN UMUT VERİCİ DOĞAL YENİ GIDA KATKI MADDELERİ

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ABSTRACT

Objective: *Food additives are widely used in industry to improve the appearance, quality and safety of food during processing, storage and packaging. The sunflower (Helianthus annuus L.) is an important agricultural product that is cultivated worldwide for seeds. Its seeds are one of the largest sources of vegetable oil. In this study, the biological activity of sunflower seeds as well as various plant parts, which are agricultural by-products and mostly treated as waste, was tested and their potential for use as a food additive was determined.*
Material and Method: *The antiproliferative effect of six different plant parts (seeds, ray florets, disc florets, leaves, stems and receptacle) of four sunflower varieties (DERAY, SY GRANIT, P64 LP 130, TR 2242 CL) against the healthy cell line L929 was investigated using the MTT method and the concentration range that can be safely used was determined. The antioxidant capacity was determined using the DPPH, TEAC and CUPRAC methods. Inhibition of the enzyme tyrosinase was investigated to prevent enzymatic browning of food.*
Result and Discussion: *It was found that even at concentration of 400 and 800 µg/ml, safe use is possible. In all methods, disc floret and ray floret showed a strong antioxidant effect. In the DPPH free radical scavenging effect of the ray floret of the TR 2242 CL showed the highest value with 101.40 mg gallic acid/g extract. Disc and ray floret showed strong inhibition of the tyrosinase enzyme in all varieties. The enzyme inhibition of methanol extracts of TR 2242 CL ray and disc floret was found to be 60.42 and 151.25 mg KAE/g extract, respectively. The lack of cytotoxicity against healthy cells, the high antioxidant capacity and the strong anti-browning activity suggest that sunflower agro-food byproducts may be a new, non-toxic, cost-effective and recyclable source to be used in the food industry instead of food additives that have negative side effects on health.*
Keywords: *Antioxidant, antiproliferative, food additives, Helianthus annuus L., tyrosinase inhibition*

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ÖZ

Amaç: *Gıda katkı maddeleri, işleme, depolama ve paketleme sırasında gıdanın görünümünü, kalitesini ve güvenliğini iyileştirmek amacıyla endüstride yaygın olarak kullanılmaktadır. Ayçiçeği (Helianthus annuus L.), dünya çapında tohumluk olarak yetiştirilen önemli bir tarım bitkisidir. Tohumları en büyük bitkisel yağ kaynaklarından biridir. Bu çalışmada tarımsal yan ürün olan ve çoğunlukla atık olarak nitelendirilen ayçiçeğinin tohumlarının ve çeşitli bitki kısımlarının biyolojik etkileri test edilmiş ve gıda katkı maddesi olarak kullanım potansiyelleri belirlenmiştir.*

Gereç ve Yöntem: *Dört ayçiçeği çeşidinin (DERAY, SY GRANIT, P64 LP 130, TR 2242 CL) altı farklı bitki kısmının (tohum, dil çiçekleri, disk çiçekleri, yaprak, gövde ve tabla) L929 sağlıklı hücre hattına karşı antiproliferatif etkisi MTT yöntemi kullanılarak araştırıldı ve güvenle kullanılabilecek konsantrasyon aralığı belirlendi. Antioksidan kapasite DPPH, TEAC ve CUPRAC yöntemleri kullanılarak belirlendi. Gıdanın enzimatik esmerleşmesini önlemek için tirozinaz enzim inhibisyonu araştırıldı.*

Sonuç ve Tartışma: *400 ve 800 µg/ml konsantrasyonlarda dahi güvenli kullanımın mümkün olduğu tespit edildi. Tüm yöntemlerde disk çiçeği ve dil çiçeği güçlü antioksidan etki gösterdi. DPPH serbest radikal süpürücü etki tayininde TR 2242 CL'nin dil çiçeği 101.40 mg gallik asit/g ekstrakt ile en yüksek etkiyi gösterdi. Disk ve dil çiçeği tüm çeşitlerde en güçlü tirozinaz enzim inhibisyonu gösterdi. TR 2242 CL dil ve disk çiçeği metanol ekstraktlarının enzim inhibisyonu sırasıyla 60.42 ve 151.25 mg KAE/g ekstrakt olarak bulundu. Ayçiçeği tarım-gıda yan ürünlerinin sağlıklı hücrelere karşı sitotoksisitesinin olmaması, yüksek antioksidan kapasitesi ve güçlü enzimatik kararma önleme aktivitesi; gıda endüstrisinde kullanılabilecek, sağlığa olumsuz yan etkileri olan gıda katkı maddelerinin yerine geçebilecek yeni, toksik olmayan, uygun maliyetli ve geri dönüştürülebilir bir kaynak olabileceğini düşündürmektedir.*

Anahtar Kelimeler: *Antioksidan, antiproliferatif, gıda katkı maddeleri, Helianthus annuus L., tirozinaz inhibisyonu*

INTRODUCTION

Food additives and colorants are important ingredients that ensure that the taste, appearance and quality of food remain unchanged throughout the entire process from preparation to consumption [1,2]. Food additives can be divided into different groups depending on their function, e.g. bleaching agents, sweeteners, antioxidants, preservatives, colorants and thickeners [3]. Tyrosinase is a key enzyme responsible for enzymatic browning and melanogenesis in mammals. It is found in many plants such as mushrooms, apples, bananas, potatoes and avocados as well as in shrimps. This metalloenzyme, which carries copper in its core, is responsible for the enzymatic browning in foods [4]. Enzymatic browning leads to color changes, softening, texture deterioration and taste changes in foods. This negatively affects the taste and appearance of food. Inhibitors targeting the tyrosinase enzyme are used as food additives to prevent enzymatic browning. As a result, foods such as vegetables, fruit and shrimp retain their fresh taste and appearance without browning [1].

Colorants are additives that are added to foods to correct the colors of foods that change during the production process or to make them look more attractive and bright. There are both natural colorants, which are obtained from animals, plants or minerals, and artificial colorants in synthetic form [5].

Synthetic food dyes are often used for coloring because of their low cost. However, many of them have toxic side effects with long-term use and cause numerous health problems such as anemia, asthma, eczema, urticaria, pathological lesions in various organs, cancer and mental retardation [6]. Tartrazine is a synthetic azo dye that is often used in the food industry to produce a bright yellow color. Its use in large quantities has negative effects on human health. Studies have reported negative effects such as increased oxidative stress, damage to cells, carcinogenic, mutagenic and reproductive toxicity [7].

Natural and synthetic antioxidants are used in the food industry to extend the shelf life of foods without them losing their appearance and nutritional value. Vitamins C, E and spices such as basil, rosemary, pepper and thyme are used as natural antioxidants. Butylated hydroxylanisole, butylated hydroxyltoluene and propyl gallate, which are synthetic antioxidants, are among the most commonly used and very effective food additives. However, various studies show that synthetic antioxidants in particular can lead to obesity, excessive sweating, asthma, stomach, eye and skin diseases with

prolonged use [8]. Rodents exposed to high levels of butylated hydroxyanisole (BHA) in the diet developed forestomach tumors classified by the IARC (International Agency for Research on Cancer) as Group 2B ("possibly carcinogenic to humans") [9]. Sulphites are synthetic food additives that are widely used in the food industry for their anti-browning, color-stabilizing, antimicrobial and antioxidant properties and are added to fruits and vegetables, seafood, some beverages and meat products. However, a high dietary intake of sulphites can cause various negative side effects such as allergic diseases and vitamin deficiencies and have a negative impact on the microbiota [10].

In view of all these negative side effects, it seems that synthetic food additives in particular are not welcome by consumers. Natural products, which are a good alternative to increase the safety, quality and attractiveness of food, can also be used to support physiological functions with their high nutritional and mineral content. This situation has shown that there is a need to replace synthetic compounds used as food additives with natural bioactive sources.

The sunflower (*Helianthus annuus* L.) is a plant of global importance, as it is consumed as food and animal feed. Although it is native to South America, the sunflower is cultivated worldwide as it adapts to different climates and soils. Its seeds are consumed as food due to their high nutritional value, used in the kitchen to produce cooking oil or offered to consumers as nuts [11].

In this study, the antioxidant, anti-browning and antiproliferative effects against healthy fibroblast cells of extracts obtained from different parts of 4 registered sunflower plants grown within the scope of the adaptation project were examined and their potential as an effective, low-cost, new food additive that can be used safely was determined.

MATERIAL AND METHOD

Plant Material

DERAY, P64 LP 130, TR 2242 CL and SY GRANIT genotypes used in the study were obtained from Edirne Trakya Agricultural Research Institute within the scope of the project "Determination of Adaptations of Oil and Snack Sunflower Genotypes Suitable for Eastern Anatolia Region" and tested at Erzurum Eastern Anatolia Agricultural Research Institute sites in 2023. The plant material was provided by the project leader, agricultural engineer, M.sc Aysema Tazegul Cavusoglu.

Preparation of Extracts from Different Parts of 4 Varieties of *Helianthus annuus L.*

H. annuus seeds, ray florets, disc florets, leaves, stems and receptacle were dried and extracted separately with MeOH three times at 40℃. The extracts were combined and evaporated under vacuum to obtain the main extracts. After freeze-drying, they were stored at 4 °C to be used for biological activity studies.

Antiproliferative Effect

To evaluate the effect of the extracts on the viability of the L929 cell line (mouse fibroblasts), cells were plated at a density of 1×10^5 cells per well and cultured for 24 hours under appropriate conditions in an incubator. Then the cells were incubated for 48 hours with extracts in the concentration range of 0-800 µg/ml. After incubation, 10 µl of MTT solution (5 mg/ml in PBS) was added and incubated for 4 hours. Formazan crystals were then dissolved in 100 µl DMSO and the absorbance values were measured at 570 nm using a microplate reader. The antiproliferative effect was expressed as percentage viability [12].

DPPH Radical Scavenging Effect

The DPPH radical scavenging effect was determined by spectroscopic evaluation of the color change of the methanolic 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution, which changed from purple to yellow [13]. The analyzes were repeated three times with gallic acid as standard. The results were expressed as gallic acid equivalents.

Trolox Equivalent Antioxidant Capacity (TEAC)

The ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity of

methanol extracts obtained from different parts of *H. annuus* was evaluated by spectrophotometric measurement of color change at 734 nm. The unit of total antioxidant activity was expressed as mg Trolox/g extract [14].

Copper Reducing Antioxidant Capacity (CUPRAC)

The antioxidant capacity, which reduces copper ions, was determined according to the method of Özyürek et al. The change in absorbance values was measured at 450 nm [15]. A standard curve was prepared with different concentrations of the standard compound Trolox. The unit of total antioxidant activity was expressed as mg Trolox/g extract.

Mushroom Tyrosinase Enzyme Inhibition Assay

The anti-browning effect of the extracts was determined by tyrosinase inhibition test. The method developed by Kim et al. was modified in some respects [16-18]. In this method, in which kojic acid was used as a standard compound (positive control), L-tyrosine was determined as a substrate and the dopachrome extinction was measured spectrophotometrically at a wavelength of 475 nm, which results from the reaction of substrate and enzyme.

Statistical Analysis

All experiments were performed in triplicate and data are presented as mean ± SD. Statistical analysis of the results was performed using one-way ANOVA (analysis of variance) followed by Duncan's test using SPSS-22 software, and $p < 0.001$ was considered significant.

RESULT AND DISCUSSION

Antiproliferative Effect Evaluation of the Extracts

The antiproliferative effect of methanol extracts from seeds, ray florets, disc florets, leaves, stems and receptacles of the 4 registered varieties *H. annuus* (DERAY, SY GRANIT, P64 LP 130, TR 2242 CL) on normal L929 fibroblast cells was investigated using the MTT method and the maximum non-toxic concentration range was determined. The extracts were applied in a concentration range of 0-800 µg/ml, and cell viability was close to 100% even at the highest concentration of 800 µg/ml. Receptacle methanol extract of TR 2242 CL methanol extract inhibited cell viability by 12.04% only at the highest concentration (800 µg/ml), but was found not to affect cell viability as the concentration decreased. Cell viability at the highest concentration of methanol extracts from the DERAY and SY GRANIT were 93%. Leaf methanol extracts were found to reduce cell viability at 800 µg/ml in all varieties. However, the cytotoxic effect was found to disappear 400 µg/ml (Figure 1-4).

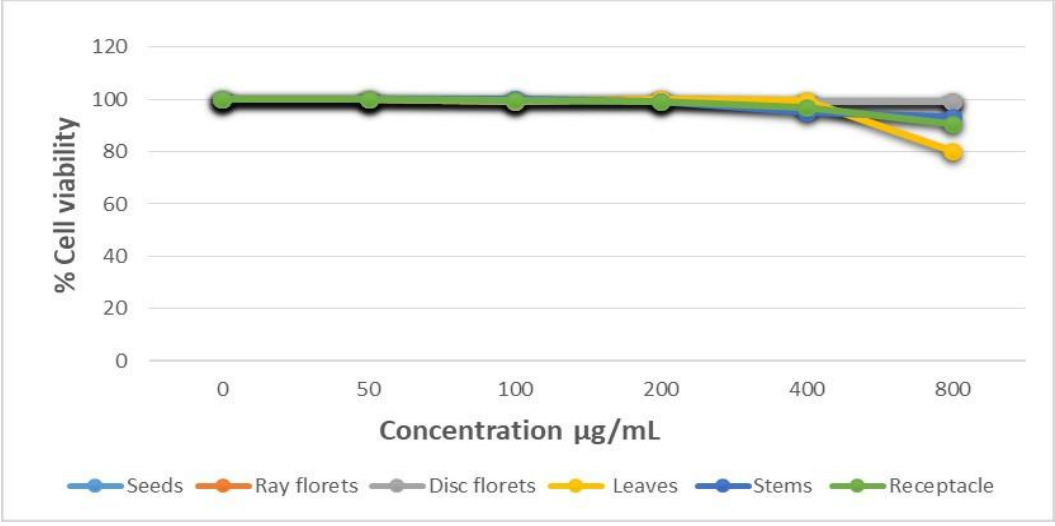

Figure 1. Antiproliferative effect of the DERAY extracts against L929

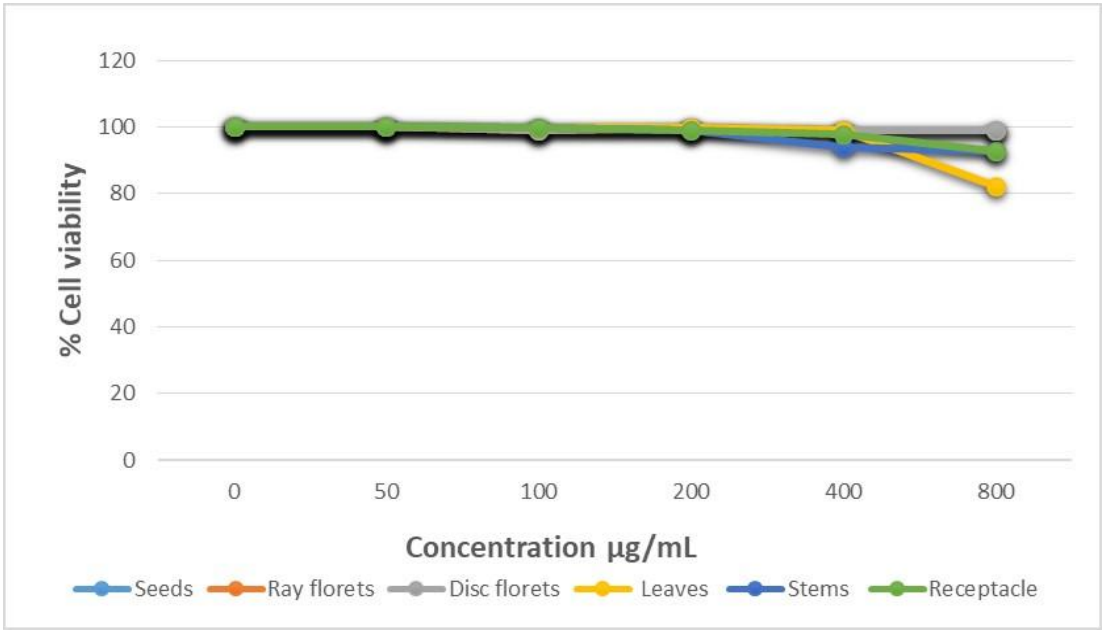

Figure 2. Antiproliferative effect of the SY GRANIT extracts against L929

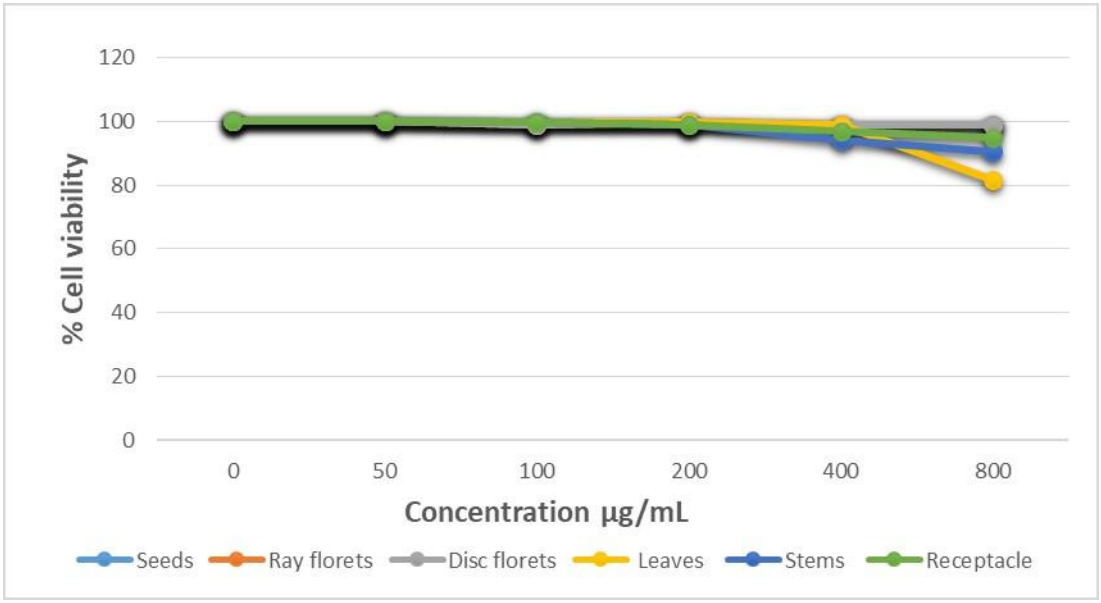

Figure 3. Antiproliferative effect of the P64 LP 130 extracts against L929

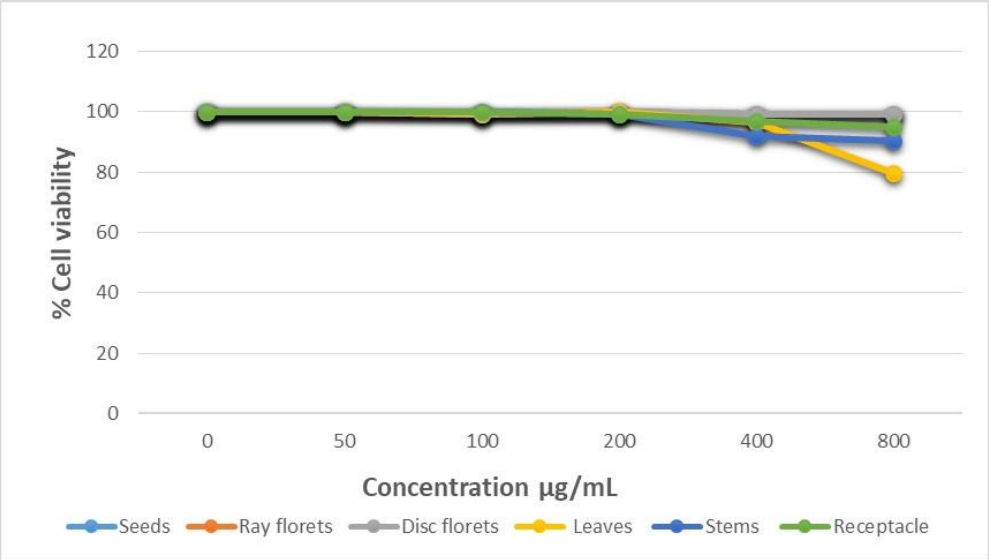

Figure 4. Antiproliferative effect of the TR 2242 CL extracts against L929

Antioxidant Capacity of the Extracts

The antioxidant capacity of methanol extracts of 4 different *H. annuus* varieties was determined by DPPH, TEAC and CUPRAC analyses. While all three types of methods, disc floret and ray floret, were found to be the most effective, the weakest effect was seen in stem methanol extracts (Table 1-4). In the radical scavenging effect of DPPH, where gallic acid was used as the standard compound, ray floret of the TR 2242 CL variety showed the highest effect with a value of 101.40 mg gallic acid/g extract. DERAY disc floret with 98.12 mg trolox/g extract value in the CUPRAC method and SY GRANIT disc floret with 83.80 mg trolox/g extract value in the TEAC method were found to be the most effective extracts.

Table 1. Antioxidant capacity of DERAY extracts

Plant Part	DPPH[a]	CUPRAC[b]	TEAC[b]
Seeds	48.32 ± 0.94	62.29 ± 1.84	53.57 ± 1.46
Ray floret	98.16 ± 0.90	86.24 ± 1.24	79.60 ± 1.82
Disc floret	94.24 ± 1.84	98.12 ± 1.28	73.22 ± 2.34
Leaves	58.14 ± 2.40	60.25 ± 1.84	69.46 ± 1.42
Stems	13.42 ± 0.62	19.18 ± 0.64	19.68 ± 0.84
Receptacle	24.32 ± 0.99	20.55 ± 0.94	26.56 ± 1.02

Data are presented as mean ± SD, n=3 experiments, ($p < 0.001$)
a: mg gallic acid/g extract**,** b: mg trolox/g extract

Table 2. Antioxidant capacity of SY GRANIT extracts

Plant Part	DPPH[a]	CUPRAC[b]	TEAC[b]
Seeds	58.122 ± 1.06	84.15 ± 2.01	69.05 ± 1.26
Ray floret	99.14 ± 1.96	77.26 ± 1.68	75.43 ± 1.22
Disc floret	92.26 ± 1.44	78.80 ± 1.28	83.80 ± 1.64
Leaves	60.18 ± 1.80	63.49 ± 1.84	59.05 ± 1.42
Stems	14.12 ± 0.84	10.17 ± 0.94	14.07 ± 0.94
Receptacle	28.12 ± 0.98	24.81 ± 0.74	24.75 ± 1.22

Data are presented as mean ± SD, n=3 experiments, ($p < 0.001$)
a: mg gallic acid/g extract**,** b: mg trolox/g extract

Table 3. Antioxidant capacity of P64 LP 130 extracts

Plant Part	DPPH[a]	CUPRAC[b]	TEAC[b]
Seeds	62.02 ± 1.46	63.81 ± 2.01	53.71 ± 1.64
Ray floret	98.20 ± 2.24	66.58 ± 1.88	70.01 ± 1.42
Disc floret	99.88 ± 1.04	68.88 ± 2.08	78.28 ± 1.84
Leaves	54.28 ± 1.84	47.02 ± 1.14	59.14 ± 1.02
Stems	11.08 ± 0.94	9.07 ± 0.84	12.07 ± 0.98
Receptacle	30.82 ± 0.98	32.04 ± 0.94	31.99 ± 1.20

Data are presented as mean ± SD, n=3 experiments, ($p < 0.001$)
a: mg gallic acid/g extract**,** b: mg trolox/g extract

Table 4. Antioxidant capacity of TR 2242 CL extracts

Plant Part	DPPH[a]	CUPRAC[b]	TEAC[b]
Seeds	60.32 ± 1.86	44.66 ± 1.81	46.66 ± 1.24
Ray floret	101.40 ± 2.64	76.72 ± 1.08	70.01 ± 2.42
Disc floret	99.08 ± 1.08	78.73 ± 2.68	75.61 ± 1.64
Leaves	58.24 ± 1.64	36.70 ± 1.40	53.71 ± 2.02
Stems	14.28 ± 0.96	11.90 ± 0.88	15.69 ± 1.02
Receptacle	34.84 ± 1.02	30.81 ± 0.98	31.81 ± 1.20

Data are presented as mean ± SD, n=3 experiments, ($p < 0.001$)
a: mg gallic acid/g extract, b: mg trolox/g extract

Mushroom Tyrosinase Inhibition Activity

By studying the inhibition of the enzyme tyrosinase, which is responsible for the enzymatic browning of food, the potential of the extracts for use as food additives was determined. The results were calculated using the standard compound kojic acid and are given as mg KAE/g extract. The highest activity to inhibit the mushroom tyrosinase enzyme was observed in methanol extracts of disc floret, followed by ray floret. While no activity was observed in the leaf extracts of all varieties, low inhibition was observed only in SY GRANIT and P64 LP 130 in the stem extracts (Table 5).

Table 5. Mushroom tyrosinase inhibition of *H. annuus* extracts (mg KAE/g extract)

Varieties	Ray floret	Leaves	Stems	Disc floret	Seeds	Receptacle
DERAY	60.01 ± 0.98	na	na	125.31 ± 1.44	55.70 ± 0.82	30.61 ± 1.68
SY GRANIT	55.48 ± 1.42	na	8.57 ± 0.84	106.12 ± 1.86	49.59 ± 1.06	41.02 ± 1.24
P64 LP 130	50.43 ± 0.88	na	5.46 ± 0.86	147.23 ± 1.64	46.53 ± 0.98	40.06 ± 1.06
TR 2242 CL	60.42 ± 0.98	na	na	151.25 ± 1.20	30.68 ± 1.04	34.32 ± 0.86

* Data are are means±S.D. of three parallel measurements ($p < 0.05$). KAE, kojic acid equivalents. na: not active

The growing population and changing lifestyles are leading to significant changes in the composition of food. In addition, changing eating habits have led to a significant change in the demand for food. The food industry is striving to increase the shelf life and quality of food by using additional food additives and preservatives [3,19].

In the food industry many compounds used as additives to extend the taste, appearance and shelf life of food have undesirable side effects on health. All these negative effects show that agricultural food by-products, which are renewable raw materials, can be one of the possible solutions.

The sunflower is one of the oilseeds that play an important role worldwide as a source of first-class oil and fiber, which are of great benefit to human health. The products of the sunflower (*H. annuus*), a plant cultivated worldwide, are mostly consumed as food in the kitchen or marketed as animal feed. Thanks to the sunflower's adaptation to different climatic and soil conditions, its importance as an oilseed crop has increased worldwide [11].

In Türkiye, about half of the vegetable oil demand is covered by sunflowers. Some of the main reasons why production is so high are its drought and cold tolerance compared to other oil-producing crops, its good adaptability to all soil types and its adaptability to different ecosystems [20].

In this study, the antioxidant, antityrosinase and antiproliferative effects of 4 different sunflower genotypes were investigated and the use of different plant parts as potential food additives and the seeds used as food were tested. Since both safe use and efficacy were sought, the cytotoxic effects of 24 different extracts on L929 healthy fibroblast cells were investigated. The antiproliferative activity of the extracts at different concentrations (0-800 µg/ml) was investigated, and the viability of all extracts was calculated to be over 90% at a concentration of 400 µg/ml.

The antioxidant effect was tested using 3 different methods. Disc floret and ray floret extracts showed the highest activity in all methods. Compared to other varieties in ray floret of TR 2242 CL cultuvar showed the highest antioxidant capacity with 101.40 mg gallic acid/g extract.

Enzymatic browning, one of the biggest problems in the food industry, was tested with the inhibition of the mushroom tyrosinase enzyme. The results were parallel to the results of the determination of antioxidant capacity. The TR 2242 CL varieties, which has the highest antioxidant effect, was also found to have the highest enzyme inhibition (151.25 mg KAE/g extract).

In a study by Özcan et al., the antioxidant effect of DERAY seeds was examined using the DPPH method and a value of 1.73 mmol Trolox (TE)/kg extract was determined [21].The antioxidant activity of various extracts from the disc and ray florets of *H. annuus* was investigated by a few methods, including the free radical scavenging effects of DPPH and ABTS. It was found that 90% (v/v) aqueous methanol floret extracts had the highest phenolic content and antioxidant capacity [22]. In a study comparing the antioxidant and enzyme-inhibiting activities of oils from the seeds of seven new sunflower (*Helianthus annuus* L.) lines, only the seed oils of the APO41, APO42, APO43 and BOH3 lines showed tyrosinase enzyme inhibition [23]. In the seed methanol extracts of the same lines, all lines showed a significant inhibition of the tyrosinase enzyme in the range of 52.94–60.43 mg kojic acid equivalent/g and considerable antioxidant activity. The antioxidant capacity of the methanol extracts of the seeds of the lines ranged between 22.60-40.42 mg TE/g in the DPPH method, 19.46-31.90 mg TE/g in the ABTS method and 59.98-117.86 mg TE/g in the CUPRAC method. [24]. Our antioxidant capacity results were similar to the previous studies and the tyrosinase enzyme inhibition of the seeds was found to be in the range of 30.68-55.7 mg kojic acid equivalent/g extract.

In a study by Mutiah et al. the antiproliferative effect of sunflower leaf, stem and root extracts against HeLa cells was determined using the MTT method. While the IC_{50} value of the root and stem extracts was >1000 µg/ml, it was found to be 153.76 µg/ml and 126.6 µg/ml for the seeds and leaves, respectively [25]. In another study, the antiproliferative effect of 3 different meal extracts obtained from sunflower seeds on L929 cells in the concentration range of 6.25-100 mg/ml was investigated and it was found that they did not damage healthy cells in this concentration range. The ABTS radical scavenging activity was 709.48-736.40 mg Trolox/100 g and the DPPH radical scavenging activity was 1320.12-1597.60 mg Trolox/100 g [26]. In our results, the antiproliferative effect against L929 cells in the range of 50-800 µg/ml was investigated, and similar to the studies of Adascălului et al., it was found that the healthy viability of the cells was continued almost without damage.

Sunflower is cultivated worldwide to meet nutritional, medicinal and industrial needs. The seeds, which play an important role in the food industry and nutrition, are rich in antioxidants, proteins, vitamins and trace elements and are used for oil extraction. Usually, only the seeds of the plant are in the foreground, while other parts are considered as agricultural by-products. In our study, the antioxidant, the antibrowning and the antiproliferative effect were carried out for the first time on six different parts of sunflowers belonging to four genotypes and the potential of the plant parts as food additives was investigated. Thus, by-products were obtained that may contain value-added compounds with high functionality and/or bioactivity for the agriculture- food industry.

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AUTHOR CONTRIBUTIONS

Concept: Z.B.G., A.T.Ç.; Design: Z.B.G.; Control: Z.B.G.; Sources: Z.B.G., A.T.Ç.; Materials: Z.B.G., A.T.Ç.; Data Collection and/or Processing: Z.B.G.; Analysis and/or Interpretation: Z.B.G.; Literature Review: Z.B.G., A.T.Ç.; Manuscript Writing: Z.B.G.; Critical Review: Z.B.G., A.T.Ç.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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