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# Antioxidant, anti-tyrosinase activities and characterization of phenolic compounds for some plants from the Marmara Region, Türkiye

**Zehra İlke MERİÇ [1], Ebru ÖZDEMİR NATH [2], Ahmet DOĞAN [3], Leyla BİTİŞ [1]***

[1] Department of Pharmacognosy, Faculty of Pharmacy, Marmara University, Başıbüyük 34854 İstanbul, Turkey.
[2] Department of Pharmaceutical Botany, Faculty of Pharmacy, Altınbaş University, Bağcılar 34217 İstanbul, Turkey.
[3] Department of Pharmaceutical Botany, Faculty of Pharmacy, Marmara University, Başıbüyük 34854 İstanbul, Turkey.
* Corresponding Author. E-mail: lbitis@yahoo.com, leylabitis@marmara.edu.tr (L.B.).

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**ABSTRACT**: In this study, antioxidant, anti-tyrosinase, and sun protection factor (SPF) values of 26 extracts obtained from 24 plants naturally grown in the Marmara Region were investigated, and phenolic compound characterization of 8 active plants was performed. All of the plants mentioned in this study have been evaluated for their Sun Protection Factor (SPF) values for the first time, as well as 3 of them evaluated for antioxidant activity and 15 of them evaluated for tyrosinase inhibition for the first time. The results showed that the plant extracts generally exhibited high antioxidant activities. In terms of DPPH radical scavenging activity, *Cota tinctoria* (L.) J. Gay exhibited a very close $IC_{50}$ value (0.038 mg/mL) to the standard compounds, ascorbic acid and quercetin. *Plantago major* L. subsp. *intermedia* (Gilib.) Lange demonstrated the highest CUPRAC radical scavenging activity (0.187 mM ascorbic acid equivalent). *Hypericum perforatum* L. was determined to have the highest total phenolic content (0.268 mg GAE g/extract). Among the plant extracts, *Sambucus ebulus* L. fruit extract exhibited the highest tyrosinase inhibition ($IC_{50}$ 0.08 mg/mL), showing a similar effect to the standard compound kojic acid. The extract with the highest SPF value was calculated *Inula oculus-christi* L. extract, with a value of 28.55. The phenolic compound analysis of eight plants, which have been determined to exhibit high efficacy in both antioxidant activities and tyrosinase inhibition, was conducted. Some of phenolic compounds obtained from these eight plants were novel for these species. According to the experiments conducted in this study, *Euphorbia helioscopia* has high potential as natural sources of antioxidants and skin whiteners.

**KEYWORDS**: Skin whitening; Total phenolic content; Sun protection factor (SPF); phenolic compounds.

## 1. INTRODUCTION

Although oxygen is an essential element for life, it can cause significant damage to living organisms through oxidative processes. Humans require approximately 21% oxygen in the atmosphere to sustain their metabolic and, especially, mental activities. In contrast, plants can be exposed to 100% oxygen concentration in the chloroplasts during the illuminated process of photosynthesis, which can lead to considerable oxidative damage. Oxidative damage can be defined as an imbalance between oxidants and antioxidants. To protect against this damage, plants have developed antioxidant defense mechanisms, such as tocopherols, flavonoids, carotenoids, high levels of ascorbic acid, and other phenolic compounds [1,2]. Oxidative stress can cause damage through processes such as lipid peroxidation, protein oxidation, and nucleic acid oxidation [3,4]. This damage can lead to various health problems, including autoimmune, cerebrovascular, endocrine, kidney diseases; cardiovascular, gastrointestinal disorders; cancer, and infertility [5–14]. Consequently, there has been an increase in the use of antioxidant preparations in recent times.

Tyrosinase is an enzyme used in various fields, such as health, cosmetics, food, and environmental technology. Tyrosinase inhibition is a method used to investigate natural sources with potential effects, such as skin spot removal, prevention of browning in fruits and vegetables, development of enzymatic sensors for agricultural pesticides, pathogens and genetically modified organisms (GMO) detection, and reduction of neurotoxicity associated with Parkinson's disease [15–18].

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Tyrosinase plays a significant role in melanin synthesis. Melanin, produced by melanocytes is the pigment that determines skin and hair color. Tyrosinase converts the amino acid tyrosine into DOPA and subsequently catalyzes the synthesis of melanin, leading to the formation of the melanin pigment [18–20]. Due to the cultural and social media-driven perception that flawless and light skin tone is more beautiful and healthier, there has been a high demand for cosmetic products that can remove skin spots and lighten the skin color. Some inhibitors, such as hydroquinone, have been used as skin whitening agents; however, they have disadvantages, including irritation, erythema, chromosomal abnormalities, potential mutagenicity for mammalian cells, toxic effects, and side effects such as contact dermatitis. These risks have led to strict regulations on the use of such products in many countries, accelerating the search for natural substances with low side effect risks and high efficacy [21–24].

For centuries, humanity has regarded the sun as a God due to its provision of life, warmth, and brightness. Although sun worship rituals continue, the harmful effects of the sun on the skin have also been recognized. The Egyptians were the first civilization to notice this and started using photoprotective methods around 4000 BC. Information has been passed down to the present day regarding the use of rice, jasmine, and bitter bean in the composition of modern-day skin protectants. In ancient Greece, athletes preparing for the Olympic Games in 500 BC reportedly protected themselves from the sun by covering their bodies with a mixture of oil and sand (similar to the effects of zinc oxide, a physical sunblock used today) [25]. Each year, approximately one million people are diagnosed with skin cancer, and about 10,000 people die from malignant melanoma. Most skin cancers occur in areas of the body that are most exposed to the sun, such as the face, neck, head, and back of hands [26]. Sun Protective Factor (SPF) is a quantitative measure of the effectiveness of a sunscreen formulation. A broad absorption range between 290 and 400 nm is necessary for a sun-protective product to be effective in avoiding sunburn and other skin damage [26]. Ultraviolet A and B (UVA and UVB) radiations are primarily responsible for various skin pathologies, including sunburns, cutaneous degeneration, photosensitivity, phototoxicity, actinic elastosis, photoaging, and immunosuppression, and they have been identified as a contributing factor to skin cancer caused by ultraviolet radiation [26–28].

Recent trends have shown a preference for products containing low-risk botanical extracts in response to the increasing demand for skin lightening products. It would be beneficial for a potential product under development to include botanical extracts with high SPF values for protection against the harmful effects of UV radiation and antioxidant properties aimed at reducing existing skin damage.

## 2. RESULTS & DISCUSSION

### 2.1. Antioxidant Activity

In this study, 20 of the 26 extracts with DPPH radical scavenging activity were found to be very highly effective (80% and higher). Among these extracts, *Cota tinctoria* displayed remarkable efficacy, with $IC_{50}$ values very close to the standard ascorbic acid and quercetin. The $IC_{50}$ value for ascorbic acid was determined as 0.022 mg/mL, and for quercetin, it was 0.028 mg/mL. For *Cota tinctoria*, the $IC_{50}$ value was calculated as 0.038 mg/mL. The plant with the second-highest effect was *Euphorbia helioscopia*, with an $IC_{50}$ value of 0.048 mg/mL, followed by *Plantago major* subsp. *intermedia* with an $IC_{50}$ value of 0.077 mg/mL. *Astragalus angustifolius* extract and *Sambucus ebulus* fruit extracts showed the lowest radical scavenging effect and $IC_{50}$ values were calculated as 1.546 mg/mL and 1.493 mg/mL, respectively.

When CUPRAC radical scavenging activity was examined, *Plantago major* subsp. *intermedia* was determined to be the most potent extract, showing a radical scavenging effect equivalent to 0.187 mM ascorbic acid (AAEC). The second highest effect was observed *Cota tinctoria* (0.164 mM AAEC). However, despite demonstrating a relatively lower CUPRAC radical scavenging effect (0.148 mM AAEC), *Euphorbia helioscopia* exhibited the second highest effect in terms of DPPH radical scavenging.

In previous studies, the methanol extract of *Plantago major* subsp. *intermedia* and *Cota tinctoria* were evaluated for its antioxidant activity using DPPH, ABTS, and CUPRAC assays, and it was found to be effective [29]. The high antioxidant effect of *Cota tinctoria* extract is believed to be attributed to its abundant hydroxy acid derivatives (3-hydroxy 4-methoxy cinnamic acid, 4-hydroxy benzoic acid, vanillic acid) and flavonoids (apigenin, hyperoside, kaempferol, luteolin, hesperidine, galangin, rutin and quercetin) in its content, which possess significant antioxidant potential [30,31]. Another study involving *Thymbra spicat*a evaluated methanol extracts prepared using hydrodistillation and microwave-assisted extraction methods for their antioxidant activity using DPPH and FRAP assays. Both methods reported that the extracts were highly effective in terms of antioxidant activity [32]. *Euphorbia helioscopia* has been studied for its antioxidant activity using the DPPH method for both methanol and ethanol extracts, and it was found to be effective [33]. Phenolic compounds,

one of the main classes of secondary metabolites in plants, act as antioxidants to protect from oxidative degradation at low concentrations [34]. Previous studies have evaluated the fruit, leaf, and flower extracts of the *Sambucus ebulus* in terms of DPPH and CUPRAC radical scavenging activity and tyrosinase inhibition. In that study, the leaf extract demonstrated the highest antioxidant activity, and our study supports these findings [35,36]. *Oenanthe pimpinelloides*, *Calepina irregularis* and *Gallium verum* subsp. *glabrescens* extracts have been evaluated for the first time in this context, but their effects have been found to be quite low for antioxidant activity.

**2.2. Total Phenolic Content (TPC)**

A substance with a phenol residue is referred to as a phenolic compound, which indicate a significantly large chemical group. They are naturally found in every plant and exhibit a wide range of biological activities, such as antioxidant, antimicrobial, and anti-inflammatory effects [37]. When considering the total phenolic content, *Hypericum perforatum* possesses the highest amount of phenolic material with 0.268 mg gallic acid equivalent (GAE)/extract. It has been demonstrated in previous studies that *Hypericum perforatum* plant contains a high number of phenolic compounds [38–40]. The subsequent plant with the second highest phenolic content is *Plantago major* subsp. *intermedia* have 0.256 mg GAE/extract. *Euphorbia helioscopia* has also been investigated for its phenolic compounds in the literature, and the highest phenolic content has been reported in its flowers [33]. In this study, extracts obtained from the aerial parts of the plant were used, and a considerably high level of phenolic compound content (0.227 mg GAE/extract) was determined.

**2.3. Tyrosinase Inhibition**

In the study evaluating tyrosinase inhibition, the $IC_{50}$ value of the standard compound kojic acid was found to be 0.069 mg/mL, and the closest $IC_{50}$ values were exhibited by *Sambucus ebulus* fruit extract ($IC_{50}$ 0.080 mg/mL) and *Euphorbia helioscopia* ($IC_{50}$ 0.083 mg/mL). The plant with the third highest tyrosinase inhibition was *Astragalus angustifolius*, with an $IC_{50}$ value of 0.120 mg/mL. The fourth and fifth plants with the highest effects were *Viscum album* and *Centaurea benedicta*, with $IC_{50}$ values of 0.188 mg/mL and 0.220 mg/mL, respectively. While a literature search revealed that *Sambucus ebulus* had the highest effect on tyrosinase inhibition in leaf extracts obtained through subcritical water extraction [35], our study found the fruit extract to have the highest effect. Chemical analysis of *Sambucus ebulus* L. fruits revealed the presence of phenolic acids, flavonoids, and their derivatives and resveratrol-3-O-glucoside [41]. Previous studies have demonstrated that resveratrol-3-O-glucoside exhibits very low $IC_{50}$ values and is an effective compound for tyrosinase inhibition. [42]. In this study, the predominant reason for the high antioxidant effects observed in the majority of the utilized plants is could be correlated their rich content of phenolic compounds (phenolic acids, flavonoids, stilbenes). This difference in results could be attributed to variations in extraction methods or the timing of plant collection. *Euphorbia helioscopia* and *Astragalus angustifolius* have been evaluated for the first time in this context. Previous studies have demonstrated the presence of triterpenic compounds in *Euphorbia helioscopia*, and our literature review has shown the high potential of triterpenic compounds in tyrosinase inhibition [43,44]. Therefore, the high tyrosinase inhibition activity of this plant could be attributed to its content of triterpenes and phenolic compounds [33,43-45]. Earlier research has demonstrated that condensed tannins exhibit significant tyrosinase inhibition potential. It was also worth the mentioning that the *Astragalus angustifolius* plant's notable tyrosinase inhibition is attributed to the presence of tannins and flavones in its composition [46,47].

**2.4. Sun Protection Factor**

SPF is a vital numerical sorting system that quantifies a product's ability to shield against harmful sunlight. Accurate measurement of SPF values is crucial in evaluating the effectiveness of sunscreens. A higher SPF value signifies superior protection against UV radiation. In this study methanol extracts of plants were evaluated. Most of the plants evaluated in this study showed average or higher SPF values. It has been observed that four out of the calculated SPF values of plants are >20. *Inula oculus-christi* extract showed the highest SPF value 28.55. The top 4 plants with the highest SPF values were determined as follows: *Inula oculus-christi* extract, *Stachys cretica L.* 25.83, *Plantago major* subsp. *intermedia* 24.18, *Salvia amplexicaulis* Lam. 22.82. None of the plants mentioned in this study have been previously evaluated for their SPF values. Considering that even the lowest SPF value was calculated as 9, it was understood that all the plants studied were slightly protective against UV radiation.

The antioxidant, total phenolic content, antityrosinase and sun protection factor analysis results of plant extracts were expressed in Table 1.

**Table 1.** TPC, Antioxidant, Anti-tyrosinase, SPF activities of plant extracts

| Family | Species | DPPH IC50mg/mL | TPC mg GAE mg/extract | CUPRAC mM AAE | Tyrosinase Inhibition IC50 mg/mL | SPF (1mg/mL) |
|---|---|---|---|---|---|---|
| Adoxaceae | *Sambucus ebulus* (leaf) | 0.596 ± 0.004 | 0.109±0.003 | 0.058 ± 0.006 | 0.234 ± 0.009 | 13.69 ± 3.567 |
| Adoxaceae | *Sambucus ebulus* (flower) | 0.751 ± 0.002 | 0.133±0.008 | 0.043 ± 0.003 | 0.713 ± 0.022 | 11.42 ± 8.152 |
| Adoxaceae | *Sambucus ebulus* (fruit) | 1.493 ± 0.136 | 0.116±0.010 | 0.039 ± 0.003 | 0.08 ± 0.030 | 10.25 ± 0.801 |
| Apiaceae | *Oenanthe pimpinelloides* (Aerial) | 0.391 ± 0.005 | 0.126±0.002 | 0.078 ± 0.004 | 0.565 ± 0.214 | 16.1 ± 2.446 |
| Araceae | *Dracunculus vulgaris* (Aerial) | 0.747 ± 0.022 | 0.091±0.005 | 0.032 ± 0.003 | 0.348 ± 0.059 | 10.04 ± 0.821 |
| Asteraceae | *Achillea arabica* (Aerial) | 0.159 ± 0.026 | 0.176±0.011 | 0.142 ± 0.008 | 0.386 ± 0.034 | 9.11 ± 1.610 |
| Asteraceae | *Bellis perennis* (Aerial) | 0.37 ± 0.013 | 0.138±0.006 | 0.064 ± 0.008 | 0.751 ± 0.021 | 17.76 ± 0.52 |
| Asteraceae | *Centaurea benedicta* (Aerial) | 0.608 ± 0.004 | 0.123±0.003 | 0.077 ± 0.018 | 0.220 ± 0.023 | 15.47 ± 0.958 |
| Asteraceae | *Cota tinctoria* (Aerial) | 0.038 ± 0.014 | 0.225±0.027 | 0.164 ± 0.008 | 0.271 ± 0.020 | 17.11 ± 13.73 |
| Asteraceae | *Crepis sancta* (Aerial) | 0.426 ± 0.001 | 0.150±0.004 | 0.073 ± 0.001 | 0.297 ± 0.053 | 16.18 ± 0.667 |
| Asteraceae | *Inula oculus-christi* (Aerial) | 0.458 ± 0.101 | 0.137±0.003 | 0.083 ± 0.007 | 0.438 ± 0.009 | 28.55 ± 3.028 |
| Brassicaceae | *Calepina irregularis* (Aerial) | 0.892 ± 0.109 | 0.118±0.004 | 0.037 ± 0.004 | 0.468 ± 0.027 | 13.9 ± 0.902 |
| Brassicaceae | *Sinapis alba* (Aerial) | 0.528 ± 0.042 | 0.166±0.015 | 0.053 ± 0.003 | 0.340 ± 0.046 | 10.98 ± 2.891 |
| Euphorbiaceae | *Euphorbia helioscopia* (Aerial) | 0.048 ± 0.001 | 0.227±0.003 | 0.148 ± 0.015 | 0.083 ± 0.058 | 18.6 ± 0.014 |
| Fabaceae | *Astragalus angustifolius* (Aerial) | 1.546 ± 0.263 | 0.053±0.020 | 0.069 ± 0.004 | 0.120 ± 0.028 | 9.06 ± 2.02 |
| Hypericaceae | *Hypericum perforatum* (Aerial) | 0.138 ± 0.002 | 0.268±0.029 | 0.154 ± 0.012 | 0.332 ± 0.008 | 18.34 ± 11.22 |
| Lamiaceae | *Marrubium vulgare* (Aerial) | 0.285 ± 0.01 | 0.123±0.011 | 0.077 ± 0.008 | 0.327 ± 0.034 | 18.99 ± 0.106 |
| Lamiaceae | *Mentha longifolia* subsp. *typhoides* (Aerial) | 0.130 ± 0.004 | 0.162±0.011 | 0.144 ± 0.004 | 0.424 ± 0.009 | 16.47 ± 6.845 |
| Lamiaceae | *Micromeria myrtifolia* (Aerial) | 0.258 ± 0.002 | 0.137±0.009 | 0.092 ± 0.016 | 0.282 ± 0.016 | 15.66 ± 8.208 |
| Lamiaceae | *Salvia amplexicaulis* (Aerial) | 0.137 ± 0.005 | 0.168±0.004 | 0.118 ± 0.015 | 0.450 ± 0.063 | 22.82 ± 5.878 |
| Lamiaceae | *Stachys cretica* (Aerial) | 0.24 ± 0.002 | 0.143±0.004 | 0.108 ± 0.005 | 0.491 ± 0.043 | 25.83 ± 0.335 |
| Lamiaceae | *Thymbra spicata* subsp *spicata* (Aerial) | 0.097 ± 0.006 | 0.204±0.013 | 0.139 ± 0.027 | 0.307 ± 0.057 | 10.2 ± 2.239 |
| Lythraceae | *Lythrum salicaria* (Aerial) | 0.110 ± 0.002 | 0.186±0.004 | 0.106 ± 0.009 | 0.280 ± 0.025 | 17.27 ± 8.846 |
| Plantaginaceae | *Plantago major* subsp. *intermedia* (Aerial) | 0.077 ± 0.077 | 0.256±0.011 | 0.187 ± 0.025 | 0.329 ± 0.010 | 24.18 ± 1.318 |
| Rubiaceae | *Galium verum* subsp. *glabrescens* (Aerial) | 0.521 ± 0.025 | 0.139±0.018 | 0.061 ± 0.001 | 0.519± 0.077 | 15.79 ± 0.477 |
| Santalaceae | *Viscum album* (Aerial) | 0.414 ± 0.018 | 0.126±0.009 | 0.059 ± 0.006 | 0.188 ± 0.110 | 16.72 ± 0.488 |
| | Ascorbic acid | 0.022 ± 0.000 | | | | |
| | Quercetin | 0.028 ± 0.001 | | | | |
| | Kojic acid | | | | 0.069 ± 0.003 | |

(Each value in the table is represented as mean ± SD (n=3).

### 2.5. Phenolic contents by LC-MS/MS

The phenolic compound analysis of eight plants, which have exhibited high efficacy in both antioxidant activities and tyrosinase inhibition, was conducted. Those eight plant extracts were investigated qualitatively by using LC-MS/MS. The results of detected phenolic compounds were showed in Table 2.

These compounds were identified for the first time in the literature for these plants: 3-hydroxy-4-methoxycinnamic acid (or 4-hydroxy-3-methoxycinnamic acid), *trans*-3-hydroxy-4-methoxycinnamic acid from *Cota tinctoria* (L.) J.Gay; 4-hydroxybenzoic acid, apigenin, galangin from *Astragalus angustifolius* Lam.; 4-hydroxy-3-methoxycinnamic acid (or 3-hydroxy-4-methoxycinnamic acid), 4-hydroxybenzoic acid, vanillic acid, and hydroxycinnamic acid, apigenin from *Centaurea benedicta* (L.) L.; ellagic acid, trans-caffeic acid, vanillic acid, rutin, and hyperoside from *Euphorbia helioscopia* L.; 3-hydroxy-4-methoxycinnamic acid (or 4-hydroxy-3-methoxycinnamic acid), 3,4-dimethoxy cinnamic acid, protocatechuic acid, apigenin, kaempferol from *Plantago major* subsp. *intermedia* (Gilib.) Lange; vanilic acid from *Sambucus ebulus* L.; 3-hydroxy-4-methoxycinnamic acid (or 4-hydroxy-3-methoxycinnamic acid), syringic acid, rosmarinic acid, protocatechuic acid, genkwanin from *Thymbra spicata* L.; 3-hydroxy-4-methoxycinnamic acid (or 4-hydroxy-3-methoxycinnamic acid), 4-hydroxybenzoic acid from *Viscum album* L. As a result, the most common phenolic compounds found in these 8 plants were 3-hydroxy-4-methoxy cinnamic acid (or 4- hydroxy 3-methoxy cinnamic acid), 4- hydroxy benzoic acid and vanilic acid.

**Table 2.** Phenolic compound contents of eight species

| Species | Retention Time (min) | [M-H]-m/z | Fragments | Identification |
|---|---|---|---|---|
| *Astragalus angustifolius* | 9.7 | 137 | 93 | 4-Hydroxybenzoic acid |
| | 9.05 | 271 | 153 | Apigenin |
| | 9.05 | 271 | 153 | Galangin |
| *Centaurea benedicta* | 9.47 | 193 | 134 | 3-hydroxy 4-methoxy cinnamic acid / 4-hydroxy 3-methoxy cinnamic acid |
| | 9.54 | 137 | 93 | 4-hydroxy benzoic acid |
| | 8.93 | 151 | 91 | Hydroxy cinnamic acid |
| | 2.38 | 153 | 109 | Protocatechuic acid |
| | 8.98 | 167 | 108 | Vanilic acid |
| | 9.94 | 285 | 133 | Luteolin |
| | 9.14 | 271 | 153 | Apigenin |
| *Cota tinctoria* | 9.46 | 193 | 134 | 3-hydroxy 4-methoxy cinnamic acid / 4-hydroxy 3-methoxy cinnamic acid |
| | 9.52 | 137 | 93 | 4-hydroxy benzoic acid |
| | 9.45 | 193 | 134 | *Trans*-3-hydroxy 4-methoxy cinnamic acid |
| | 8.96 | 167 | 108 | Vanilic acid |
| | 9.88 | 271 | 153 | Apigenin |
| | 8.88 | 463 | 300 | Hyperoside |
| | 8.91 | 287 | 153 | Kaempferol |
| | 8.75 | 609 | 301 | Hesperidine |
| | 9.88 | 271 | 153 | Galangin |
| | 9.60 | 285 | 133 | Luteolin |
| | 8.75 | 609 | 300 | Rutin |
| | 8.96 | 301 | 151 | Quercetin |
| *Euphorbia helioscopia* | 8.97 | 301 | 145 | Ellagic acid |
| | 1.83 | 169 | 125 | Gallic acid |
| | 9.02 | 167 | 108 | Vanilic acid |
| | 9.64 | 179 | 135 | *trans*-caffeic acid |
| | 8.88 | 463 | 300 | Hyperoside |
| | 9.11 | 271 | 119 | Naringenin |
| | 8.85 | 301 | 151 | Quercetin |
| | 8.74 | 609 | 300 | Rutin |

**Table 2.** Phenolic compound contents of eight species *(Continue)*

| Species | Retention Time (min) | [M-H]-m/z | Fragments | Identification |
|---|---|---|---|---|
| *Plantago major* subsp. *inte rmedia* | 9.46 | 193 | 134 | 3-hydroxy 4-methoxy cinnamic acid / 4-hydroxy 3-methoxy cinnamic acid |
| | 9.08 | 209 | 191 | 3,4-dimethoxy cinnamic acid |
| | 2.45 | 153 | 109 | Protocatechuic acid |
| | 9.13 | 271 | 153 | Apigenin |
| | 9.13 | 271 | 153 | Galangin |
| | 8.94 | 285 | 121 | Fisetin |
| | 8.95 | 287 | 153 | Kaempferol |
| | 8.95 | 285 | 133 | Luteolin |
| *Sambucus ebulus* (fruit) | 2.45 | 153 | 109 | Protocatechuic acid(3,4- dihydroxy benzoic acid) |
| | 9.01 | 167 | 108 | Vanilic acid |
| | 8.90 | 463 | 300 | Hyperoside |
| | 8.75 | 609 | 300 | Rutin |
| *Thymbra spicata* subsp *spicata* | 9.50 | 193 | 134 | 3-hydroxy 4-methoxy cinnamic acid / 4-hydroxy 3-methoxy cinnamic acid |
| | 9.55 | 137 | 93 | 4-hydroxybenzoic acid |
| | 9.48 | 193 | 134 | *Trans* -4-hydroxy 3-methoxy cinnamic acid |
| | 2.37 | 153 | 109 | Protocatechuic acid |
| | 9.23 | 359 | 161 | Rosmarinic acid |
| | 9.23 | 197 | 123 | Syringic acid |
| | 8.94 | 167 | 108 | Vanillic acid |
| | 9.87 | 271 | 119 | Naringenin |
| | 9.05 | 609 | 301 | Hesperidine |
| | 9.32 | 283 | 268 | Genkwanin |
| | 9.05 | 301 | 151 | Quercetin |
| *Viscum album* | 9.47 | 193 | 134 | 3-hydroxy 4-methoxy cinnamic acid / 4-hydroxy 3-methoxy cinnamic acid |
| | 9.51 | 137 | 93 | 4-hydroxy benzoic acid |
| | 2.38 | 153 | 109 | Protocatechuic acid |
| | 9.61 | 179 | 135 | *Trans*-caffeic acid |
| | 9.14 | 301 | 151 | Quercetin |
| | 8.94 | 167 | 108 | Vanilic acid |

## 3. CONCLUSION

This study presents a comprehensive investigation of plant extracts, evaluating their antioxidant activities, TPC, tyrosinase inhibitory effects, and SPF values. In the assessment of antioxidant activity, it was found that most of the 26 extracts obtained from 24 different plants were highly effective. In particular, the *Cota tinctoria* extract demonstrated remarkable efficacy comparable to that of the standard compounds ascorbic acid and quercetin. The elevated antioxidant activity of plant extracts is attributed to their abundance of phenolic compounds.

Regarding tyrosinase inhibitory effects, *Sambucus ebulus* fruit extract and *Euphorbia helioscopia* were identified as the most effective plant extracts. These plant extracts hold potential for various applications, such as skin spot removal and skin lightening, as well as in the development of enzymatic sensors for agricultural purposes and neurotoxicity reduction related to Parkinson's disease.

Additionally, SPF values were calculated for the plant extracts, revealing that many of them provide effective protection against harmful sunlight. *Inula oculus-christi* displayed the highest SPF value. These findings suggest that plant extracts can serve as natural sources of sun-protective products. In conclusion, this

study comprehensively explored the various biological effects of plant extracts. The rich diversity of plant extracts shows significant antioxidant activities, tyrosinase inhibitory effects, and effective sun protection properties. These findings encourage further research on the potential use of plant extracts in cosmetics, food, and health industries, particularly in the development of natural alternatives for sun protection products. Therefore, in this study, particular attention was given to selecting plant species that are commonly found in nature while avoiding endemic species. Future studies should delve deeper into understanding the effects of plant extracts and optimizing their utilization in commercial products.

## 4. MATERIALS AND METHODS

### 4.1. Preparation of Plant Extracts

A total of 24 plants collected from the Marmara region were used. The list of plants used is given in Table 3. Some of these plants were identified and stored in the herbarium of the Department of Pharmaceutical Botany at Marmara University Faculty of Pharmacy by Dr. Ahmet Doğan, Assistant Professor, while the remaining plants were identified and stored by Dr. Ebru Özdemir Nath, Assistant Professor, at the Department of Pharmaceutical Botany, Altınbaş University Faculty of Pharmacy. The list of used species shown in Table 3. The identified plants were dried in the shade. A 10 g powder was taken from each plant sample and placed in a flask. Then, 100 mL of methanol was added, and the mixture was allowed to rest overnight. The next day, the mixture was filtered through filter paper, and an additional 100 mL of methanol was added to the plant residues. This process was repeated five times resulting in 500 mL of filtrate. The filtrate was evaporated to dryness using a rotary evaporator. Residue was stored in refrigerator until further use.

**Table 3.** The list of used species and their voucher number

| | Species | Voucher |
|---|---|---|
| 1 | *Achillea arabica* Kotschy | ISTE 109618 |
| 2 | *Astragalus angustifolius* Lam. | ISTE 109729 |
| 3 | *Bellis perennis* L. | MARE 19147 |
| 4 | *Calepina irregularis* Thell. | MARE 19144 |
| 5 | *Centaurea benedicta* (L.) L. | ISTE 109621 |
| 6 | *Cota tinctoria* (L.) J.Gay | MARE 18461 |
| 7 | *Crepis sancta* (L.) Babc. | MARE 19146 |
| 8 | *Dracunculus vulgaris* Schott | ISTE 109555 |
| 9 | *Euphorbia helioscopia* L. | MARE 19143 |
| 10 | *Galium verum* L. subsp. *glabrescens* Ehrend. | MARE 18463 |
| 11 | *Hypericum perforatum* L. | MARE 18460 |
| 12 | *Inula oculus-christi* L. | MARE 18466 |
| 13 | *Lythrum salicaria* L. | ISTE 109765 |
| 14 | *Marrubium vulgare* L. | ISTE 109848 |
| 15 | *Mentha longifolia* (L.) L. subsp. *typhoides* (Briq.) Harley | MARE 18468 |
| 16 | *Micromeria myrtifolia* Boiss. & Hohen | ISTE 109809 |
| 17 | *Oenanthe pimpinelloides* L. | ISTE 109542 |
| 18 | *Plantago major* L. subsp. *intermedia* (Gilib.) Lange | MARE 19145 |
| 19 | *Salvia amplexicaulis* Lam. | MARE 18465 |
| 20 | *Sambucus ebulus* L. | MARE 11718 |
| 21 | *Sinapis alba* L. | MARE 19142 |
| 22 | *Stachys cretica* L. | ISTE 109789 |
| 23 | *Thymbra spicata* subsp. *spicata* L. | ISTE 109780 |
| 24 | *Viscum album* L. | EÖ61* |

*EÖ61=Ebru Özdemir personal collection

### 4.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity

The method is based on the measurement of the conversion of the dark violet color of DPPH to light yellow in the UV/visible region at 517 nm [48]. In our study, the method proposed by Lu et al. was modified and used [49]. A 0.1 mM DPPH solution was prepared. Plant extracts were prepared in the range of 63 µg to 1000 µg. Quercetin and ascorbic acid were used as standards. 40 µL of extract/blank/standard and 160 µL of DPPH were added to the wells, and the mixture was incubated at room temperature in the dark for 30 minutes. Absorbance measurements were taken at 517 nm. The experiment was carried out in triplicate. The DPPH radical scavenging percentage was calculated using the following formula:

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

$A_0$: Absorbance of 0.1 mM DPPH stock solution at 517 nm
$A_1$: Absorbance of the sample and DPPH solution at 517 nm

### 4.3. Copper (II) Ion Reducing Antioxidant Capacity (CUPRAC) Method

The CUPRAC method for copper (II) ion reducing antioxidant capacity was performed by modifying the method of Apak et al. [50]. In 96-well microplates, 50 µL of 10 mM $CuCl_2$, 50 µL of 7.5 mM neocuproine, and 60 µL of $NH_4Ac$ were added and mixed. Then, 40 µL of sample/blank/standard was added, and the mixture was incubated for one hour. Absorbance measurements were taken at 450 nm. The experiment was carried out in triplicate. Ascorbic acid was used as a positive standard.

### 4.4. Determination of Total Phenolic Content

The determination of total phenolic content was carried out using a modified method by Cao et al. [51]. Briefly, a mixture of 100 µL of diluted Folin-Ciocalteu reagent (1:5), 40 µL of 1M $Na_2CO_3$, 40 µL of distilled water, and 20 µL of the sample was placed in the wells of a 96-well microplate. After 80 minutes of incubation, the absorbance was measured at 756 nm. The experiment was carried out in triplicate. The total phenolic content was expressed as equivalents of gallic acid (GAE g/extract).

### 4.5. Tyrosinase Activity Assay

The tyrosinase activity assay was performed by modifying different methods and selecting the most suitable method for our experiment [52–60]. A mixture of 40 µL of the sample, 80 µL of phosphate buffer, and 40 µL of 10 mM L-DOPA was incubated for 10 minutes, followed by the addition of 40 µL of tyrosinase (50 units/mL) and further incubation for 20 minutes. The absorbance was measured at 492 nm. The experiment was carried out in triplicate. Kojic acid was used as a standard. The activity was calculated using the following formula:

$$\text{Absorbance} = ((A_2 - (A_1 - A_0)) / A_2) \times 100$$

$A_0$= phosphate buffer + substrat + tyrosinase
$A_1$= phosphate buffer + substrat + tyrosinase + sample
$A_2$= phosphate buffer + substrat + sample

### 4.6. UV Protection Activity Study

For the UV protection activity study, 1mg/mL concentrations of each extract were prepared and dispensed into 200 µL wells of a 96-well plate. The absorbance was measured by spectrophotometer at 5 nm intervals between 290 nm and 320 nm using a microplate reader. Three replicates were performed for each sample. It was calculated using the equation developed by Mansur et al. [26].

$$SPF_{spectrometric} = CF \times \sum_{290}^{320} EE\,(\lambda) \times I(\lambda) \times Abs(\lambda)$$

EE – erythemal effect spectrum; I –solar intensity spectrum; Abs - absorbance of sunscreen product; CF – correction factor (= 10).

### 4.7. Phenolic contents by LC-MS/MS

LC-MS/MS analysis of species was performed by using Shimadzu HPLC + AB Sciex 3200 QTrap Mass Detector. The separation was achieved using an Agilent Poroshell120 SB-CB 3,0 x100 mm 2,7µm analytical column. During the experiment, the column oven temperature was set at 40 °C. The elution gradient contains ultrapure water (mobile phase A) and Formic acid (mobile phase B). To achieve a better chromatographic

differentiation and also to facilitate ionization, 0.2% formic acid were added to mobile phases A and B. The flow rate was 0.25 mL/min. Mobile phase flow method was given in Table 4. Chartacterization of phenolic compounds were analyzed by Tekirdağ Namık Kemal University Scientific and Technological Research Application and Research Center (NABİLTEM).

**Table 4.** Mobile phase flow method

| Time (min) | Mob A (%) | Mob B (%) |
|---|---|---|
| 1 | 95 | 5 |
| 2 | 95 | 5 |
| 5 | 5 | 95 |
| 10 | 5 | 95 |
| 10.01 | 95 | 5 |
| 12.00 | 95 | 5 |
| 15.01 | STOP | |

## 5. Statistical analysis

The results were analyzed by the Microsoft® Excel 16.23 program and expressed as mean ± standard deviation.

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