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Quantitative analysis and antioxidant activity of *Trifolium pratense* L. stem

Trifolium pratense L. gövdesinin kantitatif analizi ve antioksidan aktivitesi

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ABSTRACT

The use of natural products for medical and nutritional purposes dates back to ancient times and has made significant contributions to the discovery and development of drugs, thanks to the compounds they contain. In this study, quantitative analysis of phenolic compounds and antioxidant activity of *Trifolium pratense* stem were determined. Rutin (0.075 mg/g extract), salicylic acid (0.044 mg/g extract), trans-ferulic acid (0.004 mg/g extract), gallic acid (0.003 mg/g extract), and hesperidin (0.024 mg/g extract) were determined as major compounds. The antioxidant activity of the methanol extract of the stem was evaluated using the DPPH and ABTS assays. In the DPPH assay, the extract exhibited moderate activity with an IC_{50} value of 17.22 ± 0.67 µg/mL. The activities of BHA and BHT were calculated as (5.47 ± 0.23, IC_{50}, µg/mL) and (10.67 ± 0.20, IC_{50}, µg/mL), respectively. Regarding the ABTS activity, the extract displayed high activity (IC_{50}, 9.03 ± 0.11 µg/mL). The standard BHT activity was detected as 9.51 ± 0.25 µg/mL (IC_{50}). *Trifolium pratense* stem could be a valuable source of antioxidant and bioactive compounds that can be isolated from the corresponding plants.

Keywords: *Trifolium pratense* L. stem, LC-MS/MS, antioxidant activity

ÖZET

Doğal ürünlerin tıbbi ve beslenme amaçlı kullanımı çok eski çağlara dayanmakta olup, içerdikleri bileşikler sayesinde ilaçların keşfi ve geliştirilmesine önemli katkılarda bulunmuştur. Bu çalışmada, *Trifolium pratense* gövdesinin fenolik bileşiklerinin kantitatif analizi ve antioksidan aktivitesi belirlenmiştir. Rutin (0,075 mg/g ekstre), salisilik asit (0,044), trans-ferulik asit (0,0385), gallik asit (0,0329) ve hesperidin (0,024) ana ürün olarak belirlenmiştir. Sapın metanol ekstresinin antioksidan aktivitesi DPPH ve ABTS analizleri kullanılarak değerlendirilmiştir. DPPH analizinde ekstre, 17,22 ± 0,67 µg/mL'lik bir IC_{50} değeriyle orta düzeyde aktivite göstermiştir. BHA ve BHT aktiviteleri sırasıyla (5.47 ± 0.23, IC_{50}, µg/mL) ve (10.67 ± 0.20, IC_{50}, µg/mL) olarak hesaplandı. ABTS aktivitesine gelince, ekstre yüksek aktivite gösterdi (IC_{50}, 9.03 ± 0.11 µg/mL). Standart BHT'nin aktivitesi 9.51 ± 0.25 µg/mL (IC_{50}) olarak tespit edildi. *Trifolium pratense* sapı, ilgili bitkilerden izole edilebilen değerli biyoaktif bileşik kaynağı ve antioksidan olabilir.

Anahtar kelimeler: *Trifolium pratense* L. gövde, LC-MS/MS, antioksidan aktivite

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Introduction

Human beings have been using plants as a source of nutrients for years (Cragg et al., 1997; Guemidi et al., 2024; Sahin Yaglioglu et al., 2013; Topçu et al., 1999). With the development of spectroscopy in the 19th century, the importance of plants increased considerably. Bioactive compounds in plants have been isolated by chromatographic methods, and their structures have been determined by spectroscopic techniques (Zerrouki et al., 2022). Bioactive compounds have been used in the pharmaceutical and food industries (Aksit et al., 2014; Bayir et al., 2014). Thus, active compounds in plants have become a valuable resource in the pharmaceutical industry (Erenler et al., 2014; Kaya et al., 2014; Türkmen et al., 2014). Intensive studies are being conducted on the synthesis of natural compounds. These studies include total synthesis, semi-synthesis, and functionalization of natural products (Lu et al., 2014). Synthetic chemists have taken inspiration from natural products and have begun to synthesize them (Çelik et al., 2007; Erenler et al., 2007; Erenler et al., 2006; Erenler et al., 2009). Thus, new drugs have been introduced, which are a turning point in drug discovery. Quantitative analysis of phenolics is crucial for various fields, including agriculture, medicine, and industry. Accurate measurement allows for better utilization of plant resources, improved health benefits, and enhanced product quality (Erenler, Demirtas, et al., 2018; Erenler, Telci, et al., 2018; Erenler et al., 2015; Genç et al., 2020). Phenolics help plants defend against pathogens, herbivores, and environmental stresses. They contribute to the regulation of growth and signaling pathways in plants. Determining the amount of phenolic compounds in a plant with a high concentration will allow the isolation of these compounds from the relevant plant. Phenolic compounds are plant-derived secondary metabolites with significant pharmaceutical applications due to their diverse biological activities (Atalar et al., 2023; Erenler, Atalar, et al., 2023; Erenler, Geçer, et al., 2022; Erenler, Karan, et al., 2023; Erenler, Yaman, et al., 2023; Yaman et al., 2022). Their importance in medicine and drug development stems from their antioxidant, anti-inflammatory, antimicrobial, anticancer, and neuroprotective properties (Bakchiche et al., 2024; Guemidi et al., 2024; Houari et al., 2024). Phenolics neutralize free radicals, reducing oxidative stress-related diseases such as cardiovascular disorders, diabetes, and neurodegenerative diseases (e.g., Alzheimer's, Parkinson's) (Erenler et al., 2014).

Flavonoids are widely found in plants and have the benzo-γ-pyrone structure. They are synthesized via the phenylpropanoid pathway and exhibit broad biological activity. Flavonoids scavenge free radicals and chelate metal ions, thus exhibiting antioxidant effects. Flavonoids can induce human protective enzyme systems. Numerous studies have suggested that flavonoids have protective effects against various infectious diseases (bacterial and viral) and degenerative diseases, including cardiovascular diseases, cancers, and other age-related conditions (Hadjra et al., 2023; Khodja et al., 2023; Ortiz et al., 2022).

Free radicals are reactive oxygen species produced by oxidative processes in the mammalian body (Gecer, 2023; Gecer et al., 2023). The human body has many defense mechanisms against oxidative stress. Under conditions such as poor lifestyle and exposure to environmental factors such as cigarette smoke and UV radiation, the body's antioxidant enzymes become insufficient. Excess free radicals damage the structure and function of the cell membrane, leading to conditions such as Alzheimer's, aging process, acute liver toxicity, cardiovascular disease, arteriosclerosis, nephritis, diabetes mellitus, rheumatism, inflammatory process and DNA damage that can lead to carcinogenesis (Erenler & Hosaflioglu, 2023). Many antioxidant-based drug formulations are used for the prevention and treatment of such diseases (Demirtas et al., 2013). In recent years, interest in natural antioxidants for use in the food, cosmetics, and pharmaceutical industries has increased due to the carcinogenic effects of synthetic antioxidants (Aissous et al., 2023; Zaoui et al., 2022). Antioxidant phytochemicals found in vegetables, fruits, and medicinal plants have received increasing attention due to their potential roles in disease prevention (Elmastas et al., 2004).

The *Trifolium* genus, which belongs to the Leguminosae family, comprises approximately 300 species. *Trifolium pratense* L. is an important forage plant widely grown in most temperate regions due to its high seedling vigor and fast growth characteristics. *Trifolium pratense* L. has potential applications in herbal dietary supplements, the treatment of menopausal symptoms, and the maintenance of bone and cardiovascular health. *Trifolium* species have been reported to display various biological activities. *T. alexandrinum* L. displayed antioxidative, hepatoprotective, and antibacterial properties, while *Trifolium angustifolium* L. revealed an antioxidant effect. *T. resupinatum* L. exhibited anti-inflammatory properties (Kolodziejczyk-Czepas, 2012). It has also recently attracted great attention due to its positive effects on the breast and endometrium (McKenna et al., 2018).

Here, a methanol extract of *Trifolium pratense* stem was prepared, and phenolic compounds were quantitatively analyzed. Moreover, the antioxidant activity of this extract was examined.

Material and methods

Plant material

Trifolium pratense was collected from the Iğdır University campus, and its botanical identification was carried out by Dr. Belkıs Muca Yiğit. A specimen was deposited in the Iğdır University Herbarium (No: INWM00000113).

Extraction and LC-ESI-MS/MS analysis

Trifolium pratense stem was powdered and extracted with methanol for 24 hours at room temperature. After removing the solvent, the crude extract was obtained. After the dilution from stock solution (1 mg/mL), the extract was subjected to LC-MS/MS analysis to quantify the phenolic compounds (Figure 1) (Başar et al., 2024).

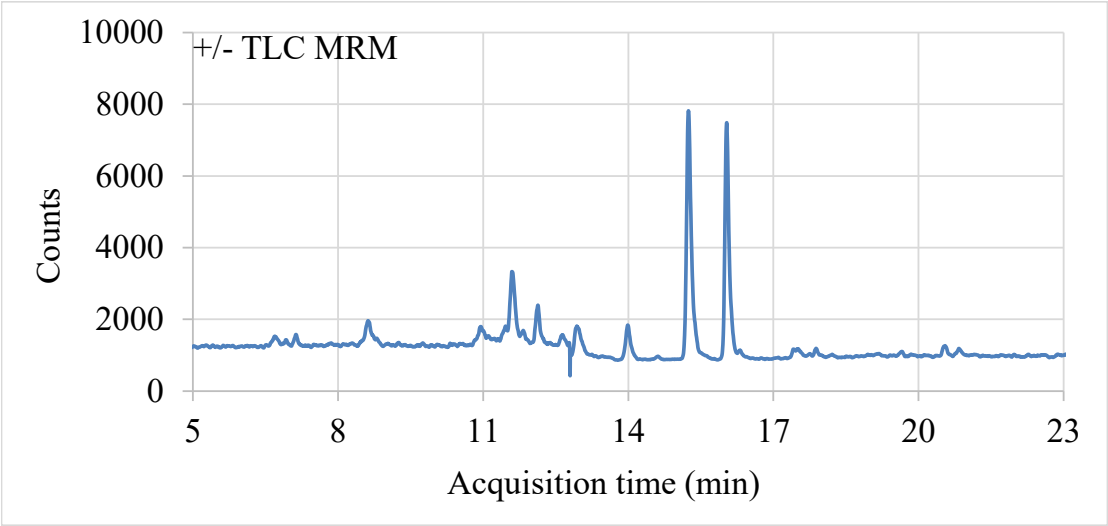

Figure 1. The MRM chromatogram *Trifolium pratense* stem

Antioxidant activity

Antioxidant activity of *Trifolium pratense* stem was carried out using the DPPH radical scavenging and ABTS cation scavenging assays. For the DPPH assay, samples at various concentrations were treated with a DPPH• solution in ethanol. Absorbance measurements were carried out at 517 nm, and the results were presented as IC_{50} values (Erenler et al., 2021). For the $ABTS^{\bullet+}$ radical cation scavenging assay, the different concentrations of samples were reacted with the $ABTS^{\bullet+}$ solution. The measurement was performed using a spectrophotometer at 734 nm. The results were calculated as IC_{50} (Genc et al., 2021).

Statistical analysis

GraphPad Prism (version 8.00) was used for the statistical analysis. A one-way ANOVA followed by Tukey multiple comparison tests was performed. The results were calculated as mean values ± standard deviation ($P < 0.05$).

Results and discussion

Quantitative analysis of phenolic compounds in plants is a significant area of research in natural product chemistry. A plant with a determined phenolic content will guide further scientific studies. Quantitative analysis of phenolic compounds in *Trifolium pratense* stem by LC-MS/MS was executed. Rutin (mg/g extract) (0.075), salicylic acid (0.044), trans-ferulic acid (0.004), gallic acid (0.003), and hesperidin (0.024) were determined as major compounds (Table 1).

Table 1. Quantitative analysis of natural compounds in *Trifolium pratense* stem by LC-MS/MS (mg/g extract)

No	Compound	RT	Quantity
1	Gallic acid	3.23	0.003
2	Chlorogenic acid	7.11	0.001
3	Hydroxybenzaldeyde	7.60	0.001
4	Caffeic Acid	7.77	0.001
5	Syringic acid	8.41	0.027
6	Vanillin	8.66	0.002
7	*o*-Coumaric acid	9.39	0.001
8	Salicylic acid	9.54	0.044
9	*t*-Ferulic acid	10.12	0.004
10	Sinapic acid	10.77	0.003
11	*p*-Coumaric acid	11.54	0.001
12	Coumarin	11.57	0.010
13	Hesperidin	11.84	0.024
14	Isoquercitrin	11.81	0.002
15	Rutin	12.39	0.075
16	Kaempferol-3-glucoside	13.29	0.003
17	Fisetin	13.44	0.002
18	Naringenin	15.07	0.004
19	Hesperetin	15.87	0.003
20	Kaempferol	16.12	0.010

nd: not detected, **RT**: retention time

Rutin is a significant natural compound found in many aromatic and medicinal plants, displaying a wide range of biological activities. The high concentration of rutin in *Trifolium pratense* stems provides the plant with high biological activity. Rutin (3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside) is a flavonol. Rutin has a large variety of biological activities such as sedative activity, anticonvulsant activity, anti-alzheimer activity, antidepressant effects, analgesic effect, antinociceptive effect, antiarthritic effects, antidiabetic effects, anti-hypercholesterolemic effects, anti-hypercholesterolemic effects, antiasthmatic activity, antiosteoporotic effect, antiosteopenic effect, anticataract effect, anticataract, anticancer effects, antibacterial activity, antifungal activities, antiviral activity, antifatigue activity, neuroprotective activity, retinoprotective activity, nephroprotective activity, wound healing activity (Ganeshpurkar et al., 2017).

The effects of salicylic acid (SA) on human health have been investigated for years. Salicylic acid, a phenolic compound, plays a vital role in plant defense against pathogens. It is found in fruits, vegetables, and spices in varying amounts. Salicylic acid is the primary metabolite and active

ingredient of acetylsalicylic acid, an anti-inflammatory drug used in clinical practice for over 100 years. In recent years, scientific studies have determined that acetylsalicylic acid is effective in preventing cardiovascular disease and colorectal carcinoma (Randjelović et al., 2015).

The antioxidant activity of *Trifolium pratense* stem was investigated using the DPPH and ABTS assays. The extract revealed the moderate DPPH activity (IC_{50}, 17.22 ± 0.67 µg/mL). The standard BHA and BHT activities were recorded as 5.47 ± 0.23 (IC_{50}, µg/mL) and 10.67 ± 0.20 (IC_{50}, µg/mL), respectively. The same trend was observed for the ABTS assay. The extract activity was determined as 9.03 ± 0.11 µg/mL (IC_{50}). BHT activity was detected as 9.51 ± 0.25 µg/mL (IC_{50}) (Figure 2).

Previous studies reported that the plants had antioxidant activity due to their secondary metabolite contents. *Rubia tinctorum* L. essential oils were reported to reveal considerable antioxidant activity (Houari et al., 2024). Plants have been used for synthesis of nanoparticles (Dag, 2022; Erenler & Dag, 2022; Gecer, 2021). Bioactive compounds in plants act as reducing, stabilizing and capping agents (Erenler & Gecer, 2022a, 2022b; Gecer & Erenler, 2022a). Due to the properties of nanoparticles and bioactive compounds capped the silver ions, nanoparticles were observed to display the considerable antioxidant activity (Gecer & Erenler, 2022b; Gecer, Erenler, et al., 2022; Sahin Yaglioglu et al., 2022).

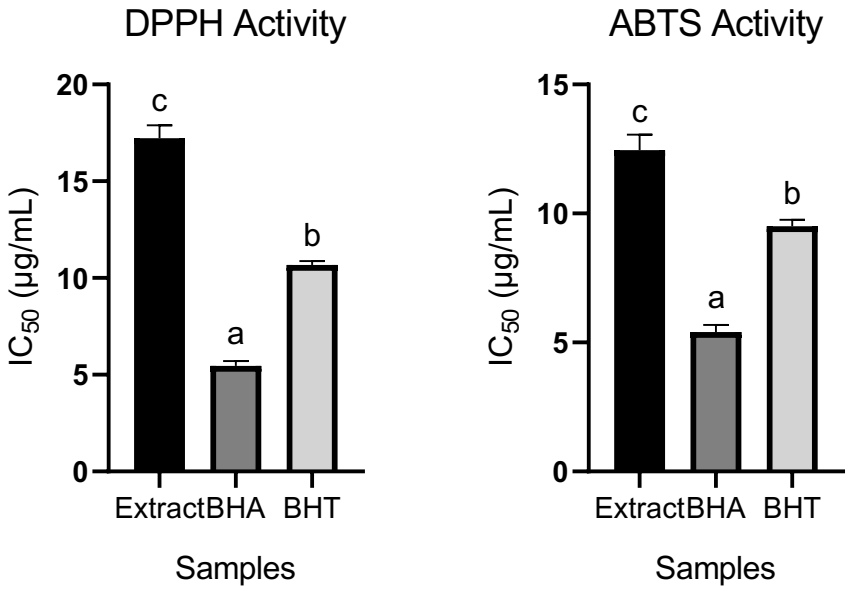

Figure 2. Antioxidant activity of *Trifolium pratense* stem. Means (three replicates) followed by different letters (a, b, and c) express a statistical difference ($P < 0.05$).

Conclusions

The phytochemistry of *Trifolium pratense* stem was evaluated. Quantitative analysis of phenolics was executed. The pharmaceutically significant compounds were determined as major products. Hence, this study will guide the synthesis chemist for further studies. The high concentrated compounds in *Trifolium pratense* stem such as rutin, salicylic acid, *t*-ferulic acid, gallic acid, and hesperidin can be isolated by chromatographic methods and their structures can be identified by spectroscopic techniques. The usability of *Trifolium pratense* stem extract in food supplement can be investigated.

Author contributions

R.E. and E.N.G. designed the study. R.E. interpreted the results and wrote the manuscript. I.Y. and S.M.C. carried out the experiment. I.H. collected and deposited the plant material.

Declaration of interests

The authors declare that there is no conflict of interest.

References

Aissous, I., Benrebai, M., Ameddah, S., Menad, A., Erenler, R., Benayache, S., & Benayache, F. (2023). The preventive effects of *Centaurea maroccana* Ball. extract against oxidative stress induced by cisplatin in mice brains: in vitro and in vivo studies. *Drug and Chemical Toxicology, 46*(6), 1162-1175. https://doi.org/10.1080/01480545.2022.2139841

Aksit, H., Çelik, S. M., Sen, Ö., Erenler, R., Demirtas, I., Telci, I., & Elmastas, M. (2014). Complete isolation and characterization of polar portion of *Mentha dumetorum* water extract. *Records of Natural Products, 8*(3), 277-280.

Atalar, M. N., Erenler, R., Turkan, F., Alma, M. H., Demirtas, I., Baran, A., Kandemir, S. I., Kekec, A. I., & Saltan, F. Z. (2023). Phytochemical analysis and biological activity of *Corchorus olitorius* L.: Quantitative analysis of bioactive compounds by LC–MS/MS, antibacterial, enzyme inhibition, and cytotoxic activities. *European Journal of Integrative Medicine, 62*, 102290. https://doi.org/10.1016/j.eujim.2023.102290

Bakchiche, B., Hadjira, G., Erenler, R., Yildiz, I., Asmaa, S. M., & El-shazly, M. A. (2024). Mineral contents, Antioxidant and Antimicrobial Activities of Algerian Terfezia claveryi extracts. [Cezayir Terfezia claveryi ekstraktlarının mineral içerikleri, Antioksidan ve Antimikrobiyal Aktiviteleri]. *Fabad Journal of Pharmaceutical Sciences, 49*(1), 19-36. https://doi.org/10.55262/fabadeczacilik.1326710

Başar, Y., & Erenler, R. (2024). Phytochemical analysis of *Silybum marianum* flowers: Quantitative analysis of natural compounds and molecular docking application. [Silybum marianum çiçeklerinin fitokimyasal analizi: Doğal bileşiklerin kantitatif analizi ve moleküler yerleştirme uygulaması]. *Turkish Journal of Biodiversity, 7*(1), 20-31. https://doi.org/10.38059/biodiversity.1450643

Bayir, B., Gündüz, H., Usta, T., Şahin, E., Özdemir, Z., Kayır, Ö., Sen, Ö., Akşit, H., Elmastaş, M., & Erenler, R. (2014). Chemical Composition of Essential Oil from *Marrubium Vulgare* L. Leaves. *Journal of New Results in Science, 6*(6), 44-50.

Cragg, G. M., Newman, D. J., & Snader, K. M. (1997). Natural products in drug discovery and development. *Journal of Natural Products, 60*(1), 52-60. https://doi.org/10.1021/np9604893

Çelik, I., Erenler, R., Akkurt, M., Ünlü, C. S., Tutar, A., & Garcia-Granda, S. (2007). Crystal structure of 1,2,3-tribromobenz[f]indane. *Analytical Sciences: X-ray Structure Analysis Online, 23*(11), x229-x230. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-70549106673&partnerID=40&md5=6742016390819e9a0382e6e7a59ef428

Dag, B. (2022). Green synthesis, characterization, and antioxidant activity of silver nanoparticles using *Stachys annua* L. subsp. *annua var. annua*. *Particulate Science and Technology, 40*(4), 512-520. https://doi.org/10.1080/02726351.2021.1966689

Demirtas, I., Erenler, R., Elmastas, M., & Goktasoglu, A. (2013). Studies on the antioxidant potential of flavones of *Allium vineale* isolated from its water-soluble fraction. *Food Chemistry, 136*(1), 34-40. https://doi.org/10.1016/j.foodchem.2012.07.086

Elmastas, M., Ozturk, L., Gokce, I., Erenler, R., & Aboul-Enein, H. Y. (2004). Determination of antioxidant activity of marshmallow flower (*Althaea officinalis* L.). *Analytical Letters, 37*(9), 1859-1869. https://doi.org/10.1081/AL-120039431

Erenler, R., Atalar, M. N., Yıldız, İ., Geçer, E. N., Yıldırım, A., Demirtas, İ., & Alma, M. H. (2023). Quantitative analysis of bioactive compounds by LC-MS/MS from Inula graveolens. *Bütünleyici ve Anadolu Tıbbı Dergisi, 4*(3), 3-10. https://doi.org/10.53445/batd.1278048

Erenler, R., & Biellmann, J. F. (2007). Synthesis of pentafluorophenyl- and pyridinyl-3 allenes. *Journal of the Chinese Chemical Society, 54*(1), 103-108. https://doi.org/10.1002/jccs.200700017

Erenler, R., & Dag, B. (2022). Biosynthesis of silver nanoparticles using *Origanum majorana* L. and evaluation of their antioxidant activity. *Inorganic and Nano-Metal Chemistry, 52*(4), 485-492. https://doi.org/10.1080/24701556.2021.1952263

Erenler, R., Demirtas, I., Buyukkidan, B., & Cakmak, O. (2006). Synthesis of hydroxy, epoxy, nitrato and methoxy derivatives of tetralins and naphthalenes. *Journal of Chemical Research*(12), 753-757. https://doi.org/10.3184/030823406780199721

Erenler, R., Demirtas, I., Karan, T., Gul, F., Kayir, O., & Karakoc, O. C. (2018). Chemical constituents, quantitative analysis and insecticidal activities of plant extract and essential oil from *Origanum onites* L. *Trends in Phytochemical Research, 2*(2), 91-96.

Erenler, R., & Gecer, E. N. (2022a). Green synthesis of silver nanoparticles from *Astragalus logopodioides* L. leaves. *Turkish Journal of Agriculture-Food Science and Technology, 10*(6), 1112-1115. https://doi.org/10.24925/turjaf.v10i6.1112-1115.5190

Erenler, R., & Gecer, E. N. (2022b). Synthesis of Silver Nanoparticles Using *Sideritis montana* L. Leaf Extract: Characterization, Catalytic Degradation of Methylene Blue and Antioxidant Activity. *Journal of Nano Research, 75*, 17-28. https://doi.org/10.4028/p-333bjm

Erenler, R., Gecer, E. N., Genc, N., & Yanar, D. (2021). Antioxidant activity of silver nanoparticles synthesized from *Tagetes erecta* L. leaves. *International Journal of Chemistry and Technology, 5*(2), 141-146. https://doi.org/10.32571/ijct.1005275

Erenler, R., Geçer, E. N., Alma, M. H., & Demirtas, İ. (2022). Quantitative Analysis of Bioactive Compounds by High-Performance Liquid Chromatography in *Origanum bilgeri*. *Journal of Integrative and Anatolian Medicine, 4*(1), 15-20. https://doi.org/10.53445/batd.1210373

Erenler, R., & Hosaflioglu, I. (2023). Green synthesis of silver nanoparticles using *Onobrychis sativa* L.: Characterization, catalytic degradation of methylene blue, antioxidant activity, and quantitative analysis of bioactive compounds. *Materials Today Communications, 35*, 105863. https://doi.org/10.1016/j.mtcomm.2023.105863

Erenler, R., Karan, T., & Hosaflioglu, İ. (2023). Phytochemical analysis of Syringa vulgaris: Quantitative analysis of natural compounds by LC-ESI-MS/MS. *Turkish Journal of Biodiversity, 6*(2), 75-78. https://doi.org/10.38059/biodiversity.1312872

Erenler, R., Telci, İ., Elmastaş, M., Akşit, H., Gül, F., Tüfekçi, A. R., Demirtaş, İ., & Kayir, Ö. (2018). Quantification of flavonoids isolated from *Mentha spicata* in selected clones of Turkish mint landraces. *Turkish Journal of Chemistry, 42*(6), 1695-1705. https://doi.org/10.3906/kim-1712-3

Erenler, R., Telci, I., Ulutas, M., Demirtas, I., Gul, F., Elmastas, M., & Kayir, O. (2015). Chemical Constituents, Quantitative Analysis and Antioxidant Activities of *Echinacea purpurea* (L.) Moench and *Echinacea pallida* (Nutt.) Nutt. *Journal of Food Biochemistry, 39*(5), 622-630. https://doi.org/10.1111/jfbc.12168

Erenler, R., Uno, M., Goud, T. V., & Biellmann, J. F. (2009). Preparation of some heterocyclic enones and ynones by isomerisation of the propargylic alcohols. *Journal of Chemical Research, 2009*(7), 459-464. https://doi.org/10.3184/030823409X464467

Erenler, R., Yaman, C., Demirtas, I., & Alma, M. H. (2023). Phytochemical Investigation of *Hypericum heterophyllum* Flowers: LC-ESI-MS/MS Analysis, Total Phenolic and Flavonoid Contents, Antioxidant Activity. *The Natural Products Journal, 13*(7), e120123212672. https://doi.org/10.2174/2210315513666230112165545

Erenler, R., Yilmaz, S., Aksit, H., Sen, O., Genc, N., Elmastas, M., & Demirtas, I. (2014). Antioxidant activities of chemical constituents isolated from *Echinops orientalis* Trauv. *Records of Natural Products, 8*(1), 32-36.

Ganeshpurkar, A., & Saluja, A. K. (2017). The Pharmacological Potential of Rutin. *Saudi Pharmaceutical Journal, 25*(2), 149-164. https://doi.org/10.1016/j.jsps.2016.04.025

Gecer, E. N. (2021). Green Synthesis of Silver Nanoparticles from *Salvia aethiopis* L. and Their Antioxidant Activity. *Journal of Inorganic and Organometallic Polymers and Materials, 31*(11), 4402-4409. https://doi.org/10.1007/s10904-021-02057-3

Gecer, E. N. (2023). Synthesis and characterization of silver nanoparticles using *Origanum onites* leaves: Cytotoxic, apoptotic, and necrotic effects on Capan-1, L929, and Caco-2 cell lines. *Green Processing and Synthesis, 12*(1), 20228126. https://doi.org/10.1515/gps-2022-8126

Gecer, E. N., & Erenler, R. (2022a). Biosynthesis of silver nanoparticles using *Dittrichia graveolens* (Asteraceae) leaves extract: characterisation and assessment of their antioxidant activity. *Turkish Journal of Biodiversity, 5*(1), 50-56. https://doi.org/10.38059/biodiversity.1090549

Gecer, E. N., & Erenler, R. (2022b). Biosynthesis of Silver Nanoparticles using *Echinacea pallida* (Nutt.) Nutt. and Antioxidant Activity Thereof. *Journal of the Chemical Society of Pakistan, 44*(6), 610-617. https://doi.org/10.52568/001187/JCSP/44.06.2022

Gecer, E. N., & Erenler, R. (2023). Biogenic synthesis of silver nanoparticles using *Echium vulgare*: Characterisation, quantitative analysis of bioactive compounds, antioxidant activity and catalytic degradation. *Journal of the Indian Chemical Society, 100*(5), 101003. https://doi.org/10.1016/j.jics.2023.101003

Gecer, E. N., Erenler, R., Temiz, C., Genc, N., & Yildiz, I. (2022). Green synthesis of silver nanoparticles from *Echinacea purpurea* (L.) Moench with antioxidant profile. *Particulate Science and Technology, 40*(1), 50-57. https://doi.org/10.1080/02726351.2021.1904309

Genc, N., Yildiz, I., Chaoui, R., Erenler, R., Temiz, C., & Elmastas, M. (2021). Biosynthesis, characterization and antioxidant activity of oleuropein-mediated silver nanoparticles. *Inorganic and Nano-Metal Chemistry, 51*(3), 411-419. https://doi.org/10.1080/24701556.2020.1792495

Genç, N., Elmastaş, M., Telci, İ., & Erenler, R. (2020). Quantitative analysis of phenolic compounds of commercial basil cultivars (*Ocimum basilicum* L.) by LC-TOF-MS and their antioxidant effects. *International Journal of Chemistry and Technology, 4*(2), 179-184. http://dx.doi.org/10.32571/ijct.795629

Guemidi, C., Ait Saada, D., Ait Chabane, O., Elmastas, M., Erenler, R., Yilmaz, M. A., Tarhan, A., Akkal, S., & Khelifi, H. (2024). Enhancement of yogurt functionality by adding Mentha piprita phenolic extract and evaluation of its quality during cold storage. *Food Science & Nutrition, 12*, 3007-3020. https://doi.org/10.1002/fsn3.3981

Hadjra, H., Yamina, B., Soulef, K., & Erenler, R. (2023). Evaluation of Antifungal Potential of *Mentha pulegium* Essential oil in Biological Control Against the Pathogen of Inflorescence Rot Disease of Date Palm (*Mauginiella scaettae*). *Jordan Journal of Biological Sciences, 16*(4), 665-672. https://doi.org/10.54319/jjbs/160412

Houari, F. Z., Brahmi, M., Erenler, R., & Hariri, A. (2024). Chemical profile, antibacterial and antioxidant properties of *Rubia tinctorum* L. essential oils. *Natural Product Research*, 165-174. https://doi.org/10.1080/14786419.2024.2356670

Kaya, G., Karakaya, R., Tilgel, E., Sandikci, M., Yucel, E., Cicek, G., Kayir, O., Aksit, H., Telci, I., & Guzel, A. (2014). Essential Oil Constituents of *Thuja orientalis* Berries. *Journal of New Results in Science, 7*(7), 1-6

Khodja, E. A. T., Abd El Hamid Khabtane, R. A., Benouchenne, D., Bensaad, M. S., Bensouici, C., & Erenler, R. (2023). In vitro assessment of antioxidant, neuroprotective, anti-urease and anti-tyrosinase capacities of *Tamarix africana* leaves extracts. *Journal of Traditional Chinese Medicine, 43*(2), 252. https://doi.org/10.19852/j.cnki.jtcm.20230105.003

Kolodziejczyk-Czepas, J. (2012). Trifolium species-derived substances and extracts—Biological activity and prospects for medicinal applications. *Journal of Ethnopharmacology, 143*(1), 14-23. https://doi.org/10.1016/j.jep.2012.06.048

Lu, J., Maezawa, I., Weerasekara, S., Erenler, R., Nguyen, T. D. T., Nguyen, J., Swisher, L. Z., Li, J., Jin, L. W., Ranjan, A., Srivastava, S. K., & Hua, D. H. (2014). Syntheses, neural protective activities, and inhibition of glycogen synthase kinase-3β of substituted quinolines. *Bioorganic and Medicinal Chemistry Letters, 24*(15), 3392-3397. https://doi.org/10.1016/j.bmcl.2014.05.085

McKenna, P., Cannon, N., Conway, J., & Dooley, J. (2018). The use of red clover (*Trifolium pratense*) in soil fertility-building: A Review. *Field Crops Research, 221*, 38-49. https://doi.org/10.1016/j.fcr.2018.02.006

Ortiz, A. d. C., Fideles, S. O. M., Reis, C. H. B., Bellini, M. Z., Pereira, E. d. S. B. M., Pilon, J. P. G., de Marchi, M. Â., Detregiachi, C. R. P., Flato, U. A. P., & Trazzi, B. F. d. M. (2022). Therapeutic effects of citrus flavonoids neohesperidin, hesperidin and its aglycone, hesperetin on bone health. *Biomolecules, 12*(5), 626. https://doi.org/10.3390/biom12050626

Randjelović, P., Veljković, S., Stojiljković, N., Sokolović, D., Ilić, I., Laketić, D., Randjelović, D., & Randjelović, N. (2015). The beneficial biological properties of salicylic acid. *Acta facultatis medicae Naissensis, 32*(4), 259-265. https://doi.org/10.1515/afmnai-2015-0026

Sahin Yaglioglu, A., Akdulum, B., Erenler, R., Demirtas, I., Telci, I., & Tekin, S. (2013). Antiproliferative activity of pentadeca-(8E, 13Z) dien-11-yn-2-one and (E)-1,8-pentadecadiene from *Echinacea pallida* (Nutt.) Nutt. roots. *Medicinal Chemistry Research, 22*(6), 2946-2953. https://doi.org/10.1007/s00044-012-0297-2

Sahin Yaglioglu, A., Erenler, R., Gecer, E. N., & Genc, N. (2022). Biosynthesis of Silver Nanoparticles Using *Astragalus flavesces* Leaf: Identification, Antioxidant Activity, and Catalytic Degradation of Methylene Blue. *Journal of Inorganic and Organometallic Polymers and Materials, 32*, 3700-3707. https://doi.org/10.1007/s10904-022-02362-5

Topçu, G., Erenler, R., Çakmak, O., Johansson, C. B., Çelik, C., Chai, H.-B., & Pezzuto, J. M. (1999). Diterpenes from the berries of *Juniperus excelsa*. *Phytochemistry, 50*(7), 1195-1199. https://doi.org/10.1016/S0031-9422(98)00675-X

Türkmen, N., Öz, A., Sönmez, A., Erol, T., Gülümser, D., Yurdakul, B., Kayır, Ö., Elmastas, M., & Erenler, R. (2014). Chemical Composition of Essential Oil from *Rosmarinus Officinalis* L. Leaves. *Journal of New Results in Science, 6*(6), 27-31.

Yaman, C., Önlü, Ş., Ahmed, H., & Erenler, R. (2022). Comparison of phytochemicals and antioxidant capacity of *Hypericum perforatum;* wild plant parts and in vitro samples. *Journal of Animal & Plant Sciences, 32*(2), 596-603. https://doi.org/10.36899/JAPS.2022.2.0459

Zaoui, H., Boutaoui, N., Menad, A., Erenler, R., Zaiter, L., Fadilab, B., & Souada, A. (2022). Phytochemical Profile, Anti-lipid peroxidation and Anti-diabetic activities of *Thymus algeriensis* Boiss. & Reut. *Egyptian Journal of Chemistry, 65*(12), 695-705.

Zerrouki, S., Mezhoud, S., Sahin Yaglioglu, A., Bensouici, C., Nuri Atalar, M., Demirtas, I., Ameddah, S., & Mekkiou, R. (2022). Antioxidant, anticancer activities, and HPLC-DAD analyses of the medicinal halophyte *Limoniastrum guyonianum* Dur. extracts. *Journal of Research in Pharmacy, 26*(3), 598-608. https://doi.org/10.29228/jrp.157