MAU J AGR NAT (2026) 6(1): 21-45
DOI: 10.59359/maujan.1846297
e-ISSN: 2822-3500

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MUŞ ALPARSLAN ÜNİVERSİTESİ
TARIM VE DOĞA DERGİSİ

MUŞ ALPARSLAN UNIVERSITY
JOURNAL OF AGRICULTURE AND NATURE

Euphorbia peplus L.'nin Antioksidan Kapasitesinde Ekstraksiyon Tipine Bağlı Değişikliklerin Araştırılması

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Please cite this paper as follows: Güçlü, G., (2026). Investigation of Extraction-Type-Dependent Changes in Antioxidant Capacity of *Euphorbia peplus* L.. *Mus Alparslan University Journal of Agriculture and Nature*, 6(1), 21-25. https://doi.org/10.59359/maujan.1846297

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi: 21.12.2025
Kabul Tarihi: 03.03.2026
Online Yayınlanma: 11.03.2026

Anahtar Kelimeler:
Euphorbia peplus L.,
Antioksidan aktivite,
Ekstrakt,
TPC,
TFC

ÖZET

Euphorbia peplus L., Kuzey Afrika, Avrupa ve Batı Asya'da bulunan, önemli toksisite özellik gösteren ve steroller, flavonoidler ve diterpenler dahil olmak üzere zengin fitokimyasal içeriği ile karakterize edilen bir türdür. Bu bileşikler, bitkiye antiinflamatuar, antimikrobiyal, antikanser ve allelopatik özellikler kazandırır. Bitkinin antioksidan kapasitesi üzerine yapılan sınırlı çalışmaları genişletmek amacıyla, bu çalışmada üç farklı çözücü (su, %80 etanol, metanol) ile hazırlanan *E. peplus* L. özütlerinin *in vitro* antioksidan kapasitesi karşılaştırmalı olarak analiz edilmiştir. DPPH yöntemi ile elde edilen radikal temizleme aktivitesi sonuçlarına göre, su ekstresi (517,5 ± 0,7 µg/mL) etanol (1165 ± 1,2 µg/mL) ve metanol (1275 ± 1,4 µg/mL) ekstrelerine göre daha yüksek antioksidan aktivite göstermiştir. Folin–Ciocalteu ve kolorimetrik yöntemler kullanılarak TPC ve TFC içerikleri incelendiğinde, en yüksek TPC (158,2 ± 0,07 mg GAE/g) ve TFC (14,03 ± 0,02 mg RE/g) değerleri etanol ekstraktında belirlenmiştir. *E. peplus* L. su ekstraktının antioksidan aktivitesi, toplam fenolik içeriği ve flavonoid içeriği ilk kez rapor edilmiştir ve bu çalışmanın, bitkinin gelecekteki çalışmalarında antioksidanların aktif bileşenlerinin belirlenmesinde çözücü seçimi için potansiyel bir kaynak sağlayabileceği düşünülmektedir.

Investigation of Extraction-Type-Dependent Changes in Antioxidant Capacity of *Euphorbia peplus* L.

Research Article

Article History
Received: 21.12.2025
Accepted: 03.03.2026
Online Published: 11.03.2026

Keywords:
Euphorbia peplus L.,
Antioxidant activity,
Extract,
TPC,
TFC

ABSTRACT

Euphorbia peplus L. is a species found in North Africa, Europe, and Western Asia, characterized by significant toxicity capacity and a rich phytochemical content including sterols, flavonoids, and diterpenes. These compounds provide the plant with anti-inflammatory, antimicrobial, anticancer, and allelopathic properties. To expand the limited studies on its antioxidant capacity, this study comparatively analyzed the in vitro antioxidant capacity of *E. peplus* L. extracts prepared with three different solvents (water, 80% ethanol, methanol). According to the radical scavenging activity results obtained by the DPPH method, the water extract (517.5 ± 0.7 µg/mL) showed higher antioxidant activity than the ethanol (1165 ± 1.2 µg/mL) and methanol (1275 ± 1.4 µg/mL) extracts. When TPC and TFC contents were examined using the Folin–Ciocalteu and colorimetric methods, the highest TPC (158.2 ± 0.07 mg GAE/g) and TFC (14.03 ± 0.02 mg RE/g) values were determined in the ethanol extract. The antioxidant activity, total phenolic content, and flavonoid content of *E. peplus* L. water extract have been reported for the first time, and it is thought that this study could provide a potential source for solvent selection in determining the active components of antioxidants in future studies of the plant.

1. Introduction

The Euphorbiaceae family, one of the most widespread and largest plant families worldwide, is remarkably rich in secondary metabolites. It exhibits a strong profile in terms of glycosides, phenolic and flavonoid compounds, and tannins, with its largest genus being *Euphorbia* (Webster, 1987). This genus, comprising over 2,000 species, includes 91 species native to Turkey (Radcliffe-Smith and Tutin, 1982).

Species of *Euphorbia* have long been used in folk medicine for their medicinal and aromatic properties. They are particularly effective in treating skin wounds, digestive system disorders, respiratory ailments, and various chronic inflammatory conditions. Although the latex they contain is generally highly toxic, it is widely used in dermatological diseases (Lazreg Aref et al., 2014; Hua et al., 2017). Recent pharmacological and phytochemical studies have provided results supporting the scientific basis of these traditional uses, thereby increasing interest in the potential therapeutic applications of *Euphorbia* species (Ghramh et al., 2019; Widyananda et al., 2024).

Euphorbia peplus L., a species with high toxicity distributed across Europe, North Africa, and Western Asia (Barla et al., 2006), contains phytochemicals such as sterols, triterpene alcohols, diterpenes, dihydroflavonol 3-O-monoglucosides, C- and O-glucosides, rutin, kaempferol, quercetin and myricetin (Khafagy et al., 1975; Noori et al., 2009; Benjamaa et al., 2022). These phytochemicals confer anti-inflammatory, antimicrobial, and anticancer activities to the plant, in addition to enabling allelopathic effects (Ali et al., 2013; Kačániová et al., 2020; El-Sakhawy et al., 2025).

Plants generally exhibit high antioxidant activity due to phenolic compounds, flavonoids, and other secondary metabolites produced as natural defense mechanisms against oxidative stress. However, the determination of antioxidant capacity is influenced not only by the plant's collection period and biotic/abiotic stress mechanisms but also by extraction methods and different solvents used in in vitro studies.

In this study, a comparative analysis was conducted on the antioxidant capacity and total phenol-flavonoid contents of *Euphorbia peplus* L. extracts prepared using three different solvents (water, 80% ethanol, and methanol). This approach aimed to elucidate the effects of the extraction solvent on the plant's antioxidant potential, thereby shedding light on its prospective biological and pharmaceutical applications.

2. Material and Methods

2.1. Plant Material and Extracts Preparations

The aerial parts of the *E.peplus* L. was collected from Nardüzü, Hatay (36°33′ N; 36°09′ E, 42 m) in March, 2025. Freshly harvested aerial parts were dried at room temperature, after which the dried material was ground. Subsequently, 10 g of the powdered samples were extracted with water, 80% ethanol, and methanol at room temperature for 72 hours.

2.2. Determination of *in vitro* antioxidant activity

DPPH Assay

To determine the free radical scavenging activity of different extracts of *E. peplus* L., the 2,2-diphenyl-1-picrylhydrazil (DPPH) method reported by Salihoğlu et al. (2022) was adapted with minor modifications. In this method, the radical scavenging activity of an antioxidant was determined by measuring the decrease in the absorbance of DPPH at 517 nm. This method was applied to plant extracts at decreasing concentrations (2000, 1000, 500, 250, 50 μg/mL). Gallic acid was used as a reference substance at the same concentrations.

The DPPH scavenging effect was calculated using the following equation:

DPPH Scavenging (%) = [(Abs_control − Abs_sample) / Abs_control] × 100

Total phenol (TPC) and flavonoid (TFC) content

The total phenolic contents of *E.peplus* L. extracts were determined using the Folin–Ciocalteu method. Extracts diluted with DMSO were mixed with distilled water and Folin–Ciocalteu reagent. The mixture was left to stand at room temperature for 5 minutes, then a 7.5% Na_2CO_3 solution was added and incubated for 1 hour, and the absorbance values were measured at 650 nm. DMSO was used as a blank sample and gallic acid as a standard.

The total flavonoid content was determined using the aluminum chloride colorimetric method. On a microplate, the prepared extract solutions were mixed with 2% $AlCl_3$ and incubated for 15 minutes. At the end of the incubation period, the absorbances were measured at 435 nm, and the results were expressed as rutin equivalents (RE/g).

3. Results and Dıscussıon

3.1. DPPH radical scavenging activity

The IC_{50} values obtained from the DPPH radical scavenging assay clearly demonstrate the antioxidant activity of ethanol, methanol, and water (aqueous) extracts, as well as gallic acid used as a positive control. The IC_{50} value for the ethanol extract was calculated as 1165 ± 1.2 μg/mL, for the methanol extract as 1275 ± 1.4 μg/mL, and for the aqueous

extract as 517.5 ± 0.7 µg/mL. The IC_{50} value of gallic acid, used as a reference, was determined to be 1.995 ± 0.4 µg/mL. Accordingly, *E. peplus* L. exhibited the strongest antioxidant activity within aqueous extract (Figure 1).

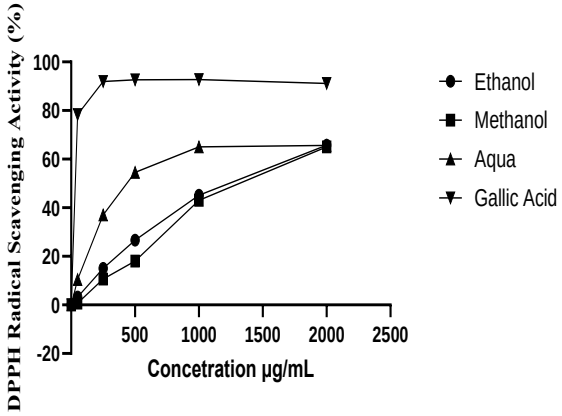

Figure 1. DPPH radical scavenging activity of aqueous, 80% ethanol, and methanol extracts of *E.peplus* L. Gallic acid was used as a reference.

Examination of DPPH antiradical activities in various *Euphorbia* species has revealed that antioxidant activity increases with higher concentrations. Although the ethanol extract of *E. eriophora* exhibited lower antioxidant activity compared to the reference compound ascorbic acid, it demonstrated significant activity with increasing concentration (Akgül et al., 2022). A study on the ethanol extract of *Euphorbia hirta* similarly reported a dose-dependent increase in antioxidant activity (Praveen et al., 2024). The methanolic extract of *E. rigida* displayed good antioxidant activity when evaluated against the reference compound BHT (Torunoğlu and Akdağ, 2025). Likewise, the water extract of *E. gaillardotii* was reported to exhibit strong DPPH antioxidant activity (Husunet et al., 2025). While these findings indicate that different solvents yield varying antioxidant activities, data enabling direct solvent comparisons remain limited.

Previous studies on *E. peplus* L. have investigated its DPPH radical scavenging activity in methanol and ethanol extracts, concluding that the methanol extract exhibited stronger antioxidant activity than the ethanol extract when benchmarked against catechin and BHT as reference compounds (Amin et al., 2021). In another study using ascorbic acid as the reference, the EtOH extract of *E. peplus* L. demonstrated concentration-dependent radical scavenging activity (Alruhaimi et al., 2023). Taken together, these data suggest that *E. peplus* L. extracts display stronger antioxidant activity at higher concentrations, and extracts prepared with solvents such as ethanol or methanol exhibit lower activity compared to water extracts. To date, no literature reports have documented the antioxidant activity of *E. peplus* L. water extracts.

3.2. TFC and TPC content

When analyzing the total phenolic content (TPC) and total flavonoid content (TFC) results, significant differences were observed between the extracts. TPC values were determined as 158.2 ± 0.07 mg GAE/g for ethanol extract, 110.9 ± 0.02 mg GAE/g for aqueous extract, and 97.8 ± 0.05 mg GAE/g for methanol extract. These results indicate that the ethanol extract is enriched in phenolic compounds compared to the other extracts. In the TFC analysis, the flavonoid content was found to be 14.03 ± 0.02 mg RE/g in the ethanol extract, followed by the aqueous extract with 4.5 ± 0.002 mg RE/g, and the methanol extract with 2.01 ± 0.02 mg RE/g (Figure 2). The significantly higher flavonoid content of the ethanol extract compared to the other samples indicates that this sample may have stronger antioxidant potential.

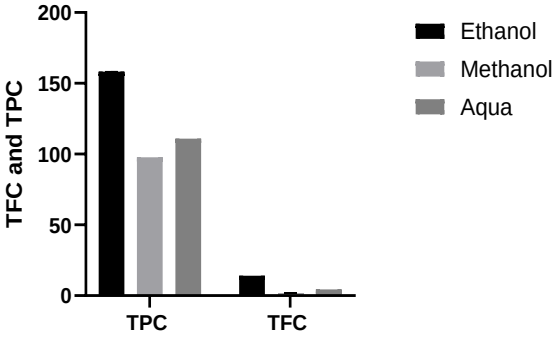

Figure 2. Total flavonoid and phenol content of aqueous, 80% ethanol, and methanol extracts of *E.peplus* L..

Although no literature data exist on the TPC and TFC contents of *E. peplus* L., studies on other *Euphorbia* species have reported varying levels of total phenol and flavonoid contents. Examination of *E. splendida* revealed significant TPC and TFC in both water and ethanol extracts, and their sub-fractions (Kefayati et al., 2017). Similarly, in *E. macroclada*, the ethanol extract exhibited higher TFC and TPC compared to the aqueous extract, consistent with the findings of this study (Farhan et al., 2013). In *Euphorbia hyssopifolia*, extracts prepared with ethanol, methanol, n-hexane, and ethyl acetate also showed higher TFC and TPC values relative to the water extract (Azaat et al., 2022).

In the antioxidant activity data of *E. peplus*, the aqueous extract demonstrated superior DPPH radical scavenging activity, while total phenol and flavonoid contents were higher in the ethanol extracts. When considered alongside previous studies, these findings indicate that high-polarity extracts such as water and ethanol exhibit greater antioxidant activity compared to low-polarity extracts. This can be explained by the greater solubility of phenolic compounds in alcohol-based solvents (Dai and Mumper, 2010).

4. CONCLUSION

Euphorbia peplus L. exhibits a wide distribution across North Africa, Europe, and Western Asia, including Turkey. The plant is known for its high therapeutic activity, with its latex having been used in wound treatments from ancient times to the present. This study demonstrates the antioxidant activity of *E. peplus* L. using three different extracts (water, ethanol, and methanol). No previous studies on the aqueous extract of this species have been reported. Furthermore, the total phenol and flavonoid contents are documented here for the first time within this context. Conducting phytochemical analyses to support these findings would be beneficial for identifying the active antioxidant compounds of this plant in future research.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' Contributions

Corresponding author, conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

Conflict of Interest

The author declares that there is no conflict of interest

Ethical approval

For this type of study, formal consent is not required.

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