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Evaluation of Biological Activities of Various Extracts of *Glaucium alakirensis*, *Marrubium bourgaei*, and *Peucedanum alpinum* from Türkiye

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ABSTRACT

Plant species contain many secondary metabolites, and these compounds differ from species to species. These differences in the concentrations of these compounds have many health implications. Today, studies on plants' antioxidant and antibacterial effects are gaining importance. In particular, the adverse effects of some existing antibiotics and the constant development of bacterial resistance are leading to the search for new natural antimicrobial agents. In this study, methanol, ethanol, ethyl acetate, acetone, and chloroform extracts were obtained from the aerial parts of *Marrubium bourgaei* Boiss and *Glaucium alakirensis* Aykurt, K.Yıldız & A.Özçandır, and *Peucedanum alpinum* B.L.Burtt & Davis, species which are naturally distributed in Türkiye. The antioxidant activity of the extracts was determined by the DPPH (2,2 Difenil-1-pikrihidrazil) and ABTS (2,2' azino-bis(3-ethylbenz-thiazoline-6-sulfonic-acid)) methods, the total phenolic content by Folin-Ciocalteu method, the total flavonoid content by aluminium chloride colorimetric method, and the antibacterial activity against ten bacteria by the disc diffusion method. According to the results, methanol, ethanol, and acetone extracts had higher antioxidant activity, total phenolic, and total flavonoid contents than other extracts. However, the total flavonoid content of *M. bourgaei* was higher in the ethyl acetate extract. When evaluated for their antibacterial activity, ethanol, chloroform, and ethyl acetate in *P. alpinum*, chloroform in *M. bourgaei*, and methanol, chloroform, and ethyl acetate in *G. alakirensis* extracts showed antibacterial activity against more bacteria than others. This is the first study to evaluate and compare the total phenolic and flavonoid content, and antioxidant and antibacterial activities of 5 different extracts of these plants.

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Türkiye'den *Glaucium alakirensis*, *Marrubium bourgaei*, and *Peucedanum alpinum*'un Çeşitli Ekstraktlarının Biyolojik Aktivitelerinin Değerlendirilmesi

ÖZET

Bitki türleri birçok ikincil metabolit içerir ve içermiş oldukları bu bileşikler türden türe farklılık göstermektedir. Bitkilerin ikincil metabolitlerinin konsantrasyonlarındaki bu farklılıkların sağlık üzerinde farklı etkileri vardır. Bitkilerin antioksidan ve antibakteriyel etkileri üzerine yapılan çalışmalar günümüzde yeniden önem kazanmaktadır. Özellikle mevcut bazı antibiyotiklerin olumsuz etkileri ve sürekli olarak artan bakteriyel direnç, yeni ve doğal antimikrobiyal ajan arayışına neden olmaktadır. Bu çalışmada, Türkiye'de doğal olarak yayılış gösteren *Marrubium bourgaei* Boiss, *Glaucium alakirensis* Aykurt, K.Yıldız & A.Özçandır ve *Peucedanum alpinum* B.L.Burtt & Davis türlerinin toprak üstü kısımlarından metanol, etanol, etil asetat, aseton ve kloroform kullanılarak ekstraktlar elde edilmiştir. Bu ekstraktların antioksidan aktiviteleri DPPH (2,2 Difenil-1-pikrihidrazil) ve ABTS (2,2' azino-bis(3-etilbenz-tiazolin-6-sülfonik-asit)) yöntemleriyle, toplam fenolik madde içerikleri Folin-Ciocalteu yöntemiyle, toplam flavonoid madde içerikleri ise alüminyum klorür

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kolorimetrik yöntemi ile belirlenmiştir. Ayrıca elde edilen bu bitkisel ekstraktların antibakteriyel aktiviteleri disk difüzyon yöntemi kullanılarak on bakteri için değerlendirilmiştir. Elde edilen sonuçlara göre, bitkilerin metanol, etanol ve aseton ekstraktları diğer ekstraktlarından daha yüksek antioksidan aktivite, toplam fenolik ve flavonoid madde içeriğine sahiptir. Ancak, *M. bourgaei*'nin toplam flavonoid madde içeriği etil asetat ekstraktında daha yüksek bulunmuştur. Ekstraktların antibakteriyel aktiviteleri değerlendirildiğinde, *P. alpinum*'da etanol, kloroform ve etil asetat, *M. bourgaei*'de kloroform, *G. alakirensis*'de ise metanol, kloroform ve etil asetat ekstraktları diğer ekstraktlara göre daha fazla bakteriye karşı etkilidir. Gerçekleştirilen bu çalışma, kullanılan bu üç bitkinin beş farklı ekstraktının toplam fenolik madde içeriğini, toplam flavonoid madde içeriğini, antioksidan aktivitelerini ve antibakteriyel aktivitelerini değerlendiren ve karşılaştıran ilk çalışmadır.

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INTRODUCTION

Since the existence of humanity, plants have been used both as food and for medicinal purposes (Dubick, 1986; Johns, 1990; Balick & Cox, 1996). In recent years, bioactive compounds from plants have been increasingly preferred, mainly because of the potential side effects of synthetic compounds (Naveed et al., 2018). The unnecessary and unconscious use of antibiotics has increased bacterial resistance to antibiotics and led to numerous side effects and various clinical problems in patients (Hughes & Andersson, 2017). Therefore, in recent years, the search for new antimicrobial agents from plants and the elaboration of information on the possibilities of using phytochemicals in this context has gained renewed attention (Savoia, 2012; Vaou et al., 2021).

One of the plants used in this study, *Glaucium alakirensis* was recently discovered in the Alakır Valley (Kumluca, Antalya) and has a minimal distribution range (Aykurt et al., 2017). Moreover, there is very limited information on the pharmacological and phytochemical properties of *G. alakirensis*. However, numerous studies have been conducted on closely related species. For example, *G. flavum* Crantz is one of the most intensively researched species within this genus. Various medicinal properties are attributed to this plant, such as antioxidant, bronchodilator, antitussive, and hypoglycemic effects (Arafa et al., 2016).

Marrubium bourgaei, which is native to Türkiye, is only found in high mountainous regions. The species of this genus are recognized for their various health-promoting effects and are used in traditional medicine to treat ailments such as asthma, hypotension, and pain relief (Bardai et al., 2001). Furthermore, several species of this genus contain various secondary metabolites of biological importance, including diterpenes, polyphenols, steroids, phenylpropanoids, and flavonoids (Hamedeyazdan et al., 2014).

Peucedanum alpinum B.L.Burtt & Davis is only known from the regions of Antalya (Türkiye) and Crete (Greece). The specialised roots of the genus *Peucedanum* L. have been used in traditional Chinese medicine for over 1500 years to cure heat and congestion in the lungs (Skalicka-Woźniak et al., 2010). The leaves of some species are also said to have healing properties for tissue injuries (Danna et al., 2022). Information on *P. alpinum* species is very limited; there are no studies on their phytochemical or pharmacological properties.

In this study, methanol, ethanol, ethyl acetate, acetone, and chloroform extracts were obtained from *P. alpinum* and the species *G. alakirensis* and *M. bourgaei* endemic to Türkiye. In selecting the plant species for this study, certain factors were considered that make these plants particularly interesting. First of all, these plants are endemic to Türkiye indicates that they have a potential uniqueness in terms of their chemical composition and bioactive compounds. Secondly, these species belong to plant families known for their medicinal properties: Lamiaceae (Uritu et al., 2018), Apiaceae (Sayed-Ahmad et al., 2017), Papaveraceae (Zielińska et al., 2021) and literature studies have shown that close relatives of these plants also show good results in terms of their health properties. Finally, the antioxidant activity, the total content of flavonoids and phenols, and the antibacterial activity of the five different extracts of these three species were analyzed and compared for the first time in this study.

MATERIALS and METHODS

Collection of Plant Materials

The plant materials used in this study were collected in the alpine and sub-alpine zones of the Western Taurus Mountains. *P. alpinum* (M. Gülben 1040 & E. Delik, AKDU 6365) and *G. alakirensis* (M. Gülben 1042 & E. Delik, AKDU 6367) were collected from screes and slopes between the Dibek Nature Reserve and the Kırkmuar Plateau at an altitude of 1330 m in Kumluca (Antalya/Türkiye), which is also the type location of *G. alakirensis.* Meanwhile, *M. bourgaei* (M. Gülben 1041 & E. Delik, AKDU 6366) was collected at the entrance of the high mountain plain of the Kırmuar Plateau (2000 m), 6-7 km southwest of the other two species' location. The identities of the plants were determined by the plant systematists C. Aykurt and M. Gülben.

Plant Extraction

The protocols used by Berber et al. (2013) and Gonelimali et al. (2018) were used to prepare the plant extracts with minor modifications. Fresh plant samples brought from the field to the laboratory were allowed to dry at room temperature (RT) in the shade. The samples dried for about 15-20 days were pulverised using a mechanical grinder. The pulverised plant samples (10 g) were placed in 100 mL of solvent (Merck, Germany) (ethanol, methanol, acetone, ethyl acetate, chloroform) and shaken gently overnight in a shaker. Then each solvent was filtered separately with filter paper (Whatmann No:1), and the samples were placed in a fume hood to evaporate the solvents. After drying, the obtained residues were stored at +4°C to be used for the experiments. In each experimental study, the extracts were prepared at concentrations of 1 mg mL^{-1} by dissolving in dimethyl sulphoxide (DMSO) (Merck, Germany).

Antioxidant Measurements with ABTS Free Radical Scavenging Method

The plant extracts were subjected to the ABTS radical scavenging assay according to the protocol described by Xiao et al. (2014). The ABTS reagent was prepared by reacting 7 mM aqueous ABTS solution (Sigma Aldrich, USA) and 2.45 mM $K_2S_2O_8$ (Sigma Aldrich, USA) for 16 h at RT in the dark. Then the ABTS+ stock solution was diluted with pure ethanol until it reached an OD of 0.70 at λ_{734}. 4 mL of the $ABTS^{\cdot+}$ stock solution was added to 1 mL of the sample and incubated at RT for 6 min. Then the absorbance value of the samples was measured at 734 nm and the radical scavenging capacity (%) was calculated using Equation (1).

$$\%Inhibiton = \frac{(Absorbance_{Control} - Absorbance_{Sample})}{Absorbance_{Control}} \times 100 \quad (1)$$

Antioxidant Measurements with DPPH Free Radical Scavenging Method

The plant extracts were subjected to the DPPH radical scavenging assay according to the protocol described by Subhasree et al. (2009). Initially, a $6x10^{-5}$ M methanol solution of DPPH (Sigma Aldrich, USA) was prepared. Subsequently, 3 mL of the methanol (Merck, Germany) of DPPH was added to 100 µL of the samples and mixed well. The prepared samples were stored at RT in a dark environment for 15 min. Then, the absorbance values of the samples were measured at 516 nm and the % DPPH radical scavenging capacity was calculated using Equation (1).

Evaluation of Total Phenolic Substance Content

The total phenolic substance content in the plant extracts was analyzed by a colourimetric method based on the Folin-Ciocalteu (FC) reagent (Škerget et al., 2005). The FC reagent was formulated according to the procedure described by Singleton & Rossi, (1965) outlined. 2.5 mL of the FC reagent (diluted 1:10) was added to 500 µL of the samples and incubated for 2 min. Then, 2 mL of Na_2CO_3 (7.5%) (Sigma Aldrich, USA) was added to the samples, and the samples were vortexed for 30 s. The vortexed samples were kept at 50°C for 5 min. The absorbance values of the samples were measured at λ_{760}, and the gallic acid equivalents of the samples (mg mL^{-1} GAE) were calculated.

Evaluation of Total Flavonoid Substance Content

The total flavonoid content in the plant extracts was assessed using the aluminum chloride colourimetric method (Ghasemi et al., 2009). 1.5 mL of methanol (Merck, Germany), 100 µL $AlCl_3$ (10%) (Sigma Aldrich, USA), 100 µL CH_3CO_2K (1 M) (Sigma Aldrich, USA), and 2.8 mL distilled water were added to 500 µL of each of the plant extracts, and the samples were incubated at RT for 30 min. After incubation, the absorbance values of the samples were measured at λ_{415}, and the quercetin equivalents (mg QE mL^{-1}) were calculated.

Determination of Antibacterial Activity

The antibacterial activity of the plant extracts was determined using the disc diffusion method (Bauer, 1966). In the experiments, *Klebsiella pneumoniae* (ATCC 13883), *Escherichia coli* (K55), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (PY79), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cereus* (DSM 22648), *Salmonella enterica* (LT2), and clinical isolates of *Proteus mirabilis* and *Listeria monocytogenes* were used. The clinical bacterial isolates were provided by Prof. Dr. Meral Dilara Öğünç (Akdeniz University, Antalya, Türkiye).

The bacteria were adjusted to 0.5 McFarland in NaCl (0.85%) solution (Merck, Germany) and spread on Mueller-Hinton Agar medium (MHA) (Merck, Germany). 30 µL plant extracts containing antibiogram discs (Bioanalyse, Türkiye) were placed on inoculated MHA. In the experiments, kanamycin discs (30 µg) (Cayman Chemical, USA) were used as positive controls, and antibiogram discs impregnated with 30 µL DMSO as negative controls. The Petri dishes were placed in an incubator at 37°C for 24 h and then the zone diameters were measured.

Statistical Evaluation

Experiments were conducted in triplicate, and statistical analyses were carried out utilizing one-way analysis of variance (ANOVA) (IBM SPSS 22 software was used (SPSS, USA)). The Tukey test was used to perform multiple comparisons. Data were presented as mean ± standard deviation and statistical significance was determined at $p<0.05$.

RESULTS AND DISCUSSION

Antioxidant Measurements with ABTS Free Radical Scavenging Results

The ABTS assay is an antioxidant assay based on the formation of the blue-green ABTS+ radical and can be applied to both hydrophilic and lipophilic antioxidant systems (Kim et al., 2002). This study investigated the ABTS radical scavenging capacity of the plant extracts obtained with different solvents (Figure 1). When the ABTS activities of the plants were analyzed, the highest ABTS activities were found in the methanol, ethanol, and acetone extracts of the plants. *P. alphinum* had the highest ABTS activity in all 3 extractions.

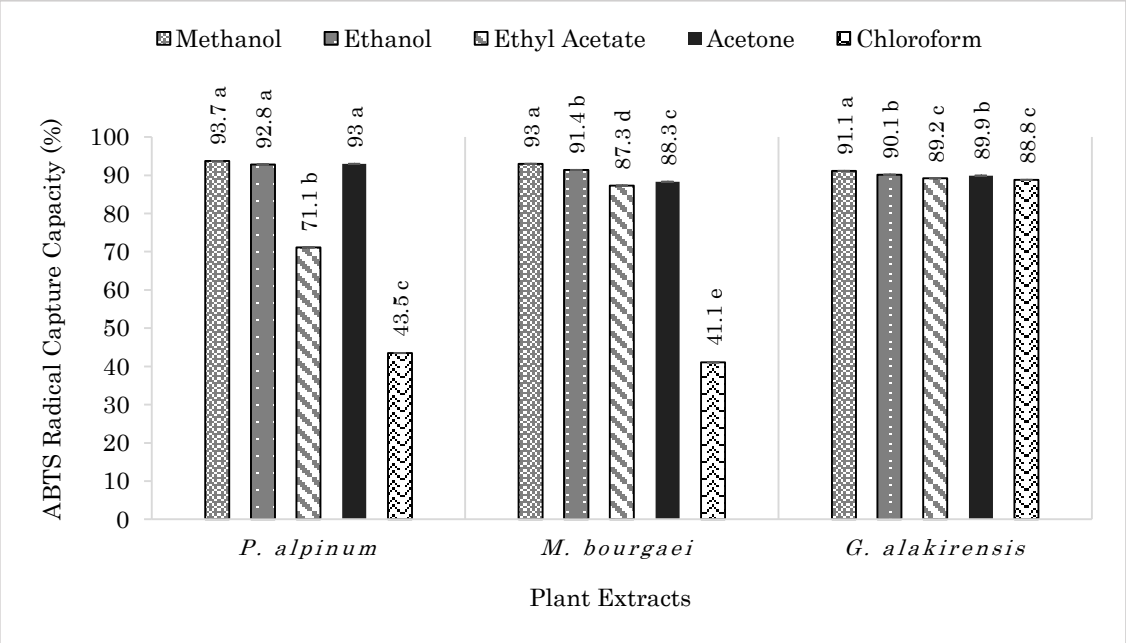

Figure 1. ABTS radical scavenging capacity of plant extracts (%) (different superscripts indicate a statistically significant difference ($p<0.05$))

Şekil 1. Bitki ekstraktlarının ABTS radikal temizleme kapasitesi (%) (farklı üst simgeler istatistiksel olarak anlamlı bir farkı gösterir (p<0.05))

It was found that the chloroform extracts of *M. bourgaei* had the lowest value in terms of ABTS scavenging capacity ($p<0.05$), while the methanol extract of *M. bourgaei* had the highest ABTS radical scavenging capacity ($p<0.05$). Yumrutaş and Saygıdeğer (2010), in their study with *M. parviflorum* Fisch. & Mey. and *Lamium amplexicaule* L., found that the ABTS activity of methanol extracts of the plants was higher than that of hexane extracts. In another study, Okur et al. (2019) reported that methanol extracts of *M. vulgare* L. had higher ABTS activity compared to trolox. Hayat et al. (2020) reported that ethanol extracts of *M. vulgare* from two different regions in north-eastern Morocco showed higher ABTS activity. They also stated that extracts prepared with polar solvents such as ethanol and methanol would give better results in ABTS and DPPH experiments than extracts prepared with intermediate or weakly polar solvents.

According to the findings, there was no statistical difference between methanol, ethanol, and acetone extracts of *P. alpinum* ($p>0.05$); however, the chloroform extract of the plant had the lowest ABTS scavenging capacity compared to the other extracts. The lowest ABTS scavenging capacity of the

chloroform extract may be because chloroform has the lowest polarity of the solvents used. In their study, Matejić et al. (2013) found that the highest ABTS activity was found in water extracts of *P. longifolium* Waldst. & Kit. and *P. aegopodioides* (Boiss.) Vandas, ethyl acetate extract of *P. alsaticum* and acetone extract of *P. officinale*, but there was no ABTS activity in ethyl acetate and acetone extracts of *P. aegopodioides* species. Moreover, Danna et al. (2022) showed that the ethanol extracts of the leaf had higher ABTS activity than the ethanol extracts of the root of *P. ostruthium* (L.) Koch. Furthermore, they stated that this is because the plant's fresh leaves have more phenolic and flavonoid compounds than the roots.

It was found that the samples from methanol extractions of *G. alakirensis* were significantly higher than the other extraction samples ($p<0.05$). Ozsoy et al. (2018), in their study of the methanolic extract of *G. grandiflorum* Boiss. & A.Huet var. *grandiflorum*, stated that ABTS scavenging activity was close to that of the routine used as a control. Alali et al. (2007) reported in their study in Jordan that the ABTS activity of water and methanol extracts of *G. aleppicum* Boiss. & Hausskn. showed the highest value among 95 species.

Antioxidant Measurements with DPPH Free Radical Scavenging Results

When the DPPH activities of the samples were evaluated, a similar pattern was observed as for the ABTS scavenging activity, and the samples from the chloroform extraction showed a lower DPPH scavenging capacity than the other extracts for all plants (Figure 2) ($p<0.05$). When the DPPH scavenging capacity of the plants was evaluated, the highest DPPH scavenging capacity was found in the methanol, ethanol, and acetone extracts of the plants. *G. alakirensis* had the highest DPPH scavenging capacity in methanol and ethanol extracts. In addition, *P. alpinum* had the highest DPPH scavenging capacity among the acetone extracts of the plants.

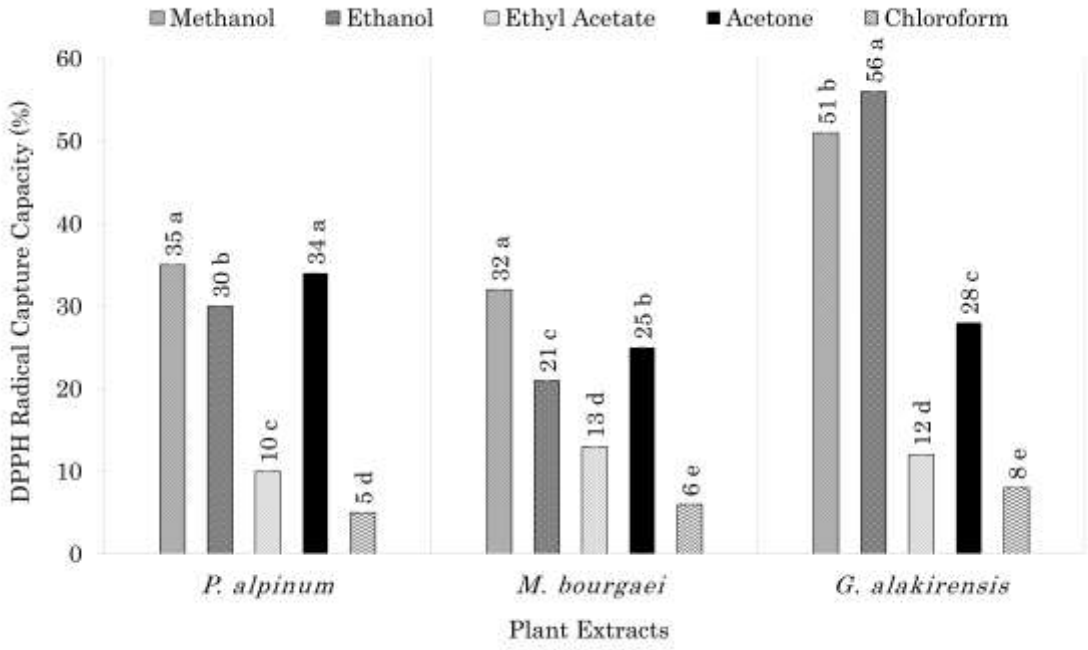

Figure 2. Ability of plant extracts to bind DPPH radicals (%) (different superscripts indicate a statistically significant difference ($p<0.05$))

Şekil 2. Bitki ekstraktlarının DPPH radikallerini bağlama yeteneği (%) (farklı üst simgeler istatistiksel olarak anlamlı bir farkı gösterir ($p<0.05$))

DPPH experiments use a radical that dissolves in organic solvents (such as alcohol). Therefore, DPPH is only applicable to hydrophobic antioxidant systems (Kim et al., 2002). Few studies are addressing the effects of different extraction solvents on the biological activities of the plant samples used in this study. In particular, too much attention has been paid to publications using the essential oils of *Marrubium*. However, studies on the essential oil of *Marrubium* are widely available and it is found that the methanol extract of *Marrubium* has better biological activities. For example, Sarikurkcu et al. (2008) evaluated the antioxidant properties of the methanol extracts and essential oil of *M. globosum* Montbret & Aucher and reported that the lowest DPPH radical scavenging activity was found in the essential oil and the strongest antioxidant activity was found in the methanol extract. Similarly, in this study, the methanol extract of *M.*

bourgaei showed the highest DPPH scavenging activity ($p<0.05$). Yumrutaş and Saygıdeğer (2010) stated that the antioxidant effect of methanol extracts might be related to the presence of various compounds such as polar thermolabile and/or thermostable phenols. Chemsa et al. (2016) reported in their study with the methanol extracts and essential oil of *M. deserti* (de Noé) Coss. that the methanol extract showed high antioxidant activity in DPPH analysis, while the essential oil showed weak activity. Furthermore, their findings indicated that polar extracts exhibited greater antioxidant activity compared to non-polar extracts. According to Kumoro et al. (2009), the polarities of the solvents used in this study can be listed as methanol, acetone, ethanol, ethyl acetate, and chloroform from strong to weak, and therefore methanol extracts of *P. alpinum* and *M. bourgaei* are thought to have higher antioxidant activity. Sarikurkcu et al. (2020) investigated the enzyme inhibitory and antioxidant activities of water, methanol, and ethyl acetate extracts of *M. lutescens* Boiss. & Heldr. and reported that the water extract exhibited the highest antioxidant capacity, followed by the methanol extract.

In this study, there was no significant difference in DPPH activity of methanol and acetone extracts of *P. alpinum* ($p>0.05$). Kim et al. (2018) evaluated the antioxidant properties of water, hexane, ethyl acetate, and ether extracts of the plants *Saposhnikovia divaricata* (Turcz. ex Ledeb.) Schischk., *P. japonicum* Thunb., and *Glehnia littoralis* (A.Gray) F. Schmidt and found that ether and ethyl acetate extracts of *P. japonicum* had higher DPPH scavenging activity compared to the other extracts. Similarly, Sarkhail et al. (2013) investigated the antioxidant activities of hydroalcoholic extracts of *P. knappii* Bornm. (n-hexane, dichloromethane, ethyl acetate, and water) and reported that ethyl acetate exhibited the highest antioxidant activity. They also isolated two flavonol glycosides, isorhamnetin-3-O-ß-D-glucopyranoside, and rhamnetin-3-O-ß-D-glucopyranoside, which are considered to be effective tyrosinase inhibitors and antioxidants from the ethyl acetate extract of *P. knappii*. However, in the study, the antioxidant activity of the methanol and acetone extracts of *P. alpinum* was higher. Al et al. (2012) evaluated the antibacterial and antioxidant activity of the methanol extract of *P. zenkeri* Engl. and reported that the DPPH scavenging capacity of 1 mg mL^{-1} methanol extract was 93.39%. They also stated that this high antioxidant activity could be due to phenolic compounds, anthraquinones, flavonoids, tannins, and anthocyanins. However, in this study, the methanol extract of *P. alpinum* was found to have a DPPH scavenging capacity of 35%. The antioxidant activity of the plant varies depending on the developmental stage of the plant, plant tissue, plant species, and environmental factors such as light, temperature, and water stress (Upadrasta et al., 2011; Zlatić et al., 2019). Therefore, these factors may have caused differences in biological activity between plant species belonging to the same genus.

In this study, the ethanol extract of *G. alakirensis* had the highest DPPH scavenging capacity ($p<0.05$). Özçandır et al. (2024) showed that the ethanolic extract of *G. alakirensis* had higher antioxidant activity than rosmarinic acid and caffeic acid, which were used as positive controls. They also reported that the ethanolic extract of the plant contained phenolic substances such as chlorogenic acid (32.95 ppm), catechin (39.08 ppm), hydroxybenzoic acid (28.37 ppm), gallic acid (5.57 ppm), and quercetin (0.73 ppm). In their research with *G. grandiflorum*, Ozsoy et al. (2018) showed that the methanol extract of the plant had significant DPPH activity, but this capacity was lower than that of rutin, which they used as a control. In their study with *G. flavum*, Boulaaba et al. (2019) showed that the ethanol extract of the plant had a higher radical scavenging capacity than the ethyl acetate extract. In this study, the ethanol extract of *G. alakirensis* also had a higher antioxidant activity than the ethyl acetate extract. It is assumed that this is due to the the polarity of ethanol, which is higher than that of ethyl acetate (Kumoro et al., 2009).

Evaluation of Total Phenolic Substance Content Results

Various antioxidant compounds, especially phenolic compounds, are closely associated with the antioxidant activity of a plant (Balasundram et al., 2006). Phenolic compounds are prominent substances with antioxidant functions for human health and are widely distributed in plants (Karaman et al., 2022; Şahin et al., 2022). When the total phenolic content of the plants was evaluated in this study, all extracts of *G. alakirensis* had higher total phenolic content than *P. alpinum* and *M. bourgaei*. There are no studies in the literature investigating the effects of the different extraction methods on the antioxidant activities of the plants used in this study. This study is the first to determine and compare the effects of 5 different extraction methods on the total phenolic content of these plants (Figure 3). However, similar studies on closely related plant species can be found in the literature. In their study, Sarikurkcu et al. (2018) evaluated the antioxidant properties of the essential oil and hexane, dichloromethane, methanol, ethyl acetate, and water extracts of *M. parviflorum* and reported that the water extract had the highest total phenolic content. They also found that there was a significant correlation between the total phenolic content and the DPPH experiments of the extracts and that the antioxidant activities of the extracts were directly dependent on the amount of phenolic compounds. However, in this

study, the methanol extract of *M. bourgaei* had the highest phenolic content ($p<0.05$). Similar to the study Sarikurkcu et al. (2020) investigated the enzyme inhibitory and antioxidant effects of methanol, ethyl acetate, and water extracts of *M. lutescens* and reported that the methanol extract had the highest total phenolic content (54.80 ± 0.52). They also stated that different results obtained by using different solvents may be related to the type of solvent used, the analytical techniques, and the characteristics of the samples..

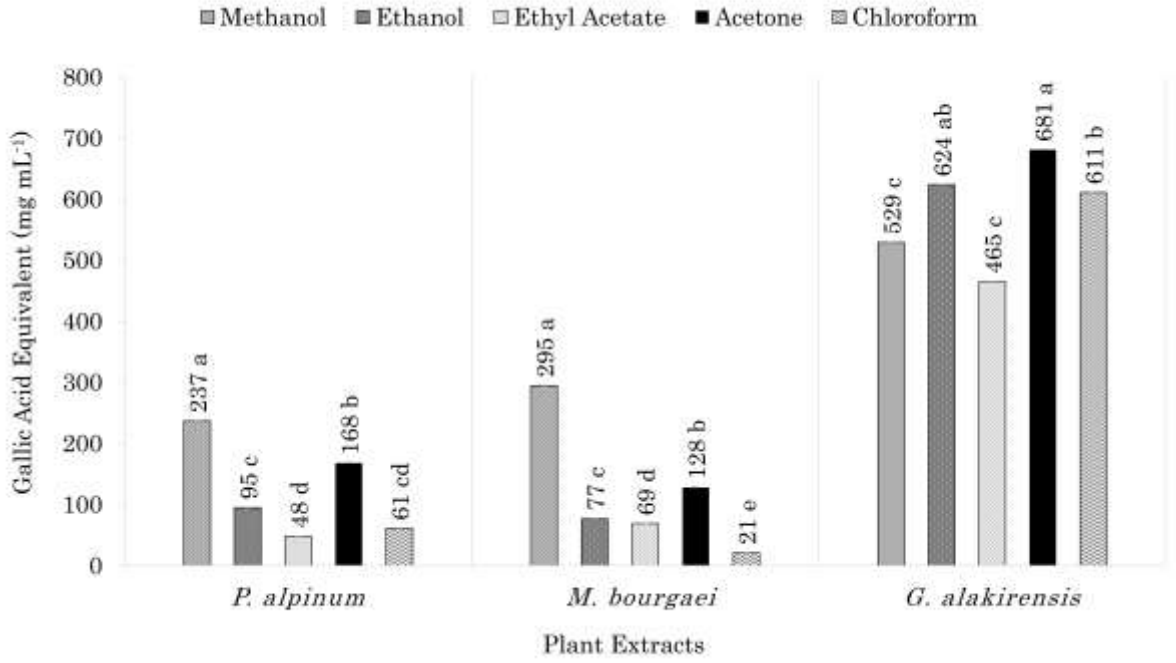

Figure 3. Total phenolic content of plant extracts (mg GAE mL^{-1}) (different superscripts indicate a statistically significant difference ($p<0.05$))

Şekil 3. Bitki ekstraktlarının toplam fenolik madde içerikleri (mg GAE mL^{-1}) (farklı üst simgeler istatistiksel olarak anlamlı bir farkı gösterir ($p<0.05$))

In this study, the methanol extract of *P. alpinum* had the highest phenolic content ($p<0.05$). Kim et al. (2018), in their study on *P. japonicum* extracts obtained with different solvents (water, hexane, ethyl acetate, ether), reported that the ether and ethyl acetate extracts of the plant had higher total phenolic content. They also stated that there was a correlation between the total phenolic substance and the antioxidant activities of the extracts. Matejić et al. (2013) compared the acetone, methanol, ethyl acetate, and water extracts of four *Peucedanum* species and reported that the total phenolic content of the extracts ranged from 52.18-118.32 mg GAE g^{-1}. In the study, the total phenolic content of *P. alpinum* extracts ranged between 48-237 mg GAE mL^{-1}.

It was found that the acetone extract of *G. alakirensis* had the highest phenolic content in the study ($p<0.05$). In the study by Kocanci et al. (2017), the total phenolic content of methanol extracts of *G. acutidentatum* Hausskn. & Bornm. and *G. corniculatum* (L.) Curtis was higher than that of the water extracts, but it was not statistically different. Boulaaba et al. (2019) reported in their study with *G. flavum* that the total phenolic content of the plant's ethanol extract was higher than that of the ethyl acetate extract. They also found that the number of phenolic compounds identified in the ethanol extract such as caffeic acid, syringic acid, isoquercitrin, trans-hydroxycinnamic acid, catechin hydrate, and chlorogenic acid was higher than the number of total compounds identified in ethyl acetates such as trans-hydroxycinnamic acid, catechin hydrate, and chlorogenic acid and that catechin hydrate and isoquercitrin were the major components

Evaluation of Total Flavonoid Substance Content Results

Flavonoids are secondary compounds commonly found in plants with diverse bioactivities and potential health benefits (Fu et al., 2021). This study is the first to evaluate and compare the total flavonoid content of 5 different plant extracts. The data on the total flavonoid content of the herbal extracts are shown in Figure 4. The acetone extract of *G. alakirensis* had the highest total flavonoid content of all plants ($p<0.05$).

No studies were found that demonstrate the effects of the extracts from the plants used in this study on biological activity. However, similar studies in the literature on closely related plant species may be

useful to compare the results. Interestingly, in this study, the highest values for ABTS, DPPH, and total phenolic content were found in the methanol extract, while the highest value for total flavonoid content was found in the ethyl acetate extract of *M. bourgaei* ($p<0.05$). Sarikurkcu et al. (2018) evaluated the antioxidant properties of essential oil and solvent extracts (hexane, methanol, ethyl acetate, dichloromethane, and water) of *M. parviflorum* and reported that the methanol extract had the highest content of total flavonoids. Sarikurkcu et al. (2020) investigated the antioxidant and enzyme-inhibitory effects of water, methanol, and ethyl acetate extracts of *M. lutescens* and reported that the water extract had the highest total flavonoid content (27.20 ± 0.81), while the methanol extract had the highest total phenolic content (54.80 ±0.52). It is known that these changes in total flavonoid content depend on the climatic conditions and geography of the area where the plant is collected, as well as the polarity of the solvent and the type of extract (Hayat et al., 2020; Bouterfas et al., 2016).

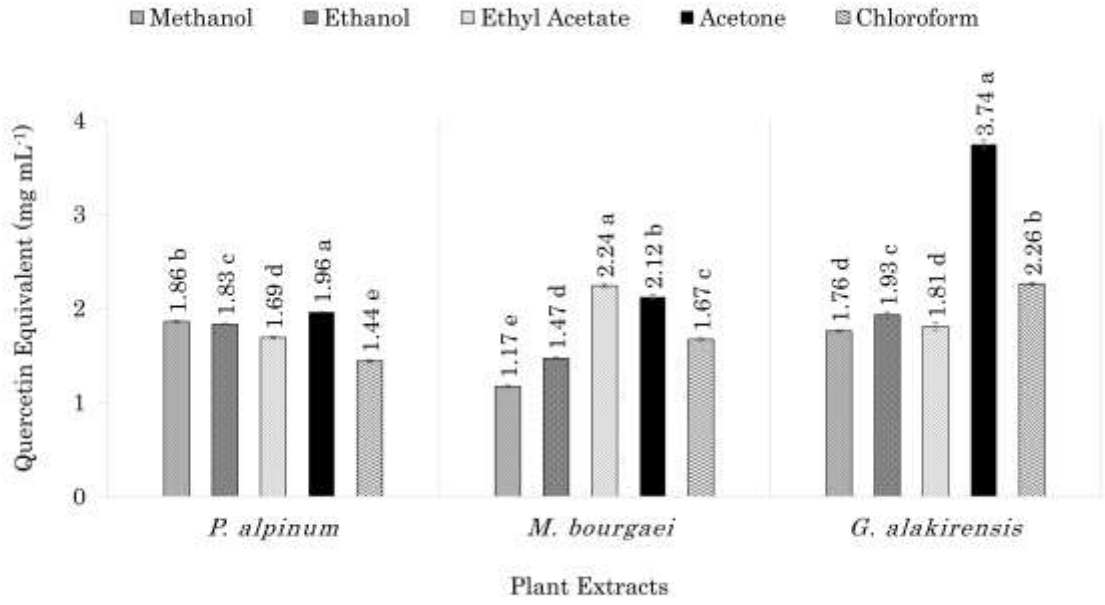

Figure 4. Total flavonoid content of plant extracts (mg KE mL^{-1}) (different superscripts indicate a statistically significant difference ($p<0.05$))

Şekil 4. Bitki ekstraktlarının toplam flavonoid madde içerikleri (mg KE mL^{-1}) (farklı üst simgeler istatistiksel olarak anlamlı bir farkı gösterir ($p<0.05$))

The total phenolic content of plant extracts varies depending on the temperature and time during the extraction process, the polarity of the extraction solvents, and the solubility of the phenolic substances in the solvent used for the extraction process (Abozed et al., 2014; Özer et al., 2021). In the present study, the acetone extract of *P. alpinum* had the highest phenolic content ($p<0.05$). Matejić et al. (2013) evaluated the antioxidant activity of extracts from four *Peucedanum* species (methanol, ethyl acetate, acetone, and water) and reported that the total flavonoid content of the extracts ranged from 4.43 to 234.67 mg quercetin equivalent (Qu) g^{-1} extract. Sarkhail et al. (2013) investigated the bioactivity of *P. knappii* extracts (dichloromethane, ethyl acetate, n-hexane, and water) and reported that ethyl acetate had the highest total phenolic and flavonoid content.

Similar to the results of total phenolic content, the acetone extract of *G. alakirensis* had the highest total flavonoid content ($p<0.05$). Boulaaba et al. (2019) investigated the antioxidant properties of petroleum ether, ethanol, and ethyl acetate extracts of *G. flavum* and reported that the ethanol extract had the highest total flavonoid content. Shaghaghi et al. (2019) investigated the antioxidant properties of plants of the genera *Papaver* and *Glaucium* from different geographical regions of Iran and reported that the highest total flavonoid content was found in *G. mathiolifolium* Mobayen. Moreover, they reported that the antioxidant activity and total phenolic and flavonoid content varied among species as well as among different organs of the plants.

Determination of Antibacterial Activity Results

Certain medicinal plants are promising as potential reservoirs for new antibacterial agents (Koné et al., 2004). In the study to demonstrate the antibacterial

activity of the different plant extracts, kanamycin was used as a positive control and was effective on all bacterial strains; DMSO, which was used as a negative control, was not effective on any bacterial strain (Table 1). In this study, while all extracts of *M. bourgaei* showed antibacterial activity against *B. cereus*, *P. aeruginosa*, and *E. coli,* they were not effective against *P. mirabilis* and *L. monocytogenes* (Table 1). Antibacterial studies on *Marrubium* essential oil are more common in the literature. However, the methanol extract of the plant was found to have better antibacterial activity than the essential oil extracts. For example, Chemsa et al. (2016) evaluated the activities of the essential oil and methanol extract of *M. deserti* in terms of biofilm formation and anticholinesterase and reported that the antibiofilm activity of the methanol extract against six bacteria (*S. epidermidis, B. cereus, Micrococcus luteus, Streptococcus mutans*, *S. aureus*, *B. subtilis*) and *Candida albicans* was higher than that of the essential oil. Laouer et al. (2009), in their study, analysed the chemical composition, antimicrobial and antioxidant activity of the essential oil of *M. deserti* and did not observe any antimicrobial activity against *P. aeruginosa*, *S. aureus,* and *E. coli* strains. In this study, however, the methanol and ethyl acetate extracts of *M. bourgaei* were particularly effective against these three strains. Golmakani et al., (2016) investigated the essential oil composition and antibacterial properties of *M. duabense* Murata essential oils and found that they only observed antibacterial activity against *Clostridium perfringens* and no effect against *S. aureus*, *Salmonella*, and *E. coli*. However, in this study, the methanol extract of *M. bourgaei* was effective against these three strains. Based on the results of this study, it can be said that the extracts of *M. bourgaei* are at least as worthy of research as their essential oil. It is known that small hydrophilic molecules can easily pass through the outer membrane of Gram-negative bacteria, a property required for molecules with antibacterial activity. Methanol is also a small molecule that can penetrate the outer membrane and cause bacterial death (Kang et al., 2011). In our study, we did not find that methanol extracts were more effective against Gram-negative bacteria. This may suggest that the difference may be due to the selective effect of the secondary metabolites extracted from the plant species on the bacterial cells and not to the effect of the extraction method alone. The antimicrobial activity of plant extracts and essential oils varies according to their secondary metabolites (such as alkaloids, polyphenols, phenolic/flavonoid compounds, sulfur-containing compounds, terpenes, and coumarins). The antimicrobial activity exhibits a wide range depending on the alkylation of the glycosidic bonds of the OH groups, the number, structure, and position of the substituent groups, the type of plant, the topography, and the climate of the country where the plant was collected (Vaou et al., 2021). Similarly, plant species used in this study may have different antimicrobial activities due to secondary metabolites.

In this study, ethanol, ethyl acetate, and chloroform extracts of *P. alpinum* were effective against all bacteria except *S. enterica*. The methanol extract was effective against all bacteria except *S. enterica* and *K. pneumoniae*. Acetone extract had the lowest antibacterial activity and was not effective against *B. cereus*, *S. epidermidis*, *S. enterica,* and *B. subtilis* strains (Table 1). Similarly, Schillaci et al. (2003) evaluated the pharmacological activities of acetone extracts of *P. nebrodense* (Guss.) Nyman and observed no antibacterial activity against the strains they used (*B. subtilis*, *S. aureus*, *Streptococcus agalactiae*, *P. aeruginosa*, *E. coli*, *Candida albicans*, and *Candida tropicalis*). Kim et al. (2018) investigated the antimicrobial activities of *P. japonicum*, *S. divaricata*, and *G. littoralis* extracts (water, ethyl acetate, hexane, ether) and reported that only ether and ethyl acetate extracts of *P. japonicum* showed antimicrobial activity. Madumelu et al. (2013) evaluated methanol extracts of *P. winkleri* H. Wolff. for its phytochemical component and antimicrobial properties and reported that the extract inhibited the growth of pathogens and was comparable to the standard drugs used. However, they were unable to demonstrate antibacterial activity against *S. pyogenes*, *P. aeruginosa*, and *Candida krusei*.

G. alakirensis all extracts were effective against *K. pneumoniae*, *E. coli*, *S. aureus*, *P. mirabilis*, and *L. monocytogenes*. In addition, ethyl acetate and acetone extracts of the plant formed the same zone diameter as the positive control for *P. mirabilis*. According to Özçandır et al. (2024), *G. alakirensis* ethanol extracts were effective against *S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *C. albicans,* and *C. tropicalis* at concentrations of 50-200 µg mL^{-1}. Plant extracts were effective against microorganisms at concentrations of 50-200 µg mL^{-1}. However, Mojdeh et al. (2017) reported that alkaloid fractions and methanol extracts of *G. aucheri* and *G. vitellinum* were not effective against bacteria and also *Candida albicans*. Morteza-Semnani et al. (2005) investigated the antibacterial activity of extracts from three *Glaucium* species and found that methanol extracts were effective against Gram-negative microorganisms, while chloroform extracts were most effective against all strains tested. They also reported that 10 and 50 µg methanol and chloroform extracts of the plants were not effective against *S. aureus* (except chloroform extract of *G. oxylobum*). However, in this study, methanol and chloroform extracts (30 µg $disc^{-1}$) of *G. alakirensis* were found to be effective against *S. aureus*. Tosun et al. (2006) worked with 14 different plants used in traditional medicine and reported that the methanol

extract of *G. grandiflorum* was not effective against any of the bacteria and fungi (except *C. krusei*) they used. However, in this study, the antibacterial activities of *G. alakirensis*, especially the chloroform, methanol, and ethyl acetate extracts, were found to be quite broad-spectrum.

Table 1. Mean zone diameter of the antibacterial effects of the extracts (in mm)
Çizelge 1. Ekstraktların antibakteriyel etkilerinin ortalama zon çapı (mm)

	EC	KP	BC	SA	PA	SE	SEN	BS	PM	LM
DMSO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
KAN	15±0	16±0.04	15±0.04	16±0.04	15±0	16±0	17±0.04	19±0.2	10±0.09	18±0.1
Pa-EtOH	9±0	8±0.05	8±0.05	9±0	9±0	7±0	ND	7±0	9±0	10±0.05
Pa-MeOH	10±0	ND	8±0.05	8±0.05	8±0.05	7±0	ND	8±0.05	9±0.05	11±0.05
Pa-Chl	8±0	8±0.05	9±0.05	9±0.05	9±0.05	8±0.05	ND	9±0.05	9±0	13±0.05
Pa-Ace	8±0	8±0.05	ND	9±0.05	8±0	ND	ND	ND	7±0	14±0.05
Pa-AcOEt	9±0	9±0.05	9±0.05	10±0.05	9±0	8±0.05	ND	7±0	8±0.05	11±0.05
Mb-EtOH	7±0	7±0	8±0.05	ND	9±0.05	ND	9±0	ND	ND	ND
Mb-MeOH	7±0	7±0	7±0.05	7±0	9±0	ND	9±0.05	ND	ND	ND
Mb-Chl	8±0	7±0	7±0.04	ND	7±0	8±0.05	8±0.01	10±0	ND	ND
Mb-Ace	8±0.01	ND	7±0.09	ND	9±0.02	ND	8±0.01	8±0.01	ND	ND
Mb-AcOEt	8±0.04	ND	7±0.05	9±0.02	8±0.05	7±0.01	ND	9±0	ND	ND
Ga-EtOH	8±0	7±0	7±0	10±0	ND	ND	7±0	8±0	8±0	8±0
Ga-MeOH	8±0	8±0	8±0	10±0	7±0	10±0	7±0	ND	9±0	10±0
Ga-Chl	9±0	7±0	7±0	12±0.05	9±0	10±0	ND	9±0	9±0	9±0
Ga-Ace	7±0	10±0.02	ND	9±0	ND	ND	8±0	ND	10±0	9±0
Ga-AcOEt	9±0	9±0	9±0	10±0.05	9±0	9±0	7±0	ND	10±0	8±0

The diameter of the discs used in the study is 6 millimetres. (ND: Not Detected, EC: *E. coli*, BC: *B. cereus*, KP: *K. pneumoniae*, SA: *S. aureus*, PA: *P. aeruginosa*, SE: *S. epidermidis*, SEN: *S. enterica*, BS: *B. subtilis*, PM: *P. mirabilis*, LM: *L. monocytogenes*, DMSO: Dimethyl sulfoxide, KAN: Kanamycin, EtOH: Ethanol, MeOH: Methanol, Chl: Chloroform, Ace: Acetone, AcOEt: Ethyl acetate, Pa: *P. alpinum* Mb: *M bourgaei*, Ga: *G alakirensis*)

CONCLUSION

In this study, the antioxidant activity, total phenolic content, total flavonoid content, and antibacterial activity of extractions with different solvents of *P. alpinum*, *G. alakirensis*, and *M. bourgaei* were investigated, and it was found that the use of different solvents for extraction may have various effects on the biological activity of the samples. The best antioxidant activity and total phenolic and flavonoid content generally varied between ethanol, methanol, and acetone extracts. However, in *M. bourgaei,* ethyl acetate showed the highest total flavonoid content compared to the other extracts. Ethanol, methanol, chloroform, and ethyl acetate extracts showed the highest antibacterial activity. It is extremely important to identify these plants growing naturally in Türkiye and to investigate their potential use in medical and industrial fields. There are no or insufficient extraction studies with these species used in this study. From this point of view, this study can fill an important gap in the literature and provide a starting point for research on the important pharmacological properties of species of these genera, but the results of this *in vitro* study cannot be transferred to the clinical field without an *in vivo* study.

Author's Contributions

Eda DELİK, Berfin EROĞLU and Asst. Prof. Burcu Emine TEFON ÖZTÜRK has designed the study and collected the data. The collection of plant materials was done by Mertcan GÜLBEN and Eda DELİK and the identifications were made by Prof. Candan AYKURT and Mertcan GÜLBEN. Eda Delik, Berfin EROĞLU and Asst. Prof. Burcu Emine TEFON ÖZTÜRK executed the experiment. Eda DELİK, Berfin EROĞLU, and Mertcan GÜLBEN wrote the article, and critically reviewed it by Asst. Prof. Burcu Emine TEFON ÖZTÜRK and Prof. Candan AYKURT.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

Ethical Approval

Not required

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