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Research Article

A study on antioxidant and antimicrobial activities of *Phlomis amanica, Echinops viscosus* and *Ruscus aculeatus* from Hatay, Türkiye

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Abstract: This study aimed to examine the antioxidant and antimicrobial activities of *Ruscus aculeatus, Phlomis amanica* and *Echinops viscosus* plants growing in Hatay, Türkiye. The antioxidant effect was evaluated by utilizing the Folin-Ciocalteu, DPPH free radical scavenging, and β-carotene-linoleic acid emulsion methods. Findings were compared to BHA and BHT. Antimicrobial effect was evaluated by the disk diffusion agar method using *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,* and *Candida albicans* fungi. Phenolic substance content was highest in *Phlomis amanica* (859.1 mg/g GAE), followed by *Echinops viscosus* (453.5 mg/g GAE) and *Ruscus aculeatus* (229.7 mg/g GAE). According to the results of both DPPH free radical scavenging and β-carotene-linoleic acid emulsion methods, *Phlomis amanica* showed the highest antioxidant activity because of IC_{50} (0.013 mg/mL) and % inhibition values (62.07%) among the plants examined. Considering antimicrobial analysis, it was determined that all plants were the least effective against *Escherichia coli* and the most effective against *Staphylococcus aureus. Phlomis amanica* exhibited the strongest antimicrobial activity with an inhibition zone of 21 mm.

1. INTRODUCTION

Plants are the origin of many of the drugs used today, thanks to the compounds and the bioactive substances with pharmacological properties in their structure (Nissen & Evans, 2012). Accordingly, three plants belonging to the genera *Phlomis, Echinops,* and *Ruscus,* known for their bioactivity potential, were investigated in this study.

The Lamiaceae family contains 46 genera and 782 taxa in Türkiye. Besides, 346 taxa are endemic. Lamiaceae are important because of their commercial and therapeutic utility (Çalış, & Başer, 2021). The genus *Phlomis* L. (Lamiaceae) has 113 species, especially in the Mediterranean area, Asia, and China (Khitri *et al.,* 2020; Merouane *et al.,* 2019). Some species, including *Phlomis* have typical flavor and are used as herbal tea in folk medicine (Sarikurkcu *et al*., 2013). The *Echinops* genus is a member of the Asteraceae family and consists of 120 species. These species are distributed throughout the Mediterranean, Asia, Africa, and Europe. Different species have also emerged in India (Bouzabata *et al.,* 2018; Elseragy *et al.,* 2024). *Echinops* is utilized for the treatment of inflammation, pain, cough, and sore throat due to its bioactive compounds (Bitew & Hymete, 2019; Sweilam *et al.,* 2022; Rafay *et al.,* 2021). The

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genus *Ruscus* (Asparagaceae) is distributed in the Mediterranean and Europe. Especially, *Ruscus aculeatus* is known as butcher's broom and evaluated in the Asparagaceae family (Masullo *et al.,* 2016). *Ruscus aculeatus* is used for skin diseases, varicose veins, and inflammation as a herbal medicine (Hadˇzifejzovi´c *et al.,* 2013; Luís *et al.,* 2011; Rodrigues *et al.,* 2021; Thomas, & Mukassabi, 2014). Reactive Oxygen Species (ROS) cause oxidative stress (Suaza-Gaviria *et al.,* 2023). This situation is dangerous for human health and leads to the development of various diseases such as cancer (Moutawalli *et al.,* 2024). Antioxidants must be used to protect the human body from diseases caused by ROS (Ahmad *et al.,* 2024). The bioactive compounds like flavonoids and phenolics can be utilized as antioxidants to protect human health (Mwangi *et al.,* 2024).

With the beginning of the COVID-19 epidemic, research on plants, especially as sources of both natural antioxidants and antimicrobial substances, has attracted significant global attention. This is due to the crucial role of these substances in human health, serving as both protective and defensive agents (Ahmad *et al.,* 2024; Güneş *et al.,* 2024; Jokovi´c *et al.,* 2024; Koirala *et al.,* 2024; Shehata *et al.,* 2024; Walasek-Janusz *et al.,* 2024). In this context, it is thought that *Phlomis amanica, Ruscus aculeatus,* and *Echinops viscosus* can also be utilized as natural antioxidants and antimicrobial sources.

In this study, antioxidant and antimicrobial activities of *Phlomis amanica* (endemic), *Ruscus aculeatus,* and *Echinops viscosus* plants growing in Hatay, Türkiye were examined. BHA and BHT are known as comparative standards for antioxidant activity investigations. Thus, they are used for this purpose. Although there are various studies on natural products in the literature, there isn't any research on these plants collected from the Hatay region of Türkiye, adds originality to this study. The results obtained from this research can highlight the potential importance of the examined plants in human health and food industry.

2. MATERIAL and METHODS

2.1. Plant Material

The plant materials of the research were collected from various regions of Hatay, Türkiye (*Ruscus aculeatus;* Şenköy District of Hatay's Yayladağı Town, *Phlomis amanica;* Höyük District of Hatay's Arsuz Town, and *Echinops viscosus;* Kıcı District of Hatay's Belen Town) in June 2021. After the plants were dried at room temperature, their aerial parts were ground into powder. To prepare the samples to be used in the analysis, 20 g of the powdered plants was weighed. Methanol was added to the samples, and they were extracted at 40ºC for 3 hours. This process was continued by filtering each time until the methanol ceased giving color. The filtrates were combined, and the solvent was removed by an evaporator under vacuum at 40ºC.

2.2. Antioxidant Activity

2.2.1. *Folin-Ciocalteu assay for total phenolic content*

The Folin-Ciocalteu reagent used in the Folin-Ciocalteu assay is reduced by phenolic compounds. It is expected that the phenolic contents of plants can also reduce the Folin-Ciocalteu reagent. Thus, the antioxidant capacity of plants should be evaluated by determining their total phenolic content (Aydın *et al.,* 2016). Folin-Ciocalteu process was carried out to determine the total phenolic content of samples (Kahkonen *et al.,* 1999). 0.25 mL of the sample solution with a concentration of 0.4 mg/mL was taken in a test tube. Then, 1.25 mL of Folin reagent diluted 1:10 and 3.75 mL of 10% Na_2CO_3 solution were added to this sample, the tube was closed, and incubated for two hours. The same procedures were applied for gallic acid. To construct a standard curve, five different concentrations (0.025-0.4 mg/mL) were analyzed. After incubation, the absorbance of all samples was measured at 765 nm by a UV/VIS spectrophotometer (Hitachi U-1900). The total phenolic content of the samples was determined from the calibration curve of gallic acid. Results are presented as milligrams of gallic acid equivalent (GAE) per 1 gram of dry extract.

2.2.2. *DPPH free radical scavenging activity*

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a free radical that changes color from purple to yellow as it is reduced when it encounters an antioxidant. Because of this color change, DPPH behaves as an indicator for antioxidants. This is the basis of the Brand-Williams' method (Brand-Williams *et al.,* 1995). The radical scavenging effects of the samples were determined using the Brand-Williams' method (Brand-Williams *et al.,* 1995). Five different concentrations of the sample solutions (0.4 mg/mL) were prepared by diluting them 1:1. Then, 3.75 mL of a $6x10^{-5}$ M DPPH solution was added to 1.25 mL of the sample solution. The sample solutions were kept for half an hour at room temperature and in the dark. Then, the absorbance of the samples was measured at 517 nm by a UV/VIS spectrophotometer (Hitachi U-1900). % inhibition values were calculated with the following formula.

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad (1)$$

The same procedures were carried out for BHA and BHT synthetic antioxidants. Using the % inhibition values, the sample concentrations required to scavenge half of the DPPH free radical (IC_{50} values) were found and compared with the results of BHT and BHA antioxidants.

2.2.3. *β-carotene–linoleic acid assay*

A β-carotene linoleic acid emulsion system was used for this analysis (Kivrak *et al.,* 2009). To prepare the β-carotene linoleic acid emulsion solution, 0.2 mg β-carotene was dissolved in 1 mL of chloroform. 0.02 mL of 60% linoleic acid solution and 200 mg of Tween 40 were added to the mixture and mixed. Then, chloroform was completely removed by an evaporator under vacuum at 40ºC and dissolved in 100 mL deionized water by saturating with oxygen. The samples were dissolved in 70% methanol as 2 mg/mL. 0.2 mL of the sample was taken into the test tubes, and 4.8 mL of the prepared emulsion solution was added. Methanol was used for control purposes. Then, 5 mL of β-carotene linoleic acid emulsion solution was added to 0.2 mL of methanol and mixed. The absorbances of the experimental solutions were read at 470 nm (A_o). Then, samples were incubated in a water bath at 50ºC. At the end of 120 minutes, the absorbances of the incubated solutions were measured by a UV/VIS spectrophotometer (Hitachi U-1900) (A_t). Absorbance change ratio (AO) and % inhibition values were calculated from the equations below:

$$AO = \ln (A_0 / A_t) / t \quad (2)$$

$$\% \text{ Inhibition} = [(AO_{control} - AO_{sample}) / AO_{control}] \times 100 \quad (3)$$

2.3. Antimicrobial Activity

Antimicrobial activities of the samples were determined according to the disk diffusion agar method. For this analysis, solutions to the samples were prepared with a concentration of 100 mg/mL. Dimethyl sulfoxide (DMSO) was used as a solvent. *Candida albicans* (ATCC 140539), *Escherichia coli* (ATCC 35218), *Klebsiella pneumoniae* (ATCC 700603), and *Staphylococcus aureus* (ATCC 29213) strains were obtained from Hatay Mustafa Kemal University Research Hospital, and they were first allowed to grow in Nutrient Broth (8 g/L) medium. After 24 hours of incubation at 37ºC, bacterial concentrations were adjusted according to the McFarland 0.5 turbidity standard. After inoculation in Müller Hinton medium, the discs containing 30 µl of sample were placed in the petri dishes and incubated at 37ºC for 24 hours. The effects of the samples against microorganisms were determined by measuring inhibition zone diameters formed around the discs in millimeters. Antibiotic chloramphenicol was also used as a positive control (Yazdi & Behbahani, 2013).

2.4. Statistical Analysis

All assays were performed in triplicate, and the data were indicated as mean±standard deviation with *p value* <0.05.

3. RESULTS and DISCUSSION

3.1. Antioxidant Activity

3.1.1. *Total phenolic content*

The calibration curve of gallic acid is shown in Figure 1. The total phenolic contents of the samples are given as mg gallic acid equivalents (GAE) per gram of dry extract in Table 1. Phenolic compounds are good antioxidants (Nigussie *et al.,* 2023). Therefore, as the number of polyphenolic compounds found in the plant increases, the antioxidant effect also increases. As can be seen in Table 1, the total phenolic substance amounts of the samples are quite different from each other. According to Table 1, *Phlomis amanica* has the highest total phenolic content (859.1±78.0 mg/g GAE) while *Ruscus aculeatus* has the lowest (229.7±18.0 mg/g GAE). Aydın *et al.,* (2016) reported the maximum value of the total phenolic content of *Echinops ritro* as 92.24 mg GAE/g. Consequently, the results in this study were more remarkable than those reported in the literature.

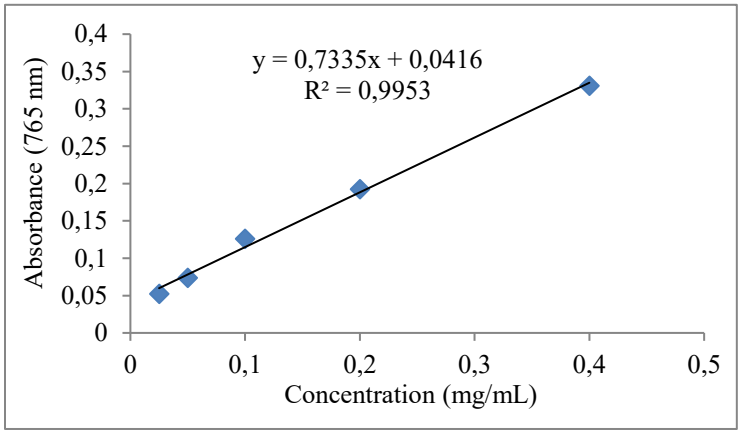


Figure 1. The calibration curve of gallic acid.

Table 1. Total phenolic content of the samples.

Samples	mg GAE/g dry extract
Phlomis amanica	859.1 ± 78.0
Echinops viscosus	453.5 ± 23.9
Ruscus aculeatus	229.7 ± 18.0

3.1.2. *DPPH free radical scavenging activity method*

The radical scavenging activity of the samples were determined by DPPH assay. As a result, IC_{50} values of the samples were calculated. Also, BHA and BHT are examined as standard antioxidants. The low IC_{50} value indicates that the antioxidant effect is high, because IC_{50} expresses the amount of the antioxidant required to scavenge half of DPPH (Mahdi *et al.,* 2023). IC_{50} values of the samples are given in Table 2. These values are important to compare the antioxidant activities of the samples. According to Table 2, BHA has the highest radical scavenging activity with the lowest IC_{50} value (0.009±0.001 mg/mL) because there is an opposite correlation between the antioxidant capacity of the sample and IC_{50} value. The highest activity among the plant samples belongs to *Phlomis amanica*, which has the lowest IC_{50} value (0.013±0.003 mg/mL). Phenolic content of plants has free radical inhibition effects (Kil *et al.,* 2009). Besides, *Phlomis amanica*, which has a very low IC_{50} value, has a radical scavenging activity as high as the synthetic antioxidant BHA. Lower IC_{50} values of all plant samples compared to the synthetic antioxidant BHT highlight their stronger radical scavenging activity (Sofiane *et al.,* 2017). As reported by Mahdi *et al.* (2023), IC_{50} value of *Phlomis floccosa* was 0.025 mg/mL. According to this result, *Phlomis amanica,* tested in this study, is more bioactive than *Phlomis floccosa* in the mentioned literature.

Table 2. IC_{50} values of the samples.

Samples	IC_{50} (mg/mL)
BHA	0.009 ± 0.001
BHT	0.365 ± 0.024
Phlomis amanica	0.013 ± 0.003
Echinops viscosus	0.048 ± 0.004
Ruscus aculeatus	0.209 ± 0.018

3.1.3. *β-carotene–linoleic acid method*

% Inhibition values of the samples are shown in Table 3. Greater % inhibition value means higher antioxidant activity (Kivrak *et al.,* 2009). β-Carotene-lineolic acid emulsion method experimental results showed that the % inhibition values of the plants were quite high and very close to each other. Considering the results, it was determined that the antioxidant effect of the samples was high according to the β-carotene linoleic acid emulsion system method. Similar to the data of the DPPH method, *Phlomis amanica* showed the highest inhibition effect among the samples, with the % inhibition value (62.07±2.20 %) closest to BHA (66.08±3.10 %) and BHT (67.93±2.30 %) according to Table 3. While the % inhibition values of *Phlomis amanica* and *Ruscus aculeatus* are close to each other, *Echinops viscosus* has the lowest. Then, there are some different results among the β-carotene–linoleic acid and the other antioxidant activity assays in this research because other components, except the phenolic compounds affect the antioxidant capacity too (Karadeniz-Pekgöz *et al.,* 2024). In the literature, the methanol extract of *Echinops ritro* showed similar antioxidant activity to *Phlomis amanica* in this study (Aydın *et al.,* 2016).

Table 3. % Inhibition values of the samples.

Samples	% Inhibition
BHT	67.93 ± 2.30
BHA	66.08 ± 3.10
Phlomis amanica	62.07 ± 2.20
Echinops viscosus	43.88 ± 4.95
Ruscus aculeatus	58.57 ± 1.35

3.2. Antimicrobial Activity

Antimicrobial activity was determined by measuring the diameters of the inhibition zones created by the plants against each microorganism as millimeters. In addition, the inhibition zone diameters of chloramphenicol as a positive control, used in the tests against microorganisms were also measured, and the results are presented in Table 4.

Table 4. Antimicrobial activity of the samples.

Samples	Zone of inhibition (mm)			
	Candida albicans	*Escherichia coli*	*Klebsiella pneumoniae*	*Staphylococcus aureus*
Phlomis amanica	12±1	8±0	12±1	21±1
Echinops viscosus	10±2	9±1	10±0	15±1
Ruscus aculeatus	11±1	6±0	10±1	15±1
Chloramphenichol	-	29±1	14±0	45±1

Table 4 shows that the samples have different activities against each microorganism. It was observed that the samples formed the highest inhibition zones against *Staphylococcus aureus*. According to these results, all samples showed the highest antimicrobial effect against

Staphylococcus aureus and the lowest against *Escherichia coli.* According to the results obtained, it can be said that the antimicrobial activities of plants generally increase with the increase in total phenolic content. Similar results were found in the literature (Tabti *et al.,* 2014). Antimicrobial activities of the plants against *Eschericha coli* (E), *Klebsiella pneumonie* (K), *Staphylococcus aureus* (S), and *Candida albicans* (C) are shown in Figure 2-5. Abbreviations of the Turkish names of the plants in figures are respectively: *Phlomis amanica* (Ç), *Echinops viscosus* (A), and *Ruscus aculetus* (T). "An" represents the chloramphenicol antibiotic**.**

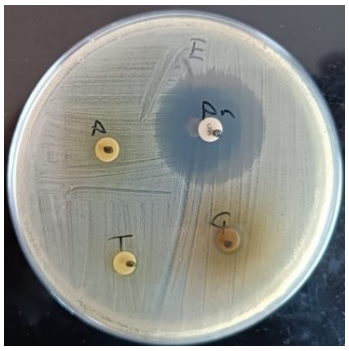


Figure 2. Inhibition of *Eschericha coli* by the plants.



Figure 3. Inhibition of *Klebsiella pneumonie* by the plants.

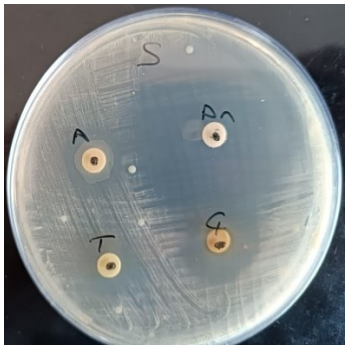


Figure 4. Inhibition of *Staphylococcus aureus* by the plants.



Figure 5. Inhibition of *Candida albicans* by the plants.

4. CONCLUSION

According to the results obtained, the antioxidant and antimicrobial effects of the samples are quite strong. The research results show that these plants have high antioxidant capacity and have strong antimicrobial effects, especially against *Staphylococcus aureus*. So, it can be suggested that *Phlomis amanica, Echinops viscosus* and *Ruscus aculeatus* grown in Hatay, Türkiye should be evaluated as important herbal resources both in terms of health and economy by supporting their potential medical and industrial applications. The results of this study can make an important contribution to the evaluation of the biodiversity and natural resources of the Hatay region, Türkiye.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Sezer Göycıncık: Investigation, laboratory studies, validation, writing and reviewing. **Halil Dolap**: Laboratory studies.

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