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MISTLETOE (*LORANTHUS EUROPAEUS* JACQ.): ANTIOXIDANT, ANTIMICROBIAL AND ANTICHOLINESTERASE ACTIVITIES

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ABSTRACT

Plants are natural resources responsible for different biological activities. Determining the biological activities of plants is very important. In our study, some biological activities and total phenolic and flavonoid contents of *Loranthus europaeus* Jacq. were determined. In this context, ethanol and methanol extracts of the plant were obtained using the soxhlet device. Total antioxidant, total oxidant, and oxidative stress statuses were determined using Rel Assay kits. Antimicrobial activity was tested against standard bacterial and fungal strains using the agar dilution test. Anticholinesterase activity was determined by detecting acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities. Determination of total phenolic content was determined using the Folin-Ciocalteu reagent. Total flavonoid quantification was performed using an aluminum chloride assay. As a result of the study, TAS values (Total antioxidant status) of ethanol and methanol extracts of the plant were determined as 5.620 ± 0.134 and 6.384 ± 0.134 mmol/L, respectively. TOS values (Total oxidant status) were determined as 10.997 ± 0.183 and 13.368 ± 0.222 $\mu\text{mol/L}$, respectively. OSI values (Oxidative stress index) were measured as 0.196 ± 0.004 and 0.209 ± 0.003 , respectively. TPC (Total phenolic content) value was determined as 34.49 ± 1.78 and 55.82 ± 2.34 mg/g, respectively, and TFC (Total flavonoid content) value was determined as 46.10 ± 2.40 and 55.49 ± 1.15 mg/g, respectively. Ethanol and methanol extracts of the plant were found to be effective against bacterial and fungal strains at concentrations between 50-200 $\mu\text{g/mL}$. In addition, the anti-AChE values of ethanol and methanol extracts were determined as 13.51 ± 0.81 and 22.79 ± 1.86 $\mu\text{g/mL}$, respectively, and the anti-BChE values were determined as 27.84 ± 0.62 and 33.08 ± 1.63 $\mu\text{g/mL}$, respectively. According to the results obtained, it was determined that *L. europaeus* has antioxidant, antimicrobial, and anticholinesterase activity. In this context, it is thought that it can be used as a natural material in pharmacological designs.

KEYWORDS: Antioxidant, medicinal plants, mistletoe, natural products.

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1. Introduction

Today, many natural products are used within the scope of complementary or alternative medicine. Before the development of modern medicine, people turned to natural products to combat different diseases [1]. In this context, natural products such as mushrooms, plants, and animals have become a solution for people to fight against diseases. Among natural products, plants are the most important materials with a history equivalent to human

history [2]. Plants have been used in many communities for many purposes such as animal and human nutrition, shelter, heating, making equipment, tools, and fighting diseases. Plants that stand out with their nutritional properties are widely used, especially in the fight against diseases [3]. In studies conducted by different researchers, it has been reported that plants have different biological activities such as anticancer, antimicrobial, antioxidant, anti-inflammatory, antiproliferative, antiaging, hepatoprotective, and DNA

protective [2,4-9]. In this context, determining the biological activities of plants is very important in terms of their usage potential [10]. *Loranthus europaeus* was used as material in this study. The plant's antioxidant, antimicrobial, anticholinesterase activities, and total phenolic and flavonoid contents were determined.

Loranthus is a genus of parasitic plants that grows on the branches of woody trees. In different beliefs, mistletoe is considered a cure for infertility in animals, an antidote against poison, and is considered sacred when it grows on oak trees [11]. It is known as *Loranthus europaeus* (Loranthaceae) in Europe: European yellow mistletoe, Summer mistletoe, in Italy: Vischio quercino, in Arabic: Hib el-debgh [12-14]. It is distributed in Central and Southeastern Europe and the Eastern Mediterranean region, as well as in some parts of Asia Minor [15]. *L. europaeus* is a deciduous plant with dull brown whorls. It blooms in May and June. The fruits are yellow and round in shape. Additionally, its fruits ripen towards the end of autumn [16].

2. Materials and Methods

Loranthus europaeus specimens were collected from Hatay/Turkey on *Quercus* sp. The species identification of the plant was made using Flora of Turkey and the East Aegean Islands. Vol. 7 [17]. Plant herbarium samples are kept in Osmaniye Korkut Ata University, Biology Laboratory. All parts of the plant were used for analyses. Plant samples were dried in a laboratory environment away from direct sunlight. The dry samples were then ground into powder. 30 g of the samples were weighed and filled into soxhlet cartridges. It was then subjected to extraction with 250 mL of ethanol at 50 °C for approximately 6 hours. The process was repeated to obtain methanol extracts.

2.1. Total Antioxidant and Oxidant parameters

Total antioxidant and oxidant levels of the samples were determined using Rel Assay kits. Total antioxidant values were determined using TAS (Total antioxidant status) kits and expressed as mmol Trolox equivalent/L. Total oxidant values were measured with TOS (Total oxidant status) kits and expressed as μmol hydrogen peroxide equivalent/L. The tests were carried out following the manufacturer's protocol [18,19]. The OSI value (Oxidative stress index), which shows the percentage of oxidant compounds suppressed by antioxidant compounds, was measured by equalizing the units of the TOS value and the TAS value and taking the percentage by proportioning them [20].

2.2. Antimicrobial activity tests

The antimicrobial activities of ethanol and methanol extracts of the plant were tested against standard bacterial and fungal strains. Stock solutions were prepared at concentrations between 12.5-800 $\mu\text{g/mL}$ by dilution method. Bacterial strains were precultured in Muller Hinton Broth medium. Fungal strains were pre-cultured in RPMI 1640 Broth medium. The findings were expressed in $\mu\text{g/mL}$ [21-24].

Bacterial strains used: *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC 19606.

Fungal strains used were: *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135 and *C. glabrata* ATCC 90030.

2.3. Anticholinesterase activity test

Anticholinesterase activity of the extracts was determined using the Ellman method [25]. Extracts were diluted to concentrations ranging from 3.125-200 $\mu\text{g/mL}$. 130 μL of 0.1 M pH=8 phosphate buffer, 10 μL of stock solution, and 20 μL of enzyme (acetylcholinesterase (AChE) or butyrylcholinesterase (BChE) enzyme solution) were added to the microplate. The resulting mixture was incubated at 25 °C in the dark for 10 min, and then 20 μL of DTNB (5,5"-dithiobis-(2-nitrobenzoic acid)) solution and 20 μL of a substrate (acetylcholine iodide or butyrylcholine iodide) were added to the solution. Then, the reading was done at 412 nm. IC50 values (half-maximal inhibitory concentration) of the results were calculated and expressed as $\mu\text{g/mL}$.

2.4. Total Phenolic and Total Flavonoid Analysis

1 mL stock solutions were prepared from the extracts and 1 mL of Folin-Ciocalteu reagent (1:9, v/v) was added and mixed. Then, 0.75 mL of 1% Na_2CO_3 was added to this solution. Then, it was incubated at room temperature for 2 hours and the reading was taken at 760 nm. According to the calibration curve of the gallic acid standard solution, the total phenolic content was expressed in mg/g [26].

Total flavonoid levels of the extracts were measured by aluminum chloride test [27]. For the test, 0.1 mL 10% $\text{Al}(\text{NO}_3)_3$, 0.1 mL 1 M $\text{NH}_4\text{CH}_3\text{COO}$, 4.3 mL methanol, 0.5 mL Quercetin and 0.5 mL plant extract were mixed. The mixture was then incubated for 40 minutes. Then, absorbance was measured at 415 nm. Total flavonoid content was expressed in mg/g.

3. Results and Discussion

3.1. Total phenolic and total flavonoid contents

Plants produce secondary metabolites as a result of their defense systems. These compounds do not have nutritional properties but are medically important compounds [28,29]. In our study, the total phenolic and flavonoid contents of the ethanol and methanol extracts of *L. europaeus* are shown in Table 1.

Table 1. TAS, TOS, OSI, TPC and TFC values of *Loranthus europaeus*

| Parameters | Solvents | |
|---------------------------|--------------------|--------------------|
| | Ethanol | Methanol |
| TAS (mmol/L) | 5.620 \pm 0.134 | 6.384 \pm 0.134 |
| TOS ($\mu\text{mol/L}$) | 10.997 \pm 0.183 | 13.368 \pm 0.222 |
| OSI (TOS/(TASx10)) | 0.196 \pm 0.004 | 0.209 \pm 0.003 |
| TPC (mg/g) | 34.49 \pm 1.78 | 46.10 \pm 2.40 |
| TFC (mg/g) | 55.82 \pm 2.34 | 55.49 \pm 1.15 |

As a result of the study, the TPC value (Total phenolic content) of the methanol extract of the plant was found to be higher than the ethanol extract. In addition, the TFC value (Total flavonoid content) of the ethanol extract of the plant was found to be higher than the methanol extract. In the literature, in a study conducted in Tunisia, it was reported that the total phenol value of the methanol extract obtained from *L. europaeus* was

between 72.59-83.98 mg GAEs/g and the total flavonoid value was between 63.61-139.73 mg QEs/g [30]. In a study conducted in Iraq, it was reported that the flavonoid value of the methanol extract obtained from the fruit of *L. europaeus* was 2.545 mg/g [31]. In a study conducted in Greece, it was reported that the total phenol value of the methanol extract obtained from the aerial part of *L. europaeus* was 94-320 mg/g and the total phenol value of the methanol extract obtained from the root part was 197 mg/g. It has also been reported that the total phenol value of the ethyl acetate extract obtained from the aerial part is 61-313 mg/g and the total phenol value of the ethyl acetate extract obtained from the root part is 246 mg/g [32]. In our study, it was observed that the total phenolic and flavonoid contents of the ethanol extract of *L. europaeus* were 34.49 mg/g and 55.82 mg/g, while the methanol extract was 46.10 mg/g and 55.49 mg/g. When we compared it with literature studies, it was seen that the results we found were within normal values. As a result, it is thought that *L. europaeus* plant may be an important source of phenolic and flavonoid content.

3.2. Antioxidant activity

Oxidant compounds are free radicals routinely produced as a result of metabolic activities [33]. While low levels of these compounds can be tolerated by the body, they can have harmful effects as levels increase [34]. The antioxidant defense system suppresses or eliminates the effects of oxidant compounds [35]. However, in some cases, the balance between antioxidant and oxidant compounds is disrupted. In this case, oxidative stress occurs. As a result of oxidative stress, serious diseases such as cardiological disorders, Parkinson's disease, Alzheimer's disease, multiple sclerosis and cancer may occur in humans [36,37]. Supplemental antioxidants can be used to reduce the negative effects of oxidative stress. In this context, plants are the natural products with the highest antioxidant potential. In our study, the antioxidant potential of *L. europaeus* was determined. In this context, TAS, TOS and OSI values of the ethanol and methanol extracts of the plant were determined and shown in Table 1. In literature reviews, it has been reported that *L. europaeus* has antioxidant activity using different methods [30,32,38]. In literature research, no study was found on the TAS, TOS and OSI values of *L. europaeus*. With our study, TAS, TOS and OSI values of *L. europaeus* were determined for the first time. It was measured that the TAS, TOS and OSI values of the methanol extract of *L. europaeus* used in our study were high when compared to the results found in the ethanol extract. In TAS, TOS and OSI studies conducted on different plant species, TAS values of *Helianthemum salicifolium*, *Silybum marianum*, *Rumex scutatus*, *Asparagus officinalis*, *Galium aparine* and *Salvia absconditiflora* were reported as 9.490, 5.767, 8.656, 6.238, 5.147 and 7.350 mmol/L, respectively. TOS values were reported as 14.839, 12.144, 4.951, 13.892, 18.679 and 8.501 μ mol/L, respectively. OSI values have been reported as 0.157, 0.211, 0.057, 0.221, 0.346 and 0.116, respectively [39-44]. The TAS value of the ethanol extract of *L. europaeus* used in our study was determined to be higher than that of *G. aparine* and lower than that of *H. salicifolium*, *S. marianum*, *R. scutatus*, *A. officinalis* and *S. absconditiflora*. The TAS value of the methanol extract of *L. europaeus* was determined to be lower than that of *H. salicifolium*, *R. scutatus* and *S. absconditiflora*,

and higher than that of *S. marianum*, *A. officinalis* and *G. aparine*. TAS value shows the totality of antioxidant compounds produced within the plant [45]. When the TAS values of *L. europaeus* used in our study were examined, it was seen that it had antioxidant potential. TOS values are an indicator of the totality of oxidant-active compounds produced within natural products [45]. The TOS value of the ethanol extract of *L. europaeus* used in our study was determined to be lower than that of *H. salicifolium*, *S. marianum*, *A. officinalis* and *G. aparine*, and higher than that of *R. scutatus* and *S. absconditiflora*. The TOS value of the methanol extract of *L. europaeus* was determined to be lower than that of *H. salicifolium*, *A. officinalis*, *G. aparine*, and higher than that of *S. marianum*, *R. scutatus* and *S. absconditiflora*. In this context, it can be seen that the TOS values of *L. europaeus* are generally high. OSI value is used to express the percentage of oxidant compounds suppressed by antioxidant compounds [45]. To use natural products, the OSI value must be low. The OSI value of both ethanol and methanol extracts of *L. europaeus* used in our study was determined to be higher than *H. salicifolium*, *R. scutatus*, and *S. absconditiflora*, and lower than *S. marianum*, *A. officinalis* and *G. aparine*. In this context, it has been observed that the ability of *L. europaeus* to suppress oxidant compounds is at normal levels. As a result, it was determined that *L. europaeus* has antioxidant potential.

3.3. Antimicrobial activity

Nowadays, the number of microbial diseases is increasing. The number of resistant microorganisms due to pollution of nature and unconscious use of antibiotics triggers the increase in the number of these diseases [46]. The antimicrobial drugs used are insufficient. In this context, researchers have turned to the discovery of new antimicrobial drugs [47,48]. Plants occupy an important place among natural antimicrobial sources. In our study, the effect of *L. europaeus* against standard bacterial and fungal strains was investigated. The findings obtained are shown in Table 2.

Table 2. Antimicrobial potential of *Loranthus europaeus*

| Microorganisms | Solvents (μ g/mL) | |
|-----------------------|------------------------|----------|
| | Ethanol | Methanol |
| <i>S. aureus</i> | 200 | 200 |
| <i>S. aureus MRSA</i> | 100 | 200 |
| <i>E. faecalis</i> | 100 | 100 |
| <i>E. coli</i> | 100 | 200 |
| <i>P. aeruginosa</i> | 100 | 200 |
| <i>A. baumannii</i> | 50 | 100 |
| <i>C. glabrata</i> | 50 | 200 |
| <i>C. albicans</i> | 100 | 200 |
| <i>C. krusei</i> | 100 | 200 |

50, 100, 200 μ g/mL - the lowest extract concentrations that prevent the growth of microorganisms

Considering the research in the literature, a study conducted in Iran investigated the antimicrobial status of the essential oil and hydroalcoholic extract of *L. europaeus* against *Acinetobacter baumannii*, *Staphylococcus aureus*

and *Pseudomonas aeruginosa*. In the study, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were examined. Among the strains used, it was reported that the best MIC value was 6 µg/mL in *S. aureus* and the best MBC value was 196 µg/mL in *S. aureus* [49]. In a study conducted in Italy, the antimicrobial effect of the protein extract obtained from the sample of *L. europaeus* on *Aspergillus niger*, *Alternaria* spp., *Penicillium* spp., *Botrytis cinereus*, *Listeria monocytogenes*, *S. aureus*, *Salmonella typhimurium* and *Escherichia coli* strains was investigated. As a result of the study, it was reported that the best MIC value among bacterial strains was 0.50 mg/mL in *S. typhimurium* and the best MBC value was 0.38 mg/mL in *L. monocytogenes*. Additionally, among fungal strains, it has been reported that the colony diameter result is 2.0-3.5 cm with the best effect in the sporulation of *A. niger* [50]. In a study conducted in Iraq, the antimicrobial effect of the aqueous and alcohol extract of *L. europaeus* on *S. aureus* was examined. As a result of the study, it was reported that the best effect in aqueous extract was the inhibition zone value of 25 mm at a concentration of 200 mg/mL, and the best effect in the alcohol extract was 17 mm at the concentration of 200 mg/mL [51]. In our study, ethanol and methanol extract of *L. europaeus* were used. As a result of the study, it was seen that the ethanol extract of the plant was effective against *S. aureus*, one of the bacterial strains, at a concentration of 200 µg/mL. It was also determined to be effective against *P. aeruginosa*, *S. aureus* MRSA, *E. faecalis* and *E. coli* at concentrations of 100 µg/mL and against *A. baumannii* at concentrations of 50 µg/mL. Additionally, it was observed that 100 µg/mL was effective against *Candida albicans* and *C. krusei*, and 50 µg/mL was effective against *C. glabrata*. Methanol extract was found to be effective against *S. aureus* and *S. aureus* MRSA, *P. aeruginosa* and *E. coli* at concentrations of 200 µg/mL, and against *E. faecalis* and *A. baumannii* at concentrations of 100 µg/mL. It has also been shown to be effective against *C. albicans*, *C. glabrata* and *C. krusei* at a concentration of 200 µg/mL. As a result, according to our findings, it is thought that *L. europaeus* can be used as a natural antimicrobial source.

3.4. Anticholinesterase activity

In recent years, the incidence and lethality of neurodegenerative diseases have been increasing. Diseases such as Parkinson's and Alzheimer's are among the most common neurodegenerative diseases. In addition, the incidence of these diseases is higher in people over the age of 65 [52]. In this context, it is thought to reduce the effects of neurodegenerative diseases by using natural products. In our study, acetyl and butyrylcholinesterase activities of ethanol and methanol extracts of *L. europaeus* were determined. The obtained IC50 values are shown in Table 3.

Table 3. Anti-AChE and anti-BChE values of *Loranthus europaeus*

| Parameters | Solvents | | Standard |
|--------------|------------|------------|-------------|
| | Ethanol | Methanol | Galantamine |
| AChE (µg/mL) | 13.51±0.81 | 22.79±1.86 | 9.43±0.46 |
| BChE (µg/mL) | 27.84±0.62 | 33.08±1.63 | 20.16±0.87 |

When the literature studies were examined, it was seen that there were no anticholinesterase studies on *L.*

europaeus. In our study, the anticholinesterase activity of *L. europaeus* was measured for the first time. In our study, it was observed that the IC50 value of the anti-AChE activity of ethanol extract was 13.51 µg/mL, and the anti-AChE activity of methanol extract was 22.79 µg/mL. Among the extracts, ethanol extract had a better value. It was also measured that the anti-AChE activity in both extracts used was lower than that of galantamine used as a control. When studies conducted at the genus level were examined, a study conducted in Bangladesh reported that the AChE inhibition IC50 values of different extracts obtained from the bark part of *L. globosus* were between 64.987-171.533 µg/mL. Additionally, BChE inhibition IC50 values have been reported to be between 85.270-391.633 µg/mL [53]. Compared to this study, it was measured that the anti-AChE activity of both the ethanol extract and methanol extract of *L. europaeus* used in our study was higher. In a study conducted in South Korea, the IC50 value of the methanol extract obtained from the stem part of *L. parasiticus* was reported to be 1542.6 µg/mL [54]. Compared to this study, it was measured that the anti-AChE activity of both the ethanol extract and methanol extract of *L. europaeus* used in our study was higher. In a study conducted in Nigeria, it was reported that the EC50 value of the aqueous extract obtained from the leaf part of *L. bengwensis* was 241.1-390.2 µg/ml [55]. Compared to this study, it was measured that the anti-AChE activity of both the ethanol extract and methanol extract of *L. europaeus* used in our study was higher. As a result, it is thought that *L. europaeus* used in our study may play a role in the treatment of neurodegenerative diseases such as Alzheimer's disease by determining its anticholinesterase activity.

4. Conclusions

Plants are important natural resources. In our study, some biological activities and total phenolic and flavonoid contents of *L. europaeus* were determined. As a result of the analyses, it was determined that the plant has antioxidant, antimicrobial, and anticholinesterase activities. It has been observed that the plant can be a source of phenolics and flavonoids. In this context, it is thought that the plant can be used as an important natural resource in pharmacological designs.

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