ANTIOXIDANT, ANTIMICROBIAL AND ANTICHOLINESTERASE ACTIVITY OF DITTRICHA GRAVEOLENS

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ABSTRACT

Plants are responsible for many different biological activities. In our study, antioxidant, antimicrobial and anticholinesterase activities of Dittrichia graveolens (L.) Greuter were determined. In addition, the total phenolic and flavonoid contents of the plant were measured. The aerial parts of the plant were extracted with ethanol. Antioxidant activities of the extracts were measured with Rel assay kits. Antimicrobial activity was determined using the agar dilution method. Anticholinesterase activity was determined by measuring the inhibition of acetyl (AChE) and butyrylcholinesterase (BChE) activities. Determination of total phenolic content was determined using the Folin-Ciocalteu reagent. Total flavonoid quantification was performed using aluminum chloride assay. As a result of the study, the TAS (total antioxidant status) value of the plant was determined as 6.933±0.121 mmol/L, the TOS (total oxidant status) value was 12.535±0.244 µmol/L and the OSI (oxidative stress index) value was 0.181±0.006. It was determined that the plant extract was effective against microorganisms at concentrations of 50-400 µg/mL. Additionally, the anti-AChE IC50 value was found to be 25.88±1.73 µg/mL and the anti-BChE IC50 value was 45.32±2.26 µg/mL. Total phenolic content was determined as 86.42±2.72 mg/g, and total flavonoid content was determined as 117.96±1.93 mg/g. As a result, it was determined that D. graveolens has antioxidant, antimicrobial and anticholinesterase activity.

KEYWORDS: Antialzheimer, Antimicrobial, Antioxidant, Medicinal plants.

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1. Introduction

Many natural products used in traditional medicine have been used by people in the treatment of different diseases [1]. Among these natural products, plants are the most widely used. Plants are at the top of people's diet lists [2]. In addition to their nutritional properties, plants are also very important natural materials from a medicinal point of view [3]. The literature has shown that different plant species have different biological activities such as anticancer, antioxidant, antimicrobial, antiproliferative, DNA protective, antiaging, hepatoprotective and antiallergic [4-9]. In this context, it is very important to determine the biological activities of plants due to the possible side effects of synthetic drugs. In our study, some biological activities of Dittrichia graveolens (L.) Greuter were determined.

D. graveolens (Asteraceae) is known as stinkwort or stinking fleabane. This cosmopolitan plant is considered a noxious weed in some regions. This plant is an aromatic, fragrant, branching, short shrub that can grow up to 150 cm. The leaves are long and narrow, with glandular hairs on their surfaces have pointed tips, and have a sticky resin. The flowers of the plant are yellow [10]. In this study, the antioxidant, antimicrobial, antiallergic, total phenolic and total flavonoid contents of D. graveolens were determined.

2. Materials and Methods

Plant samples were collected from Duhok (Iraq). Herbarium samples are kept in the herbarium of Zakho University, Faculty of Arts and Sciences, Department of Biology. Plant samples were ground into powder. 30 g of
the powder samples were weighed and extracted with 250 mL of ethanol in a Soxhlet extractor at 50°C for approximately 6 hours. Then, solvents were removed using a rotary evaporator and the extract was stored at +4°C until experiments were performed.

2.1. Antimicrobial activity tests

The activities of the plant extract against the bacterial and fungal strains used in our study were measured by the agar dilution method. The bacterial strains used in our study were *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. The fungal strains used in our study were *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135, *C. glabrata* ATCC 90030. Bacteria were precultured in Muller Hinton Broth medium. Fungi were pre-cultured in RPMI 1640 Broth medium. Stock solutions were prepared from the plant extract at concentrations ranging from 12.5-800 μg/mL. The lowest concentrations of these stock solutions that prevented the growth of microorganisms were determined. The findings were expressed in μg/mL [11-14].

2.2. Antioxidant parameters

Total antioxidant (TAS) and oxidant (TOS) values of *D. graveolens* (L.) Greuter extract were determined using Bel assay kits. The manufacturer's protocol included in the kits was followed. TAS values were expressed as mmol trolox equivalent/L. TOS values were expressed as μmol hydrogen peroxide equivalent/L [15,16]. Oxidative stress index (OSI) value shows the percentage of oxidant compounds suppressed by antioxidant compounds. Therefore, when calculating the OSI value, the units of the TOS value and the TAS value were equalized. Then, the TOS value was proportioned to the TAS value and the percentage was taken [17].

2.3. Anticholinesterase tests

Anticholinesterase activity of the ethanol extract of the plant was determined using the Ellman method [18]. Solutions at concentrations between 3.125-200 μg/mL were prepared from the dry plant extract using distilled water. 130 μL of 0.1 M pH=8 phosphate buffer, 10 μL of extract solution, and 20 μL of enzyme (AChE or BChE enzyme solution) were added to the microplate. The solution was incubated at 25°C in the dark for 10 min. After incubation, 20 μL of DTNB (5,5”-dithiobis-(2-nitrobenzoic acid)) solution and 20 μL of substrate (acetylcholine iodide or butyrylcholine iodide) were added to the solution. The absorbance was then read at 412 nm. The extract was studied in triplicate. IC50 values of the results were calculated and expressed as μg/mL.

2.4. Total Phenolic and Total Flavonoid Analyzes

A 1 mL stock solution was prepared from the ethanol extract of the plant. Then, 1 mL of Folin-Ciocalteu reagent (1:9, v/v) was added to this solution and mixed. 0.75 mL of 1% Na2CO3 was added to the mixture. Then it was incubated for 2 hours at room temperature. After incubation, measurements were made at 760 nm. According to the calibration curve of the gallic acid standard solution, the total phenolic content (TPC) was expressed in mg/g [19].

The total flavonoid content of the ethanol extract of the plant was determined by the aluminum chloride test [20]. 0.1 mL 10% Al(NO3)3, 0.1 mL 1 M NaH2C2O4, 4.3 mL methanol, 0.5 mL quercetin and 0.5 mL plant extract solution were mixed. The solution was incubated for approximately 40 minutes. Then, absorbance was measured at 415 nm. Total flavonoid content (TFC) was expressed in mg/g.

3. Results and Discussions

3.1. Antimicrobial activity

The emergence of resistant microorganisms due to unconscious use of antibiotics has made the treatment of infectious diseases difficult [21]. Due to the resistance of pathogens to today's antimicrobial drugs and the possible side effects of synthetic drugs, there is an increasing trend towards natural products that are thought to be effective against microorganisms. In this context, determining the antimicrobial potential of plants is very important [22]. In our study, the lowest extract concentration of *D. graveolens* that prevented the growth of test microorganisms was determined. The findings obtained are shown in Table 1.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Extract concentration (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>200</td>
</tr>
<tr>
<td><em>S. aureus</em> MRSA</td>
<td>200</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>400</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>200</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>400</td>
</tr>
<tr>
<td><em>A. baumannii</em></td>
<td>100</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>50</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>100</td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>100</td>
</tr>
</tbody>
</table>

It has been reported in the literature that the essential oils of *D. graveolens* samples collected from Poland are effective against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus parasticipus*, *Candida glabrata* and *Candida albicans* [23]. It has been reported that the essential oils of *D. graveolens* collected from Montenegro are effective against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli*, and *Aspergillus niger* [24]. Unlike these studies, the ethanol extract of the plant was used in our study. As a result, it was determined that Dittricia graveolens extract was effective against *Enterococcus faecalis* at a concentration of 400 μg/mL and against *Staphylococcus aureus*, *S. aureus* MRSA and *Escherichia coli* at a concentration of 200 μg/mL. It was also found to be effective against *Acinetobacter baumannii*, *Candida krusei* and *C. albicans* at a concentration of 100 μg/mL, and against *C. glabrata* at a concentration of 50 μg/mL. It is thought that the plant may be a natural antimicrobial source against the test microorganisms used in our study.

3.2. Antioxidant activity
High levels of oxidant compounds produced as a result of metabolic activities can cause serious damage to living organisms [25]. The antioxidant defense system functions by suppressing oxidant compounds. In some cases, the antioxidant defense system may be insufficient [26]. In this case, oxidative stress occurs. As a result of oxidative stress, serious diseases such as cancer, cardiological disorders, Alzheimer’s disease, Parkinson’s disease, and multiple sclerosis may occur in humans [27,28]. Supplementary antioxidants can be used to prevent the possible effects of oxidative stress. In this context, plants have the potential to be important supplementary antioxidants [29]. In our study, the antioxidant potential of the ethanol extract of D. graveolens was determined. The findings obtained are shown in Table 2.

Table 2. TAS, TOS, OSI, TPC and TFC values of Dittrichia graveolens

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS (mmol/L)</td>
<td>6.93±0.12</td>
</tr>
<tr>
<td>TOS (µmol/L)</td>
<td>12.53±0.24</td>
</tr>
<tr>
<td>OSI (TOS/ (TAS*10))</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>TPC (mg/g)</td>
<td>86.42±2.72</td>
</tr>
<tr>
<td>TFC (mg/g)</td>
<td>117.96±1.93</td>
</tr>
</tbody>
</table>

*Values are presented as mean±SD, n=3

TAS, TOS and OSI values of D. graveolens have not been reported before in the literature. They were determined for the first time in our study. In studies conducted on different plant species, TAS values of Hesperis pendula, Alcea kurdica, Rumex scutatus, Helianthemum salicifolium, Galium aparine and Viola odorata were reported as 5.71, 3.298, 8.656, 9.490, 5.147 and 6.752 mmol/L, respectively. TOS values were reported as 21.65, 8.312, 4.951, 14.839, 18.679 and 7.886 µmol/L, respectively. OSI values have been reported as 0.380, 0.252, 0.057, 0.157, 0.346 and 0.117, respectively [30-35]. Compared to these studies, the TAS value of D. graveolens was determined to be higher than that of H. pendula, A. kurdica, G. aparine and V. odorata, and lower than that of R. scutatus and H. salicifolium. The TAS value shows the totality of antioxidant-active compounds produced within the plant [36]. It has been reported in the literature that D. graveolens has antioxidant activity by different methods [37-39]. The D. graveolens used in our study was observed to have significant antioxidant potential due to its high TAS value. TOS value is an indicator of the totality of oxidant compounds produced within the plant [36]. The TOS value of D. graveolens used in our study was measured to be lower than H. pendula, H. salicifolium and G. aparine, and lower than A. kurdica, R. scutatus and V. odorata. TOS values are expected to be low due to the use of natural products [36]. It was observed that the TOS value of D. graveolens used in our study was at normal levels compared to other studies reported in the literature. OSI value shows the percentage of oxidant compounds suppressed by antioxidant compounds [36]. When recommending the consumption of a natural product, a low OSI value is expected. The OSI value of D. graveolens used in our study was measured to be lower than H. pendula, A. kurdica and G. aparine, but higher than R. scutatus, H. salicifolium and V. odorata. In this context, D. graveolens was observed to have average OSI values.

3.3. Anticholinesterase activity

In recent years, the incidence of neurodegenerative diseases has been increasing. Oxidative stress-related diseases such as Alzheimer’s disease are common, especially in people over the age of 65. It is thought that this number will increase exponentially in the coming years [40]. In our study, antiacetyl- and butyrylcholinesterase inhibition by D. graveolens was determined. In this context, the plant’s potential against neurodegenerative diseases such as Alzheimer’s disease has been determined. IC50 values of the findings are shown in Table 3.

Table 3. IC50 values of Dittrichia graveolens against AChE and BChE (µg/mL)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AChE</th>
<th>BChE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract</td>
<td>25.88±1.73</td>
<td>45.32±2.26</td>
</tr>
<tr>
<td>Galantamine</td>
<td>11.54±0.99</td>
<td>16.45±1.09</td>
</tr>
</tbody>
</table>

The literature says that the essential oil of D. graveolens from France has an IC50 value of 0.27 mg/mL (270 µg/mL) for its antiacetylcholinesterase activity [41]. Compared to this study, the anti-AChE activity of the ethanol extract of D. graveolens used in our study was found to be higher. In a different study, it was reported that the IC50 value of the acetylcholinesterase activity of the essential oil of D. graveolens was collected from Tunisia in different months varied between 5.01-8.12 µg/mL [42]. Compared to this study, the ethanol extract of D. graveolens used in our study was found to have lower anti-AChE activity. It is thought that this may be due to the difference in the solvent used when determining activity and the region where the plant was collected. In a different study, it was reported that the IC50 value of the acetylcholinesterase activity of different extracts of D. graveolens collected from Tunisia varied between 17.03-38.00 µg/mL [43]. It was observed that similar results were obtained compared to this study. In addition, it was observed that the ethanol extract of D. graveolens had lower anti-AChE and anti-BChE activity compared to galantamine used as control. Investigating the presence of enzymes that can cause diseases and suppressing the activity of these enzymes are very important in the treatment of diseases [44]. It is thought that D. graveolens used in our study can be used in the treatment of neurodegenerative diseases due to its potential anticholinesterase activities.

3.4. Total phenolic and total flavonoid contents

Plants produce secondary metabolites with different properties as a result of their defense systems. These compounds are responsible for many biological activities [45]. In our study, the total phenolic and flavonoid contents of the ethanol extract of D. graveolens were measured and shown in Table 2. In the literature, it has been reported that the methanol extract of D. graveolens collected from Morocco has a total phenolic content of 86.19 mg/g and a total flavonoid content of 9.72 mg/g [46]. Compared to this study, the total phenolic content and total flavonoid content of the ethanol extract of Dittrichia graveolens used in our study were determined to be higher. The main reason for this is thought to be due to the difference in the solvents used and the regions
where the plant is collected. In this context, it is thought that the plant may be an important source of phenolic and flavonoid content.

4. Conclusions

In this study, antioxidant, anticholinesterase and antimicrobial activities of *D. graveolens* were investigated. Additionally, total phenolic and flavonoid contents were determined. As a result of the studies, it has been determined that plant extract can be a natural agent against neurodegenerative diseases. In addition, it is thought that it can be used as an antioxidant and antimicrobial natural product.

**Author Contributions:** Conceptualization, N.K., F.S.M., I.U. and M.S.; methodology, N.K., F.S.M., I.U. and M.S.; investigation, N.K., F.S.M., I.U. and M.S.; resources, N.K., F.S.M., I.U. and M.S.; data curation, N.K., F.S.M., I.U. and M.S.; writing—original draft preparation, N.K., F.S.M., I.U. and M.S.; writing—review and editing, N.K., F.S.M., I.U. and M.S.; All authors have read and agreed to the published version of the manuscript.

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