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Hesperis matronalis subsp. matronalis'in Alzheimer ve Mikrobiyal Rahatsızlıklar için Terapötik Potansiyeli

The Therapeutic Potency of *Hesperis matronalis* subsp. *matronalis* for Alzheimer's and Microbial Diseases

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ÖZET

Bu çalışmanın amacı *Hesperis matronalis subsp. matronalis'in* topraküstü kısımlarının methanol ekstresinin, Alzheimer hastalığı ve mikrobiyal rahatsızlıklar için tedavi edici potansiyelinin belirlenebilmesi için, asetilkolinesteraz (AChE) ve butirilkolinesteraz (BuChE) inhibitor etkilerinin ve antimikrobiyal etkisinin ortaya çıkarılmasıdır. Alzheimer hastalığı, nörodejeneratif hasarla doğrudan ilişkili hafıza bozukluğuna yol açması nedeniyle ciddi bir sağlık sorunu olarak değerlendirilmektedir. Mikroorganizmalar insan sağlığı üzerine olumsuz etkilere yol açabilen, birçok rahatsızlıkla doğrudan ilişkilidir. *Hesperis* cinsi Türkiye'de 28 tür ile temsil edilmektedir. *Hesperis* cinsine ait türler arasından, çalışma için *H. matronalis subsp. matronalis* türü bu çalışma kapsamında seçilmiştir. *H. matronalis subsp. matronalis'in* AChE ve BuChE inhibitör aktivitesi kolorimetrik Ellman'ın yöntemiyle belirlenirken, antimikrobiyal aktivitesi ise agar difüzyon metodu kullanılarak belirlenmiştir. Çalışma sonucunda elde edilen veriler ışığında, *Escherichia coli, Yersinia pseudotuberculosis, Klebsiella pneumonia subp. pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Bacillus cereus, Mycobacterium smegmatis, Candida albicans ve Saccharomyces cerevisiae* mikroorganizmaları üzerinde *H. matronalis subsp. matronalis'in* antimikrobiyal aktivitesinin olduğu gözlenmiştir. Çalışma kapsamında *H. matronalis subsp. matronalis'in* AChE ve BuChE inhibitör aktivitesi için IC_{50} değerleri ise, sırasıyla 316.23 ± 2.15 ve 74.13 ± 0.85 µg mL^{-1} olarak bulunmuştur. *H. matronalis subsp. matronalis* AChE ve BuChE'nin inhibitör etkileri ve potansiyel antimikrobiyal aktivitesi nedeniyle, *H. matronalis, subsp. matronalis* Alzheimer'ın yanısıra birçok mikrobiyal hastalığın tedavisinde alternatif bir doğal ilaç kaynağı olabilir.

Anahtar Kelimeler: Antimikrobiyal Aktivite, Kolinesteraz İnhibisyonu, *Hesperis matronalis.*
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ABSTRACT

The goal of the present study, to investigate for the methanolic extract from the aerial parts of *Hesperis matronalis* subsp. *matronalis* in terms of acetylcholinesterase (AChE) and butyrylcholinesterase inhibitory and antimicrobial effects to detect therapeutic potential of Alzheimer's and microbial diseases. Alzheimer's disease is a serious health problem because of its memory defect effect related with neurodegenerative damage. Microorganisms are associated with many ailments that cause negative effects on human health. *Hesperis* genus is represented by 28 genus in Turkey. *H. matronalis* subsp. *matronalis* was selected for the study among from the genus. The inhibitory activities of AChE and BuChE of *H. matronalis* subsp. *matronalis* were determined by colorimetric Ellman's method and the antimicrobial activity was executed using agar diffusion method. As a result of the study, the antimicrobial activity of *H. matronalis* subsp. *matronalis* was observed on *Escherichia coli, Yersinia pseudotuberculosis, Klebsiella pneumonia* subp. *pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Bacillus cereus, Mycobacterium smegmatis, Candida albicans* and *Saccharomyces cerevisiae.* The IC_{50} values for inhibitory activities of AChE and BuChE of *H. matronalis* subsp. *matronalis* have been found as 316.23 ± 2.15 and 74.13 ± 0.85 µg mL^{-1}, respectively. Because of significiant inhibitory effects on AChE and BuChE and potential antimicrobial activity, *H. matronalis* subsp. *matronalis* can be a candidate alternative natural drug source in the treatment of Alzheimer's and many microbial diseases.

Keywords: Antimicrobial Activity, Cholinesterase İnhibition, *Hesperis Matronalis*

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1. Introduction

Alzheimer's disease (AD) is knowed is a prevalent neurodegenerative illness related with memory defect. Reduction of acetylcholine levels in nerve cells is one of the biochemical change in the disorder (1). AChE and BuChE are enzymes in charge of hydrolysis of acetylcholine in synapse. Cholinesterase inhibitors possess a therapeutic value in AD (2). Nowadays, tacrine, rivastigmine and galantamine are used as cholinesterase inhibitors to treat of AD. On the other hand, adverse impacts like gastrointestinal and hepatotoxicity problems of the agents limit usage of the agents in AD treatment (3, 4). So, natural products have become important to obtain safe and effective cholinesterase inhibitors.

Microbial diseases are another health area that increases the importance of using natural compounds because of the resistance of synthetic drugs (5). *Escherichia coli* is a gram negative type of bacterium that causes digestive system diseases, urogenital system diseases, meningitis, pneumonia and other various diseases in humans. *Yersinia pseudotuberculosis* is a pathogen from the class of *Yersinia* that causes symptoms such as swelling of lymph nodes, septicemia, typhoidal clinical effects in humans. *Pseudomonas aeruginosa* is a gram negative type of bacterial pathogen capable of produce lots of acute and chronic infections. *Staphylococcus aureus* is a gram-positive pathogen induces infection in many tissues such as skin, bone and soft tissue (6-7). *Enterococcus faecalis* is a gram-positive bacterium brings about important diseases such as surgical wound infection and urinary tract infection. *Bacillus cereus* is a spore, gram positive and symtomps like diarrhea, abdominal pain like cramp are observed infected people with the bacterium (8). *Mycobacterium smegmatis* is a gram (+) bacterium, induces symtomps associated with immunosuppression (9). *Candida albicans* is a yeast type fungus, causes candidiasis infection by creating oral and vaginal infections in humans (10). *Saccharomyces cerevisiae* is a fungemia causes lots of invasive infection (11).

Hesperis genus belongs to Brassicaceae family is represented by 56 species in the world. It has been recorded that *Hesperis* have been 28 species (33 taxa) in Turkey and the endemism is nearly 82 % (12). It has known that *Hesperis* species have been invasive characteristics, so agricultural fields can be destroyed (13). However, some *Hesperis* species have been used perfumery industry and as diaphoretic and diuretic in medicine (14).

There have been a lot of studies about the botanic characteristic of *Hesperis* species in Turkey (13-20). However, sufficient studies for the biological activities of the species have not been found.
Hesperis matronalis L. have being grown in northern and western Europe (15). Also, *H. matronalis* has been cultivated for its essential oil, and spread in Black Sea region in Turkey. The objective of the current research is to investigate antimicrobial and anticholinesterase effects of *H. matronalis* subsp. *matronalis* growing in Turkey.

2. Materials and Methods

Chemicals and Instrumentation

Acetylcholinesterase, Butyrylcholinesterase, DTNB (5,5′-Dithiobis(2-nitrobenzoic acid)) and galantamine were used in cholinesterase inhibition studies were procured from Sigma-Aldrich (USA). Methanol was obtained from Merck (Germany). Ampicillin and Fluconazole drugs were used in antimicrobial activity purchased from Mustafa Nevzat and Pfizer, respectively.

Plant Material and Preparation of Extract

H. matronalis subsp. *matronalis* specimens were collected from Kars/Ardahan/Göle, Turkey. All specimens were deposited in the Karadeniz Technical University herbarium (herbarium number: KATO: 16791). 30 g of dried plant mixed with 300 mL methanol. This mixture was subjected to overnight at room temperature. Thereafter, this mixture was filtered and this process was repeated 3 times. Filtrate was evaporated using by rotary evaporator (Heidolph, Germany). This extract acquired was used for anticholinesterase and antimicrobial activity studies.

Antimicrobial Activity

B. cereus 702 ROMA, *E. coli* ATCC 25922, *K. pneumonia* subp. *pneumonia* ATCC18883, *Y. pseudotuberculosis* ATCC 911, *P. auroginosa* ATCC 27853, *S. aureus* ATCC 25923, *M. smegmatis* ATCC607, *E. faecalis* ATCC 29212, *C. albicans* ATCC 60193 and *S. cerevisiae* RSKK 251 were used in the antimicrobial activity experiment. All micro-organisms were procured from the Hifzissihha Institute of Refik Saydam in Turkey.
The agar-well diffusion method with modifications was used for susceptibility testing (21-22). Sabouraud dextrose agar (SDA) (Difco, Detriot, MI) and brain heart infusion agar (BHA) were used in the case of yeast-like fungi and for *M. smegmatis* (23), respectively. Wells 5 mm in diameter were opened in the agar with the help of a sterile glass pipe, and 8900- 12800 µg/50 µL of the extract substances was placed into these. The plates were subsequently subjected to incubation for 18 h (35 °C). Ampicillin and fluconazole were preferred a as standard drugs, and dimethylsulfoxide as a control. Finally, Inhibition Zone Diameter (IZD) (mm) was calculated.

Cholinesterase Inhibition Activity

Cholinesterase inhibition activities (AChEI and BChEI) were studied using colorimetric Ellman's method with some modifications (24). Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were used as enzymes during the experiment. Acetylthiocholine iodide and butyrylthiocholine iodide were used as substrates for AChEI and BChEI studies, respectively. Also, DTNB was preferred as colouring agent.
The concentration of 25-200 µg mL^{-1} of the control, standart and test compounds were prepared with sodium phosphate buffer. Firstly, 10 µL of the control, standart and test compounds and then 20 µL of the enzyme were added in a plate. The plate was incubated during 10 minutes at 25 ºC. After incubation, 20 µL of DTNB and 20 µL of substrate were added to all wells. Galantamine was used as standart. The absorbance was read at 412 nm at ELISA reader. Afterwards, % inhibition and IC_{50} values were assessed.

3. Results

Antimicrobial Activities of *Hesperis matronalis* subsp. *matronalis*

Inhibition zone diameter of extract was used to detect the antimicrobial activity of *H. matronalis* subsp. *matronalis* extract against the bacteria tested (Table 1). The methanolic extract of aerial parts of the plant exhibited antimicrobial effects against *E. coli* (IZD = 8 mm), *Y. pseudotuberculosis* (IZD = 9 mm), *K. pneumonia* subp. *pneumonia* (IZD = 8 mm), *P. aeruginosa* (IZD = 6 mm), *S. aureus* (IZD = 8 mm), *E. faecalis* (IZD = 8 mm), *B. cereus* (IZD = 8 mm), *M. smegmatis* (IZD = 15 mm), *C. albicans* (IZD = 8 mm) and *S. cerevisiae* (IZD = 6 mm).

Table 1. Inhibition zone diameter (mm) of extract of Hesperis matronalis subsp. matronalis

Tested Compounds	**Microorganisms and Inhibition Zone Diameter (mm)**									
	Gram negative				**Gram positive**			**No gram**	**Fungi**	
	Ec	**Yp**	**Kp**	**Pa**	**Sa**	**Ef**	**Bc**	**Ms**	**Ca**	**Sc**
Methanolic Extract										
	8	9	8	6	8	8	8	15	8	6
Ampicillin	10	10	10	18	35	10	15	-	-	-
Streptomycin								35		
Fluconazole									25	25

Ec: *E. coli* ATCC 25922, Yp: *Y. pseudotuberculosis* ATCC 911, Kp: *K. pneumonia* subp. *pneumonia* ATCC18883, Pa: *P. aeruginosa* ATCC 27853, Sa: *S. aureus* ATCC 25923, Ef: *E. faecalis* ATCC 29212, Bc: *B. cereus* 702 Roma, Ms: *M. smegmatis* ATCC607, Ca: *C. albicans* ATCC 60193, Sc: *S. cerevisiae* RSKK 251, (-): no activity

Table 2. % Inhibition of acetylcholinesterase inhibitor activities

Samples	25 µg mL^{-1}	50 µg mL^{-1}	100 µg mL^{-1}	200 µg mL^{-1}
Methanolic extract	9.22 ± 0.23	21.31 ± 1.26	26.28 ± 1.02	42.09 ± 2.34
Galantamine	55.63 ± 1.65	74.26 ± 2.45	81.31 ± 1.54	86.75 ± 2.32

Table 3. IC_{50} values of acetylcholinesterase inhibitor activities

Samples	IC_{50} (µg mL^{-1})
Methanolic extract	316.23 ± 2.15
Galantamine	13.03 ± 1.32

Table 4. % Inhibition of Butyrylcholinesterase inhibitor activities

Samples	25 µg mL^{-1}	50 µg mL^{-1}	100 µg mL^{-1}	200 µg mL^{-1}
Methanolic extract	33.14 ± 1.25	44.63 ± 1.31	52.41 ± 2.47	66.39± 2.38
Galantamine	40.53 ± 2.13	59.71 ± 1.42	71.73 ± 2.71	78.35 ± 2.14

Table 5. IC_{50} values of butyrylcholinesterase inhibitor activities

Samples	IC_{50} (µg mL^{-1})
Methanolic extract	74.13 ± 0.85
Galantamine	35.24 ± 1.52

Anticholinesterase Activities of *Hesperis matronalis* subsp. *matronalis*

We examined the effect of the methanolic extract of the plant on the anticholinesterase activities. % inhibition of AChE inhibitor effect of methanolic extract was 9.22 ± 0.23, 21.31 ± 1.26, 26.28 ± 1.02 and 42.09 ± 2.34 µg mL^{-1} for 25, 50, 100 and 200 µg mL^{-1}, respectively. % inhibition of AChE inhibitor effect of galantamine was 55.63 ± 1.65, 74.26 ± 2.45, 81.31 ± 1.54 and 86.75 ± 2.32 µg mL^{-1} for 25, 50, 100 and 200 µg mL^{-1}, respectively (Table 2). The IC_{50} values for inhibitory effect of AChE assay of the plant has been found as 316.23 ± 2.15 µg mL^{-1} (Galantamine IC_{50}=13.03 ± 1.32 µg mL^{-1}) (Table 3). % inhibition of BChE inhibitor effect of methanolic extract was 33.14 ± 1.25, 44.63 ± 1.31, 52.41 ± 2.47 and 66.39 ± 2.38 µg mL^{-1} for 25, 50, 100 and 200 µg mL^{-1}, respectively. % inhibition of BChE inhibitor effect of galantamine was 40.53 ± 2.13, 59.71 ± 1.42, 71.73 ± 2.71 and 78.35 ± 2.14 µg mL^{-1} for 25, 50, 100 and 200 µg mL^{-1}, respectively (Table 4). The IC_{50} values for inhibitory effect of BChE assay of the plant has been found as 74.13 ± 0.85 µg mL^{-1} (Galantamine IC_{50}=35.24 ± 1.52 µg mL^{-1}) (Table 5).

4. Discussion

Nowadays, new therapeutic agents have been investigated because of the resistance of bacteria to synthetic drugs. For this purpose, researchers have focused on the antimicrobial effects of natural sources. Although there are not enough studies on the biological activities of *H. matronalis* subsp. *matronalis*, a few studies are avaliable about antimicrobial effect of *H. matronalis*. Aqueous extract of *H. matronalis* leaves was effective against *Salmonella typhimurium* (IZD = 11.1 mm) was reported (25). Another study showed that, aqueous ethanolic extract of *H. matronalis* was effective on *S. aureus* (IZD = 14 mm) but impotent on *E. coli*, *P. aeruginosa* and *C. albicans* (26). Also, Aerial parts of *H. matronalis* L. subsp. *matronalis* ethanolic extract produced antimicrobial effect *on Mycobacterium tuberculosis* (27). The seed of *H. matronalis* methanolic extract was impotent on *E. coli, P. aeruginosa, S. aureus* and *C. albicans* (28).

In this study, it was observed that dry methanolic extract of aerial parts of *H. matronalis* subsp. *matronalis* was effective against microorganisms like *E. coli*, *Y. pseudotuberculosis*, *K. pneumonia subp. pneumonia*, *P. aeruginosa*, *S. aureus*, *B. cereus*, *E. faecalis*, *M. smegmatis, C. albicans* and *S. cerevisiae.* Comparing with previous literatures, difference of effect on some microbial organisms of the same species may be probably related with subspecies differences, selections of different parts of the plants and extraction solvents, climatic, seasonal or geographical factors. *H. matronalis* subsp. *matronalis* owned preservative effect on these microorganisms caused various diseases have been hopeful for further studies. Thus, *H. matronalis* subsp. *matronalis* can be potantial natural source of antimicrobial agents.

In recent years, interest of using natural resources has increased in studies of novel cholinesterase inhibitors to treatment Alzheimer's disease (29). Therefore, anticholinesterase activities of the species have been evaluated with this study. According to this study, the results showed that *H. matronalis* subsp. *matronalis* had moderate AChE inhibitory activity and high BuChE inhibitory activity compared with galantamine. It is the first study about AChE and BuChE inhbition of the species. The results are quite promising and this study may guide to further studies for Alzheimer's disease

5. Conclusion

In this study, antimicrobial activity and anticholinesterase activity of metanolic extract of *H. matronalis* subsp. *matronalis* have been investigated. Consequently, it is shown that the plant posses significiant antimicrobial and anticholinesterase properties. These plant extract may represent a natural source of antimicrobial and anticholinesterase agents.

6. Authors' Contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

7. Competing Interests

Authors have declared that no competing interests exist.

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