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Original Article

BRANCHED HORSETAIL (*EQUISETUM RAMOSISSIMUM*): SOME BIOLOGICAL ACTIVITIES AND TOTAL PHENOLIC AND FLAVONOID CONTENTS

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ABSTRACT

Today, many plant species come to the fore with their medicinal potential. Plants are medically important natural resources. In our study, the biological activities of *Equisetum ramosissimum* were determined. Additionally, total phenolic and flavonoid contents were measured. The aboveground parts of the plant were extracted with ethanol and methanol. Antioxidant activities of the extracts were measured with Rel Assay TAS and TOS kits. Antimicrobial activity was determined against test bacteria and fungi using the agar dilution method. The anticholinesterase potential (anti-AchE and anti-BchE) of the extracts was determined by measuring acetyl- and butyrylcholinesterase activities. 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, TAS values of ethanol and methanol extracts of the plant were determined as 4.802±0.090 mmol/L and 5.350±0.008 mmol/L, respectively. TOS values were determined as 7.643±0.189 µmol/L and 11.608±0.145 µmol/L, respectively. OSI values were determined as 0.159±0.005 and 0.217±0.006, respectively. It was determined that the plant extract was effective against bacterial strains at concentrations of 50-400 µg/mL and against fungal strains at concentrations of 50-200 µg/mL. In addition, the anti-AChE values of ethanol and methanol extracts were determined as 20.45±0.54 µg/mL and 16.70±0.43 µg/mL, respectively, and the anti-BChE values were determined as 40.07±0.90 µg/mL and 36.94±0.86 µg/mL, respectively. Total phenolic contents of ethanol and methanol extracts were determined as 161.30±3.51 mg/g and 199.11±1.96 mg/g, respectively, and total flavonoid contents were determined as 119.00±1.60 mg/g and 103.61±2.39 mg/g. As a result, it has been determined that *Equisetum ramosissimum* has a high biological potential and can be used as a natural material in pharmacological designs.

KEYWORDS: Anticholinesterase, Antimicrobial, Antioxidant, Medicinal plants.

1. Introduction

It is known that plants have been used by people for different purposes since ancient times [1]. Different communities use plants for food, religious rituals, fuel, shelter, making tools and equipment, and in the fight against diseases [2]. In addition to their different features, plants are very important natural resources, especially from a medical point of view [3]. Many studies have shown that plants have different biological activities such as antioxidant, anticancer, antiproliferative, antimicrobial, hepatoprotective, antiallergic, and DNA protective [4-10]. In this context, determining the biological activities of plants is very important in terms of their potential for medicinal use. In our study, the antioxidant, antimicrobial, antialzeihmer, total phenolic and total

flavonoid contents of *Equisetum ramosissimum* Desf. were determined.

Equisetum ramosissimum is in the Equisetaceae family. When we look at the research in the literature, it is known locally by different names. These names are: **English:** common horsetail; **Arabic:** kinbat, kinbat-el-hokol, thailel-faras, thanbel-faras; **Spanish:** cola de an caballo; **Swedish:** akerfraken; **Japanese:** sugi-na, tsukushi; **French:** prele des champs; **German:** akerschactelhalm [11-12]. Geographically, it is distributed in Southeast Asia, the South Pacific Islands, Fiji, from the north of Europe to southern Germany, Mongolia, Korea, the Azores and the Mediterranean region. *E. ramosissimum* is known for its uniform aerial stems, sterile and fertile branched stems, and strobili-producing branches [11].

2. Matherials and Methods

Equisetum ramosissimum samples were collected from Türkiye (Mersin). Herbarium samples of the plant are kept in the laboratory of Osmaniye Korkut Ata University, Department of Biology. The aboveground parts of the plant were dried in the laboratory environment, away from direct sunlight. The dry samples were then ground into powder and 30 g of the samples were weighed. Then, the samples added to the Soxhlet apparatus were extracted with 250 mL of ethanol at 50 ºCfor approximately 6 hours. The process was repeated to obtain methanol extracts. Then, solvents were removed using a Rotary Evaporator and stored at 4 ºC until experiments were performed.

2.1. Antimicrobial activity tests

Solutions of ethanol and methanol extracts of the plant were prepared with the dilution method at concentrations between 12.5 and 800 µg/mL. The lowest concentrations of these prepared solutions that completely prevented the growth of standard fungal and bacterial species were determined. *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 were used as bacterial strains. *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135 and *C. glabrata* ATCC 90030 were used as fungal strains. Bacteria were pre-cultured in Muller Hinton Broth medium, and fungi were pre-cultured in RPMI 1640 Broth medium. The findings were expressed in µg/mL [13-16].

2.2. Antioxidant parameters

The antioxidant potentials of ethanol and methanol extracts of the plant were determined using Rel Assay kits. Total antioxidant values were measured with TAS kits, and total oxidant values were measured with TOS kits. Tests were performed following the manufacturer's protocol. TAS values were expressed as mmol trolox equivalent/L, and TOS values were expressed as µmol hydrogen peroxide equivalent/L [17-18]. The OSI value, 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 [19].

2.3. Anticholinesterase tests

Anti-acetyl- and anti-butyrylcholinesterase activities of plant extracts were determined using the Ellman method [20]. Solutions of plant extracts were created at concentrations ranging 3.125-200 µg/mL. 130 µL of 0.1 M pH=8 phosphate buffer, 10 µL of extract solution, 20 µL of enzyme (AChE or BChE enzyme solution) were added to the microplate and the mixture was incubated at 25 °C in the dark for 10 min. Then, 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. Then, absorbance reading was done 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

1 mL of stock solutions were prepared from plant extracts. Then, 1 mL of Folin-Ciocalteu reagent (1:9, v/v) was added to the solution and vortexed. 0.75 mL 1% Na_2CO_3 was added to the resulting solution and incubated for 2 hours at room temperature. Then, absorbance reading was taken at 760 nm. According to the calibration curve of the gallic acid standard solution, the total phenolic content (TPC) was expressed in mg/g [21].

Total flavonoid content (TFC) of plant extracts was determined using the aluminum chloride test [22]. 0.1 mL 10% $Al(NO_3)_3$, 0.1 mL 1 M NH_4CH_3COO, 4.3 mL methanol, 0.5 mL Quercetin solution and 0.5 mL plant extract were mixed. Then the mixture was incubated for 40 minutes and absorbance was measured at 415 nm. Total flavonoid content was expressed in mg/g.

3. Results and Discussion

3.1. Antimicrobial activity

In recent years, there has been an increase in the number of many diseases originating from microorganisms [23]. The increase in the number of resistant microorganisms as a result of unconscious use of antibiotics also increases the mortality and morbidity of diseases. Due to the possible side effects of synthetic drugs, people are turning to natural antimicrobial drugs [24-25]. In this context, it is very important to investigate the antimicrobial activities of plants, which are the most common and important among natural resources. In this study, the effect of ethanol and methanol extract of *E. ramosissimum* on the tested microorganisms was determined and shown in Table 1.

Table 1. Antimicrobial potential of *Equisetum ramosissimum*

Microorganism	Extract	
	Ethanol [µg/mL]	Methanol [µg/mL]
S. aureus	50	100
S. aureus MRSA	50	100
E. faecalis	200	200
E. coli	100	100
P. aeruginosa	200	400
A. baumannii	100	200
C. glabrata	50	100
C. albicans	50	200
C. krusei	50	100

In our study, the microorganisms chosen as test microorganisms were gram negative and positive bacteria. Additionally, fungi were selected as test microorganisms. The selection of test microorganisms used in our study was due to the fact that they are common bacterial and fungal strains. In previous studies, the antimicrobial status of methanol, ethanol and water extracts obtained from the aerial parts of *E. ramosissimum* samples collected from Jordan against *Bacillus subtilis Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae, Escherichia coli, Salmonella* sp., and *Erwinia carotovora* was investigated. As a result of the study, it was seen that the best inhibition zone value was 25 mm of ethanol extract, which was effective against *B. subtilis* and *E. carotovora*. It was reported that the best minimum inhibitory concentration (MIC) value was exhibited by the ethanol extract against *E. carotovora* at a concentration of 12.5 mg/mL, and the water extract against *E. coli* and *S. epidermidis* at a concentration of 12.5 mg /mL [26]. In a study conducted in Iran, the antimicrobial effect of aqueous, methanol and ethanol extract of ashes obtained by burning *E. ramosissimum* against *S. aureus, E. coli* and *Candida albicans* strains was investigated. As a result of the study, it was reported that the aqueous extract exhibited the best minimum bactericidal concentration (MBC) value against *E. coli* at a concentration of 25 mg/mL [27]. In our study, ethanol and methanol extract obtained from the aerial parts of *E. ramosissimum* were used. According to the findings, the ethanol extract of the plant was found to be effective against the bacterial strains *P. aeruginosa* and *E. faecalis* at a concentration of 200 µg/mL. It was also determined to be effective against *A. baumannii* and *E. coli* at concentrations of 100 µg/mL, and against *S. aureus* and *S. aureus* MRSA at concentrations of 50 µg/mL. In addition, it was observed that 50 µg/mL was effective against fungal strains *C. albicans, C. glabrata* and *C. krusei*. Methanol extract was effective against the bacterial strains *P. aeruginosa* at a concentration of 400 µg/mL and against *E. faecalis* and *A. baumannii* at a concentration of 200 µg/mL. In addition, it has been shown to be effective against *E. coli, S. aureus* and *S. aureus* MRSA at concentrations of 100 µg/mL. In addition, it was determined that it was effective against fungal strains at 200 µg/mL against *C. albicans* and 100 µg/mL against *C. glabrata* and *C. krusei*. When we compare both the literature and our studies, it is thought that *E. ramosissimum* may be a natural antimicrobial source against different microorganisms.

3.2. Antioxidant Activity

Free radicals are oxidant compounds produced as a result of routine metabolic activities [28]. While low levels of these compounds do not have any negative effects, serious cellular damage can occur when levels increase [29]. In suppressing the effects of oxidant compounds, the antioxidant defense system comes into play and suppresses oxidant compounds [30]. However, in some cases oxidant compounds may be more dominant. In such cases, oxidative stress occurs. As a result of oxidative stress, serious diseases such as cancer, cardiological disorders, Parkinson's disease, Alzheimer's disease and multiple sclerosis may occur in humans. Supplementary antioxidants serve to reduce the effects of oxidative stress [31-32]. Plants are natural products with high potential to be antioxidant supplements. In our study, the antioxidant potential of ethanol and methanol extract obtained from the aerial part of *E. ramosissimum* was determined. The findings obtained are shown in Table 2.

Table 2. TAS, TOS, OSI, TPC and TFC values of *Equisetum ramosissimum*

Parameters	Solvents	
	Ethanol	Methanol
TAS mmol/L	4.802±0.090	5.350±0.008
TOS µmol/L	7.643±0.189	11.608±0.145
OSI (TOS/(TASx10))	0.159±0.005	0.217±0.006
TPC mg/g	161.30±3.51	199.11±1.96
TFC mg/g	119.00±1.60	103.61±2.39

No previous studies on TAS, TOS and OSI values of *E. ramosissimum* have been found in the literature. In our study, TAS, TOS and OSI values of *E. ramosissimum* were determined for the first time. As a result of the study, it was observed that the TAS, TOS and OSI values of the methanol extract of *E. ramosissimum* were higher than these of the ethanol extract. In different studies conducted in the literature on the TAS, TOS and OSI values of different plant species, the 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. In the same studies, TOS values were reported as 14.839, 12.144, 4.951, 13.892, 18.679 and 8.501 µmol/L, respectively. In the same studies, OSI values were reported as 0.157, 0.211, 0.057, 0.221, 0.346 and 0.116, respectively [33-38]. Compared to these studies, the TAS value of the methanol extract of *E. ramosissimum* was found to be higher than in case of *G. aparine* and lower than in case of *H. salicifolium, S. marianum, R. scutatus* and *A. officinalis*. In addition, the TAS value of the ethanol extract of *E. ramosissimum* was found to be lower than that of *H. salicifolium, S. marianum, R. scutatus, G. aparine* and *A. officinalis*. TAS value shows the totality of antioxidant effective compounds present within natural products [39]. It has been reported in the literature that *E. ramosissimum* showed antioxidant activity in other different tests [40-44]. In this context, it was observed that *E. ramosissimum* used in our study also had antioxidant potential. TOS values are an indicator of the totality of oxidant-active compounds produced within a natural product [39]. The TOS value of the methanol extract of *E. ramosissimum* used in our study was determined to be lower than that of *H. salicifolium, S. marianum, A. officinalis* and *G. aparine*, and lower than the TOS value of *R. scutatus* and *S. absconditiflora*. In addition, the TOS value of the ethanol extract of *E. ramosissimum* was determined to be higher than that of *R. scutatus* and lower than that of *H. salicifolium, S. marianum, A. officinalis, G. aparine* and *S. absconditiflora*. In this context, the TOS values of *E. ramosissimum* used in our study were found to be at normal levels. The OSI value is used to express the percentage of oxidant compounds suppressed by antioxidant compounds [39]. The OSI value of the ethanol extract of *E. ramosissimum* used in our study was determined to be higher than for *H. salicifolium,*

R. scutatus and *S. absconditiflora*, and lower than *S. marianum, A. officinalis* and *G. aparine*. In addition, the OSI value of the methanol extract of *E. ramosissimum* was determined to be higher than in case of *H. salicifolium, S. marianum, R. scutatus* and *S. absconditiflora*, and lower than *A. officinalis* and *G. aparine*. As a result, it was seen that *E. ramosissimum* has antioxidant potential.

3.3. Anticholinesterase activity

Nowadays, the mortality and morbidity of neurodegenerative diseases are increasing. The incidence is increasing. Although diseases such as Parkinson's and Alzheimer's generally occur with different effects, oxidative stress is one of these factors. The increasing incidence of Alzheimer's disease, which is very common in patients over the age of 65, worries people [45]. In this context, it is very important to identify natural products to be used in reducing the effects of neurodegenerative diseases. In our study, anti-acetyl and anti-butyryl-cholinesterase activities of *E. ramosissimum* were determined. IC50 values of the findings are shown in Table 3.

Table 3. Anti-AChE and anti-BChE values (IC50) of *Equisetum ramosissimum*

Parameters	Solvents		Standart
	Ethanol	Methanol	Galantamine
AChE µg/mL	20.45±0.54	16.70±0.43	6.75±0.25
BChE µg/mL	40.07±0.90	36.94±0.86	18.10±0.12

When the literature was examined, it was seen that there was no anticholinesterase activity study on *E. ramosissimum*. In our study, the anti-AChE activity of *E. ramosissimum* was determined for the first time. When studies conducted at the genus level were examined, a study conducted in Morocco reported that the IC50 value of the ethanol extract obtained from the aerial part of *E. arvense* was 3.134 mg/mL [46]. Compared to this study, the anti-AChE activity of both the ethanol and methanol extracts of *E. ramosissimum* used in our study was found to be lower. In our study, it was observed that the IC50 value of the anti-AChE activity of ethanol extract was 20.45 µg/mL, and the anti-AChE activity of methanol extract was 16.70 µg/mL. It was determined that methanol extract had better value among the extracts. In addition, it was determined that the anti-AChE activity of both extracts used was lower than that of galantamine used as control. As a result, it is thought that *E. ramosissimum* used in our study can be used in the treatment of neurodegenerative diseases thanks to its potential anticholinesterase activity.

3.4. Total Phenolic and Total Flavonoid Contents

Phenolic compounds including flavonoids are secondary plant metabolites. Secondary metabolites serve many different functions for plants. Among these tasks, biological activity comes first [47-49]. In our study, the total phenolic and flavonoid contents of the ethanol and methanol extract of *E. ramosissimum* were measured and shown in Table 2. In the literature, in a study conducted in Jordan, it was reported that the total phenolics content of the ethanol extract obtained from the leaf of *E. ramosissimum* was 0.032 mg/mg. Additionally, in the same study, it was reported that the major components of the leaf part of the *E. ramosissimum* plant were catechin, caffeic acid, isoferulic acid, kaempferol-3-O-rutinoside, 2,4-dihydroxyacetophenone, kaempferol-3-O-neohesperidoside, kaempferol-3-O-glucoside and kaempferol [50]. In a study conducted in Iraq, it was reported that the total flavonoid content of the methanol extract obtained from the root of *E. ramosissimum* was 831.8 µg/ml. Additionally, in the same study, it was reported that the major components of the root part of the *E. ramosissimum* plant were kaempferol, kaempferol-3-O-glycoside, leutolin, myrcetin, querctin and rutin [51]. In a study conducted in Siberia, it was reported that the total flavonoid content of the methanol extract obtained from the aerial part of *E. ramosissimum* was 1.751 mg/g [40]. In a study conducted in India, it was reported that the total phenolic and flavonoid content of the methanol extract obtained from the root part of *E. ramosissimum* were 600.02 mgGAE/g and 631.38 mgQE/g, respectively [52]. When we look at other different studies conducted on the phenolic compound contained in the *E. ramosissimum* plant, it has been reported that the major components in the aerial part used are isoferulic acid, iso-orientin, myristic acid, linoelaidic acid, rutin, 3-Glu-7-Rha quercetin, kaempferol-3,7-O-diglucoside, vanillin, ferulic acid and tannic acid [53-54]. In our study, it was observed that the total phenolic and flavonoid content of the aerial part of *E. ramosissimum* in ethanol extract was 161.30 mg/g and 119.00 mg/g, and in methanol extract it was 199.11 mg/g and 103.61 mg/g. It was observed that the total phenolic value was higher in methanol extract and the total flavonoid value was higher in ethanol extract. When we compared it with literature studies, it was seen that the results we found were generally high. As a result, it is thought that *E. ramosissimum* may be an important source of phenolic and flavonoid content.

4. Conclusions

In this study, the biological activities and total phenolic and flavonoid contents of *E. ramosissimum* were determined. As a result of the studies, it was seen that the plant extract has antioxidant, antimicrobial and anticholinesterase potential. In addition, it has been determined that it may be a natural source of total phenolics and flavonoids. As a result, it has been determined that *E. ramosissimum* can be an important natural resource in pharmacological designs.

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