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Antioxidant, Anticholinesterase and Antibacterial Activities of *Jurinea consanguinea* DC.

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Abstract: The aim of this study was to investigate *in vitro* antioxidant, anticholinesterase and antibacterial activities of the petroleum ether, chloroform and methanol extracts obtained from the aerial parts of *Jurinea consanguinea* DC. (Asteraceae). Total phenolic and flavonoid contents of these crude extracts were determined as pyrocatechol and quercetin equivalents, respectively. The methanol extract which possessed almost the same effect with the chloroform extract in β-carotene-linoleic acid system exhibited higher free radical scavenging activity than a standard compound, BHT, at 100 and 200 µg/mL concentrations. The petroleum ether extract showed the highest acetylcholinesterase inhibitory activity among the tested extracts. The methanol extract exhibited higher butyrylcholinesterase inhibitory activity than galantamine at all concentrations (94% inhibition at 200 µg/mL). The antibacterial activity of the extracts was determined by the disc diffusion method. The chloroform extract showed moderate activity against *B. subtilis*, *P. aeruginosa* and *S. aureus*.

Keywords: *Jurinea consanguinea*; Asteraceae; antioxidant; anticholinesterase; antibacterial.

1. Introduction

The genus *Jurinea* Cass. (Asteraceae) comprising about 250 species is widespread in South-west and Central Asia, and the Mediterranean region. This genus is represented by 19 taxa in Turkey, seven of them are endemic [1]. Recently a new endemic species is described in Turkey and named *Jurinea turcica* B. Dogan & A. Duran, from North-west Anatolia [2]. The limited phytochemical studies on *Jurinea* species revealed that their main constituent was the sesquiterpene lactones, which is the chemotaxonomic marker for the Asteraceae family (Compositae) [3, 4, 5]. The germacranolides oxygenated at C-14 and C-15 are characteristic for this genus [6]. However, the pentacyclic triterpenes

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were also found in the roots of *J. albicaulis* L., and in the fruits of *J. anatolica* Boiss. and *J. consanguinea* DC. [7, 8]. No pharmacological and biological researches on *Jurinea* species have been previously performed, except for the antimicrobial activity of *J. ancyrensis* Bornm. [9]. In India, *J. dolomiaea* has been used as aphrodisiac [10].

Chemical and biological studies on medicinal and edible plants have been increased to find natural active compounds since many researches have been shown that synthetic drugs and additives may possess toxic effects and cause serious diseases [11]. Furthermore, consumers have been also more interested in natural products, especially natural antioxidants, to protect their health. The consumption of fruits and vegetables rich in vitamins and phenolic compounds which have a strong antioxidant effect may reduce incidence of chronic diseases. Dietary antioxidants can help to prevent oxidative damage to cellular components [12]. Some studies showed also that antioxidants may retard the progression of Alzheimer's disease which is frequently seen among elderly people all around the world [13]. Many plants have been used as antibacterial in traditional medicine all around the world since ancient times. Studies on medicinal plants have been increased to find antibacterial agents because of the resistance building by pathogenic microorganisms against the antibiotics [14].

A perennial plant, *Jurinea consanguinea* DC., is distributed in Turkey, Bulgaria and Greece [1]. In the present study, total phenolic and flavonoid contents of the petroleum ether, chloroform and methanol extracts prepared from the aerial parts of *J. consanguinea* were determined as pyrocatechol and quercetin equivalents, respectively. These crude extracts were tested *in vitro* for their antioxidant activity by using two methods namely β-carotene-linoleic acid test system and DPPH free radical scavenging assay, their anticholinesterase activity by Ellman method and their antibacterial activity by disc diffusion method. The antibacterial activity was investigated against Gram-positive (*B. subtilis*, *S. aureus*) and Gram-negative (*K. pneumoniae*, *P. vulgaris*, *P. aeruginosa*) bacteria. This study examined the antioxidant, anticholinesterase and antibacterial activities of *J. consanguinea* for the first time.

2. Materials and Methods

2.1. Plant material

The aerial parts of *Jurinea consanguinea* DC. were collected from Edirne (Turkey) in May 2008, and identified by Assis. Prof. Dr. Ciler Meric. A voucher specimen was deposited in the Herbarium of Faculty of Science, Trakya University (EDTU 9651).

2.2. Preparation of plant extracts

The aerial parts of *J. consanguinea* were dried at room temperature (25ºC) and chopped into small pieces. They were separated to three parts (each 50 g) for macerating with 200 mL petroleum ether, chloroform and methanol at room temperature three times (24 h x 3), indivudually. After filtration of each extract, the solvents were evaporated to dryness *in vacuo*, and the crude extracts were obtained, separately. The yield of petroleum ether, chloroform and methanol extracts were 2.4, 5.4 and 13.9 % (w/w), respectively.

2.3. Determination of total phenolic content

The concentrations of phenolic content in the crude extracts were expressed as micrograms of pyrocatechol equivalents (PEs), determined with FCR according to the method of Slinkard and Singleton [15]. The solution (100 µL) of the crude extracts in methanol was added to 4.6 mL of distilled water and 100 µL of FCR (Folin-Ciocalteu's Reagent), and mixed thoroughly. After 3 min, 300 µL sodium carbonate (2%) was added to the mixture and shaken intermittently for 2 h at room temperature. The absorbance was read at 760 nm. The concentration of phenolic compounds was calculated according to the following equation that was obtained from the standard pyrocatechol graphic:

Absorbance = 0.1493 pyrocatechol (μg) - 0.0753 (R^2 = 0.9974)

2.4. Determination of total flavonoid content

Measurement of flavonoid content of the crude extracts was based on the method described by Park et al. [16] with a slight modification and results were expressed as quercetin equivalents. An aliquot of 1 mL of the solution (contains 1 mg of crude extract in methanol) was added to test tubes containing 0.1 mL of 10% aluminium nitrate, 0.1 mL of 1 M potassium acetate and 3.8 mL of methanol. After 40 min at room temperature, the absorbance was determined at 415 nm. The concentration of flavonoid compounds was calculated according to following equation that was obtained from the standard quercetin graphic:

Absorbance = 0.0732 quercetin (μg) + 0.0153 (R^2 = 0.9972)

2.5. Antioxidant activity

The antioxidant activity was determined by using two methods namely β-carotene-linoleic acid test system and DPPH (Diphenyl picryl hydrazyl) free radical scavenging assay.

2.5.1. Determination of the antioxidant activity by the β-carotene bleaching method

The antioxidant activity of the crude extracts was evaluated using the β-carotene-linoleic acid test system [17] with slight modifications. β-Carotene (0.5 mg) in 1mL of chloroform was added to 25 μL of linoleic acid, and 200 mg of Tween-40 emulsifier mixture. After evaporation of chloroform under vacuum, 100 mL of distilled water saturated with oxygen, were added by vigorous shaking. 4 mL of this mixture was transferred into different test tubes containing different concentrations of the sample. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a 96-well microplate reader (SpectraMax 340PC, Molecular Devices, USA). The emulsion system was incubated for 2 h at 50ºC. A blank, devoid of β-carotene, was prepared for back ground subtraction. BHT and α-tocopherol were used as standards.
The bleaching rate (R) of β-carotene was calculated according to the following equation:

$$R = \frac{\ln \frac{a}{b}}{t}$$

Where: ln=natural log, *a*=absorbance at time zero, *b*=absorbance at time *t* (120 min).
The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control, using following equation:

$$AA\ (Inhibition\%) = \frac{R_{Control} - R_{Sample}}{R_{Control}} \times 100$$

2.5.2. DPPH free radical scavenging activity test

The free radical scavenging activity of the crude extracts was determined by the DPPH assay described by Blois [18] with slight modification. In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.1 mM

solution of DPPH in methanol was prepared and 4 mL of this solution was added to 1 mL of sample solutions in methanol at different concentrations. Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated by using the following equation:

$$\text{DPPH Scavenging Effect (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \text{ x } 100$$

2.6. Anticholinesterase activity test

Acetyl- and butyryl-cholinesterase inhibitory activities were measured, by slightly modifying the spectrophotometric method developed by Ellman et al. [19]. Electric eel AChE and horse serum BChE were used, while acetylthiocholine iodide and butyrylthiocholine iodide were employed as substrates of the reaction. DTNB (5,5′-dithio-bis(2-nitrobenzoic)acid) were used for the measurement of the cholinesterase activity. Ethanol was used as a solvent to dissolve test samples and the controls. Briefly, 150 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of sample solution in ethanol at different concentrations and 20 µL AChE (5.32 x 10-3 U) or BChE (6.85 x 10-3 U) solution were mixed and incubated for 15 min at 25ºC, and and 10 µL of 0.5 mM DTNB was added. The reaction was then initiated by the addition of 10 µL of acetylthiocholine iodide (0.71 mM) or butyrylthiocholine iodide (0.2 mM), in that order. The hydrolysis of these substrates were monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine iodide, respectively, at a wavelength of 412 nm utilising a 96-well microplate reader (SpectraMax 340PC, Molecular Devices, USA). The measurements and calculations were evaluated by using Softmax PRO software. Percentage of inhibition of AChE or BChE was determined by a comparison of the rates of reaction of samples relative to blank sample (ethanol in phosphate buffer pH 8) using the formula (E - S)/ E x 100, where E is the activity of enzyme without test sample, and S is the activity of enzyme with test sample. The experiments were carried out in triplicate. Galantamine was used as a reference compound.

2.7. Antibacterial activity

The following strains of bacteria were used: *Bacillus subtilis* ATCC 6633, *Klebsiella pneumoniae* ATCC 33495, *Proteus vulgaris* ATCC 13315, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923. The bacteria were obtained from the culture collection of the Bacteriology Laboratory of Medical Faculty, Trakya University. The antibacterial activity of the crude extracts was assayed by the standard disc diffusion method [20]. Empty sterilised discs of 6 mm (Oxoid) were each impregnated with 50 µL of the sample solution. All the bacteria mentioned above were incubated at 37 ± 0.1°C for 24 h by inoculation into nutrient agar (Acumedia). The culture suspensions were prepared and adjusted by comparing against 0.5 Mac-Farland turbidity standard tubes. Nutrient agar (NA) (15 mL) were poured into each sterile Petri dish (90 mm diameter) after injecting cultures (0.1 mL) of bacteria and distributing medium into Petri dishes homogeneously. The discs, injected with samples, were placed on the inoculated agar by pressing slightly. Petri dishes were kept at 4°C for 2 h, the bacteria were incubated at 37°C for 18-24 h. At the end of the period, inhibition zones formed on the medium were evaluated in millimeter. Studies performed in duplicate and the inhibition zones were compared with those of reference disc which was Ofloxacin (5 µg).

2.8. Statistical analysis

The results were mean ± SD of three parallel measurements. All statistical comparisons were made by means of Student's *t*-test, p values < 0.05 were regarded as significant.

3. Results and Discussion

The petroleum ether, chloroform and methanol extracts prepared from the aerial parts of *J. consanguinea* were screened for their possible antioxidant activity by using four complementary methods, namely the amount of total phenolic and flavonoid contents, β-carotene bleaching and DPPH free radical scavenging assays. As seen in Table 1, the phenolic and flavonoid contents of the methanol extract are higher than those of the petroleum ether and chloroform extracts. The phenolic contents of the tested extracts are higher than their flavonoid contents. β-carotene-linoleic acid system and DPPH free radical scavenging assay were carried out at four different concentrations. Although the petroleum ether and chloroform extracts exhibited over 50% inhibition of lipid peroxidation by β-carotene bleaching method at 200 µg/mL, they were found to be inactive at all concentrations in DPPH free radical scavenging assay (Fig. 1 and 2). While the methanol extract possessed almost the same effect with the chloroform extract in β-carotene bleaching method at all concentrations, it exhibited higher free radical scavenging activity than a standard compound, BHT, at 100 and 200 µg/mL (Figure 1 and 2).

Table 1. Total phenolic and flavonoid contents of *J. consanguinea* extracts[a]

Extracts	Phenolic content (µg PEs/mg extract)[b]	Flavonoid content (µg QEs/mg extract)[c]
Petroleum ether	35.77 ± 0.74	6.07 ± 1.22
Chloroform	38.13 ± 1.98	15.53 ± 1.52
Methanol	47.95 ±1.18	31.76 ± 0.76

[a] Values expressed are means ± S.D. of three parallel measurements ($p < 0.05$)
[b] PEs, pyrocatechol equivalents
[c] QEs, quercetin equivalents

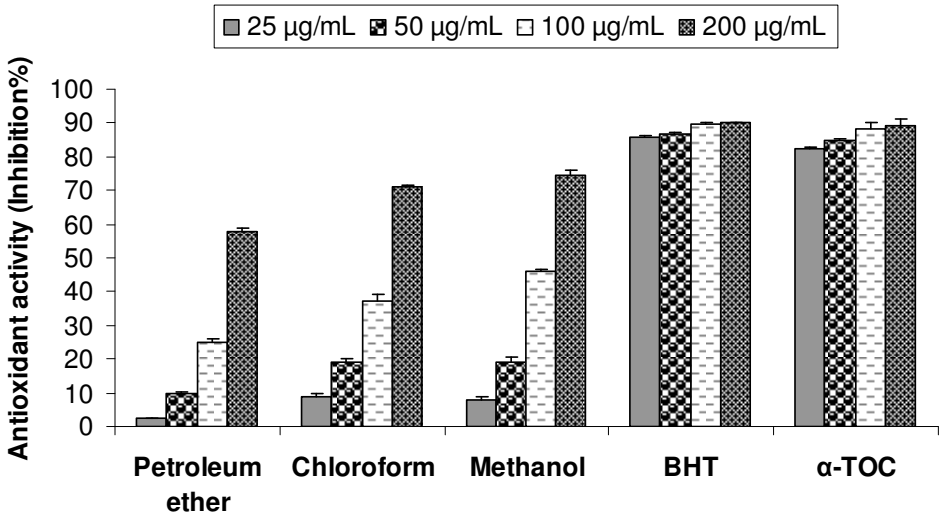


Figure 1. Inhibition (%) of lipid peroxidation of *J. consanguinea* extracts, BHT and α-TOC by β-carotene bleaching method. Values are mean ± SD, $n = 3$, $p < 0.05$, significantly different with Student's *t*-test (BHT: butylated hydroxytoluene, α-TOC: α-tocopherol)

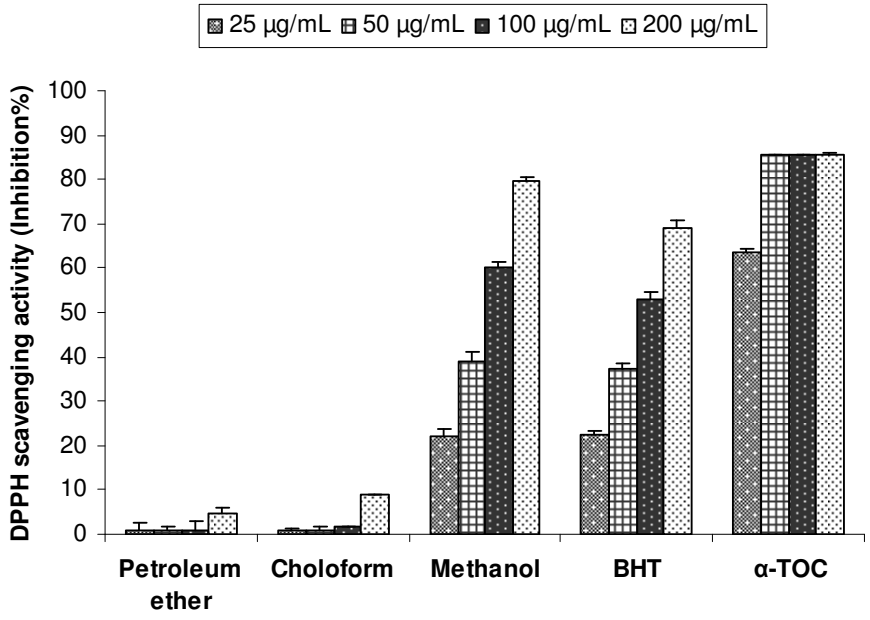
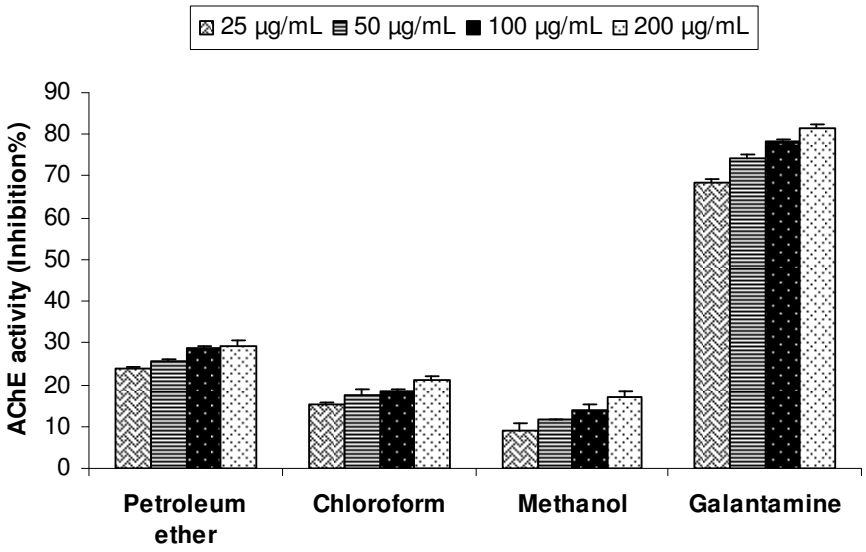


Figure 2. Free radical scavenging activity of *J. consanguinea* extracts, BHT and α-TOC. Values are mean ± SD, $n = 3$, $p < 0.05$, significantly different with Student's *t*-test

(A)



(B)

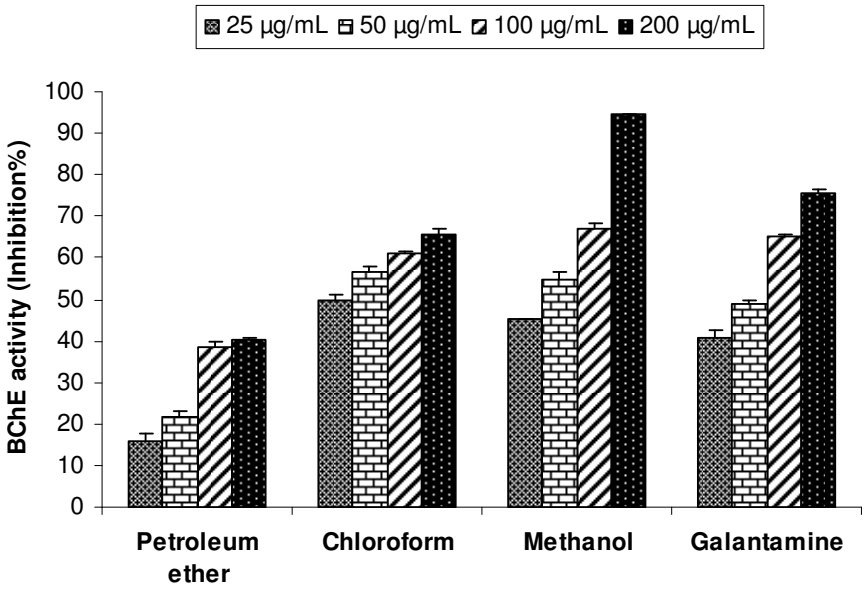


Figure 3. Acetyl- (A) and butyrly-cholinesterase (B) activities of *J. consanguinea* extracts and galantamine. Values expressed are mean ± SD of three parallel measurements ($p < 0.05$)

Although the highest inhibition percentage (24-28 % inhibition) against the enzyme acetylcholinesterase was observed for the petroleum ether extract of *J. consanguinea*, it showed the least inhibition against the enzyme butyrylcholinesterase (Figure 3A and 3B). While the chloroform extract exhibited higher inhibition than a reference compound, galantamine, at 25 and 50 μg/mL, the methanol extract showed higher butyrylcholinesterase inhibitory activity than galantamine at all concentrations (94% inhibition at 200 μg/mL).

As shown in Table 2, the inhibition zones of disc for strains were in the ranges 8.0-15.0 mm. The petroleum ether, chloroform and methanol extracts were found to be inactive against Gram-negative bacteria, *K. pneumoniae* and *P. vulgaris*. The chloroform extract showed moderate activity against *B. subtilis*, *P. aeruginosa* and *S. aureus*, the methanol extract exhibited almost the same activity with the chloroform extract against *P. aeruginosa*. When comparing the antibacterial activity of the tested extracts to that of reference antibiotic, ofloxacin, their inhibitory potency was not found to be significant.

The present study is the first report on antioxidant, anticholinesterase and antibacterial activities in *J. consanguinea*. As seen in Table 1, *J. consanguinea* extracts were not rich in phenolic and flavonoid compounds. The sesquiterpene lactones, which are the principal component of *Jurinea* species exhibited potent several pharmacological activities especially cytostatic [21,22] might be related to their antioxidant and anticholinesterase activities. Further phytochemical and biological studies are needed to characterize the antioxidant and anticholinesterase active constituents from the methanol extract of *J. consanguinea*. *Jurinea* species may be a new source for natural antioxidant and anticholinesterase agents.

Table 2. Antibacterial activity of *J. consanguinea* extracts

	Bacterial strain [a]				
Samples	*B. subtilis* ATCC6633	*K. pneumoniae* ATCC33495	*P. vulgaris* ATCC13315	*P. aeruginosa* ATCC27853	*S. aurens* ATCC25923
Petroleum ether ext.	8	-	-	-	9
Chloroform ext.	11	-	-	8	15
Methanol ext.	-	-	-	9	10
Ofloxacin [b]	28	30	37	16	28

[a] Inhibition zone, including diameter of the paper disc (6 mm)
[b] Reference antibiotic

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