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In vitro Anticancer, Antimicrobial and Antiradical Properties and Bioactive Compounds of Endemic *Wiedemannia orientalis* Fisch. & Mey. Flowers

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ABSTRACT

W. orientalis is a one-year herbaceous plant, located in the Lamiaceae family, and is called Ballıbaba. The antimicrobial and anticancer properties of flowers extracts of endemic *W. orientalis* were investigated for the first time. Also, it was investigated the antiradical activity and phytochemical contents of this plant extract. According to our study results, endemic *W. orientalis* flowers extract shows very high anticancer activity against MCF-7, HCT-116 and LNCaP cancer cell lines, high antiradical activity against ABTS radicals, and effective antimicrobial activity against some microorganism caused infection in humans. The results show that endemic *W. orientalis* can be used as an anticancer and antimicrobial agent and this plant can be the subject of further studies in the field of herbal medicine.

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1. Introduction

Plants have held an important place in human life for thousands of years for both nutrition and treatment. They are used for antiradical, antimicrobial and anticancer purposes due to their contained bioactive properties, such as vitamins, phenolic compounds, sterols, carotenoids, flavonoids, etc. which are called phytochemical compounds. In addition, due to these properties, they are at the center of many scientific studies. Synthetically derived compounds may show antioxidant, antimicrobial and anticancer properties, but they may have unwanted side effects as well as therapeutic properties. Therefore, as an alternative to synthetic compounds, herbal products and/or plant-based drugs are still preferred for the treatment of some diseases. Especially plants that are consumed as food and/or used for medicinal purposes among the public are the focus of such studies [1-3].

It has been shown by various studies that the bioactive compounds (vitamins, sterols, fatty acids, flavonoids, proanthocyanidins, phenolics, etc.) found in the stem, shell, seed, fruit, flowers, leaf, etc. of plants can be exhibited natural antioxidant, antimicrobial and anticancer properties [4-6].

The Lamiaceae family is represented in the world with approximately 200 genera and 3300 species. In flora of Turkey, there are about 45 genera and 550 species [7]. These family plants are very rich in phytochemical compounds, such as essential oils, monoterpenes, diterpenes, triterpenes, saponins, pyridine and pyrrolidine alkaloids, polyphenols, tannins, iridoids, quinones, furanoids, cyclitols, coumarins, stachyose and raffinose sugars [8]. In addition, past studies have shown that plants in the Lamiaceae family have strong antioxidant, antiradical and antimicrobial properties [9].

Wiedemannia genus is a one-year herbaceous plant, located in the Lamiaceae family. This genus is represented by two species, one of these species which is endemic (*W. orientalis*) in Turkey (other *W. multifada*) [10]. *W. orientalis* plant is called Ballıbaba in Turkish, and the antioxidant and antimicrobial properties of the methanol extract of this plant with *W. multifada* were examined in a previous study [9]. In addition, the flavonoid, iridoid and phenylethanoid glycosides derivatives of this plant [11] and essential oil compositions [10] were tried to be determined. In Turkey, *W. orientalis* flowers are used and consumed as an anti-constipation and potentiating agent [12].

The aim of this study is to determine the antiradical, antimicrobial, anticancer properties and bioactive contents of *W. orientalis* flowers water, ethanol, methanol and acetone extracts.

2. Materials and Methods

2.1. Plant Materials and Extraction Procedures

The flowers of endemic *W. orientalis* were collected in April-May 2016 from Sivrice/Elazig in Turkey. The voucher specimen number is Turkoglu 4900. This specimen was stored in the herbarium of Firat University, Faculty of Science, Department of Biology, Elazig/Turkey. The flowers were dried at dark and room temperature. Dried flowers were pulverized using a mechanic grinder, and then 25 g of the sample was extracted with 250 mL of solvent (water, ethanol, acetone and methanol). All the extracts were centrifuged. After centrifuging and filtrating of solvents, the supernatant was concentrated. The dried extract was dissolved in DMSO (µg/mL) [13].

2.2. Determination of Radical Scavenging Activities (RSAs)

The DPPH, $ABTS^{\bullet+}$ and hydroxyl (OH) radical scavenging activities (RSAs) were determined by the methods of Brand-Williams et al. [14], Re et al. [15] and Halliwell et al. [16], respectively. All tests were repeated three times and the average values were calculated. The radical scavenging activity percentages (RSA%) for each sample was calculated by the following equation:

$$RSA\% = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

A_0: control absorbance; A_1: sample absorbance.

2.3. Determination of Phytochemical Components

The determination of total phenolic contents (gallic acid used as standard), total flavonoid contents (catechin used as standard), total proanthocyanidin content (catechin used as standard) were performed according to the methods of Slinkard and Singleton [17], Kim et al. [18] and Amaeze et al. [19], respectively. The determination of flavonoid and phenolic acids was performed according to the method of Zu et al. [20] in the *W. orientalis* by HPLC. Rutin, myricetin, morin, quercetin, kaempferol, naringenin, resveratrol, vanillic acid, gallic acid, hydroxycinnamic acid, caffeic acid, ferulic acid and rosmarinic acid were quantified in the *W. orientalis* flowers by HPLC. Fatty acids in the endemic *W. orientalis* flowers were analyzed according to Christie's method [21] by Gas Chromatography (GC). The fatty acids analyses results were expressed as a percent of samples. The lipid-soluble vitamins and sterols were analyzed according to the method of Sánchez-Machado et al. [22] and Lopez-Cervantes et al. [23] by High Performance Liquid Chromatography (HPLC) from the *W. orientalis* flowers. The results of the analyses were expressed as µg/g.

2.4. Determination of Antimicrobial Properties

E. coli ATCC 25922, *B. megaterium* DSM 32, *B. subtilis* IMG 22, *P. vulgaris* FMC 1, *P. aeruginosa* DSM 50071, *L. monocytogenes* SCOTTA, *K. pneumoniae* FMC 5, *S. aureus* COWAN 1 bacteria and *C. albicans* FMC 17 fungus were used as test organisms. The antimicrobial activity tests were performed according to Collins and Lyne's method [24] by the disc diffusion method. Streptomycin sulfate (10 mg/disc) was used as standard antibiotic for the bacteria, and nystatin (30 mg/disc) was used as standard antibiotic for fungus.

2.5. Determination of Anticancer Properties

2.5.1. Cell Culture

The cell lines of MCF-7 human breast cancer HCT-116 (human colon cancer), and LNCaP (human prostate cancer) were used the anticancer studies. These cells were retrieved from American Type Culture Collection (ATCC).

2.5.2. MTT Test

The *W. orientalis* extracts (water, acetone, methanol and ethanol) were studied for anticancer activity against to the LNCaP, HCT-116 and MCF-7 cell lines. The viability of the cells was determined using 0.4% trypan blue. Effects of the % cell viability of extracts were evaluated by MTT test [25,26].

2.6. Statistical analyses

The anticancer activity results were evaluated using Kolmogorov Smirnov test ($p<0.05$); for antiradical activity tests were evaluated using the Duncan's multiple range test (DMRT) and the analysis of variance (ANOVA) by the SPSS Statistics 22.0 software. The IC_{50} values were calculated by using % cell viabilities of extracts.

3. Results and Discussion

3.1. Antiradical Properties

The antiradical activity results of *W. orientalis* flowers extracts are presented in Table 1. *W. orientalis* water, ethanol and methanol (94.96%, 98.99%, 99.03%, respectively) extracts were exhibited higher ABTS radical scavenging activity than standard antioxidant Trolox (96.33%). For the DPPH and OH radical scavenging tests, standard antioxidant Trolox (97.28%, 95.32%, respectively) had the highest radical scavenging activity among all the extracts.

Albayrak and Aksoy [9] and Turkoglu [27] showed that *W. orientalis* flowers extracts high ratio scavenged DPPH radicals. In addition, Turkoglu [27] determined ethanol and water extracts of *W. orientalis* flowers destroy ABTS radicals by 92.5% and 85.6%, respectively. In our study, *W. orientalis* flowers ethanol and water extracts scavenged ABTS radicals by 98.99% and 94.96%, respectively.

3.2. Phytochemical Composition

The phytochemical contents of *W. orientalis* extracts are presented in Table 1 and Table 2. *W. orientalis* water, ethanol, methanol and acetone extracts of total flavonoid amounts were 3999.56, 3305.44, 6509.62, and 1985.50 µg CE/g extract, respectively; total proanthocyanidin amounts were 1254.26, 873.00, 1943.00, and 1718.56 µg CE/g extract, respectively; total phenolic compounds amounts were 123.95, 110.33, 124.54, and 61.08 mg GAE/g extract, respectively.

Flavonoid amounts of *W. orientalis* were rutin (14.85 µg/g), myricetin (1.90 µg/g), morin (1.05 µg/g), quercetin (0.05 µg/g), kaempferol (0.05 µg/g), naringenin (0.05 µg/g) and resveratrol (0.30 µg/g); the phenolic acid amounts of *W. orientalis* were vanillic acid (4.30 µg/g), gallic acid (2.15 µg/g), hydroxycinnamic acid (0.05 µg/g), caffeic acid (567.55 µg/g), ferulic acid (28.45 µg/g) and rosmarinic acid (4.45 µg/g).

The lipid-soluble vitamins of *W. orientalis* were retinol (0.05 µg/g), α-tocopherol (1.60 µg/g), δ-tocopherol (0.05 µg/g), vitamin K (0.15 µg/g) and vitamin D (0.35 µg/g); the phytosterols of *W. orientalis* were ergosterol (0.75 µg/g), stigmasterol (9.10 µg/g). The fatty acids content in *W. orientalis* were 10.29% palmitic acid (16:0), 7.65% palmitoleic acid (16:1), 9.84% stearic acid (18:0), 10.76% oleic acid (18:1), 26.02% linoleic acid (18:2), 35.44% linolenic acid (18:3), 20.13% total saturated fatty acids, 79.87% total unsaturated fatty acids.

Table 1. $ABTS^{+\bullet}$, $OH^{\bullet}$, $DPPH^{\bullet}$ radicals scavenging activities, total flavonoid, total proanthocyanidin and total phenolic contents of *Wiedemannia orientalis* flowers extracts

Samples	$ABTS^{+\bullet}$ Scavenging (%)	$OH^{\bullet}$ Scavenging (%)	$DPPH^{\bullet}$ Scavenging (%)	Total Flavonoid (µg CE/g)	Total Proanthocyanidin (µg CE/g)	Total Phenolic (mg GAE/g)
W. orientalis water	94.96±0.22[b]	56.93±1.37[c]	63.11±1.06[d]	3999.56±4.55	1254.26±0.99	123.95±0.41
W. orientalis ethanol	98.99±0.10[a]	72.98±0.89[b]	80.20±0.87[c]	3305.44±3.74	873.00±0.81	110.33±0.53
W. orientalis methanol	99.03±0.08[a]	69.59±1.31[b]	91.60±0.05[b]	6509.62±5.49	1943.00±1.67	124.54±0.97
W. orientalis acetone	81.99±0.46[c]	44.76±1.43[d]	36.47±3.45[e]	1985.50±1.97	1718.56±1.75	61.08±0.19
Trolox	96.33±0.17[b]	95.32±0.34[a]	97.28±0.21[a]	-	-	-

Within a column, different superscript letters are significantly different at $p<0.001$. The antiradical activity results were calculated for 500 µg/mL extract concentrations. Total flavonoid and total proanthocyanidin contents were expressed as µg catechin equivalent/g extract, and total phenolic content were expressed as mg gallic acid equivalent/g extract.

Table 2. Contents and composition of flavonoids, phenolic acids, vitamins, phytosterols and fatty acids in *Wiedemannia orientalis* flowers

Flavonoids and Phenolic Acids	**(µg/g)**
Rutin	14.85±0.55
Myricetin	1.90±0.15
Morin	1.05±0.05
Quercetin	0.05±0.00
Kaempferol	0.05±0.00
Naringenin	0.05±0.00
Resveratrol	0.30±0.05
Vanillic Acid	4.30±0.20
Gallic Acid	2.15±0.10
Hydroxycinnamic Acid	0.05±0.00
Caffeic Acid	567.55±3.35
Ferulic Acid	28.45±0.80
Rosmarinic Acid	4.45±0.25
Vitamin and Phytosterols	**(µg/g)**
Retinol	0.05±0.00
δ-Tocopherol	0.05±0.00
α-Tocopherol	1.60±0.10
Vitamin K	0.15±0.00
Vitamin D	0.35±0.05
Ergosterol	0.75±0.10
Stigmasterol	9.10±0.20
Fatty Acids (FA)	**(%)**
16:0	10.29±0.87
16:1	7.65±0.64
18:0	9.84±1.03
18:1	10.76±1.17
18:2	26.02±2.59
18:3	35.44±2.75
Saturated FA	20.13
Unsaturated FA	79.87

When the studies with phytochemical content related to *W. orientalis* were examined, Albayrak and Aksoy [9] found that *W. orientalis* contained 9.53 mg GAE/g total phenolic compound, 422.88 µg/g catechin, 44.67 µg/g naringenin, 605.18 µg/g kaempferol, 984.58 µg/g rutin, 448.61 µg/g ferulic acid and 155.61 µg/g gallic acid. In another study, it was determined that *W. orientalis* flowers water and ethanol extracts contain 12.54 mg GAE/g extract and 11.95 mg GAE/g extract total phenolic compounds (respectively) [27].

3.3. Antimicrobial Properties

The antimicrobial properties of *W. orientalis* flowers extracts are presented in Table 3. According to these results, it was observed that *W. orientalis* water, ethanol, methanol and acetone extracts have antimicrobial property on *E. coli*, *P. vulgaris*, *P. aeruginosa*, *L. monocytogenes*, *K. pneumonia*, *B. subtilis*, *B. megaterium*, *S. aureus* bacteria and *C. albicans* yeast. When the antimicrobial studies on the *W. orientalis* flowers are evaluated; Albayrak and Aksoy [9] were showed that *W. orientalis* has antimicrobial activity on *Aeromonas hydrophila*, *K. pneumoniae*, *P. aeruginosa*, *B. brevis*, *B. cereus*, *L. monocytogenes* and *S. aureus* bacteria.

Table 3. The antimicrobial activities of *Wiedemannia orientalis* flowers extracts (mm zone)

Microorganisms	*W.orientalis* water	*W.orientalis* ethanol	*W.orientalis* methanol	*W.orientalis* acetone	Standard Antibiotics
Escherichia coli	10	11	12	11	10
Proteus vulgaris	8	9	9	8	10
Pseudomonas aeruginosa	8	9	10	8	15
Listeria monocytogenes	8	9	10	8	8
Klebsiella pneumoniae	8	9	10	8	9
Bacillus subtilis	9	10	12	9	9
Bacillus megaterium	8	10	12	8	12
Staphylococcus aureus	8	9	10	8	12
Candida albicans	8	10	11	8	10

Streptomycin sulfate (10 mg/disc) was used as standard antibiotic for bacteria, and Nystatin (30 mg/disc) was used as standard antibiotic for fungus. The diameter of the paper discs was 6 mm.

3.4. Anticancer Properties

The IC_{50} values of anticancer properties of *W. orientalis* extracts on the MCF-7, LNCaP, and HCT-116 cancer cell lines are presented in Table 4. *W. orientalis* ethanol extract (9.14 µg/mL) has better anticancer activity for the LNCaP cell lines than all the other extracts; *W. orientalis* ethanol extract (11.35 µg/mL) has better anticancer activity for the HCT-116 cell lines than all the other extracts; *W. orientalis* acetone extract (8.01 µg/mL) has better anticancer activity for the MCF-7 cell lines than all the other extracts. All these results show that *W. orientalis* flowers extracts have high anticancer activity. To our best knowledge, there is no report about anticancer activities of *W. orientalis*. For this reason, the present study can be the first report about the anticancer activities of *W. orientalis*.

Table 4. The IC_{50} values of *W. orientalis* flowers extracts against MCF-7, LNCaP and HCT-116 cancer cell lines for the anticancer activity assay

Samples (µg/mL)	MCF-7	LNCaP	HCT-116
W. orientalis water	16.53	41.09	21.86
W. orientalis ethanol	12.71	9.14	11.35
W. orientalis methanol	12.29	23.52	19.04
W. orientalis acetone	8.01	33.99	18.89

Conclusion

This study can be first report about the anticancer properties of endemic *W. orientalis* flowers extracts. Also, the present work showed that endemic *W. orientalis* has antimicrobial, anticancer, antiradical properties and highly bioactive contents. In light of all these results, it can be clearly said that this endemic *W. orientalis* flowers has very important potential for health and medicine.

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