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## Biological activities of *Wiedemannia multifida* (Linnaeus) Bentham and *Wiedemannia orientalis* Fisch. & Mey.

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## PEER REVIEW

**Peer reviewer**

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**Comments**

This study is a good start for other potential of both plants extract in living human. Since they are originated in Turkey, the cost of obtaining biological agent from natural sources might be lower than using synthetic or other imported agent. Authors could further the studies on these particular plants by purifying the active component of the plants.

**(Details on Page 200)**

## ABSTRACT

**Objective:** To evaluate total phenolic, antioxidant and antimicrobial activities of methanolic extracts of the *Wiedemannia multifida* (*W. multifida*) and *Wiedemannia orientalis* (*W. orientalis*). **Methods:** Phosmomolybdenum assay, DPPH radical scavenging activity and  $\beta$ -carotene–linoleate bleaching were used to evaluate the antioxidant capacity. **Results:** The total phenolics were found to be  $(22.45 \pm 0.60)$  and  $(9.53 \pm 0.00)$  mg gallic acid equivalents (GAE)/g in *W. multifida* and *W. orientalis* extracts, respectively. The predominant phenolic compounds identified by HPLC–DAD in the both extracts were rutin+ellagic acid and kaempferol. Total antioxidant capacity and DPPH radical scavenging activity of *W. multifida* were higher than *W. orientalis*. In  $\beta$ -carotene–linoleic acid system, both extracts exhibited strong inhibition against linoleic acid oxidation. Antimicrobial activity was assessed by the agar diffusion method against fifteen microorganisms. Both extracts exhibited remarkable antibacterial activity. **Conclusions:** The present study suggests that methanolic extracts of *W. multifida* and *W. orientalis* could be a good source of antioxidant and antimicrobial agents in foods, pharmaceuticals preparations.

## KEYWORDS

*Wiedemannia*, Antimicrobial activity, Antioxidant activity, Phenolic**1. Introduction**

Oxidative damage is a crucial etiological factor implicated in several chronic human diseases such as carcinogenesis, cardiovascular disease, diabetes mellitus, atherosclerosis, arthritis, drug toxicity, reperfusion injury, neurodegenerative diseases and also in the ageing process<sup>[1,2]</sup>. Many synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are commercially available and widely used but they may possess some side effects and toxic properties to human health<sup>[1–3]</sup>.

Attention has therefore been directed toward the development/isolation of natural antioxidants from

botanical sources, especially plants. Sources of natural antioxidants are primarily plant phenolics. Plant phenolics are multifunctional and can act as reducing agents (free radical terminators), metal chelators and singlet oxygen quenchers<sup>[4–6]</sup>.

The genus *Wiedemannia* (Lamiaceae) is represented by two species in the flora of Turkey. *W. orientalis* Fisch. & Mey. is an endemic species and is widespread throughout Anatolia<sup>[7]</sup>. The Lamiaceae family includes a large number of plants that are well-known for their strong antioxidant and antimicrobial properties. Plants belonging to the Lamiaceae family are rich in polyphenolic compounds with over 160 polyphenols having been identified, some of which

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are unique to the genus<sup>[8,9]</sup>. Although, as antimicrobial and antioxidant compounds, many plants belonging to Lamiaceae and their derivatives have been studied in detail<sup>[8,10–12]</sup>, there are only two study concerning the chemical constituents of *W. orientalis*<sup>[7,13]</sup>. However, the antimicrobial and antioxidant effects of *W. multifida* and *W. orientalis* have not been studied until now.

In this study, it was aimed to determine total phenolic compounds, phenolic compositions, and antioxidant and antimicrobial activities of methanolic extracts of *W. multifida* and *W. orientalis* which is Turkish endemic plants.

## 2. Materials and methods

### 2.1. Chemicals

Folin–Ciocalteu reagent, 2,2–diphenyl–1–picrylhydrazyl (DPPH), sodium carbonate, gallic acid, ascorbic acid, nutrient agar, nutrient broth, malt extract agar and malt extract broth were purchased from Merck. The other chemicals and solvents used in this experiment were analytical grade, also purchased from Merck.

### 2.2. Plants

The aerial parts (flower and leaf) of *W. multifida* (Linnaeus) Bentham (Erzincan, the way of Refahiye–Çatalçam, the eastern part of Turkey in Jun in 2009 at altitudes of 1620 m) and *W. orientalis* Fisch. & Mey. (Kayseri, the middle part of Anatolia of Turkey in Jun in 2009) locally endemic for Turkey were collected during the flowering stage. Plants were authenticated by Prof. Dr. Ahmet Aksoy from the Department of Biology Faculty of Art and Science, Erciyes University, Kayseri, Turkey. The voucher specimens were deposited at the Herbarium of the Department of Biology, Erciyes University, Kayseri, Turkey (Voucher no.: AAksoy2344 and AAksoy2339 for *W. multifida* and *W. orientalis*, respectively).

### 2.3. Extraction

Dried aerial parts of the plant at room temperature were crushed in a coffee grinder for 2 min. At 15 seconds intervals, the process was stopped for 15 seconds to avoid over–heating the sample. Then the powdered plant material (10 g) was extracted using a Soxhlet type extractor with 100 mL methanol (MeOH) at 60 °C for 6 h. Thereafter, the extracts were filtered through Whatman No. 1 filter paper and evaporated to dryness in a vacuum at 40 °C with a rotary evaporator (Rotavator, Buchi, Switzerland; T<40 °C). After determining the yield, the prepared extracts were stored at 4 °C until further analysis.

### 2.4. HPLC analysis of phenolic compounds in the extracts

The extracts were dissolved in methanol at a concentration of 10 mg/mL. A high performance liquid chromatograph

(Shimadzu) was equipped with HPLC pumps (LC–10ADvp) and a DAD detector (278 nm). Eclipse XDB–C18 (5 µm) column (250 mm×460 mm) (Agilent) was used. The flow rate was 0.8 mL/min and the injection volume 20 µL. The analyses of the phenolic compounds were carried out at 30 °C using two linear gradients of methanol. Catechin, 3–4 dihydroxy benzoic acid, epicatechin, eriodictyol, ferulic acid, gallic acid, hesperidin, luteolin, naringenin, o–coumaric acid, 4–hydroxybenzoic acid, kaempferol, quercetin, resveratrol and rutin+ellagic acid were used as standard. Identification and quantitative analysis were done by comparison with standards.

### 2.5. Determination of total phenolics

The total phenolic contents in the plant extracts were estimated by a colorimetric assay based on procedures described by Singleton & Rossi<sup>[14]</sup>. In short, a 40 µL aliquot of plant extracts dissolved in the same solvent was pipetted into a test tube containing 2.4 mL of distilled water. After mixing the contents, 200 µL of Folin Ciocalteu’s phenol reagent and 600 µL of a saturated sodium carbonate solution (20% Na<sub>2</sub>CO<sub>3</sub>) were added. The contents were vortexed for 15 seconds and then left to stand at room temperature for 2 h. Absorbance measurements were recorded at 765 nm using a Shimadzu 1240 spectrophotometer and gallic acid was used in the construction of the standard curve. Estimation of the phenolic content was carried out in triplicate. The results were mean values and expressed as mg of gallic acid equivalents (GAE)/g of dry extract.

### 2.6. Determination of antioxidant activity

#### 2.6.1. Total antioxidant activity (Phosphomolybdenum assay)

The total antioxidant activities of the plant extracts were determined by the phosphomolybdenum method of Prieto *et al*<sup>[15]</sup>. About 0.4 mL of the plant extract (1 mg/mL) was mixed with 4 mL of the reagent solution (0.6 mol/L sulphuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate). The tubes were capped and incubated in a water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the green phosphomolybdenum complex was measured at 695 nm. In the case of the blank, 0.4 mL of solvent was used in place of the sample. The antioxidant activity was determined using a standard curve with ascorbic acid solutions as the standard. The mean of three readings was used, and the reducing capacities of the extracts were expressed as mg of ascorbic acid equivalents (AAE)/g extract.

#### 2.6.2. 2,2–diphenyl–1–picrylhydrazyl assay

The hydrogen atom or electron donation abilities of the plant extracts were measured by bleaching the purple coloured DPPH methanol solution. This spectrophotometric assay uses the stable radical 2,2–diphenyl–1–picrylhydrazyl (DPPH) as a reagent<sup>[16]</sup>. Fifty microliters of various

concentrations (0.25–5 mg/mL) of the plant extract in the same solvent was added to 1 mL of 0.1 mmol/L DPPH methanol solution. After a 30 min incubation period at room temperature, absorbance was read against a blank at 517 nm. IC<sub>50</sub> (the concentration required to scavenge 50% DPPH free radicals) values of the plant extracts were determined graphically. The same procedure was repeated with BHT (Butylated hydroxytoluene) as a positive control. The measurements were performed in triplicate, and the results were averaged.

Radical scavenging activity was expressed as a percentage inhibition of the DPPH radical and was calculated by following equation:

$$\text{Inhibition\%} = (\text{A}_{\text{blank}} - \text{A}_{\text{sample}} / \text{A}_{\text{blank}}) \times 100$$

Where A<sub>blank</sub> is the absorbance of the control reaction (containing all reagents except the test compound) and A<sub>sample</sub> is the absorbance of the test compound.

### 2.6.3. $\beta$ -Carotene bleaching assay

The extracts ability to inhibit the bleaching of the  $\beta$ -carotene–linoleic acid emulsion was determined [17].  $\beta$ -carotene (10 mg) was dissolved in 10 mL of chloroform (CHCl<sub>3</sub>). An aliquot (0.2 mL) of this solution was added to a boiling flask containing 20 mg of linoleic acid and 200 mg of Tween 40. The chloroform was removed using a rotary evaporator at 40 °C for 5 min. Distilled water (50 mL) was slowly added to the residue and mixed vigorously to form an emulsion. The emulsion (5 mL) was added to a tube containing 0.2 mL of the extract solution. The test emulsion was incubated in a water bath at 50 °C for 2 h, at which point the absorbance was measured at 470 nm. In the negative control, the extract was substituted with an equal volume of ethanol. BHT was used as the positive controls.

### 2.7. Determination of antimicrobial activity

The following microorganisms, obtained from the Department of Food Engineering, Erciyes University, Kayseri, Turkey, were used in this study: *Aeromonas hydrophila* (*A. hydrophila*) ATCC 7965, *Bacillus brevis* (*B. brevis*) FMC 3, *Bacillus cereus* (*B. cereus*) RSKK 863, *Bacillus subtilis* (*B. subtilis*) ATCC 6633, *Escherichia coli* (*E. coli*) ATCC 25922, *Klebsiella pneumoniae* (*K. pneumoniae*) FMC 5, *Listeria monocytogenes* (*L. monocytogenes*) 1/2B, *Morganella morganii* (*M. morganii*), *Proteus mirabilis* (*P. mirabilis*) BC 3624, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853, *Salmonella typhimurium* (*S. typhimurium*) NRRLE 4463, *Staphylococcus aureus* (*S. aureus*) ATCC 29213, *Yersinia enterocolitica* (*Y. enterocolitica*) ATCC 1501, *Candida albicans* (*C. albicans*) ATCC 1223 and *Saccharomyces cerevisiae* (*S. cerevisiae*) BC 5461.

Antimicrobial activity assays of the extracts was carried out using the agar–well diffusion method [18]. Each microorganism was suspended in sterile nutrient broth. Test yeasts (*C. albicans*, *S. cerevisiae*) were suspended in malt

extract broth. Suspensions of microorganisms, adjusted to 10<sup>6</sup>–10<sup>7</sup> cfu/mL, were placed in flasks containing 25 mL of sterile nutrient or malt extract agar at 45 °C. The mix was poured into Petri plates (9 cm in diameter). The agars were then allowed to solidify at 4 °C for 1 h. The wells (4 mm in diameter) were cut from the agar. The extracts were prepared at 30 mg/mL concentrations in the same solvent and 50  $\mu$ L of the extract solutions were then applied to the wells. The absolute methanol, absolute ethanol and water without herb extract were used as a control. *Y. enterocolitica*, *C. albicans* and *S. cerevisiae* were incubated at 25 °C for 24–48 h in an inverted position. The other microorganisms were incubated at 37 °C for 18–24 h. At the end of the incubation period, all plates were examined for any zones of growth inhibition, and the diameters of these zones were measured in millimeters. Ampicillin (AMP–10  $\mu$ g/disc), Carbenicillin (CAR–100  $\mu$ g/disc), Gentamycin (CN–10  $\mu$ g/disc), Oxacillin (OX–1  $\mu$ g/disc) and Rifampicin (RD–5  $\mu$ g/disc) (Oxoid) standard antibiotics were used as positive controls. All the tests were performed in duplicate, and the results were presented as averages.

## 3. Results

In this study, the phenolic compositions, antioxidant and antimicrobial activities of *W. multifida* and *W. orientalis* extracts were determined. The percent yields of methanolic extracts of *W. multifida* and *W. orientalis* were 20.0% and 24.0%, respectively.

The phenolic acids and flavonoids of *W. multifida* and *W. orientalis* extracts were determined by the HPLC method. The data from the qualitative and quantitative analysis of the extracts made using HPLC coupled with photodiode array DAD detection, is presented in Table 1. The amount of each compound was demonstrated as  $\mu$ g/g dry residue. Catechin, 3–4 dihydroxy benzoic acid, ferulic acid, gallic acid, hesperidin, luteolin, naringenin, 4–Hydroxy benzoic acid, kaempferol, quercetin and rutin+ellagic were identified in the *W. multifida* extract, while catechin, 3–4 dihydroxy benzoic acid, ferulic acid, gallic acid, hesperidin, naringenin, 4–Hydroxy benzoic acid, kaempferol and rutin+ellagic were identified in the *W. orientalis* extract by comparison with the retention times and UV spectra of authentic standards analyzed under identical analytical conditions. The quantitative data was calculated from their respective calibration curves. The major component present in *W. multifida* extract was identified as rutin+ellagic acid (10894.18  $\mu$ g/g) followed by luteolin (996.09  $\mu$ g/g) and kaempferol (697.03  $\mu$ g/g). The major component present in *W. orientalis* extract was identified as rutin+ellagic acid (984.58  $\mu$ g/g) followed by kaempferol (605.18  $\mu$ g/g) and ferulic acid (448.61  $\mu$ g/g). Epicatechin, eriodictyol, o–coumaric acid and resveratrol could not be identified in the both extracts tested. Results demonstrated that *W. multifida* and *W. orientalis* extracts possess similar composition, but *W. multifida* showed higher amount than *W. orientalis* extract.

**Table 1**

The quantity of some phenolic compounds ( $\mu\text{g/g}$ ) determined in *W. multifida* and *W. orientalis* extracts by HPLC.

Compounds	<i>W. multifida</i>	<i>W. orientalis</i>
Catechin	440.93	422.88
3–4 dihydroxy benzoic acid	56.45	72.14
Epicatechin	–	–
Eriodictyol	–	–
Ferulic Acid	75.12	448.61
Gallic Acid	150.54	155.61
Hesperidin	120.99	103.67
Luteolin	996.09	–
Naringenin	–	44.67
o–Coumaric acid	–	–
4–Hydroxy benzoic acid	107.89	121.45
Kaempferol	697.03	605.18
Quercetin	117.26	–
Resveratrol	–	–
Rutin+ellagic acid	10 894.18	984.58
Total	13 656.48	2 958.79

–: Not detected.

**Table 2**

The percent yield, total phenolic content (mg GAE/g extract), total antioxidant activity (mg AAE/g extract),  $\beta$ -carotene bleaching activity (%) and  $\text{IC}_{50}$  value ( $\mu\text{g/mL}$ ) of *W. multifida* and *W. orientalis* extracts.

Characteristics	<i>W. multifida</i>	<i>W. orientalis</i>
Yield	20.00	24.00
Total phenolic content	22.45 $\pm$ 0.60	9.53 $\pm$ 0.00
Total antioxidant activity	181.63 $\pm$ 0.50	127.69 $\pm$ 0.50
$\beta$ -carotene bleaching activity	77.60 $\pm$ 1.30	86.28 $\pm$ 1.10
$\text{IC}_{50}$	39.28	87.22

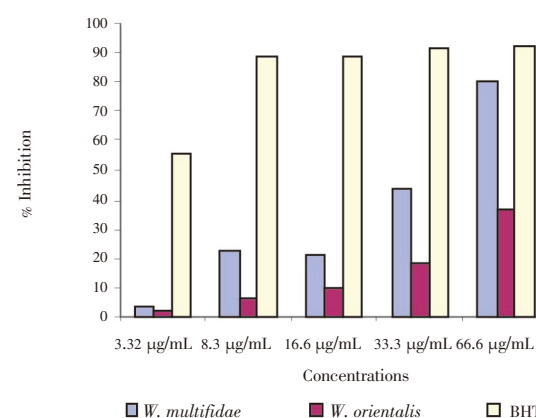
Values expressed are mean $\pm$ SD of three experiments.

Total phenolic contents of *W. multifida* and *W. orientalis*

extracts were shown in Table 2. The total phenolic contents of methanolic extracts determined using Folin–Ciocalteu method expressed as gallic acid equivalents. As seen in Table 1, total phenolics contents of *W. multifida* [(22.45 $\pm$ 0.60) mg GAE/g extract] extract were higher than those of *W. orientalis* extract [(9.53 $\pm$ 0.00) mg GAE/g extract].

Total antioxidant activities of the extracts measured by phosphomolybdenum assay, are given in Table 2. Both extracts had strong total antioxidant activity. Total antioxidant activities of *W. multifida* and *W. orientalis* were estimated as (181.63 $\pm$ 0.50) mg and (127.69 $\pm$ 0.50) mg ascorbic acid equivalent (AAE)/g dry extract, respectively.

DPPH molecule that contains a stable free radical has been widely used to evaluate the radical scavenging ability of antioxidants. Free radical scavenging activities of the extracts were measured in DPPH assay were shown Figure 1.



**Figure 1.** %Inhibition values of *W. multifida* and *W. orientalis* extracts by DPPH free radical scavenging activity assay.

*W. multifida* and *W. orientalis* extracts showed potent free radical scavenging activity. Free radical scavenging activities of both extracts increased with increasing extract concentration (Figure 1). At all of the concentrations tested,

**Table 3**

Antimicrobial activities of *W. multifida* and *W. orientalis* extracts at different concentrations (inhibition zones, mm).

Microorganisms	% Concentration of the extracts								Standard antibiotics ( $\mu\text{g}$ )				
	<i>W. multifida</i>				<i>W. orientalis</i>				AMP	CAR	CN	OX	RD
Gram (–)	10.0	5.0	2.5	1.0	10.0	5.0	2.5	1.0	27.0	35.0	8.5	15.0	17.0
<i>A. hydrophila</i>	10.0	9.0	8.5	7.0	9.0	8.0	7.5	7.0	6.5	6.5	9.0	–	10.0
<i>E. coli</i>	–	–	–	–	–	–	–	–	–	18.0	–	–	10.0
<i>M. morgani</i>	–	–	–	–	–	–	–	–	–	18.0	–	–	10.0
<i>K. pneumoniae</i>	–	–	–	–	7.0	6.0	–	–	14.0	12.0	6.5	–	11.0
<i>P. mirabilis</i>	–	–	–	–	–	–	–	–	26.0	30.0	8.0	–	10.0
<i>P. aeruginosa</i>	8.0	7.5	7.0	–	9.0	8.0	8.0	7.0	25.0	31.0	12.0	–	13.0
<i>S. typhimurium</i>	–	–	–	–	–	–	–	–	24.0	26.0	8.0	–	12.0
<i>Y. enterocolitica</i>	9.0	–	–	–	–	–	–	–	8.0	9.0	9.0	–	12.0
Gram (+)	11.0	10.0	8.0	–	10.0	9.0	8.0	–	8.0	9.0	16.0	–	10.0
<i>B. brevis</i>	11.0	9.0	7.0	6.0	10.0	9.0	7.5	7.0	31.0	38.0	11.0	20.0	17.0
<i>B. cereus</i>	–	–	–	–	–	–	–	–	24.0	24.0	12.0	19.0	18.0
<i>B. subtilis</i>	–	–	–	–	–	–	–	–	24.0	24.0	12.0	19.0	18.0
<i>L. monocytogenes</i>	11.0	9.0	–	–	10.0	9.0	–	–	28.0	31.0	13.0	–	24.0
<i>S. aureus</i>	–	–	–	–	8.0	7.5	7.0	–	16.0	13.0	7.0	–	11.0
Yeasts	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>C. albicans</i>	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>S. cerevisiae</i>	–	–	–	–	–	–	–	–	–	–	–	–	–

Inhibition zones include diameter of hole (5 mm), sample amount 50  $\mu\text{L}$ , –: ineffective Ampicillin (AMP–10  $\mu\text{g}$ /disc), Carbenicillin (CAR–100  $\mu\text{g}$ /disc), Gentamycin (CN–10  $\mu\text{g}$ /disc), Oxacillin (OX–1  $\mu\text{g}$ /disc), Rifampicin (RD–5  $\mu\text{g}$ /disc).



*W. multifida* extract showed significantly stronger activity than *W. orientalis* extract. The extract concentrations providing IC<sub>50</sub> of DPPH radical are given in Table 2. The lower IC<sub>50</sub> value reflects better protective action. The IC<sub>50</sub> of *W. multifida* at 39.28 mg/mL was significantly lower than the IC<sub>50</sub> of *W. orientalis* at 87.22 mg/mL, suggesting that *W. multifida* was a significantly stronger scavenger for DPPH radicals.

In β-carotene/linoleic acid assay, oxidation of linoleic acid was effectively inhibited by *W. multifida* and *W. orientalis* extracts (Table 2). *W. multifida* and *W. orientalis* extracts were found to possess maximum antioxidant activities of 77.60% and 86.28%, respectively. The inhibition rate of *W. orientalis* was higher than the synthetic antioxidant BHT (84.26%).

The antimicrobial activities of the extracts at 10.0%, 5.0%, 2.5% and 1.0% concentrations on the test microorganisms are presented in Table 3. Food pathogens were selected as target microorganisms for the preliminary antimicrobial screening of the extracts in the present study. The extracts caused different inhibition zones on the tested microorganisms. Pure methanol (control) used as solvent had no inhibitory effects on the microorganism tested. In this study, the antimicrobial activities of the extracts are compared with standard antibiotics. Both extracts showed similar potencies against all tested microorganisms. The results showed that *W. multifida* and *W. orientalis* extracts had antibacterial effect against Gram (+) and Gram (–) bacteria. The antibacterial effects of *W. multifida* and *W. orientalis* extracts were increased by the amount of extracts. No activity was found with both extracts against *E. coli*, *M. morgani*, *S. typhimurium* and *B. subtilis*. *K. pneumoniae* and *S. aureus* was inhibited by only *W. orientalis* while *Y. enterocolitica* was inhibited by only *W. multifida*. *B. brevis*, *B. cereus* and *L. monocytogenes* were most sensitive bacteria for both extracts. Also, both extracts had no an inhibition effect on yeasts tested (*C. albicans* and *S. cerevisiae*).

#### 4. Discussion

No references concerning the total phenolic content, antioxidant and antimicrobial activities of *Wiedemannia* species could be found despite the thorough literature survey. This is also the first study concerning the chemical composition of *W. multifida*. As far as we know, only two reports had been published on the chemical constituents of *W. orientalis*[7,13]. In the report of Başer *et al.*, water distilled essential oil from fresh aerial parts of *Wiedemannia orientalis* was analyzed by GC and GC–MS, and 31 compounds were identified with germacrene D (38.94%), geijerene (14.60%), and pregeijerene (12.90%) as the major constituents[13]. In other study, five iridoid glycosides, lamiide, ipolamiide, ipolamiidoside, 6β-hydroxyipolamiide, and 5-hydroxy-8-epi-loganin; 5 flavonoid glycosides,

apigenin 7-O-β-glucopyranoside, luteolin 5-O-β-glucopyranoside, isorhamnetin 3-O-rutinoside, quercetin 3-O-rutinoside, and apigenin 7-O-(6β-O-trans-p-coumaroyl) β-glucopyranoside; and a phenylethanoid glycoside, acteoside (verbascoside) were isolated and identified from the aerial parts of *W. orientalis*[7].

There are many reports dealing with antioxidant and antimicrobial activity of vary species belonging to *Lamiaceae*. For instances, Salah *et al.* reported that *Salvia aegyptiaca* (IC<sub>50</sub> 43.6 mg/mL), *Salvia verbenaca* (IC<sub>50</sub> 86.9 mg/mL) and *Salvia argentea* (IC<sub>50</sub> 374.4 mg/mL) extracts showed antioxidant activity in DPPH assay[8]. These values were lesser than that of *Wiedemannia* species tested. Sokmen *et al.* studied that antioxidant activity of the polar subfraction of the methanol extract of *Thymus spathulifolius* and they found that it was able to reduce the stable free radical DPPH with an IC<sub>50</sub> of (16.15±0.50) μg/mL, which was lower than that of *Wiedemannia* species tested[10].

As similar to our results, Sagdic *et al.* reported that *T. argaeus* extract had antimicrobial activity and were ineffective against *B. subtilis*, *E. coli* and *M. morgani*. *C. albicans* and *S. cerevisiae* were not inhibited by *W. multifida* and *W. orientalis* extracts[12]. That finding is in agreement with that of Sokmen *et al.* who showed that methanolic extract of *Thymus spathulifolius* had no anticandidal activity[10].

In conclusion, *W. multifida* and *W. orientalis* extracts have shown strong antioxidant and antibacterial activity. Therefore, the present study suggests that methanolic extracts of *W. multifida* and *W. orientalis* are a potential source of natural antioxidant and antimicrobial agents. This is the first study concerning the chemical compositions, antioxidant and antimicrobial activities of *W. multifida* and *W. orientalis*. After this screening experiment, further works should be performed to the isolation and identification of individual phenolic compounds and also describes the antioxidant and antimicrobial activities in more detail as *in vivo*.

#### Conflict of interest statement

We declare that we have no conflict of interest.

#### Acknowledgements

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#### Comments

#### Background

This study is about the bioactivity of *W. multifida* and *W.*

*orientalis* extracts from Lamiaceae family which have been claimed to obtain antioxidant and antimicrobial activities. Total phenolic content, antioxidant and antimicrobial assay of both plant were done to prove the traditional usage among local people.

### Research frontiers

Research was done to prove the antioxidant and antimicrobial activity of *W. multifida* and *W. orientalis* methanol extracts. Either than that, total phenolic content of these plants extract were also been identified using HPLC. The assays used for antioxidant and antimicrobial activity were not a novel or improved methods, but were enough to prove both bioactivities of the plants. However, the applications of both plants in bioassay were a new study in order to obtain a natural antioxidant and antimicrobial agent from local source.

### Related reports

A series of studies have been done for centuries to discover the potential of certain plant in certain bioactivity, as such done in this study, besides others which exploring the anticancer, antiproliferative, antidiabetic *etc.* The extract of both plants have not been tested for any biological assay and this study is considered novel in their specific field.

### Innovations and breakthroughs

Methanol extract of both plants have a high potential to be applied in pharmaceutical industry since they possessed a good and significant antioxidant and antimicrobial activity.

### Applications

The reported plant extracts are beneficial in pharmaceutical industry as an antioxidant and antimicrobial agent from natural resources, which are able to replace synthetic agents. However, studies on toxicity of the plant extracts should be done in order to obtain the safe dose for human.

### Peer review

This study is a good start for other potential of both plants extract in living human. Since they are originated in Turkey, the cost of obtaining biological agent from natural sources might be lower than using synthetic or other imported agent. Authors could further the studies on these particular plants by purifying the active component of the plants.

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