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Original Article

Antioxidant, antimicrobial and anticancer effects of different extracts from wild edible plant *Eremurus spectabilis* leaves and roots

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Abstract: This study was conducted to determine the *in vitro* antioxidant, antimicrobial, anticancer and apoptotic assays of aqueous and organic extracts of edible *Eremurus spectabilis (E. spectabilis)* leaves and roots. The total phenolic contents of *E. spectabilis* extracts were evaluated by the Folin-Ciocalteu method. Aqueous, acetone and ethanol extracts were evaluated for antioxidant potential by the DPPH radical scavenging and lipid peroxidation assays. The antimicrobial activities of *E. spectabilis* extracts were investigated by the disk diffusion method. Anticancer activities of the extracts were determined by the MTT colorimetric assay in the prostate cancer cell line (PC-3). The WST-1 assay was used to test the extracts on the proliferation of the prostate cancer cell line, PC-3. The radical and hydroxyl scavenging assays and total antioxidant capacity indicated that *E. spectabilis* possesses potent antioxidant potential. Moreover, *E. spectabilis* extracts have the potential to inhib*it the microbial gro*wth of *L. monocytogenes*, *S. cerevisiae*, *S. aureus* and *E. coli*. Acetone extract of leaves had the highest phenolic and flavonoid content and antioxidant activity with 3703.25 µg ascorbic acid/g (dw). Up-regulation of Bax and caspase-3 and down-regulation of Bcl-2 mRNA in treated PC-3 with *E. spectabilis* indicated mitochondria-mediated apoptosis of PC-3 cells in comparison with control. The results of this study show that *E. spectabilis* have antioxidant, antimicrobial and antiproliferative effects against prostate cancer cells.

Keywords: *Eremurus spectabilis*, antioxidant, anticancer activity, prostate cancer

Introduction

Prostate cancer is the second common form of cancer and sixth leading cause of cancer deaths in men around the world [1]. There were 14.1 million new cases and 8.2 million deaths from cancer worldwide in 2012 [2]. This problem is further estimated to increase with over 75 million prevalent cases, 27 million incident cases and 17 million cancer deaths expected globally by 2030 [2]. Recently, phytochemicals such as lycopene, a compound present in tomato, have been found to significantly prevent prostate cancer [3]. The genus Eremurus (Liliaceae) comprising about 50 species are widely distributed in the mountains of central and western Asia [4] and the Middle East including Afghanistan, Iran, Tajikistan, Lebanon and Turkey [5]. They are used in folk medicine to cure several diseases [6]. *Eremurus spectabilis (E. spectabilis)* (Bieb). Fedtsch is a wild vegetable, grows in spring and localized in the east of Turkey. It has been used for many purposes as food and food additives [7]. Its root has traditionally been used to cure jaundice, liver disorders, stomach irritation, pimples, diabetes, dermal infection, sticking and antihyperlipidemic [8]. Published reports on *E. spectabilis* features are limited up to now to human health. Therefore, this study was carried out to evaluate the medicinal property of aqueous and organic extracts (acetone and ethanol) of *E. spectabilis* in terms of antioxidant, antimicrobial activity as well as anticancer in the literature.

This study was also conducted to investigate the effects of the proliferation of the prostate cancer cell line (PC-3) and apoptotic potentials of aqueous as well as organic extracts of *E. spectabilis*.

Materials and methods

Material

Leaves and roots of *E. spectabilis* were collected from Bingol province (Turkey). The voucher specimens of the species were air dried at room temperature and deposited in the herbarium of Bingol University (Bingol, Turkey). Dried and powdered leaf and root materials (10 g) were used in the preparation of extracts. For extraction, 150 ml acetone was used in an amber flask (500 ml). The extract was mixed by a magnetic stirrer at 150 rpm for 2 h. The mixture was left at room temperature for 24 h for maceration. Then, the extract was filtered using a blue band filter paper and Buchner funnels to obtain particle free extract. The residue was re-extracted twice with acetone and filtered. All extraction solvents were evaporated under reduced pressure. The same procedure was applied for water and ethanol extraction. They were stored in dark at 4°C until use. Total phenolic content (TPC) of *E. spectabilis* extracts was measured using the Folin-Ciocalteu method [9]. Total antioxidant capacity of the samples was evaluated according to the phosphomolybdenum method [10]. Antioxidant activities of the *E. spectabilis* extracts were estimated by measuring their scavenging capacity against the DPPH radical [11]. Hydroxyl radical scavenging was analyzed by using the Fenton reaction [12]. The metal chelation of ferrous ions by the extract was estimated by the method of Dinis et al. [13].

Cell culture studies

Human prostate cells (PC-3) were purchased from American Type Culture Collection (ATCC, USA). Intracellular reactive oxygen species (ROS) were determined according to the study of Shen et al. [14]. The fluorescence intensity was monitored on a Perkin-Elmer spectrofluorometer LS-55 by means of well plate reader with excitation wavelength at 485 nm (bandwidth 5 nm) and emission wavelength at 525 nm (bandwidth 5 nm). Lipid peroxidation of PC-3 treated with the extracts was determined using the study of Gomez-Monterrey et al. [15]. Antimicrobial activity tests were performed using the agar disc diffusion method described in Radji et al. [16]. WST-1 cell proliferation assay kit (Clontech, USA) was used for analyzing cell proliferation and viability. Cells were grown in T-25 flasks and harvested. Cells were counted by using Thoma hemocytometer. 5×10^3 cells/well in 100 µl medium were seeded in 96 well plates. After 24 h, various concentrations of the extracts of *E. spectabilis* were added to wells in 100 µl medium. Cells were treated with 250 and 500 µg/ml concentrations of the extracts. After 48 h, 5 µl of WST-1 reagent was added to each well and after 4 h incubation; absorbance was measured at 450 nm by SpectraMax plus 384 Microplate Reader (Molecular Devices, CA, USA).

Western blotting analysis

PC-3 cells were cultured and treated with 3 different phases from *E. spectabilis* leaves and roots for 24 h. Total cell protein lysates were performed according to standard protocol using ice-cold RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 0,1% SDS, 1 mM EDTA and 1 mM PMSF). Proteins were separated on a 12% polyacrylamide mini-protean TGX gel and moved onto 0.2 µm nitrocellulose membrane (Bio-Rad, Hercules, CA, USA) by Trans-Blot Turbo Transfer System. All membranes were blocked with 5% (w/v) BSA in TBS-Tween 20 (TBS-T; 0.05%) for 60 min. For analyzing the effect of 3 different phases from *E. spectabilis* leaves and roots on protein expression, membranes were incubated for 3 h at room temperature with the primary polyclonal or monoclonal antibodies for Bax (1:1000 dilution); Bcl-2 (1:100 dilution); caspase-9 (1:1000 dilution); caspase-3 (1 µg/ml); cytochrome c (1:1000 dilution). Additionally, β-actin (1:1000 dilution; ab8227, Abcam, Cambridge, UK) was chosen for loading control of protein expressions. After three items of washing for 5 min in TBS-T, the nitrocellulose membranes were incubated with secondary antibodies (1:1,000 dilution; ab97069, Abcam, Cambridge, UK) and (1:1,000 dilution; sc- 2005, Santa Cruz Biotechnology, Heidelberg, Germany) for 90 min at room temperature. After three items of washing for 5 min in TBS-T, the immune-reactive bands were developed with DAP western blotting system. The signal quantity of proteins was measured by using the Gel Doc EZ Imaging System

Table 1. Total phenolic and flavonoid content of different extracts of *E. spectabilis*

Eremurus spectabilis	Acetone Soluble	Ethanol Soluble	Water Soluble
Total Phenolic Content, Leaves (μg gallic acid/g)	2042.60 ± 76.15[a]	490.26 ± 5.92[b]	161.29 ± 4.80[c]
Total Phenolic Content, Root (μg gallic acid/g)	981.85 ± 8.70[a]	707.09 ± 22.43[b]	512.15 ± 53.9[c]
Total Flavonoid Content, Leaves (μg catechin/g)	3.01 ± 0.01[a]	1.08 ± 0.05[b]	0.33 ± 0.05[c]
Total Flavonoid Content, Root (μg catechin/g)	0.42 ± 0.02[b]	0.11 ± 0.06[c]	1.85 ± 0.01[a]

[a-c]Values followed by different letters are significantly different at P < 0.05 as determined by Tukey's multiple comparison tests.

and analyzed using Image Lab software (Bio-Rad, Berkeley, CA, USA). Relative expressions of Bax, Bcl-2, cytochrome c, caspase-9, and caspase-3 were standardized with the intensity of referenced protein (β-actin, Abcam, Cambridge, UK). The ratio was calculated with the following formula; = intensity of target protein band/intensity of referenced (β-actin) protein band.

Quantitative real-time PCR analysis

The expression levels of apoptotic genes were analyzed by using a Bio-speedyTM real time relative gene expression assay kit (Bioeksen, Sarıyer, Istanbul, Turkey) within Rotor-Gene Q (Qiagen GmbH, Hilden, Germany). The total RNA was isolated from cell culture by exerting RNeasy Plus Mini Kit (Qiagen GmbH, Hilden, Germany) before bio-speedyTM first chain cDNA synthesis kit (Bioeksen, Istanbul, Turkey) was used to reverse transcriptase mRNA to cDNA. The quality of total RNA was previously measured for cDNA conversion by nanodrop (SpectraMax Plus 384 Microplate Reader, Molecular Devices, CA, USA). The mRNA levels of Bax, Bcl-2, cytochrome c, caspase-9 and caspase-3 in addition to them β-actin as a reference housekeeping gene were assayed. The quantitative data of RT-PCR were analyzed by a comparative threshold (Ct) method and the fold change of treated samples was calculated by comparing with the control sample. β-Actin was preferred as a housekeeping gene to normalize expression of apoptosis-related genes. The Ct values were used to define the mRNA levels in PC-3 cells and controlled cells treated with a various extract of *E. spectabilis* leaves and roots in water, ethanol and acetone for 48 h.

Statistical analysis

Experimental results are presented as the means ± SD of data obtained from in triplicate experiments. The statistically significant differences between the means of the samples were determined using one-way ANOVA (Analysis of variance) by Tukey's multiple comparison tests, and the values were considered significantly different at *P* values < 0.05. All the statistical analyses were performed using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Total phenolic and flavonoid content

The total phenolic content of the *E. spectabilis* extracts was determined through a linear gallic acid standard curve and expressed as mg GAE/g dry weight of extracts. The highest total phenolic content was detected in the acetone fraction (2042.60 ± 76.15 μg GAE/g), whereas the lowest content was found in the water fraction (161.29 ± 4.80 μg GAE/g). The acetone fractions of the leaf have approximately 4.2 and 12.7 times more phenolic contents than ethanol and water fractions, respectively. The phenolic contents of acetone fractions of root have approximately 1.4 and 1.9 times higher than ethanol and water fractions, respectively. As shown in **Table 1**, the acetone fractions of leaf contain approximately 2.8 and 9.1 times higher total flavonoid contents (TFC) than ethanol and water fractions. In contrary to leaf extracts, water fractions of root indicated the highest values according to acetone and ethanol fractions of the root, and it provided approximately 4.4 and 16.8 times higher total flavonoid contents than acetone and ethanol.

Assay of DPPH and hydroxyl radical scavenging activities

The DPPH scavenging potential of the extracts of *E. spectabilis* improved with an increasing concentration of the extracts as shown in **Figure 1A**. The percentage inhibition of DPPH by leaf and root extracts was recorded in increasing from 26.0758 to 81.7623% and

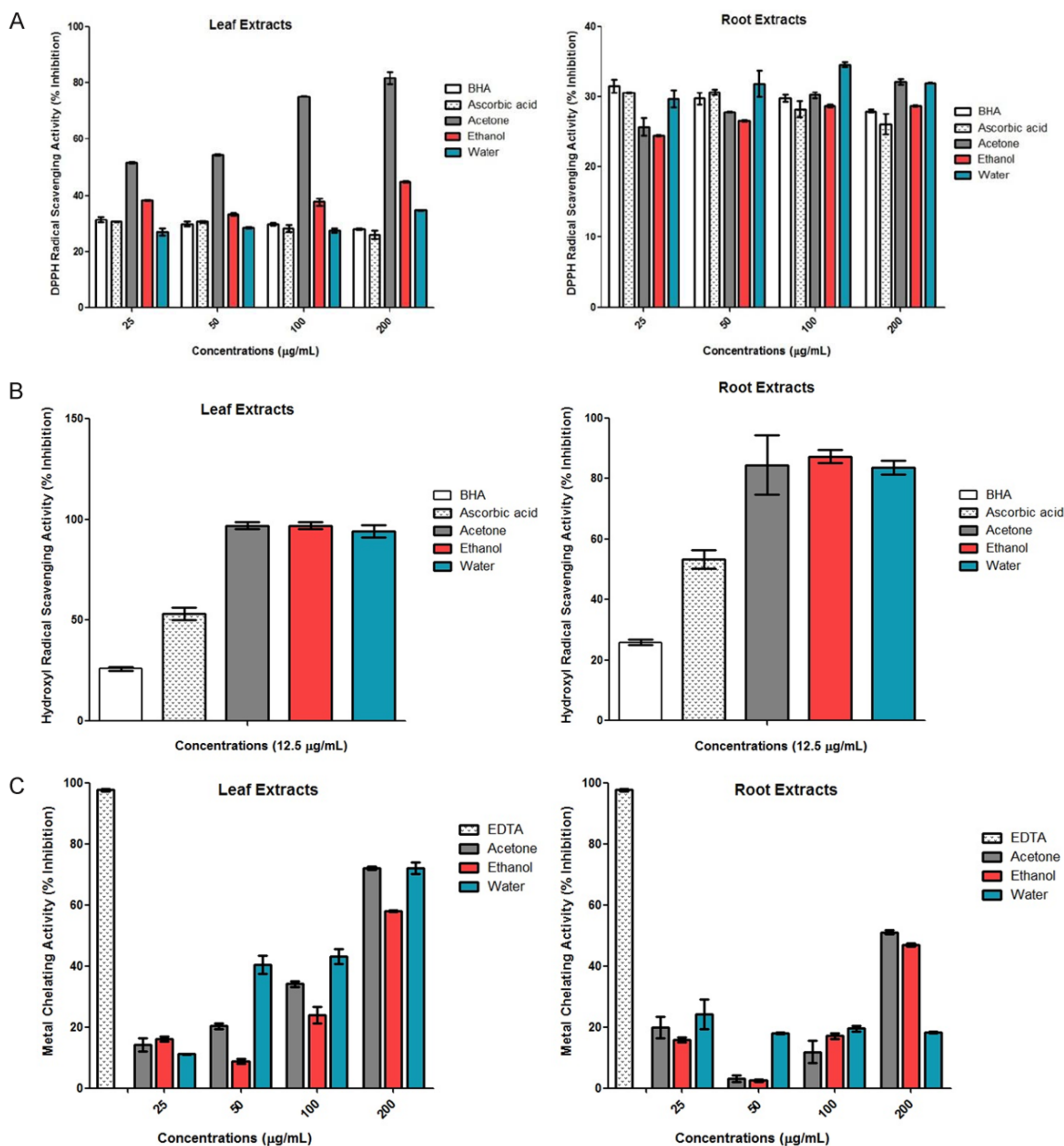

Figure 1. DPPH (A), hydroxyl (B) radical scavenging and metal chelating activities (C) of leaf and root extracts of *E. spectabilis* extracted with different solvents. Each of the bars given shows the means of triplicate replications with standard deviations.

24.4302 to 34.5312%, respectively (**Figure 1A**). The % inhibition of DPPH of the leaf acetone fraction (200 μg/ml) was the highest compared with BHA, ascorbic acid and other fractions ($P < 0.05$).

The scavenging activity of the leaf extract of *E. spectabilis* against hydrogen peroxide is shown in **Figure 1B**. The % inhibition of hydroxyl radical scavenging activity of BHA, ascorbic acid, and the leaf fractions at 12.5 μg/ml concentrations was in the order of 25.8616, 53.3333, 96.8553, 96.9182, 94.1509, respectively (**Figure 1B**). Similarly, the % inhibition of root fractions was 25.8616, 53.3333, 84.4025, 87.2956, 83.6478%, respectively. The average values of leaf fractions displayed an approximately 3.7 fold higher % inhibition than BHA and 1.8 fold higher % inhibitions than ascorbic acid; whereas root fractions indicated an approximately 3.3 fold higher % inhibition than BHA and 1.6 fold higher % inhibition than ascorbic acid. In terms of leaf fractions, there were significant differences ($P < 0.0001$) among

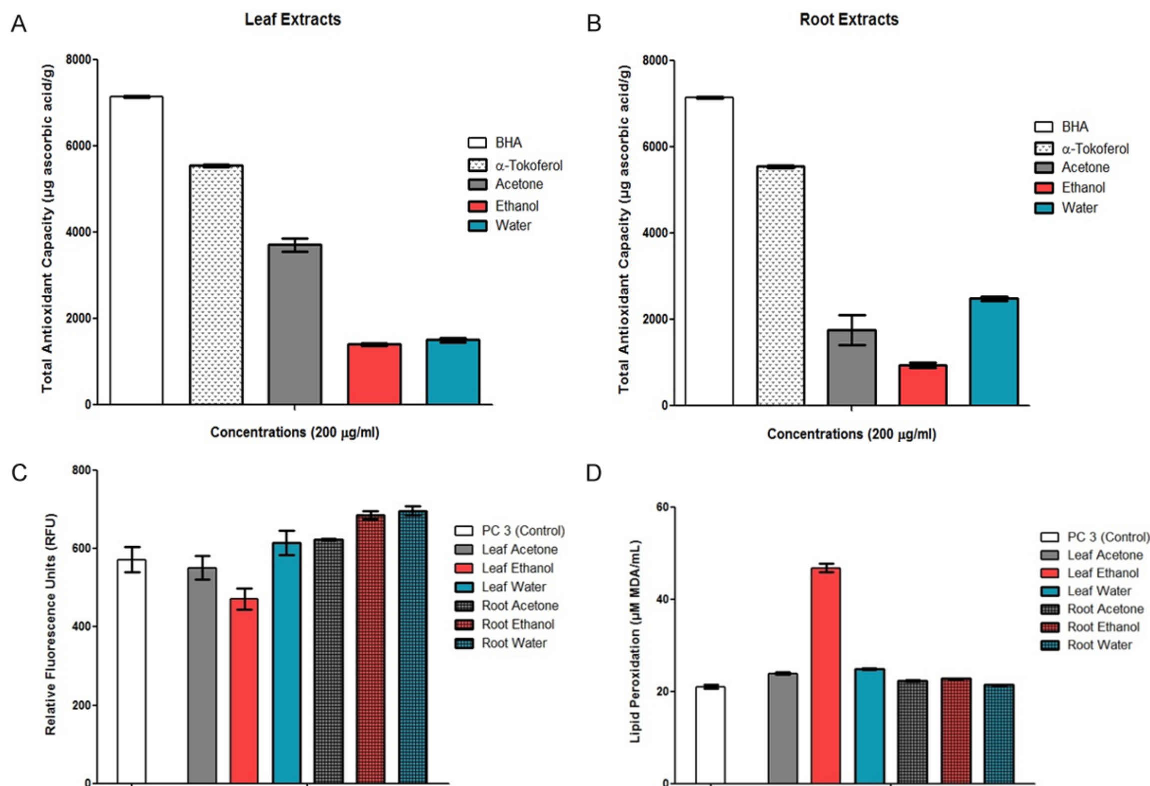

Figure 2. Total antioxidant capacity of leaf (A) leaf and root (B) exctracts, and the reactive oxygen species (ROS) (C) and lipid peroxidation (D) of leaf and root extracts of *E. spectabilis* extracted with different solvents. Each of the bars given shows the means of triplicate replications with standard deviations.

BHA, ascorbic acid and these antioxidants with leaf fractions, but there were no significant differences ($P > 0.05$) among leaf fractions when they compared each other.

Assay of metal chelating activity

The potential of the plant extracts to reduce metal chelating activities is an indication of their antioxidant ability. The metal chelating activity (%) of extracts was tested at 25, 50, 100 and 500 μg/mL concentration (**Figure 1C**). The percentage inhibition of metal chelating activities of leaf and root fractions of the *E. spectabilis* increased from 8.8106 to 72.1786 and 2.5096 to 51.0932%, respectively (**Figure 1C**). Acetone extract showed the highest iron chelating activity while water extract exhibited lowest chelating activity. Also, all fractions of leaf root and their different concentration levels showed statistically significant differences when compared with EDTA ($P < 0.001$).

Total antioxidant capacity

Antioxidant capacity of BHA, α-tocopherol, and leaf fractions was 7139.4, 5541.43, 3703.25, 1400.08, 1501.67 μg ascorbic acid/g (d.w.), while the antioxidant capacity of root fraction was 7139.4, 5541.43, 1758.02, 937.381, 2481.83 μg ascorbic acid/g (d.w.), respectively (**Figure 2A**, **2B**). Acetone fractions of leaf exhibited approximately 2.6 and 2.5 fold higher values than ethanol and water fractions of the leaf. Water fractions of root demonstrated 1.41 and 2.65 fold higher values than acetone and ethanol fractions of the root. The findings show the strong positive relationship between the total flavonoid contents of leaf and root extracts and total antioxidant capacity (r = 0.95 and 0.93).

Intracellular ROS activity and lipid peroxidation effect of plant extracts

Intracellular reactive oxygen species (ROS) assays were used to determine in vivo oxidative stress in PC-3 treated with leaf and root fractions of *E. spectabilis.* The data showed a significant difference from the solvent extract used. The plant had the highest inhibitory activity against ROS in PC-3 cells, with a value of 571.793 RFU in untreated cells, while acetone,

Table 2. Antimicrobial activities of leaves and root extracts of *E. spectabilis* performed with different solvents against microbial test organism

Microorganism	DMSO	Ampicillin	Acetone Extract	Ethanol Extract	Water Extract
	Zone of inhibition (mm)				
Leaves					
B. subtilis	-	8.26 ± 0.25	-	-	-
E. coli	-	7.60 ± 0.36	8.20 ± 0.20	8.10 ± 0.17	8.07 ± 0.06
L. monocytogenes	-	12.86 ± 1.03	10.83 ± 1.04	6.33 ± 0.58	8.17 ± 0.29
S. cerevisiae	-	6.16 ± 0.15	8.07 ± 0.12	-	-
S. aureus	-	6.06 ± 0.06	9.13 ± 0.23	-	-
Root					
B. subtilis	-	8.03 ± 0.55	6.67 ± 0.29	6.33 ± 0.29	6.26 ± 0.25
E. coli	-	7.67 ± 0.61	-	6.57 ± 0.12	6.43 ± 0.06
L. monocytogenes	-	6.97 ± 0.06	-	-	-
S. cerevisiae	-	7.07 ± 0.40	-	-	-
S. aureus	-	7.30 ± 0.30	-	-	-

Values are mean ± SD of three parallel measurements - = No zone of inhibition

ethanol and water leaf and root extracts were 550.987, 471.617, 614.697 and 623.087, 684.583, 696.443 RFU, respectively (**Figure 2C**). The scavenging of lipid peroxides by leaf and root extracts of *E. spectabilis* is presented in **Figure 2D**. The leaf extracts had the highest inhibitory activity against lipid peroxidation in PC-3 cells, with values of acetone, ethanol and water extracts 23.9545, 46.8182, 24.9091 μM MDA/ml, while acetone, ethanol and water extracts of root were active with 22.4318, 22.8409, 21.4545 μM MDA/ml values, respectively.

Antimicrobial activity test

The antimicrobial impacts of extracts applied to the discs against the microorganisms in respect to zone diameter ranged from 6.26 ± 0.25 to 10.83 ± 1.04 mm. Ethanol and water extracts of leaves showed significant lethal effects on only *B. subtilis* IM 622 and *E. coli* ATCC 25922, while acetone extract of leaves showed lethal effects only against *B. subtilis* IM 622. Furthermore, the extracts of leaves indicated the absence of the antimicrobial effects against *L. monocytogenes* NCTC 5348, *S. cerevisiae*, and *S. aureus* ATCC 29213. The acetone extract from the root of *E. spectabilis* indicated a quite good lethal effect against the *E. coli* ATCC 25922, *L. monocytogenes* NCTC 5348, *S. cerevisiae*, and *S. aureus* ATCC 29213, whereas the acetone extract from root could not indicate antimicrobial effect against the *B. subtilis* IM 622. Ethanol and water extracts of root could indicate antimicrobial effect only against the *E. coli* ATCC 25922 and *L. monocytogenes* NCTC 5348. On the contrary of the root extracts, the extracts of leaves were observed that were effective against the *B.subtilis* IM 622. The acetone extract from root had the most powerful lethal effect against *L. monocytogenes* and *S. aureus* with 10.83 ± 1.04 mm and 9.13 ± 0.23 mm zone diameter, respectively (**Table 2**).

Effects of various extracts of E. spectabilis on PC-3 cell proliferation, apoptosis and cytochrome c, caspase-3 and -9 activities

The proliferation of PC-3 cells treated with *E. spectabilis* aqueous and organic extracts of leaves and roots was assessed using the MTT test (**Figure 3**). In general, extracts of *E. spectabilis* decreased PC-3 cell proliferation at concentrations of 250 and 500 μg/ml when compared to control cells ($P < 0.001$) (**Figure 3A-F**). The proliferation level of PC-3 cells was diminished to 80% in comparison to the control, after 48 h of treatment, for most of the extracts tested. After 48 h of treatment, the inhibitory effect was stronger, and 250 and 500 μl volumes of all preparations tested lowered the proliferation level. The relative level of Bcl-2, cytochrome c, caspase-9, and caspase-3 was highly increased by acetone, ethanol and water extraction of *E. spectabilis* roots (**Figure 4**). The results were pointed the high level of the mRNA ratio of Bax/Bcl-2 significantly increased both with leaf

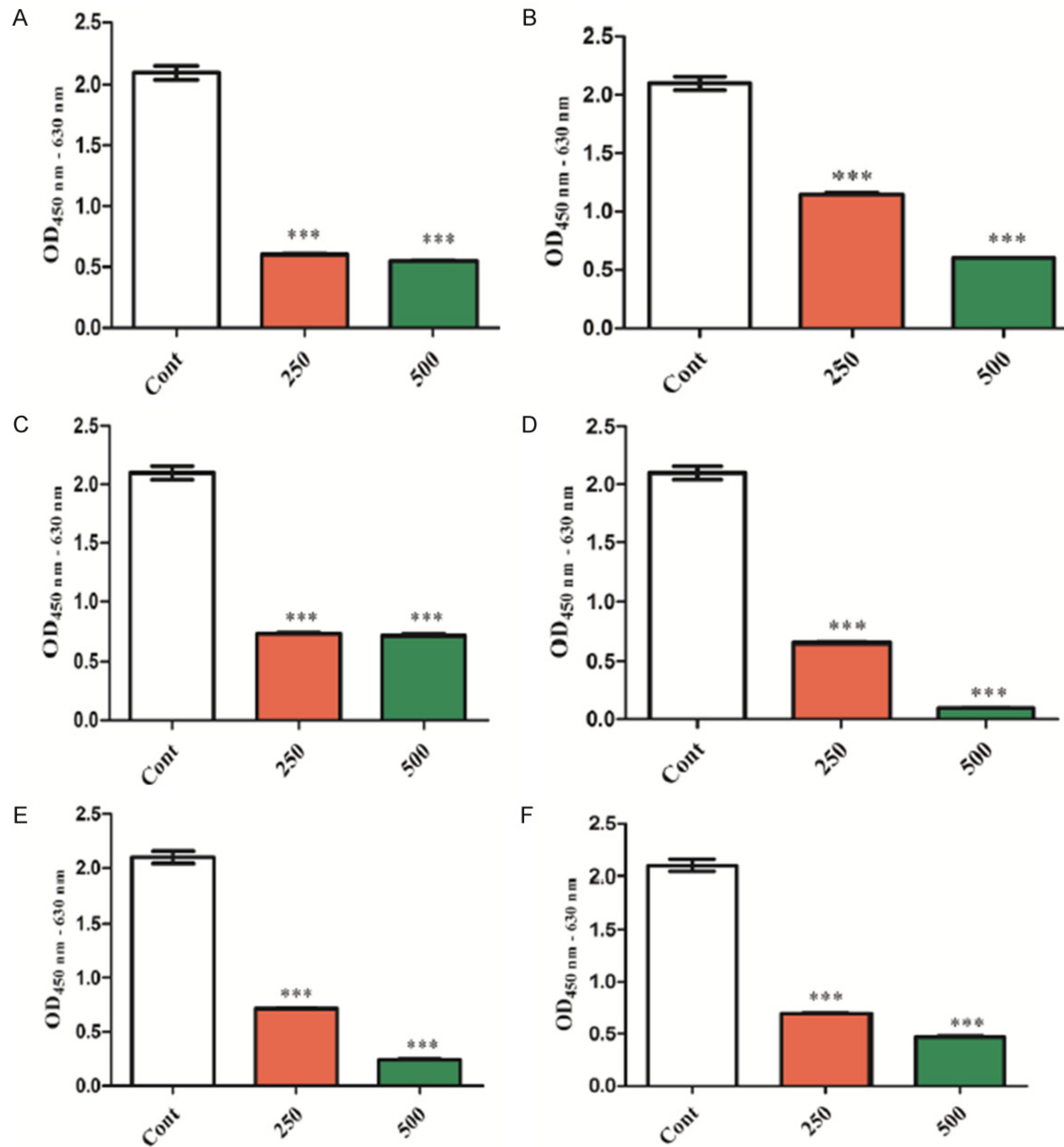

Figure 3. WST-1 results of various extracts of *E. spectabilis* on PC-3 cells. There was no agent in control (Control) cells. The extracts were applied to cells at the concentrations of 250 and 500 µg/ml for 48 h. A: Results of acetone extract of leaves, B: Ethanol extract of leaves, C: Water extract of leaves, D: Acetone extract of roots, E: Ethanol extract of roots, F: Water extract of roots of *E. spectabilis.* The data were shown as mean ± SEM (n ≥ 3). ***$P < 0.001$ control vs. 250 and 500.

(L-Acetone, L-EtOH and L-H_2O) and root (R-Acetone, R-EtOH) extracts except water extracts of root indicated the cells undergoing apoptosis in the experiment. The Bax protein expression level of acetone and ethanol extractions has no significant alteration with control. The relative level of Bcl-2, cytochrome c, caspase-9, and caspase-3 was also highly increased by the extracts of the leaf. The high ratio of Bax/Bcl-2 in mRNA level and activation of caspase-3 in protein levels compel cells to apoptotic death.

Discussion

Phenolic compounds are extensively considered for their antioxidant, antimicrobial and anti-cancer properties. In the present study, antioxidant antimicrobial and anticancer activi-

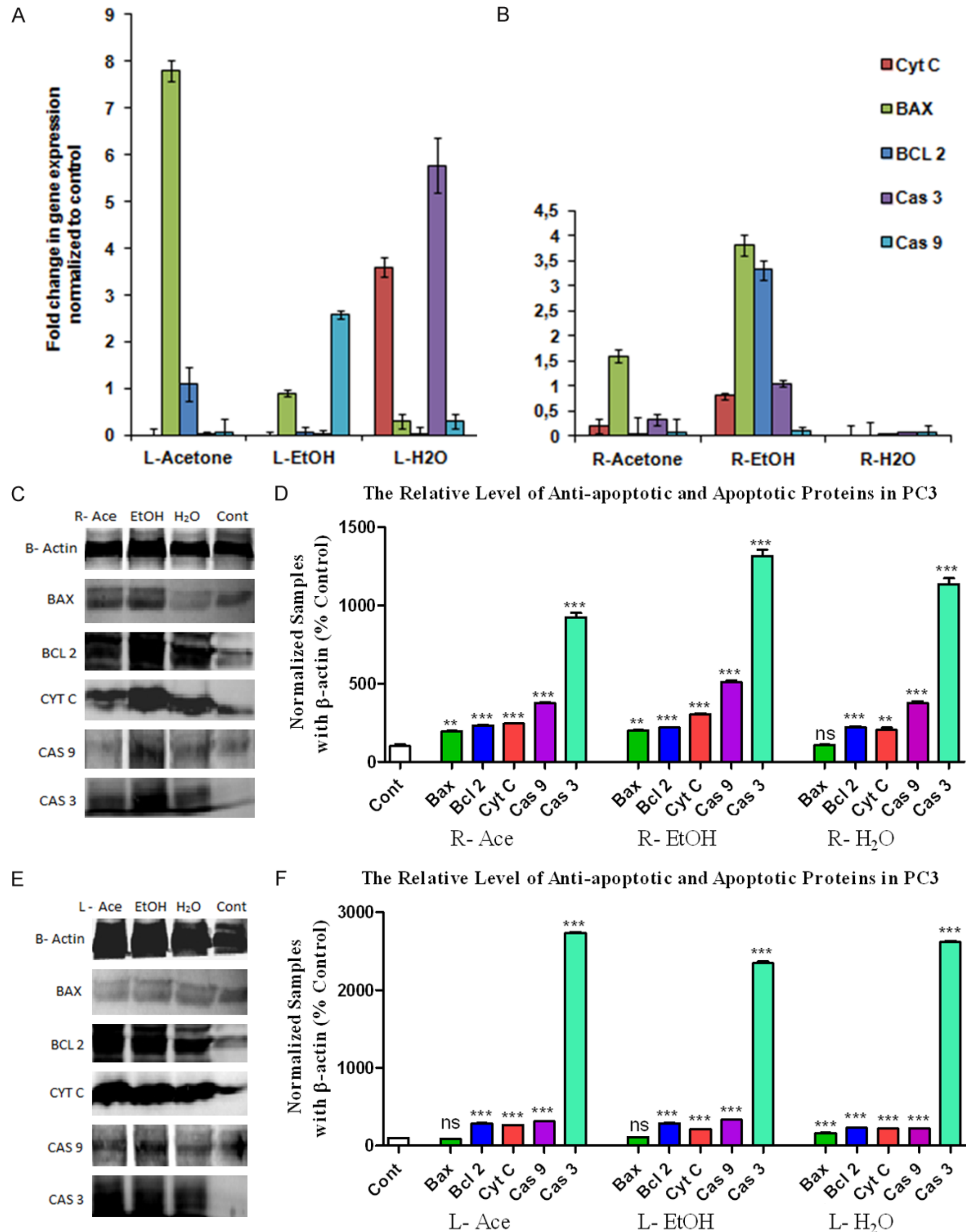

Figure 4. Expression of apoptotic proteins (A-F) of prostate cancer cell (PC3) were measured by using QRT- PCR and WB analyses according to treatment of various extraction of *E. spectabilis* (A) leaves and (B) roots. The blots were incubated with antibody against β-actin to confirm equal protein loading. ***P < 0.001, **P < 0.01 and *P < 0.05 and ns; not significant. The SEM was shown by error bars (n = 3/group).

ties refer to the ability of *E. spectabilis* to prevent damage from reactive oxygen species (ROS) and the ability to prevent propagation of these species. The in vitro antiproliferative

activities of extracted *E. spectabilis* fractions were determined by MTT assay on PC-3 cells. We demonstrated that acetone extract of *E. spectabilis* leaves and root showed the highest total phenolic content (2042.60 ± 76.15 mg GAE/g; 981.87 ± 8.70 mg GAE/g). The totally highest TPCs in leaves were quite expected and could be explained by the elevated biosynthesis of polyphenols due to light exposure in these organs. Phenolic compounds are known to serve as a filtration mechanism against UV-B radiation. Aqueous-acetone and aqueous-ethanol extracts showed higher TPCs than the water extracts suggesting that *E. spectabilis* contained mainly non-polar phenolic compounds. In a previous study, phenolic compounds are reported to be associated with antioxidant activity, anticancer effects, and other biological functions and may prevent the development of disease [17]. Karaman et al. [7] investigated the total phenolic compound of methanol, ethanol and aqueous extracts of *E. spectabilis* leaves and root and found that the methanol extract contains the highest total phenolic content (31.92 ± 0.48 mg GAE/g).

Hydroxyl radical, DPPH, considered as a good kinetic model for peroxyl radicals, metal chelating activity, total antioxidant capacity, intracellular ROS activity and lipid peroxidation assays are widely used to determine the antioxidant capacity of plant extracts due to their simplicity, stability, and reproducibility [18, 19]. In the present study, these assays provided comparable results for the antioxidant capacity measured in *E. spectabilis* extract and its fractions. In a previous study, *E. spectabilis* extract exhibited strong antioxidant activities against various oxidative systems *in vitro*. In the present study, *E. spectabilis* extract and its fractions that contained higher stronger radical scavenging effects (**Figures 1** and **2**). The correlations between the antioxidant assays, and phenolic content indicating that the four assays provided comparable values when they were used to estimate the antioxidant capacity of *E. spectabilis* extract. Similar to our results, Karaman et al. [7] also reported that the best antiradical activity determined by DPPH method was observed in ethanol extract of *E. spectabilis* (IC_{50}, 35.14 μg/ml). Many studies have shown a good positive linear correlation between antioxidant capacity and the total phenolic content of spices, medicinal herbs, and other dietary plants. Moreover, these results have also suggested that phenolic compounds are responsible for their antioxidant capacity [17, 19].

Lipid peroxidation is the oxidative destruction of polyunsaturated fatty acids, mainly of the effect of several reactive oxygen species. This reaction begins with removing one hydrogen atom from the polyunsaturated fatty acids found in membrane structure initiating a self-propagating chain reaction. The destruction of membrane lipids and the end-products of such lipid peroxidation reactions are especially dangerous for mitochondria, energy and protein synthesis and the viability of cells, even tissues. Although flavonoids and phenolic acids are known as potential antioxidants presence in consumed foods, recent studies showed that exogenous antioxidants including polyphenols have also pro-oxidant effects at high doses. The type, dosage, and matrix of exogenous antioxidants may be determining factors impacting the balance between beneficial or deleterious effects of these natural compounds. In this study lipid peroxidation, leaf and root fractions applied to PC-3 cells caused a varied increase the approximately from 1.02 fold to 2.22 fold when they compared with control. Furthermore, the ethanol fraction of leaf caused the highest increases of lipid peroxidation in PC-3 cells, with 2.22 fold the rate of increase, when compared with the others.

In the current study, in order to evaluate the cytotoxic effects of acetone, ethanol and water extracts of leaves and roots of *E. spectabilis* samples, we performed a preliminary cytotoxicity study with PC-3 cells exposed to various sample concentrations (250 and 500 μg/ml). The *E. spectabilis* acetone, ethanol and water extract (at 250 and 500 μg/mL), inhibited cell proliferation (**Figure 3**). The inhibition of cell proliferation in PC3-cells supplemented with *E. spectabilis* supports the hypothesis that this phenolic compounds might have a role in the preventing the proliferative in the PC-3 cells by its anticancer effect. There are no previous studies related to investigating the effects of *E. spectabilis* supplementation on the PC-3 cells proliferation to compare with this study. However, several studies done in cancer cells reported that phenolic compounds have similar effects [19].

It was reported that water extract from *E. spectabilis* young leaves had not any antibacterial activity against *E. coli* ATCC 25922, and they also reported that the water extract from *E. spectabilis* young leaves had a good antibacterial activity against *S. aureus* ATCC 6538. However, in the present study, the water extracts from leaves and root of *E. spectabilis* indicated a good antibacterial activity against *E. coli* ATCC 25922 with 6.43 ± 0.06 mm zone diameter as the water extracts from leaves and 8.07 ± 0.06 mm zone diameter as the water extracts from the root, respectively.

We assessed *E. spectabilis* acetone, ethanol and water extracts affected the expression of genes associated with apoptotic cell death, including the pro-apoptotic Bax, the anti-apoptotic Bcl-2, and caspases in PC-3 cells. A family of apoptosis regulator Bcl-2 known as evolutionarily related proteins govern mitochondrial outer membrane permeabilization and maintenance of homeostasis within tissues both require a tightly regulated cell death pathway and can be either positive and negative regulator. Bax is believed to interact with, and induce the opening of the mitochondrial voltage-dependent anion channel. The Bax/Bcl-2 ratio appears more important than the individual Bax or Bcl-2 level in determining a cell's vulnerability to apoptosis; high Bax/Bcl-2 ratios lead to greater apoptotic activity. This results in the release of cytochrome c and other pro-apoptotic factors from the mitochondria, often referred to as mitochondrial outer membrane permeabilization, leading to activation of initiator caspases (caspase-2, caspase-8 and caspase-9) required for activation of executioner caspase (caspase-3, caspase-6 and caspase-7). Among them, caspase-3 is a frequently activated apoptotic cysteine protease catalyzing the specific cleavage of many key cellular proteins. In the current study, to reveal the effect of *E. spectabilis* extract on the expression of Bcl-2, Bax, caspases, RT-PCR and Western blot were performed. Results from the present study demonstrated the high level of the mRNA ratio of Bax/Bcl-2 significantly increased both with leaf (L-Acetone, L-EtOH and L-H_2O) and root (R-Acetone, R-EtOH) extracts except water extracts of root indicated the cells undergoing apoptosis in the experiment. Moreover, aquatic extraction of leaves (L-H_2O) was more effective to overexpress apoptotic genes more than 3 fold changes such as cytochrome c and caspase-3 in spite of no apoptotic activity of water-soluble molecules of roots (R-H_2O). The western blot analysis indicates that the relative level of Bcl-2, cytochrome c, caspase-9, and caspase-3 were highly increased by acetone, ethanol and water extraction of *E. spectabilis* roots. Protein expression level of pro-apoptotic Bax, apoptotic cytochrome c, caspase-9 and caspase-3 were more expressed with ethanol extraction according to acetone and water extractions. The Bax protein expression level of acetone and ethanol extractions has no significant alteration with control. The relative level of Bcl-2, cytochrome c, caspase-9, and caspase-3 was also highly increased by all three extracts of the leaf. The high ratio of Bax/Bcl-2 in mRNA level and activation of caspase-3 in protein level commit cells to apoptotic death. A literature review revealed that there are no previous reports about the apoptotic cell death of *E. spectabilis* to compare these results.

Taken together, our results revealed that the *E. spectabilis* exhibits considerable antioxidant and antimicrobial in *in vitro* model and can be used as alternative medicine to prevent or treat oxidative stress. The antioxidant ability could be attributed to the phenolic compounds, especially flavonoids which possess antioxidant action. In addition, the treatment of PC-3 cells with *E. spectabilis* extract inhibited the growth of cancer cells by induction of apoptosis.

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Disclosure of conflict of interest

None.

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