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In vitro Biological Activities and Phytochemical Contents of Portulaca Oleracea L. (Purslane)

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ABSTRACT

P. oleracea is an annual herbaceous plant, belonging to the Portulaceae family is consumed as a vegetable in Turkey and grows spontaneously in wetlands, along streams, and meadows. In this study, the antiradical, antimicrobial, anticancer activities and phytochemical contents of aerial parts extracts of *P. oleracea* were investigated. According to study results, *P. oleracea* aerial part extracts show very high anticancer activity against MCF-7, HCT-116, A2780 and PC-3 cancer cell lines, high antiradical activity against OH radicals, and effective antimicrobial activity against some microorganisms. Also, it was determined that *P. oleracea* aerial parts contain highly phytochemical compounds. It can be said that the *P. oleracea* aerial parts can be used in medicine for antiradical, antimicrobial, and anticancer purposes.

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1. INTRODUCTION

Portulaca oleracea L. (purslane) is an annual herbaceous plant included in the Portulaceae family and is consumed as a vegetable in Turkey. However, this plant, which is not produced much, grows spontaneously in wetlands, along streams and meadows. In addition to being an edible vegetable, it is also used as a drug in traditional medicine. In addition to being an edible vegetable, it is also used as a drug in traditional medicine to treat bloody dysentery, snake and insect bites, asthma, ulcers, diarrhea and hemorrhoids due to its antiseptic, antispasmodic and diuretic properties [1]. Fresh purslane has been found to contain high amounts of vitamins E, C and A, carotenoid derivatives, omega 3 fatty acids, glutathione, glutamic acid, aspartate, flavonoids and phenolic compounds [2]. When making a detailed literature survey on *P. oleracea*, it is observed that the antiradical activities [3-15], protective effects against stomach cancer in rats [14], nutritional benefits [12], protective effects of phenolic compounds against DNA damage [15], antibacterial activities [5,6], some phytochemical compounds [7,8,11,13], protective effects against nephrotoxicity [16], anticancer activity of methanol extract [17] and antitumor activities of its some polysaccharides [18] were determined.

The goal of this study is to determine the antiradical ($ABTS^{+\bullet}$, $DPPH^{\bullet}$, $OH^{\bullet}$) activities; the antimicrobial properties (*Bacillus megaterium*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Staphylococcus aureus* bacteria and *Candida albicans* fungi); the anticancer properties (human breast cancer (MCF-7), human colon cancer (HCT-116), human ovarian cancer (A2780) and human prostate cancer (PC-3)) and phytochemical contents (total phenolic compounds, total proanthocyanidins, phenolic acids, lipid soluble vitamins,

flavonoids, total flavonoids, phytosterols and fatty acids) of *P. oleracea* aerial parts water, ethanol and methanol extracts.

2. MATERIALS AND METHODS

2.1. Plant Materials and Extraction

The aerial parts of *P. oleracea* were collected in July 2015 from Sivrice/Elazig in Turkey. The voucher specimen number is Turkoglu 4920. This specimen was stored in the Herbarium of Firat University, Faculty of Science, Department of Biology, Elazig/Turkey. The aerial parts were dried at dark and room temperature. Dried plant samples were pulverized using a mechanic grinder, and then 20 g of the sample was extracted with 200 mL of solvent (water, ethanol and methanol). All the extracts were centrifuged at 5000 rpm at +4 °C. After centrifuging and filtrating of solvents, the supernatant was concentrated. The dried extract was dissolved in DMSO (µg/mL) [19].

2.2. Determination of Radical Scavenging Activities

The DPPH, $ABTS^{\bullet+}$ and hydroxyl (OH) radical scavenging activities (RSAs) were determined by the methods of Brand-Williams et al. [20], Re et al. [21] and Halliwell et al. [22], respectively. All tests were repeated three times and the average values were calculated. The radical scavenging activity percentage for each sample was estimated by the following equation:

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

where A_0: control absorbance; A_1: sample absorbance.

2.3. Determination of Phytochemical Components

The determination of total phenolic contents was performed according to Slinkard and Singleton's method [23]. Gallic acid was used as a standard.

Total flavonoid content contents were determined according to Kim et al.'s [24] method. The catechin was used as a standard.

The determination of proanthocyanidin content was performed according to Amaeze et al.'s [25] method. The catechin was used as a standard.

The determination of flavonoid and phenolic acids was performed according to the method of Zu et al. [26] in the *P. oleracea* by HPLC. Rutin, myricetin, morin, quercetin, kaempferol, catechin, naringenin, resveratrol, vanillic acid, gallic acid, hydroxycinnamic acid, caffeic acid, ferulic acid and rosmarinic acid were quantified in the *P. oleracea* aerial parts by HPLC.

Fatty acids in the *P. oleracea* aerial parts were analyzed by GC according to Christie's method [27]. The fatty acids analysis results were expressed as a percent of samples.

Lipophylic vitamins and phytosterols were extracted from the *P. oleracea* aerial parts according to the method of Sánchez-Machado et al. [28] and Lopez-Cervantes et al. [29] by HPLC. The results of the analyses were expressed as µg/g. Retinol, δ-tocopherol, α-tocopherol, vitamin K, vitamin D, ergosterol and stigmasterol were quantified in the *P. oleracea* aerial parts.

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. Collins and Lyne's method [30] was used for the antimicrobial tests using the disc diffusion method. Streptomycin sulfate (10 mg/disc) and nystatin (30 mg/disc) were used as standard antibiotics.

2.5. Determination of Anticancer Properties

2.5.1. Cell Culture

The cell lines of human prostate cancer (PC-3), human colon cancer (HCT-116), human ovarian cancer (A2780) and human breast cancer (MCF-7) retrieved from American Type Culture Collection (ATCC), were employed in this study.

2.5.2. MTT Test

The ethanol, water and methanol extracts of *P. oleracea* aerial parts were screened for their anticancer properties against different types of cancer cell lines (PC-3, HCT-116, A2780 and MCF-7). The viability of the cells was determined using 0.4% trypan blue. Effects of the % cell viability of *P. oleracea* extracts were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test [31,32].

2.6. Statistical Analyses

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

3. RESULTS AND DISCUSSION

3.1. Antiradical Properties

In this study, when the antiradical properties of *P. oleracea* water, ethanol and methanol extracts were examined (Table 1), it was determined that the extracts remained at a much lower rate than the standard antioxidant BHT in scavenging of the DPPH radical. It

was observed that only *P. oleracea* water extract (97.53%) showed higher scavenging activity than BHT (93.24%) in ABTS test; the water, ethanol and methanol extracts (84.80%, 78.55%, 80.24%, respectively) showed higher scavenging activity than BHT (75.77%) in OH radical scavenging test. Uddin et al. [12] were determined that *P. oleracea* extracts scavenged 76.71% DPPH radical. In our study, *P. oleracea* water, ethanol and methanol extracts scavenged the DPPH radical by 38.10%, 33.74% and 47.13%, respectively, and it was observed that our results remained at a lower rate compared to the study of Uddin et al. [12]. In another study, DPPH and OH radical scavenging activities of *P. oleracea* water extract were examined, and it was determined that the extracts destroyed 80% DPPH radical and 12% OH radical [3]. In our study, different results were obtained in terms of the solvents with which the extraction was carried out; *P. oleracea* water extract destroyed the DPPH radical at a rate of 38.14% and the OH radical at a rate of 84.80%. Cho et al. [6] examined the DPPH and ABTS radical scavenging activities of *P. oleracea* water and ethanol extracts, and at the end of the study, they showed that the water and ethanol extracts DPPH radical scavenged at the rate of 89.2% and 72.9%, respectively; ABTS radical scavenged at the rate of 69.0% and 96.5%, respectively. These results are not similar to our results in terms of antiradical activity percentages of the extracts. According to our study results, *P. oleracea* water and ethanol extracts destroyed the DPPH radical at a rate of 38.10% and 33.74%, respectively, and the ABTS radical at a rate of 97.53% and 45.82%, respectively. Oliveira et al. [6] investigated the DPPH radical scavenging activities of water extracts of 4 different cultures of *P. oleracea* and determined that the extracts removed DPPH radicals in the range of approximately 45% to 80%. Alam et al. [13] found that the DPPH radical scavenging activities of the methanol extracts of *P. oleracea* samples collected from 13 different regions varied between 41.25% and 66.81%. These results are consistent with the 47.13% DPPH radical scavenging rate of the *P. oleracea* methanol extract in our study. Lim and Quah [4] found that methanol extract of *P. oleracea* removed very high DPPH radicals; Ercisli et al. [5] suggested that the extract in the same solvent removes less DPPH radicals than standard antioxidants BHT and BHA. Erkan [11] claimed that the methanol extract of *P. oleracea* scavenged very high DPPH and ABTS radicals.

3.2. Phytochemical Composition

P. oleracea water, ethanol and methanol extracts of total flavonoid amounts were 1277.98, 1697.49, and 1317.41 µg CE/g extract, respectively; total proanthocyanidin amounts were 597.44, 181.88, and 174.12 µg CE/g extract, respectively; total phenolic compounds amounts were 95.56, 40.54, and 75.96 mg GAE/g extract, respectively (Table 1). According to these results, it is seen that the water extract of *P. oleracea* is rich in the total phenolic compound and total proanthocyanidin, and the ethanol extract is rich in total flavonoids. When the studies on *P. oleracea* are examined, Peksel et al. [3] were found that the water extract of *P. oleracea* contains 17.91 µg of pyrocatechol equivalent total phenolic compound, 14.63 µg of catechin equivalent total flavonoid; Uddin et al. [12] were found that *P. oleracea* water, ethanol and methanol extracts contain 142.8 mg GAE/100 g, 276.8 mg GAE/100 g and 360.8 mg GAE/100 g total phenolic compound (respectively), 28.7 mg RE/100 g, 41.30 mg RE/100 g, 49.2 mg RE/100 g total flavonoids (respectively).

Table 1. $ABTS^{+\bullet}$, $OH^{\bullet}$, $DPPH^{\bullet}$ radicals scavenging activities, total flavonoid, total proanthocyanidin and total phenolic contents of *P. oleracea* aerial parts 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)
P.oleracea water	97.53±0.31^{b}	84.80±0.47^{b}	38.10±2.59^{c}	1277.98±4.24	597.44±1.34	95.56±1.09
P.oleracea ethanol	45.82±1.49^{d}	78.55±0.55^{c}	33.74±2.17^{d}	1697.49±2.96	181.88±1.13	40.54±1.53
P.oleracea methanol	73.60±0.77^{c}	80.24±1.04^{c}	47.13±1.84^{b}	1317.41±2.22	174.12±0.99	75.96±0.76
BHT	93.24±0.20^{a}	75.77±0.12^{a}	95.21±0.33^{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.

Alam et al. [13] determined the total phenolic compound amounts of the methanol extracts of 13 different cultures of *P. oleracea* in the range of 0.96-9.12 mg GAE/g, and the amount of total flavonoids in the range of 0.13-1.44 mg RE/g. Petropoulos et al. [33] determined that 6 different genotypes of *P. oleracea* contain total phenolic compounds in the range of 7.65-20.1 mg GAE/g and total flavonoids in the range of 0.12-5.30 mg CE/g. Lim and Quah [4] found that the total phenolic content of methanol extracts of 6 different cultures of *P. oleracea* in the range 127-478 mg GAE/100 g; Ercisli et al. [5] determined the total phenolic content of the same extract

of the same plant as 17.88 µg GAE/mg. Cho et al. [6] stated that *P. oleracea* water extract contains 3.05 µg/g and ethanol extract contains 6.33 µg/g total phenolic compounds; Oliveira et al. [6] found that *P. oleracea* water extracts collected from four different regions contain total phenolic compounds in the range of 211.6-633.9 mg/kg. Marrelli et al. [34] showed that the water/ethanol extract of *P. oleracea* contains 11.8 mg QE/g total flavonoids. All the researches and the results we have obtained in the study have shown that the *P. oleracea* is a very rich plant in terms of total phenolic, total flavonoid and total proanthocyanidin content.

Flavonoid amounts of *P. oleracea* were rutin (0.10 µg/g), myricetin (0.10 µg/g), morin (0.05 µg/g), quercetin (0.05 µg/g), kaempferol (0.05 µg/g), catechin (5.10 µg/g), naringenin (0.05 µg/g) and resveratrol (0.10 µg/g); the phenolic acid amounts of *P. oleracea* were vanillic acid (5.75 µg/g), gallic acid (25.05 µg/g), hydroxycinnamic acid (0.95 µg/g), caffeic acid (11.10 µg/g), ferulic acid (2.35 µg/g) and rosmarinic acid (4.70 µg/g). Erkan [11] showed that *P. oleracea* containe 55.45 mg/kg quercetin and 24.32 mg/kg kaempferol as flavonoids; 77.90 mg/kg caffeic acid, 40.99 mg/kg ferulic acid and 48.13 mg/kg rosmarinic acid as phenolic acid (Table 2).

Table 2. Contents and composition of flavonoids, phenolic acids, vitamins, phytosterols and fatty acids in *Portulaca oleracea* aerial parts

Flavonoids and Phenolic Acids	**(µg/g)**
Rutin	0.10±0.00
Myricetin	0.10±0.00
Morin	0.05±0.00
Quercetin	0.05±0.00
Kaempferol	0.05±0.00
Catechin	5.10±0.25
Naringenin	0.05±0.00
Resveratrol	0.10±0.00
Vanillic Acid	5.75±0.25
Gallic Acid	25.05±1.00
Hydroxycinnamic Acid	0.95±0.10
Caffeic Acid	11.10±0.50
Ferulic Acid	2.35±0.15
Rosmarinic Acid	4.70±0.35
Vitamin and Phytosterols	**(µg/g)**
Retinol	0.10±0.00
δ-Tocopherol	0.05±0.00
α-Tocopherol	3.30±0.20
Vitamin K	0.10±0.00
Vitamin D	2.50±0.15
Ergosterol	335.15±3.05
Stigmasterol	44.10±0.90
Fatty Acids (FA)	**(%)**
16:0	10.65±0.14
16:1	6.96±0.21
18:0	9.55±0.33
18:1	10.18±0.24
18:2	5.19±0.66
18:3	47.52±0.27
20:5	9.95±0.39
Saturated FA	27.20
Unsaturated FA	79.80

The lipid soluble vitamin levels of *P. oleracea* were retinol (0.10 µg/g), α-tocopherol (3.30 µg/g), δ-tocopherol (0.05 µg/g), vitamin K (0.10 µg/g) and vitamin D (2.50 µg/g); the phytosterol levels of *P. oleracea* were ergosterol (335.15 µg/g), stigmasterol (44.10 µg/g). The fatty acids content in *P. oleracea* were 10.65% palmitic acid (16:0),

6.96% palmitoleic acid (16:1), 9.55% stearic acid (18:0), 10.18% oleic acid (18:1), 5.19% linoleic acid (18:2), 47.52% linolenic acid (18:3), 9.95% eicosapentaenoic acid (20:5), 20.20% total saturated fatty acids, 79.80% total unsaturated fatty acids (Table 2). Simopoulus et al. [35] found that *P. oleracea* contains 170 mg/100 g α-tocopherol, 43.5 mg/100 g vitamin A precursor. Marrelli et al. [34] suggested that *P. oleracea* is rich in terms of ergosterol and stigmasterol. When the literature on the fatty acid content of *P. oleracea* is examined, Simopoulos and Salem [36] found that this plant contains 47.6% linolenic acid (18:3), Omara-Alwala et al. [37] suggested that it contains 66.4% linolenic acid (18:3). In another study, Simopoulus et al. [35] found that *P. oleracea* contains 13.45% linoleic acid (18:2), 63.78% linolenic acid (18:3), while Siriamornpun and Suttajit [8] found that the same plant contains 13.09% palmitic acid (16:0), 2.29%. stearic acid (18:0), 14.46% linoleic acid (18:2), 62.95% linolenic acid (18:3), 21.25% total saturated fatty acids, 78.75% total unsaturated fatty acids. Ercisli et al. [5] have shown that *P. oleracea* contain 9.72% palmitic acid (16:0), 4.36% stearic acid (18:0), 8.83% oleic acid (18:1), 14.01% linoleic acid (18:2), 56.3% linolenic acid (18:3), 19.12% total saturated fatty acids, and 79.17% total unsaturated fatty acids. Oliveira et al. [7] found that collected from four different regions of *P. oleracea* extracts contained in the range of 19.26-24.26% palmitic acid (16:0), in the range of 7.08-8.72% stearic acid (18:0), in the range of 11.55-19.49% oleic acid (18:1) in the range of 4.00-6.31% linoleic acid (18:2), in the range of 24.48-39.06% linolenic acid (18:3), in the range of 42.56-50.02% total saturated fatty acids, in the range of 49.99-58.03% total unsaturated fatty acids. Petropoulos et al. [33] have determined that the fatty acid content of 6 different genotypes of *P. oleracea* in the range of 23.43-26.89% palmitic acid (16:0), in the range of 4.91-8.21% stearic acid (18:0), in the range of 9.70-15.09% oleic acid (18:1), in the range of 25.09-32.90% linoleic acid (18:2), in the range of 17.91-28.40% linolenic acid (18:3), in the range of 29.27-37.06% total saturated fatty acids, in the range of 62.94-70.73% total unsaturated fatty acids. According to these results, it can be said that *P. oleracea* is a very rich food in terms of omega-3 and unsaturated fatty acids and can be consumed as a source of omega-3.

3.3. Antimicrobial Properties

It was determined that *P. oleracea* water extract has antimicrobial activity on *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *B. subtilis* and *B. megaterium* bacteria; ethanol extract has antimicrobial activity on *E. coli*, *P. vulgaris*, *P. aeruginosa*, *L. monocytogenes*, *K. pneumoniae*, *B. subtilis*, *B. megaterium* and *S. aureus* bacteria; methanol extract has antimicrobial activity on *E. coli*, *P. vulgaris*, *P. aeruginosa*, *L. monocytogenes*, *K. pneumoniae*, *B. subtilis*, *B. megaterium*, *S. aureus* bacteria and *C. albicans* fungus (Table 3). It was observed that *P. oleracea* ethanol extract (10 mm) showed higher antimicrobial activity on *B. subtilis* and methanol extract on *E. coli*, *L. monocytogenes*, *K. pneumoniae* and *B. subtilis* (11 mm, 9 mm, 10 mm, 11 mm, respectively) from standard antibiotic (10 mm, 8 mm, 9 mm, 9 mm, respectively). Ercisli et al. [5] showed that *P. oleracea* methanol extract has antimicrobial activity on *Pseudomonas syringae* pv. *tomato*, *B. subtilis*, *Vibrio cholerae* and *Yersinia pseudotuberculosis* bacteria; Cho et al. [6] determined water and ethanol extracts of this plant have antimicrobial activity on *Helicobacter pylori*, *S. epidermidis*, *S. aureus*, *E. coli* and *Streptococcus mutans* bacteria; Elkhayat et al. [38] found that *P. oleracea* methanol extract has antimicrobial activity on *E. coli*, *P. aeruginosa*, *Neisseria gonorrhea*, *S. aureus*, *B. subtilis*, *Streptococcus faecalis* bacteria and *C. albicans* fungus. These results show that *P. oleracea* has antimicrobial activity against some microorganisms that caused infection in humans.

Table 3. The antimicrobial activities of *Portulaca oleracea* aerial parts extracts (mm zone)

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

3.4. Anticancer Properties

The IC_{50} values of anticancer activities of *P. oleracea* extracts on the PC-3, A2780, MCF-7, and HCT-116 cancer cell lines are presented in Table 4. *P. oleracea* ethanol extract (6.30 µg/mL) has a better anticancer activity for the PC-3 cell lines than all the other extracts; *P. oleracea* methanol extract (2.67 µg/mL) has better anticancer activity for the A2780 cell lines than all the other extracts; *P. oleracea* water extract (23.73 µg/mL) has better anticancer activity for the MCF-7 cell lines than all the other extracts; *P. oleracea* methanol extract (6.92 µg/mL) has better anticancer activity for the HCT-116 cell lines than all the other extracts. Tan et al. [17] found that the methanol extract of *P. oleracea* has high anticancer properties on human breast cancer (MCF-7), colon cancer (HT-29), cervical cancer (HeLa) and nasopharyngeal cancer (CNE-1) cell lines. In another study, Zhao et al. [18] showed that polysaccharides obtained from *P. oleracea* cause 43.8% cancer cell death in 24 hours on cervical cancer (HeLa). In a different study, Marrelli et al. [34] studied the anticancer activity of *P. oleracea* on human breast cancer (MCF-7), human liver cancer (HepG2), and human colorectal cancer (LoVo) cell lines and they suggested that this plant exhibits anticancer properties only on LoVo cell lines. According to the results of these researches and the presented study, it can be said that *P. oleracea* has a strong anticancer activity potential.

Table 4. The IC_{50} values of *P. oleracea* aerial part extracts against PC-3, A2780, MCF-7 and HCT-116 cancer cell lines for the anticancer activity assay

Samples (µg/mL)	PC-3	A2780	MCF-7	HCT-116
P. oleracea water	6.47	3.06	23.73	7.84
P. oleracea ethanol	6.3	2.93	25.11	9.34
P. oleracea methanol	6.77	2.67	25.06	6.92

4. CONCLUSION

In this study, the antiradical, antimicrobial, anticancer activities, and phytochemical compounds of *P. oleracea* aerial parts of water, ethanol and methanol extracts. According to our results, this plant has important antiradical, antimicrobial, anticancer properties and bioactive compounds.

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