

Total Phenolics, Antioxidant, Antibacterial and Cytotoxic Activity Studies of Ethanolic Extracts *Arisarum vulgare* O.Targ.Tozz. and *Dracunculus vulgaris* Schott.

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Abstract: The present study reports the antioxidant, total phenolics, antibacterial and cytotoxic activities of *Arisarum vulgare* and *Dracunculus vulgaris* of aerial and under ground ethanol extracts. The antioxidant activity was determined by DPPH radical scavenging activity and β -carotene linoleic acid assays. Folin-Ciocalteu assay was used to detect total phenolic contents in the extract. *In vitro* cytotoxic activity was determined by the Brine shrimp lethality test. Antibacterial activity was investigated with the microdilution method. As a result, under ground parts of the extract showed higher antioxidant activity than aerial parts of the extract. Total phenolics ranged from 14.5 \pm 2.02 to 53.4 \pm 2.01 mg GAE/g, and the antioxidant activity according to the β -carotene/linoleic acid assay ranged from 70.76 \pm 1.08 % to 85.43 \pm 1.05 and according to the DPPH assay IC₅₀ values ranged from 0.089 \pm 1.02 to 1.095 \pm 1.07mg/ml. The *A. vulgare* under ground extract was tested against 2 and showed a good antibacterial activity at a concentration of <50 μ g/mL Minimum inhibitory concentration (MIC) values for the bacteria *S.aureus*. The results of the cytotoxic activities showed a very high activity of the extracts, *D.vulgaris* under ground extract (LC₅₀ = 10.6 μ g/mL).

Keywords: Antioxidant, antibacterial, cytotoxic, *Arisarum vulgare*, *Dracunculus vulgaris*,

1. Introduction

Free radical induced oxidative damage has long been thought to be the most important cause of many diseases such as diabetes, stroke, cancer, arteriosclerosis and cardiovascular diseases [1, 2]. Oxidative stress affects the prooxidants and antioxidants equilibrium in biological system which leads to the modification of DNA, proteins, carbohydrates, and lipids. Hydroxyl radicals, superoxide anion radicals, and singlet oxygen are the examples of free radicals that attack the unsaturated fatty acids in the biomembranes resulting in lipid peroxidation, decrease in fluidity, loss of enzymes and receptor activity, and damage to membrane proteins and ultimately leading to cell inactivation. Lipid peroxidation is also strongly associated with aging and carcinogenesis [1-3]. Antioxidants mitigate oxidative stress, the adverse effects of free radica. Plant products are rich sources of phytochemicals and have been found to possess variety of biological activities including antioxidant, cytotoxic, and

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hepatoprotective potential. They act as reducing agents and reverse oxidation by donating electrons and/or hydrogen ions [4, 5].

Interest in toxic plants is increasing because it is recognised that these plants contain bioactive compounds and then, have medicinal virtues. So, medicinal plants are of great importance to the health. Also, many papers have reported that the major pharmacological proprieties of medicinal plants are associated and attributed to their antioxidant activity, then; much attention is being paid to antioxidant compounds [6]. Among plant secondary metabolites, polyphenols are known to have a high capacity to scavenge free radicals due to the hydrogen and electron transfer abilities and in that way are considered as prevailing responsible for antioxidant action. High content of non-phenolic compounds (monoterpenes and sesquiterpenes) in most essential oils might be related to their weak antioxidant activity [7].

Arisarum vulgare (Araceae) is very toxic, but their tubers are eaten in times of scarcity after boiling in water. It is also known for its uses in traditional medicine to treat various diseases. For example and according to ethnobotanical investigations, it is observed that in some regions of Algeria, *A. vulgare* is used for the treatment of several diseases such as headaches, asthma, flu and it promotes healing of early wound skin lesions. [8].

The genus *Dracunculus* (Araceae) is represented with only one taxon (*D. vulgaris* Schott) in Turkey, and tubers of this plant are used external in the treatment of rheumatism and hemorrhoids [9, 10]. Leaves and tubers of the *Dracunculus* genus contain saponin and conicine alkaloids, estragole, phelandrine, methyl cavicol, iodine, rutin, tannin, flavonoid and coumarin [11]. Seed oil of this genus contains also palmitic acid, oleic acid, cis-Vaccenic acid, stearic acid and arachidic acid [12]. *D. vulgaris* Schott is a poisonous plant, and the leaves and tubers of this plant have a toxic effect on humans and animals. In the light of all informations mentioned above, this study aimed to investigate total phenolic contents, the potential antioxidant, antibacterial and cytotoxic activities of aerial and under ground parts of the ethanolic extract of *D. vulgaris* and *A. vulgare*.

2. Material and Methods

2.1. Plant Material and Preparations of Extracts

A. vulgare and *D. vulgaris* species were collected in October 2015 from Denizli-Turkey (the campus of Pamukkale University). The fresh aerial and under ground parts of the plants samples were cleaned and dried in the shadow for extraction. Dried plant parts (under ground and above ground) were pulverized. Each ground sample was transferred into a beaker. Ethanol was added in the ratio of 1:10 and they were put in water bath at 55°C for 6 h [13]. The extraction mixture was separated from the residue by filtration through Whatman No: 1 filter paper. After the filtration two extracts were combined. The residual solvent of ethanol extracts of samples were removed under reduced pressure at 48-49°C using a rotary evaporator (Rotavapor IKA VB 10, Germany). The water extract was lyophilized using a freeze dryer (Thermosavant Modulyo D, USA). Extracts were produced in duplicates and used to assay the biological activity.

2.2. Plant Extracts

Arisarum (A), under ground-ethanol (AUE), above ground-ethanol (AAE). *Dracunculus* (D), under ground-ethanol (DUE), above ground-ethanol (DAE),

2.3. Antioxidant Activities

2.3.1. β -Carotene-Linoleic Acid Assay

This test was carried out according to a described procedure [15], based on the aptitude of various extracts to decrease the oxidative discoloration of β -carotene in an emulsion. A stock solution of β -carotene-linoleic acid mixture was prepared as following: 0.5mg β -carotene was dissolved in 1 mL chloroform. 25 μ L linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 mL of distilled water was added with vigorous shaking. Also, 2.5 mL of this reaction mixture was dispensed into test tubes and 350 μ L portion (1mg/mL) of the extract was added and the emulsion system was incubated for up 2 h at 50°C. The same process was done again with synthetic antioxidant, BHT, as positive control and a blank. The absorbance of the mixtures was measured with a spectrophotometer (Shimadzu UV- 1601, Japanese) at 490 nm after the incubation period, and inhibition ratio was calculated. The antioxidant activity was measured in terms of successful bleaching of β -carotene by using the following equation [15]. The measurements were made using the equation below:

$$AA: [1 - (A_0 - A_t / A_{0o} - A_{to}) \times 100]$$

Where AA is the total antioxidant activity, A₀ is the initial absorbance of the sample, A_t is the initial absorbance of the control, A_{0o} is the sample's absorbance after 120 min, and A_{to} is the control's absorbance after 120 min.

2.3.2. DPPH Free Radical Scavenging Activity Assay

The method of Wu et al. [16] was used for determination of scavenging activity of DPPH free radical. 4 ml of the DPPH's 0.004% metanolic solution was mixed with 1 mL (0.2-1.0 mg/mL) of the extracts, and their absorbances were measured to be at 517 nm after incubation for 30 min at room temperature the absorbance value of the samples were evaluated against empty control group (where all determinants except the test compound were present). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Every test was treated three times and the averages as determined. Free radical scavenging activity was measured using the equation below:

$$\text{Scavenging activity} = [(A_0 - A_1 / A_0) \times 100]$$

Where A₀ is the absorbance of the control (blank, without extract) and A₁ is the absorbance in the presence of the extract. The extract concentration providing 50% inhibition (IC₅₀) was calculated from the graph of scavenging effect percentage against extract concentration.

2.3.3. Determination of Total Phenolic Content

The total phenolic content of extracts were determined with Folin- Ciocalteu reagent, according to the method of Slinkard and Singleton [14]. Briefly, 0.75 mL of Folin–Ciocalteu reagent (1:9; Folin-Ciocalteu reagent: distilled water) and 100 mL of sample (5 mg/mL) were put into a test tube. The mixture was allowed to stand at room temperature for 5 min. 0.75 mL of 6% (w/v) Na₂CO₃ was added to the mixture and then mixed gently. The mixture was homogenized and allowed to stand at room temperature for 90 min. Total polyphenol content was determined using a spectrophotometer at 760 nm. The Standard calibration (0.01–0.05 mg/mL) curve was plotted using gallic acid. The total phenolic content was expressed as Gallic Acid Equivalents (GAE) in mg/mL plant extract. The estimation was performed in triplicate, and the results were expressed as mean \pm SD.

2.4. Cytotoxic Activity

Brine shrimp lethality test (BSLT) was applied to analyze the possible cytotoxic activity of the extracts. *A. salina* eggs (10mg) were incubated in 500mL of seawater under artificial light at 28°C, pH 7-8. After incubation for 24h, nauplii were collected with a pasteur pipette and kept for an additional 24h under the same conditions to reach the metanauplii (mature larvae) stage. Ten nauplii were drawn through a glass capillary and placed in each vial containing 4.5mL of brine solution. In each experiment, 0.5mL of the plant extract was added to 4.5mL of brine solution and maintained at room temperature for 24h under the light and then dead nauplii were counted [17]. Experiments were conducted along with control and five different concentrations (10-1000µg/mL) of the extract in a set of three tubes per dose. Analysis of the data was performed by EPA Probit Analysis Program (version 1.5) to determine the LC₅₀ values.

2.5. Determination of Antibacterial Activity

The microorganism strains used in this study were *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). The microorganism strains used were provided by the Department of Medical Genetics of the Medicine Faculty of Pamukkale University.

The antibacterial effects of ethanolic extracts were examined using the minimum inhibition concentration with (MIC). The broth micro dilution method microdilution method was used to determine the minimum inhibitory concentrations (MICs) of the plant extracts using 96 well microtitration plates as previously described by Satyajit et al. [18]. Bacterial strains were streaked on Mueller Hinton agar plates using sterile cotton swabs. Five microlitres of dimethylsulphoxide (DMSO), loaded on sterile blank disks were placed on the agar plates and were incubated at 37°C for 24 h. One hundred and eighty-five microliter (200 µl) of the broth was added into each well in the first row of microtitration plate and 100 µl to the rest of the wells from the second row down wards. Fifteen microliter (15 µl) of the plant extracts was then added into each well on the first row (row A), starting with the positive control (Gentamicin for bacteria and Floconazole for yeast, all the antibiotics were from MAST), followed by the negative control (the 20% DMSO used to dissolve the plant extracts) and the plant extracts in the rest of the wells on that row. A twofold serial dilution was done by mixing the contents in each well of the first row and transferring 100 µl to the second well of the same column and the same was done up to the last well of the same column and the last 100 µl from the last well was discarded. Then 100 µl of yeast suspensions was added. The results were observed after 24 h incubation at 37°C, followed by the addition of 40 µl of a 0.2% Iodo Nitro Tetra the lowest concentration at which color change occurred was taken as the MIC value. All measurements of MIC values were repeated in triplicate and the most representative values were used.

3. Results and Discussion

3.1. Antioxidant Activities

The total phenolic content content of the plant extracts is shown in Table 1. The total phenolic contents of the extracts, as estimated by the Folin-Ciocalteu reagent method, ranged from 14.5±2.02 to 53.4±2.01mg GAE/g extract. The phenolic assay involving an electron-transfer reaction was evaluated by using Folin-Ciocalteu reagent [19]. Among all plant extracts, DAE had the highest phenolic content (53.4±2.01 mg GAE/g extract), followed by DUE (37.8±1.02 mg GAE/g extract).

Table 1. Total phenolic contents (mean \pm SD) of extracts from *A. vulgare* and *D. vulgaris*

Plant extracts	Total phenolic contents (mg GAE/g extract)
AUE	37.8 \pm 1.02
AAE	53.4 \pm 2.01
DUE	14.5 \pm 2.02
DAE	18.8 \pm 3.01

The total antioxidant activity of the extracts from *A. vulgare* and *D. vulgaris* plants were determined using β -carotene linoleic acid system. This system is based on the fact that β -carotene discolors when no antioxidant is present as a result of free radicals that form hydroperoxide from linoleic acid.

Under ground-Ethanol (AUE) and (DUE) extracts showed the highest antioxidant activity. The mean antioxidant activity of AUE and DUE were 85.43 \pm 1.05% and 83.43 \pm 1.03%, respectively. Both plants extracts showed slightly low, but above ground-ethanol extracts showed lowest antioxidant activity (Table 2). These results indicated that the under ground and over ground parts of the plants have the same amount of phenolic compounds during the flowering time.

Table 2. The total antioxidant activities and IC₅₀ values of plant extracts

Plant extracts	β -carotene linoleic acid bleaching assay Total antioxidant activity (%)	DPPH assay IC ₅₀ (mg/mL)
AUE	85.43 \pm 1.05	0.089 \pm 1.02
AAE	80.21 \pm 1.03	1.075 \pm 1.04
DUE	83.43 \pm 1.03	1.064 \pm 1.03
DAE	70.76 \pm 1.08	1.095 \pm 1.07
BHT	95.64 \pm 1.01	0.012 \pm 1.03

The total antioxidant activity of *A. vulgare* was observed to be higher than that of the *D. vulgaris* extracts tested in this study. The DPPH• is a stable radical and gives maximum absorbance at 517 nm. When reduced to the hydrazine derivative by an antioxidant via electron or hydrogen atom transfer reactions, this absorption maximum decreases [20]. Percent inhibition values estimated for all the extracts are presented in Table 3. The higher percent inhibition value means the higher antioxidant activity [21].

Kadri et al., reported that the methanol-water extract of *A. vulgare* seeds possesses strong antioxidative properties *in vitro*. Results confirmed by high polyphenols and flavonoids contents and corroborated by HPLC identifications [8].

Table 3. DPPH radical scavenging activity % of the *A. vulgare* and *D. vulgaris* extracts

Extracts	Concentrations			
	0.2 mg/mL	0.4 mg/mL	0.8 mg/mL	1 mg/mL
AUE	43.98	54.32	73.09	84.32
AAE	25.87	45.46	64.08	78.43
DUE	65.67	70.65	74.87	78.86
DAE	34.75	43.76	56.98	63.54
BHT	68.34	78.98	84.79	96.93

DPPH radical scavenging activities of the ethanol extracts are shown in the Table 3. DPPH free radical scavenging activities of extracts at 0.2-1 mg/mL concentrations were compared with BHT. Additionally, the concentrations of the extracts required to scavenge 50% of the DPPH radicals, the IC_{50} values, were calculated (Table 2). The most active radical scavenger was AUE ($IC_{50} = 0.089 \pm 1.02$ mg/mL). DAE exhibited the weakest antiradical activity ($IC_{50} = 1.095 \pm 1.07$ mg/mL) in this study. The results demonstrated that there is a correlation between higher radical scavenging activity and larger amount of total phenolics in the extracts. This data is supported by previous reports, which showed that phenolic compounds generally correlate with antioxidant capacities measured by DPPH assay [22-24]. Öztürk, Aslantürk *et al.* reported that potential antioxidant activity and anticancer effect of extracts from *D. vulgaris* on mcf-7 breast cancer cells [25, 26]. Previous reports suggest that the leaves and tubers of the *Dracunculus* genus contain saponin and conicine alkaloids, estragole, phelandrine, methyl cavicol, iodine, rutin, tanin, flavonoid and coumarin [12].

3.2. Antibacterial Activity

Antibacterial activity of plant extracts against Gram-positive *Staphylococcus aureus* (ATCC 25923) and Gram-negative *Escherichia coli* (ATCC 25922) was determined using broth micro dilution method. The results of the analysis of the antibacterial activity of investigated ethanol extracts, obtained by the dilution method are given in Table 4. The obtained results showed that the tested extracts possessed different antibacterial activity within the concentration range from 50 to 100 μ g/mL. Increased concentrations of extracts caused decrease in survival of bacterial cells. Very strong reduction of gram-positive *Staphylococcus aureus* growth was observed during incubation of bacteria in *A. vulgare* under-ground extracts (MIC was < 50 μ g/mL). Lower antibacterial effect was demonstrated against gram-negative strains *E. coli* (MIC was >100 μ g/mL) in *D. vulgaris* extracts.

Table 4. Antibacterial activity of *A. vulgare* and *D. vulgaris* extracts

Extracts	MIC (μ g/mL)	
	<i>S.aureus</i>	<i>E.coli</i>
AUE	<50 μ g/mL	75 μ g/mL
AAE	100 μ g/mL	100 μ g/mL
DUE	<100 μ g/mL	> 100 μ g/mL
DAE	100 μ g/mL	70 μ g/mL
Gentamycin	10 μ g/mL	-
Ampicillin	-	20 μ g/mL

Previous studies have suggested that piperazirum alkaloid isolated from n-butanol fraction of water extract of *A. palaestinum* leaves, which is from the same family as *D. vulgaris* has a significant cytotoxic effect on human lung cancer (A549), ovarian cancer (Skov-3), melanoma (SK-MEL-2) and colon cancer (HCT- 15) cells. Also, the cytotoxic activity of the extracts from this plant on MCF-7 cells in our study may be due to the presence of these chemicals, especially the presence of flavonoids and alkaloids in extracts [28].

3.3. Cytotoxic Activity

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties. Based on the results, the ethanol extract (DUE) of *D. vulgaris* has showed good toxic to brine shrimp nauplii, with LC₅₀ of 10.60 µg/ml. In addition, the degree of lethality was found to be directly proportional to the concentration of the extract (Fig. 1).

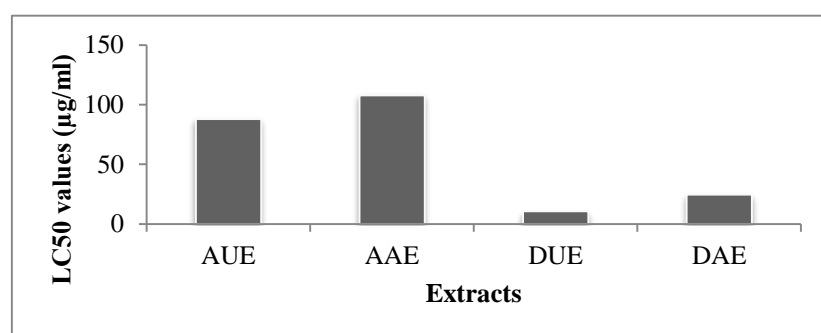


Fig 1. Cytotoxics activity of extracts by LC₅₀ values

4. Conclusion

In this study we aimed to determine the antioxidant, total phenolics, antibacterial and cytotoxic activities of *Arisarum vulgare* and *Dracunculus vulgaris* of some parts of (aerial and under ground) ethanol extracts. A further study is needed to isolate and identify the active compounds that are responsible for antioxidant activity. This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index.

5. References

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