Phytochemical analysis, phenolic content, antioxidant, antibacterial, insecticidal and cytotoxic activities of *Allium reuterianum* Boiss. extracts

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Dried bulbs and leaves of *Allium reuterianum* were extracted with different solvents and evaluated for antioxidant, antibacterial, insecticidal, *in vitro* cytotoxic activities. The obtained results indicated that the highest compound 3, 4-dihidroksi benzoic acid with 166.2 µg/g in extracts. The ethanolic extracts had the highest phenolic content and acetone extracts had the lowest fenolic content. In the β-carotene-linoleic acid test system, bulb methanol (ABM) and leaf methanol (ALM) extracts showed the highest antioxidant activity. The mean antioxidant activity of ABM and ALM were 75.76±1.22% and 73.42±1.03%, respectively. The highest ferric-reducing power of extract (ABM) was determined 12.75±0.010 trolox equivalent (mg/g). Methanolic extract (ALM) exhibited a dose-dependent scavenging of DPPH and ABTS radicals with IC50 values of 0.512±0.003 mg/mL and 0.378±0.002 mg/mL respectively. Very strong reduction of gram-positive *Staphylococcus aureus* growth were observed during incubation of bacteria in bulb extracts (MIC was 20±1.01 µg/mL). The brine shrimp lethality assay of bulb extract has showed good toxic to brine shrimp nauplii, with LC50 of 3.987 µg/mL. The bulb extract of *A. reuterianum* showed highest larvicidal activity against *Cx. pipiens* with value LC50 (6.4129 µg/mL). The results suggest that these plants could be used as a source of natural antioxidant and antibacterial agents.

**Keywords:** *Allium reuteriaum*, Antibacterial, Antioxidant, Cytotoxicite, HPLC, Insecticidal

**IPC Code:** Int. Cl.19: A01G 13/00, A61P 31/04, A61P 17/18, A61K 39/395, C07C 7/135, A01P 7/04

Extremely diverse and taxonomically difficult genus *Allium* L. is one of the largest genus in Amaryllidaceae1-7. The genus *Allium* has more than 600 species all over the world, among them just a few species have been consumed so far as vegetables, spices or ornamental plants *Allium* L. which a genus the important of between geophyta is creates a group of natural antioxidants. Since ancient times, many *Allium* species, such as onion, garlic, leek and chives, have been used as foods, spices and herbal remedies in widespread areas of the world, especially in the northern hemisphere8. *Allium* species have been used for food and medicine for thousands of years, especially *Allium* sativum (garlic) and *A. cepa* (onion), and recently interest in other species has been increasing9-11. The *Allium* genus is one of the major sources of polyphenolic compounds and the antioxidative activity of some *Allium*’s species has been reported and has been mainly attributed to a variety of organo-sulfurous compounds as wells as their precursors12,13. The many biological effects of *Allium* vegetables are mainly associated with organosulphur compounds. These compounds include four γ-glutamyl peptides: γ-l-glutamyl-S-allyl-l-cysteine (GSAC), γ-l-glutamyl-S-(trans-1-propenyl)-l-cysteine (GSPC), γ-l-glutamyl-S-methyl-l-cysteine (GSMC) and γ-glutamyl phenylalanine (γGPA). In addition, intermediate compounds in the biosynthesis of S-alk(en)yl-l-cysteine sulphoxides (ACSOs) from γ-glutamyl peptides consist of S-alk(en)yl-cysteines such as (+)-S-allyl-l-cysteine (%AC) and (+)-S-(trans-1-propenyl)-l-cysteine (SPC)14.

The species belonging to the *Allium* family have been used for a long time as a remedy for the prevention and treatment of certain diseases15. *A. reuterianum* is a perennial bulbous plant, originally from south-west Turkey. There is no report on antioxidant, antimicrobial and cytotoxic potential of *A. reuterianum* in the literature. Therefore, the aims of this study were to study phenolic acid constituent, total phenolic content and flavonoid content antibacterial, antioxidant activity, insecticidal,
cytotoxic activities of *A. reuterianum* from Turkey. Separation and quantitative determination of individual phenolic compounds was performed using high-performance liquid chromatography (HPLC).

**Materials and methods**

**Plant material**

*Allium reuterianum* Boiss (Family: Amaryllidaceae) species were collected in the spring 2015 from Kötekli locality, near Muğla province, in Turkey. The fresh bulbs and leaves of the plants samples were cleaned and dried in the shadow for extraction.

**Plant extract preparations**

Dried plant parts (bulbs and leaves) were pulverized. Each ground sample was transferred into a beaker. Ethanol, methanol and acetone were added in the ratio of 1:10 and they were put in water bath at 55ºC for 6 h. The extraction mixture was separated from the residue by filtration through Whatman No: 1 filter paper. The plant residue was re-extracted twice with ethanol, methanol and acetone. After the filtration two extracts were combined. The residual solvent of methanol, ethanol and acetone extracts of sample 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.

**Plant extracts:** Allium (A), Bulb-Methanol (ABM), Bulb-Ethanol (ABE), Bulb-Acetone (ABA), Leaf-Methanol (ALM), Leaf-Ethanol (ALE), Leaf-Acetone (ALA).

**Analysis of phenolic contents by HPLC**

Phenolic compounds were evaluated by reversed-phase High Performance Liquid Chromatography (RP-HPLC, Shimadzu Scientific Instruments). The conditions utilized were as follows: C-18 column CTO-10ASVp, 4.6 mm × 250 mm, 5 μm; mobile phase was composed of solvent A (formic acid with 3% methanol) and solvent B (100% acetonitrile); injection volume 20 μL, gradient elution from 15-100% B; run time 45 min and flow rate was 1 mL/min. For analysis, the samples were dissolved in methanol and 20 μL of this solution was injected into the column. The chromatograms were examined at 280 nm with a LC gradient detector. The phenolic compounds were recognized by comparing retention times and UV absorption spectra with those of pure standards. Gallic acid, 3,4-dihydroxy, 4-dihydroxy, chlorogenic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid and cinnamic acid were used as standard. Peaks identified by comparing retention times and UV spectra with authentic standards. The amount of each phenolic compound was expressed as μg/g of the extract.

**Total phenolic content assay**

The total phenolic content of extracts were determined with Folin- Ciocalteau reagent, according to the method of Slinkard and Singleton. 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.

**Total flavonoid content assay**

Total flavonoid content was determined using the Dowd method as adapted by Arvouet-Grand et al. (1994). For each extract, 1 mL of methanolic solution (100 μg mL⁻¹) was mixed with 1 mL of aluminium trichloride (AlCl₃) in methanol (2%). The absorbance was read at 415 nm after 10 min against a blank sample consisting of a 1 mL of methanol and 1 mL of plant extract without AlCl₃. The total flavonoid content was determined on a standard curve using quercetin as a standard. The mean of three readings was used and expressed as mg of quercetin equivalents (QE) per 100 mg of extract or fraction (mgQE/g).

**In vitro antioxidant activity**

**β-Carotene-linoleic acid assay**

The antioxidant activity of the crude extracts was evaluated using the β-carotene-linoleic acid test system with slight modifications. 0.2 mg of β-Carotene (Sigma-Aldrich) dissolved in 1 mL of chloroform was added to 20 μL of linoleic acid, and 200 mg of Tween-20 emulsifier mixture. The mixture
was then evaporated at 40°C for 10 min by means of a rotary evaporator to remove chloroform. After evaporation of chloroform, 100 mL of distilled water saturated with oxygen, 4.8 mL of this emulsion was placed into test tubes which had 0.2 mg of the sample and 0.2 units of the extract in them. For control, 0.2 mL of solvent (methanol, ethanol, acetone) was placed in test tubes instead of the extract. As soon as the emulsion was added into the test tubes, initial absorbance was measured at 470 nm with a spectrophotometer (Shimadzu UV-1601, Japanese). The measurement was carried out at 0.5 h intervals for 2 h. All samples were assayed in triplicate. BHT (Butylated hydroxy toluen) was used as standard. The antioxidant activity was measured in terms of successful bleaching of β-carotene by using the following equation:

\[
AA = \left[ 1 - \frac{(A_0 - A_t)}{(A_{0o} - A_{to})} \right] \times 100
\]

Where AA is the total antioxidant activity, A0 is the initial absorbance of the sample, At is the initial absorbance of the control, A0o is the sample’s absorbance after 120 min, and Ato is the control’s absorbance after 120 min.

**DPPH free radical scavenging activity assay**

Free radical scavenging activity of the extracts was determined using the free radical DPPH. 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 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:

\[
\text{Scavenging activity} = \left[ \frac{(A_{0} - A_{1})}{A_{0}} \times 100 \right]
\]

where \(A_0\) is the absorbance of the control (blank without extract) and \(A_1\) is the absorbance in the presence of the extract. The results were expressed as IC_{50} (the concentration required to inhibit 50% of the DPPH).

**Ferric-reducing antioxidant power (FRAP) assay**

The reducing power of the extracts was determined according to the method described by Oyaizu (1986). The different concentrations (40-150 μg/mL) of extracts were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide ([K₃Fe(CN)₆]) (1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of TCA (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm (MSE Mistral 2000, UK). The supernatant of solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of FeCl₃ (0.1%). The Fe³⁺/Fe²⁺ transformation was investigated in the presence of extracts or standards and the absorbance values were measured at 700 nm. Phosphate buffer (pH 6.6) was used as blank solution. BHT were used as standard. The FRAP was expressed as trolox equivalents in mg/g of samples used.

**ABTS free radical scavenging assay**

Experiments were performed according to Re et al. (1999) with small modifications. ABTS and potassium persulfate were dissolved in distilled water to a final concentration of 7 mM and 2.45 mM, respectively. These two solutions were mixed and the mixture was allowed to stand in the dark at room temperature for 16 h before use in order to produce ABTS radical (ABTS•⁺). For the study of phenolic compounds, the ABTS radical solution was diluted with distilled water to an absorbance of 1.00 at 734 nm. After the addition of 10 μL of sample to 4 mL of diluted ABTS solution, the absorbance was measured at 30 min. All samples were analyzed in triplicate. The ABTS radical-scavenging activity of was measured using the equation below:

\[
A\% = \left( \frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100
\]

where Acontrol is the absorbance of the blank control (ABTS solution without test sample) and Asample is the absorbance of the test sample.

**Antibacterial activity**

**Bacterial strains, culture media and growth condition**

The antibacterial activity of the plant extract was tested in vitro against the following bacteria: Gr(+) and Gr(–): *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were employed in the study. 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. The antibacterial
effect of *A. reuterianum* ethanolic extract was examined using the minimum inhibition concentration (MIC) broth micro dilution method. The microdilution method was used to determine the minimum inhibitory concentrations (MICs) of the plant extracts using 96 well microtitration plates as previously with some modification described by Ramalivhana (2014)\(^23\). One hundred and eighty-five microliter (185 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 (20% DMSO used to dissolve the plant extracts) and 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. Determination of the minimum inhibitory concentration (MIC) of the extract and cirsimarin against the test bacteria were determined by microdilution method in 96 multi-well microtiter plates\(^24\). Plates were wrapped loosely with cling film to ensure that bacteria did not become dehydrated and prepared in triplicate, and then they were placed in an incubator at 37°C for 24 h for the bacteria and at 28°C for 48 h for the yeast. Subsequently, color change was assessed visually. Any color change from purple to pink or colorless was recorded as positive. 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.

**Cytotoxic activity**

Brine shrimp lethality test (BSLT) was applied to analyze the possible cytotoxic activity of the extracts. *A. salina* eggs (10 mg) were incubated in 500 mL of seawater under artificial light at 28°C, pH 7-8. After incubation for 24 h, nauplii were collected with a pasteur pipette and kept for an additional 24 h 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 4.5 mL of brine solution. In each experiment, 0.5 mL of the plant extract was added to 4.5 mL of brine solution and maintained at room temperature for 24 h under the light and then dead nauplii were counted\(^25\). Experiments were conducted along with control and five different concentrations (10-1000 μg/mL) of the extract in a set of three tubes. The±data was performed by EPA Probit Analysis Program (version 1.5) to determine the LC\(_{50}\).

**Insectisidal activity of extracts against the larvae of *Culex pipiens* L. (Diptera: Culicidae)**

**Mosquito culture**

*Cx. pipiens* used in the assays originated from Arapsuyu, Antalya, and were collected from a pool in August 2015. The larvae were reared at 12:12 light/dark photoperiod, (60±10)% RH, and (26±2) in an insectary in the Biology Department, Akdeniz University. The third-fourth instar larvae were used for bioassays.

**Larvicidal assays**

Larvicidal activity of the extracts against *Cx. pipiens* was assessed by using the method described by Cetin and Yanikoglu\(^26\). For experimental treatment, 0.5 g of each extract was dissolved in 500 mL distilled water. A series of concentrations ranging from 100 to 1000 ppm of dissolved extract were prepared. The extract-water solution was stirred for 30 s with a glass rod. After approximately 5 min, 20 larvae taken on a strainer with fine mesh were transferred gently to the test medium by tapping. Three replicates of each concentration were run at a time. Mortality was recorded after, 24-, 48- and 72-h of exposure, during which fish food was given to the larvae. All experiments were conducted at (26±2)°C and (60±10)% relative humidity with 12:12 D:L photoperiod. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface (within a reasonable period of time) or showing the characteristic diving reaction when the water was disturbed. Larvae were also observed for discoloration, unnatural positions, uncoordination, or rigor.

**Statistical analysis**

All analyses and tests were run in triplicate and mean values recorded. All the experimental data are presented as mean ± SEM of three individual samples.
Data are presented as percentage of inhibition or radical scavenging on different concentration of *A. reuterianum*. IC$_{50}$ and LC$_{50}$ (the concentration required to scavenge 50% of free radicals) value was calculated from the dose-response curves. All of the statistical analyses were performed by means of Microsoft Office Excel 2010 software and SPSS. The results were evaluated using an unpaired t-test and one way analysis of variance ANOVA. The differences were regarded as statistically significant at $p < 0.05$.

**Result**

The phenolic compounds contained in *A. reuterianum* ethanolic extracts were characterized using HPLC methods. Of the 9 standard phenolics analyzed, 9 were identified in the extracts. The major phenolic groups included gallic acid, 3,4-dihydroxy benzoic acid, 4-dihydroxy benzoic acid, chlorogenic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid and cinnamic acid. The HPLC chromatogram of *A. reuterianum* is presented in Table 1. The obtained results indicated that the highest compound 3,4-dihydroxyksi benzoic acid with 166.2 µg/g was in *A. reuterianum* extracts. It is clear that investigated *A. reuterianum* extracts possessed higher concentrations of benzoic acid derivatives (166.2 µg/g) than derivatives cinnamic acid (p-Coumaric acid with 17.4 µg/g) (Table 1).

**Total phenolic and flavonoid contents**

The total phenolic and flavonoid content of the ethanol, methanol and acetone of bulb and leaves extracts is shown in Table 2. The ethanolic extracts had the highest phenolic content and acetone extracts had the lowest phenolic content. The phenolic assay involving an electron-transfer reaction was evaluated by using Folin-Ciocalteu reagent. Among all plant extracts, ABE had the highest phenolic content (9.46±2.025 mg GAE/mL extract), followed by ALE (6.48±3.012 mg GAE/mL extract). Other extracts phenolic contents were ABE > ALE > ALM > ABM > ABA > ALA respectively and have nearly the same amounts of phenolic contents. The results indicated that ABE (48.16±3.025 mgQE/g) has the highest and ALA (5.21±4.052 mgQE/g) has the lowest flavonoid contents. Other extracts flavonoid contents were ABE > ABM > ALM > ALE > ABA > ALA respectively.

**In vitro antioxidant activity**

**β-Carotene-linoleic acid assay**

The total antioxidant activity of the extracts from *A. reuterianum* plant was 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. Bulb methanolic (ABM) and leaf methanolic (ALM) extracts showed the highest antioxidant activity. The mean antioxidant activity of ABM and ALM were 75.76 ± 1.22% and 73.42 ± 1.03%, respectively. Both plants methanolic extracts showed slightly low, but acetonic extracts showed lowest antioxidant activity (Fig. 1). 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.

**DPPH free radical scavenging activity**

IC$_{50}$ values for DPPH scavenging activity of extracts are given in Table 3, as calculated from the percent inhibition versus concentration of extract curves. Table 3 according to the results, the leaf methanolic extract (ALM) from *A. reuterianum* exhibited good antioxidant activity with an IC$_{50}$ value (0.512±0.003 mg/mL) against DPPH.

**ABTS free radical scavenging assay**

It exhibited the lowest IC$_{50}$ value using the ABTS methods (IC$_{50}$ 0.378±0.002 mg/mL). The highest

**Table 1 — HPLC analysis of extracts for phenolic contents**

<table>
<thead>
<tr>
<th>Phenolic standard compounds</th>
<th>Standard retention time RT (min)</th>
<th><em>A. reuterianum</em> (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>7.8±0.00</td>
<td>32.1±0.00</td>
</tr>
<tr>
<td>3,4-dihidroksi benzoic acid</td>
<td>12.2±0.00</td>
<td>166.2±0.00</td>
</tr>
<tr>
<td>4-dihidroksi benzoic acid</td>
<td>16.9±0.00</td>
<td>140.2±0.00</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>19.4±0.00</td>
<td>62.2±0.00</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>21.7±0.00</td>
<td>26.6±0.00</td>
</tr>
<tr>
<td>Caffeic acid (3,4- dihydroxy cinnamic acid)</td>
<td>24±0.00</td>
<td>74.4±0.00</td>
</tr>
<tr>
<td>p-coumaric acid</td>
<td>29.3±0.00</td>
<td>17.4±0.00</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>34.7±0.00</td>
<td>38.5±0.00</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>70.7±0.00</td>
<td>115.0±0.00</td>
</tr>
</tbody>
</table>

**Table 2 — Total phenolic and total flavonoid contents of extracts**

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Total phenolic content (mg GAE/mL extract)</th>
<th>Total flavonoid content (mgQE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABE</td>
<td>9.46±2.025</td>
<td>48.16±3.025</td>
</tr>
<tr>
<td>ALE</td>
<td>6.48±3.012</td>
<td>10.55±5.032</td>
</tr>
<tr>
<td>ABM</td>
<td>4.81±5.014</td>
<td>42.22±7.071</td>
</tr>
<tr>
<td>ALM</td>
<td>5.04±4.018</td>
<td>25.47±4.059</td>
</tr>
<tr>
<td>ABA</td>
<td>3.42±1.013</td>
<td>6.51±3.042</td>
</tr>
<tr>
<td>ALA</td>
<td>2.33±4.024</td>
<td>5.21±4.052</td>
</tr>
</tbody>
</table>
activity was identified for the ALM methanolic leaves extract (Table 3).

**Ferric-reducing antioxidant power (FRAP) assay**

The Ferric-Reducing Antioxidant Power (FRAP) was expressed as trolox equivalent or known Fe(II) concentration for the extracts of *A. reuterianum*. The highest FRAP in the *A. reuterianum* extracts were determined 10.25±0.005 (ABE) trolox equivalent (mg/g) (Tables 3).

**Antibacterial activity**

Minimum Inhibitory Concentration (MIC) is defined as the highest dilution or least concentration of the extracts that inhibit growth of organisms. MICs were determined for three selected indicator strains: *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). The results of the analysis of the antibacterial activity of investigated *A. reuterianum* methanolic 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 25-50 μg/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. reuterianum* bulb extracts (MIC was 20±0.07 μg/mL).

**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 methanol extract (ABM) of *A. reuterianum* has showed good toxic to brine shrimp nauplii, with LC_{50} of 3.987 μg/mL. In addition, the degree of lethality was found to be directly proportional to the concentration of the extract (Fig. 2).

**Insectisidal activity**

Toxicities of methanol extracts from *A. reuterianum* to young (second and third) instar *Cx. pipiens* larvae were noted and the LC_{50} confidence limits for 24, 48 and 72 h were calculated. Doses were determined based on the preliminary studies that yielded between 20 and 100% larval mortalities. After a 24 h exposure, ethanolic extracts revealed various larvicidal activities according to the tested concentrations. At 1000 ppm, the extracts of plant caused 100% mortality against *Cx. pipiens* larvae (Table 5). Bulb extract was more toxic than leaf extract on young and older larval instars. The bulb extract of *A. reuterianum* showed highest larvicidal activity against *Cx. pipiens* with value LC_{50} (6.4129 units) (Table 6).

**Discussion**

*Allium* species vegetables are generally rich in secondary metabolites such as organosulfur and...
phenolic compounds. Allicin, diallyl disulphide and diallyl trisulphide appeared to be the main active ingredients of *Allium* spp. Typically many biological effects of *Allium* spp. are related to the thiosulfinates and volatile sulfur compounds. However, these compounds are not stable and give rise to product transformation. For this reason, recent studies have focused on polar compounds such as polyphenols that are more stable in cooking and storage.

Other than five *Allium* species (*A. obliquum* L., *A. senescens* L. subsp. *montanum* Holub, *A. schoenoprasum* L. subsp. *schoenoprasum*, *A. fistulosum* L. and *A. ursinum* L.) were analysed in order to determine the presence of 19 polyphenolic compounds through an HPLC method coupled with UV and mass spectrometry detection. Luteolin and apigenin were identified before and after hydrolysis of sulphur-containing compounds (alliin, γ-A. fistulosum

Table 5 — Larvicidal activity the ethanol extracts of *A. reuterianum* against *Cx. pipiens* (% Mortality±SE)

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Exposure times (h)</th>
<th>0</th>
<th>100</th>
<th>250</th>
<th>500</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulbs</td>
<td>24</td>
<td>1.3±1.3</td>
<td>30.0±5.7</td>
<td>40.0±10.0</td>
<td>60.0±5.7</td>
<td>80.0±3.3</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>2.6±2.6</td>
<td>40.0±5.7</td>
<td>46.6±6.6</td>
<td>65.0±2.8</td>
<td>86.6±3.3</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>3.6±3.6</td>
<td>53.3±3.3</td>
<td>60.0±0.0</td>
<td>76.6±6.6</td>
<td>100.0±0.0</td>
</tr>
<tr>
<td>Aerial parts</td>
<td>24</td>
<td>1.3±1.3</td>
<td>26.6±8.8</td>
<td>36.6±8.8</td>
<td>90.0±5.7</td>
<td>90.0±5.7</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>2.6±2.6</td>
<td>36.6±8.8</td>
<td>43.3±8.8</td>
<td>93.3±6.6</td>
<td>95.0±2.8</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>3.6±3.6</td>
<td>50.0±5.7</td>
<td>86.6±3.3</td>
<td>96.6±3.3</td>
<td>100.0±0.0</td>
</tr>
</tbody>
</table>

Table 6 — LC₅₀ (24, 48 and 72 h) values (µg/mL) of the extracts against *Cx. pipiens*

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Time (h)</th>
<th>LC₅₀</th>
<th>% 95 CL (LCL-UCL)</th>
<th>LC₉₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48</td>
<td>7.2156</td>
<td>461.9601-91.393</td>
<td>13.555</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>6.4129</td>
<td>511.7649-84.647</td>
<td>104.3865</td>
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<tr>
<td>Aerial part</td>
<td>24</td>
<td>146.8987</td>
<td>22.9897-746.946</td>
<td>36.9101</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>81.8139</td>
<td>0.2793-1.600</td>
<td>1.3±1.3</td>
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<tr>
<td></td>
<td>72</td>
<td>24.2661</td>
<td>736.987-632.847</td>
<td>30.0±5.7</td>
</tr>
</tbody>
</table>

Previous studies have also reported differences in the phenolic levels of different anatomical parts of several plant species. Phenolic compounds belong to a group of natural substances found in dietary products, and these compounds have gained considerable attention due to their potent antioxidant activity.

In recent years, antioxidant and antimicrobial properties of plants products have been of great interest in food industry and pharmacology. Due to the side effects of synthetic materials in human health, there is a growing tendency to use natural antioxidant compounds derived from different plant species. The present study was the first investigation of the antioxidant, cytotoxic, insecticidal and antibacterial activities of *A. reuterianum* extracts through comprehensive *in vitro* methods. The results showed that extracts had antioxidant other activities. Significant positive correlations were observed between antioxidant activities as determined by β-carotene linoleic acid, DPPH, FRAP and ABTS assays. These positive correlations indicate that the higher total phenolic contents resulted in higher total antioxidant activity. According to Nuutila et al., the linear correlation between antioxidant activity and polyphenol content underlie the fact that phenolic compounds of *Allium* plants contribute to their antioxidative effects.

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extracts species (A. nevsehirense, A. sivasicum, A. scorodoprasum subsp. rotundum and A. atrovioviolaceum), measured by DPPH, showed an IC$_{50}$ ranged between 79 and 104 μg/mL with an efficiency of 3.95 (IC$_{50}$ extract/IC$_{50}$ BHT)$^{36}$. The methods involving an electron-transfer reaction include the DPPH radical-scavenging, ferric-reducing antioxidant power and β-carotene-linoleic acid assay$^{37}$. In another study, Aydın et al. reported that total phenolic contents, antioxidant and antibacterial activities activities of A. deciduum, A. sibthorpianum and A. stylosum. According to the results of antioxidant activity, bulbs extracts exhibited higher antioxidant activity than leaves extracts from all types of solvent$^{38}$.

Reducing power was measured by direct electron donation in the reduction of Fe$^{3+}$(CN)$_6$/Fe$^{2+}$(CN)$_6$. The product was visualized by forming the intense Prussian blue color complex and then measured at 700 nm$^{39}$.

Several studies have been carried out to determine the antimicrobial activity of extracts and compounds isolated from various Allium species. Many researchers later found that oils of Alliums and their constituting sulfides have significant antimicrobial effects and are much more antifungal than antibacterial. Sökmen et al. tested the in vitro antimicrobial activities of various plants which also include Allium scorodoprasum on B. cereus, E. coli, S. aureus, Branhamella catarrhalis, Clostridium perfringens and C. albicans. A. scorodoprasum shows an inhibitor effect on only C. albicans among the test organisms, they reported that it does not have any effect on others. Sulphur and polyphenols present in garlic respond to antibacterial, antifungal and antioxidant activity was carefully studied in previous reports$^{40,41}$. Previous literature review revealed that this functionality of Allium plants extracts might be ascribed to the presence of naturally occurring products, organosulfur or sulfur-containing compounds (OSCs), especially, garlic compounds (GCs) and isothiocyanates (ITCs), represent two important and promising chemopreventive in various in vivo and in vitro models and allicin-decomposition products were shown to have potential anticancer activities and may be responsible for some beneficial properties of these plants$^{42}$.

The insecticidal activity of plant based products (extracts) against different mosquito species has been evaluated by many authors$^{43}$. This study is the first to report on the larvicidal activity of the extracts of A.reuterianum species against Cx. pipiens.

The results show that this botanical natural product could be used in mosquito control instead of synthetic larvicides. The use of botanicals in mosquito control is an alternative pest control method for minimizing the noxious effects of some pesticidal compounds on the environment.

Conclusions

In the present study, significant antioxidant and cytotoxic activities were revealed in the investigated species. It is concluded from present investigation that all the extracts/fractions (ethanol, methanol and acetone) of A. reuterianum bulbs and leaves showed considerable biological activities of plant exhibited good antioxidant and cytotoxic activity. Higher levels of total phenolics of plant are probably responsible from the biological activities observed. This finding candidates the plant as a good case for more in-depth studies and we wish our future research lead to the identification of biologically active molecules present in its extracts. The extracts of plants exhibited cytotoxic activity against the brine shrimp Artemia salina. This plant might be used as raw material for therapeutic purposes in future.

References


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