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Phytochemical analysis, phenolic content, antioxidant, antibacterial, insecticidal and cytotoxic activites 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 IC_{50} 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 LC_{50} of 3.987 µg/mL. The bulb extract of *A. reuterianum* showed highest larvicidal activity against *Cx. pipiens* with value LC_{50} (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

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Extremely diverse and taxonomically difficult genus *Allium* L. is one of the largest genus in Amaryllidaceae[1-7]. 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, northern hemisphere[8]. *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 increasing[9-11]. The *Allium* genus is one of the major sources of polypphenolic compunds 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 precursors[12,13]. 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 S-alk(en)yl-cysteines AC) and (+)-S-(trans-1-propenyl)-lcysteine (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 diseases[15]. *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,

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cytotoxic activites 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[16]. 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, vanilic 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[17]. 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) Na_2CO_3 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)[18]. For each extract, 1 mL of methanolic solution (100 µg mL^{-1}) was mixed with 1 mL of aluminium trichloride ($AlCl_3$) 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 $AlCl_3$. 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[19]. 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 40ºC 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=[1- (A0-At / A0o - Ato) x 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[20]. 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:\

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

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)[21]. 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 $[K_3Fe(CN)_6]$ (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 $FeCl_3$ (0.1%). The Fe^{3+}/Fe^{2+} 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[22]. 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% = (Acontrol – Asample)/Acontrol)×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(+); *Staphylococcus aureus* (ATCC 25923) and Gr(-); *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 37ºC for 24 h. The antibacterial

effect of *A. reuterianum* ethanolic extract was examined using the minimum inhibition concentration with (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 28ºC, 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}.

İnsectisidal 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)℃ 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, vanilic 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 GAEs/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

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

Table 2 — Total phenolic and total flavonoid contents of extracts

Plant extracts	Total phenolic content (mg GAE/mL extract)	Total flavonoid content (mgQE/g)
ABE	9.46±2.025	48.16±3.025
ALE	6.48±3.012	10.55±5.032
ABM	4.81±5.014	42.22±7.071
ALM	5.04±4.018	25.47±4.059
ABA	3.42±1.013	6.51±3.042
ALA	2.33±4.024	5.21±4.052

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).

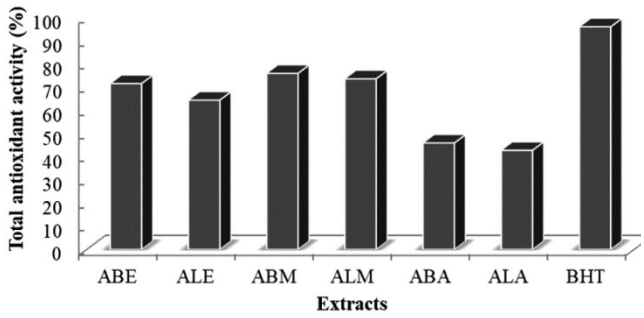

Fig. 1 — The total antioxidant activities different extracts

Table 3 — Antioxidant activity of the *A. reuterianum* extracts

	IC_{50}, mg/mL		Trolox equivalent (mg/g)
Extracts	DPPH	ABTS	FRAP
ABE	0.567±0.003	0.494±0.005	10.25±0.005
ALE	0.595±0.010	0.509±0.020	9.73±0.020
ABM	0.849±0.011	0.812±0.005	12.75±0.010
ALM	0.512±0.003	0.378±0.002	5.43±0.003
ABA	0.892±0.015	0.753±0.011	6.89±0.006
ALA	0.902±0.021	0.895±0.020	5.52±0.004

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-tümor 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).

İnsectisidal 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

Table 4 — Antibacterial activity of the *A. reuterianum* ethanol extracts

Microorganism	Extract	MIC (µg/mL)
S.aureus ATCC 25923	Aerial parts	50±0.01
	bulbs	20±0.07
E.coli ATCC 25922	Aerial parts	25±0.03
	bulbs	25±1.06

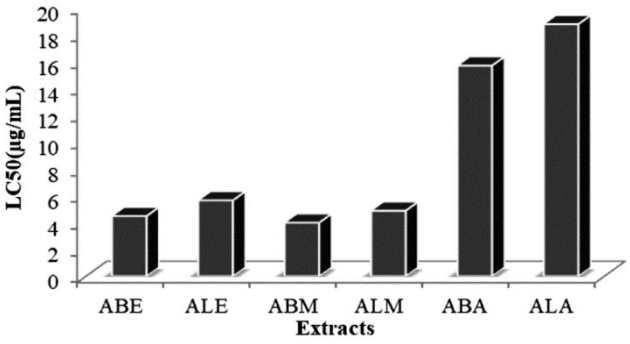

Fig. 2 — Cytotoxics activity of *A.reuterianum*

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

Extracts	Exposure times (h)	Test concentrations (ppm) 0	100	250	500	1000
Bulbs	24	1.3±1.3	30.0±5.7	40.0±10.0	60.0±5.7	80.0±3.3
	48	2.6±2.6	40.0±5.7	46.6±6.6	65.0±2.8	86.6±3.3
	72	3.6±3.6	53.3± 3.3	60.0±0.0	76.6±6.6	100.0±0.0
Aerial parts	24	1.3±1.3	26.6±6.6	36.6±8.8	86.6±3.3	90.0±5.7
	48	2.6±2.6	36.6±8.8	43.3±8.8	93.3±6.6	95.0±2.8
	72	3.6±3.6	50.0±5.7	86.6±3.3	96.6±3.3	100.0±0.0

Table 6 — LC_{50} (24,48 and 72 h) values (µg/mL) of the extracts against *Cx. pipiens*

Extracts	Time (h)	LC_{50}	% 95 CL (LCL-UCL)	LC_{90}
Bulb	24	16.2688	868.6723-19.043	602.8739
	48	7.2156	461.9601-91.393	13.555
	72	6.4129	511.7649-84.647	104.3865
Aerial part	24	146.8987	22.9897-746.946	36.9101
	48	81.8139	0.2793-1.600	1.1004
	72	24.2661	736.987-632.847	520.1906

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[27].

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 only in *A.obliquum*[28]. The antioxidant activity of *Allium* species has been attributed mainly to a variety of sulphur-containing compounds (alliin, γ-glutamylcysteine, diallyl sulfide, diallyl disulfide etc.) and proteins (lectins) as their precursors[29], but it is also related to other bioactive compounds: dietary fibres, microelements and polyphenols. Several studies have demonstrated that phytochemicals belonging to many different classes, including phenolic compounds, terpenes, polysulfides, organosulfur compounds, indole compounds, modified purines, quinones and polyamines, have longevity-extending effects[30].

Previous studies have also reported differences in the phenolic levels of different anatomical parts of several plant species[31]. 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.[32], the linear correlation between antioxidant activity and polyphenol content underlie the fact that phenolic compounds of *Allium* plants contribute to their antioxidative effects.

Previous studies have reported the quantification of bioactive sulphur compounds and their physicochemical characteristics in *Allium* vegetables. Fang et al.[33] reported a new phenylpropanoid glucoside and a chain compound from the roots of *A. tuberosum*. Additionally, Tan et al. (2015)[34] investigated the structural properties of γ- glutamyl-S-allyl-cysteine peptide isolated from fresh garlic scales (*A. sativum* L.). Also, Kim et al. (2016) reported profiling of organosulfur compounds and antioxidant activities in *A. hookeri*[35]. Previous study on the antioxidant activity of five *Allium* methanolic

extracts species (*A. nevsehirense*, *A. sivasicum*, *A. scorodoprasum* subsp. *rotundum* and *A. atroviolaceum*), 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 an 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, orgonosulfur 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 producuts was 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.

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